Phylogeny, Biogeography and Systematics of Loasaceae subfam. Loasoideae

Dissertation

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“...from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.”

Charles Darwin, The Origin of Species (1859), p. 490.

Nasa connectans, in its habitat, near the summit of the Chilola. This species is known from only two localities in El Oro Province in Southern Ecuador. It is probably endangered due to its diminutive range, like many other species *Nasa.*

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CHAPTER 1

General Introduction

1.1.Foreword

Plants represent the vast majority of the biomass on the planet and they dominate most of the terrestrial ecosystems (Bar-On, Phillips, & Milo, 2018). Human well-being depends considerably on the available biological richness (Naeem *et al.*, 2016), and particularly for plants, the importance of biodiversity cannot be overstated. The loss of biodiversity result of the, currently underway and human induced, sixth mass extinction, is considered one of the main environmental issues that humanity faces (Ceballos *et al.*, 2015). Biodiversity is not uniformly spread across Earth's surface and different areas have not only different species compositions, but also experience different outcomes from human pressure (Haddad *et al.*, 2015). The biologically richest and most endangered areas on Earth are called "hotspots" (Mittermeier *et al.*, 2004). Globally 35 hotspots are recognized (Marchese, 2015). These are defined as areas with at least 1500 endemic vascular plant species and with 30% or less of their original vegetation remaining (Brooks *et al.*, 2002; Mittermeier *et al.*, 2004). The hotspots cover just under 12% of Earth surface but harbor ca. 44% of the species of vascular plants (Mittermeier *et al.*, 2004).

It is argued that nowadays plants species are becoming extinct at rates 1000-10000 times higher than the background rate, i.e. outside mass extinction events (Pimm & Joppa, 2015). Many of the plants that may go (and may have gone already) extinct will not be described formally (Pimm & Raven, 2017), especially in the areas where most of the biodiversity is found. Coincidentally, many of the areas with the highest number of undescribed species coincide with the currently recognized biodiversity Hotspots (Joppa *et al.*, 2011)

The Tropical Andes are possibly the most species-rich of the biodiversity hotspots, and although its original vegetation covered 1 542 644 km^2, it is now reduced to 385 661 km^2 (Mittermeier *et al.*, 2004). It includes part of both the Costa Rica-Chocó (that extends from Costa Rica to northwestern Ecuador) and Tropical Eastern Andes (that extend from southeastern Colombia to northeastern Peru) centers of plant diversity, two of the five most important centers of global plant diversity with over 5000 spp / 10000 km^2 (Barthlott *et al.*, 2007).

The botanical studies in the tropical Andes have a long tradition and one of the first chroniclers of the Americas, Gonzalo Fernández de Oviedo, compiled valuable information about plants from Tierra Firme and Nueva Castilla already in the first half of the 16th century, although the information he received about Andean plants was published, for the most part, long after his death (Fernández de Oviedo, 1851). Nowadays, the knowledge about the tropical Andean flora is the most complete that it has ever been (Lasser [founding ed.], 1964-ongoing; Harling & Sparre [founding eds.], 1973-ongoing; Pinto [founding ed.], 1983-ongoing; Brako & Zarucchi, 1993; Jørgensen & León-Yánez, 1999; Hokche, Berry & Huber, 2008; Neill & Ulloa-Ulloa, 2011; Bernal, Gradstein & Celis, 2015; Jørgensen, Nee & Beck, 2015) while remaining among the least understood on a global scale, with some estimates considering that 35% of the total number of undiscovered plant species worldwide could inhabit this area (Joppa *et al.*, 2011). Considerable efforts have also been invested to catalogue the threatened flora in the Andean countries (Calderón, Galeano & García, 2002, 2005; Llamozas *et al.*, 2003; León, Pitman & Roque, 2006; León-Yánez *et al.* 2011). It seems likely, however, that these represent an underestimation of the actual number of threatened taxa, as the Tropical Andes are, very likely, one of the areas with the highest number of species at risk (Pimm & Joppa, 2015).

1.2.The Andes

1.2.1.Andean orogeny

The Andes are the longest subaerial mountain chain of the world with over 8000 km and 66 degrees of latitude in length (Borsdorf & Stadel, 2015). They extend along the entire western margin of South America, from the Caribbean in N Venezuela to the Magellan Strait (Fig. 1). They are also, by far, the most extensive mountain range in the tropics. While tropical Africa and Malesia have important mountainous areas (Rift Valley Area, Bornean Highlands, New Guinean Highlands), their high elevation surface areas are much less extensive and connected than that of the Andes (Sklenář, Hedberg, & Cleef, 2014), which have extensive areas above 3000 m elevation. This has allowed the development of the richest tropical alpine biota in the world (Sklenář, Dušková, & Balslev, 2011). The abrupt contrasts in topography, precipitation, solar exposure, geology and soils across short distances, combined with their tropical position, provide high levels of geodiversity (Barthlott *et al.*, 2005), that in turn, allow enormous biotic diversity (Josse *et al.*, 2011; Mutke *et al.*, 2014; Hughes, Nyffeler, & Linder, 2015; Mutke & Weigend, 2017).

This mountain range (specifically its tropical portion) is considered as one of the most important biodiversity hotspot for the terrestrial biota on Earth (Hughes, 2016; Lagomarsino *et al.*, 2017; Pérez-Escobar *et al.*, 2017; Bacon *et al.*, 2018) and as a major geographic feature, it plays a major role in affecting the climate on continental and planetary scales (Xu, Wang, & Xie, 2004; Poulsen, Ehlers, & Insel, 2010; Maroon, Frierson, & Battisti, 2015; Armijo *et al.*, 2015; Naiman *et al.*, 2017). This region includes a huge range of the precipitation and temperature regimes, from the Equatorial to the Cold Temperate zones, and is adjacent to both the wettest (Chocó) and driest (Atacama) areas of the New World (Borsdorf & Stadel, 2015).

The Cenozoic geology of the Andes is complex and often poorly understood (Barnes & Ehlers, 2009). Even in relatively well studied areas such as the Altiplano, the processes of uplift and development of the mountain ranges have been subject to considerable controversy, with different studies presenting contrasting and seemingly opposing results (Barnes & Ehlers, 2009) while some areas, such as the Central and Western Cordilleras of Colombia, remain very poorly studied (Richardson *et al.*, 2018).

The uplift processes must be seen as a series of often independent, yet related, episodes distributed in space and time along the western margin of South America (Gregory-Wodzicki, 2000; Graham, 2009; Bermúdez *et al.*, 2010; Eude *et al.*, 2015; Folguera *et al.*, 2016). Regardless, some major trends have generally been agreed upon by geologists, e.g., the general progression of the orogenic processes from south to north and from west to east (Gregory-Wodzicki, 2000; Graham, 2009; Folguera & Ramos, 2011; Gianni *et al.*, 2016). During the Cretaceous (ca. 100-70 Ma) compression and uplift had started at the southern Andes, caused by the westward movement of South America following the West Gondwanan break up (Gianni *et al.*, 2016) and the closure of the Rocas Verdes Basin, near the southernmost tip of the continent (Ghiglione, 2016). By the end of the

Cretaceous (ca. 70 Ma) the collision of the Caribbean Large Igneous Province appears to have caused uplift in the North Andean Block (Martin-Gombojav & Winkler, 2008; Villagómez & Spikings, 2013). Deformational events took place in the southernmost Central Andes by the Paleocene (Giambiagi *et al.* 2016), and by the Eocene (after ca. 49 Ma), the so called Incaic deformation could have affected the Western Central Andes (Gregory-Wodzicki, 2000; Herrera *et al.*, 2017), with some researchers claiming that portions of this mountain chain may have reached ca. 4 km in elevation ca. 35 Ma (Quade *et al.*, 2015), but evidence of such high elevations that early in the Cenozoic is controversial. Around at the same time, the exhumation rates of rocks in Andean Ecuador and Colombia also increased (Villagómez & Spikings, 2013). Much of the published research nowadays seems to agree that very rapid uplift pulses have happened since the Neogene. Even the rather ancient Patagonian Andes reached enough height to produce a rain shadow effect just ca. 16 Ma (Gianni *et al.*, 2016), meanwhile the major crustal shortening of the Andes of Central Chile and Argentina happened after 21 Ma, before it shifted eastwards (Giambiagi *et al.*, 2016) towards the Frontal Cordillera, the Precordillera and the Sierras Pampeanas successively. According to Gregory-Wodzicki (2000) and Graham (2009) over 50% of the current elevation of the tropical Andes was attained just in the last ca. 10 million years. In the case of the relatively well studied Altiplano, some researchers claim that the area has been subject to gradual uplift lasting 40 or more of millions of years (McQuarrie *et al.*, 2005), while others mention most of the uplift happened in the last 15-10 My (Ghosh, 2006). We must consider that there is evidence that different parts of the Altiplano had different uplift rates at different times (Lamb, 2016) and may have gone through several uplift pulses (Leier *et al.*, 2013). The Western Cordillera may have reached elevations above 2000 m as far back as 20 Ma while the Eastern Cordillera did so later, 13 Ma, in the Southern Altiplano, and just in the last ca. 5 My in its northern portion (Garzione *et al.*, 2017). The onset of the subduction of the Nazca Ridge ca. 15 Ma, appears to have been an important factor in influencing the uplift of the Cordillera Occidental in Áncash (Margirier *et al.*, 2015), which attained elevations of ca. 3500 m by the Pliocene. Evidence that topographic relief in the Marañón Fold-and-thrust system was significant as far back as the Eocene, has not been confirmed and significant surface uplift appears to have been achieved only after the Oligocene (Michalak *et al.*, 2016).

In Colombia the Northern Andes split in three branches. The Eastern Cordillera attained considerable elevation just in the last 5 million years (Gregory-Wodzicki, 2000), result of some of the most recent pulses in Andean Orogeny, but the Western and Central Cordilleras of Colombia appear to have had experienced uplift and deformation periods dating as far back as the Cretaceous when the Caribbean collided with the South American plate (see above). Paleoelevation data for the Central and Western Cordilleras are very scanty (Gregory-Wodzicki, 2000; Restrepo-Moreno *et al.*, 2009; Richardson *et al.*, 2018), and although both ranges have undergone several periods of significant exhumation (e.g. ca. 30-20 Ma), the extent of the uplift, remains difficult to determine (Restrepo-Moreno *et al.*, 2009), but rapid rock uplift and exhumation starting 15 Ma, has been suggested for parts of the Eastern Andes in Ecuador, probably result of the initial subduction of the Carnegie ridge under South America (Spikings *et al.*, 2001).

At the northernmost Andes, palynological evidence analyzed by Bermúdez *et al.*, (2017), indicates that surface elevations up to ca. 4000 m may have existed in the Cordillera de Mérida as far back as the Late Miocene (ca. 7 Ma) preceding similar elevations in the Eastern Andes of Colombia. For the Sierra de Santa Marta (usually not considered part of the Andes), the earliest evidence of exhumation and uplift dates back to the time when the Caribbean collided with Northern South America by the end of the Mesozoic. The highest exhumation rates happened between 29-16 Ma, although very rapid uplift took place in the last million years (Villagómez *et al.*, 2011).Unlike what happens elsewhere in the Andes where the subduction of the Nazca plate under the South American plate has played the most important role in mountain building (Folguera *et al.* 2016), in the northern ranges, the collision of the Caribbean plate and Panama Arc seems to have been the major cause of the uplift.

It is likely that the changing abiotic conditions resulting from some of these events, could have affected the evolution of different groups, and, as Luebert & Weigend (2014) and Richardson *et al.*, (2018) suggest, phylogenetic and historical biogeography studies could provide valuable evidence to better understand the complex history of the Andes.

1.2.2.Biogeographical outline of the Andes

A starting point for the study biogeography in the tropical Andes could be set to Humboldt & Bonpland (1805). Although the earlier works in this discipline were largely descriptive, in recent years the development of devices with enough computational power has allowed the incorporation of more complex methodologies. Quantitative historical biogeography methods (Ronquist, 1997; Ree & Smith, 2008) that take into consideration the phylogenetic reconstruction of a group (Felsenstein, 1981; Mau, Newton, & Larget, 1999) and the estimation of divergence times (Drummond *et al.*, 2012), have become customary, and it is even possible to test the performance of different models and make a selection based on their likelihoods (Matzke, 2013).

According to Luebert & Weigend (2014) the Andes have influenced plant diversification in four major ways: as sources of new habitats, as a vicariant barrier, as a latitudinal corridor and as generators of new environmental conditions in other regions (e.g., Western Amazonia and the Atacama Desert). The uplift of this mountain range and the environmental changes associated with it, have been frequently suggested as major forces influencing the distribution and cladogenesis of many groups, both in the Andes themselves and the Neotropics at large (Gentry, 1982; Hoorn *et al.*, 2010; Madriñán, Cortés, & Richardson, 2013; Lagomarsino *et al.*, 2014; Moonlight *et al.*, 2015; Sanín *et al.*, 2016; Diazgranados & Barber, 2017; Pirie *et al.*, 2018; Pouchon *et al.*, 2018). The general progression in the uplift seems to be mirrored by the inferred history and phylogenies of many plant clades (Antonelli *et al.*, 2009; Chacón *et al.*, 2012; Hughes, Pennington, & Antonelli, 2013; Jabaily & Sytsma, 2013; Murillo, Stuessy, & Ruiz, 2016; Bacon *et al.*, 2018) with the northern Andean clades having in general more recent divergence age estimates than the southern Andean clades (Luebert & Weigend, 2014). It is expected that the divergence times/onset of diversification caused by the uplift would affect first the lower elevation taxa and then, progressively higher elevation taxa; this is also reflected in many different clades in the Andean region and adjacent areas (Trénel *et al.*, 2007; Antonelli *et al.*, 2009; Roncal *et al.*, 2012; Madriñán *et al.*, 2013; Winterton *et al.*, 2014; Luebert & Weigend, 2014; Sanín *et al.*, 2016; Lagomarsino *et al.*, 2017; Pirie *et al.*, 2018; Richardson *et al.*, 2018).

The relationships of the Andean flora to other biogeographic regions, based on phylogenetic data, was reviewed by Luebert & Weigend (2014). These authors suggest especially close relationships between the Andes and Central America and southeastern Brazil (especially their mountain ranges). Recent studies on successful and widespread groups such as *Begonia* L. (Moonlight *et al.*, 2015), orchids (Pérez-Escobar *et al.*, 2017) and *Ficus* L. (Moraceae, Machado *et al.*, 2018) confirm this. Almost every other adjacent region (Amazonia, Atacama Desert, Tepuis, Patagonia) also show connections to the

Andes (Salariato *et al.*, 2016; Denham *et al.*, 2016). Links with more distant areas such as North America and Oceania were also accounted by Luebert & Weigend (2014), with long distance dispersal happening repeatedly (e.g. Wen & Ickert-Bond, 2009; Chen *et al.*, 2014); the few studied relationships of Asia-Neotropics disjunctions (Symplocaceae, Sabiaceae) have been usually explained by Boreotropical range expansion and thus connections through North and/or Central America, not by direct long distance dispersal across the tropical Pacific (Fritsch *et al.*, 2015; Yang *et al.*, 2018). It is expected that the previously mentioned patterns will be followed by most of the still unsampled clades, but new patterns or outliers would also be discovered.

Several systems to divide the Andes into Biogeographic units have been proposed (Luebert & Weigend, 2014; Morrone, 2017), but there is no universal agreement on what system would be used by different researchers, as different groups have different dispersal ecologies and are affected in different ways by the perceived barriers between units (Kessler, 2010; Albert & Reis, 2011). Traditional 'expert opinion' based units, defined mostly by the distribution of endemic taxa are still used frequently (Morrone, 2017), however these rarely include phylogenetic information from distantly related clades as independent lines of support and their biological significance has been cast into doubt by new methods (Hazzi *et al.*, 2018; Serrano *et al.*, 2018).

In agreement with volcanic and morphotectonic patterns in the South American, Nazca and Antarctic plates, it was suggested that the Andes could be divided into four main segments (Tassara & Yáñez, 2003; Folguera *et al.*, 2016), however this division does not seem to be reflected in the disribution of the biotas. Even if less satisfactory geologically, the division of the Andes into Northern (north of the Huancabamba depression), Central (between the Huancabamba depression and Central Chile and Argentina) and Southern (to the south of Central Chile and Argentina) domains (Auboin *et al.*, 1973; Gregory-Wodzicki 2000) has been used frequently in biogeography (Luebert & Weigend 2014; Fig. 1.). For some Andean taxa this division has biogeographic significance due to the relatively low elevations found in the Huancabamba depression, that would act as a barrier for high elevation taxa, while the limit between the Central and Southern Andes is supported from a climatological perspective given the shift between the summer (Central

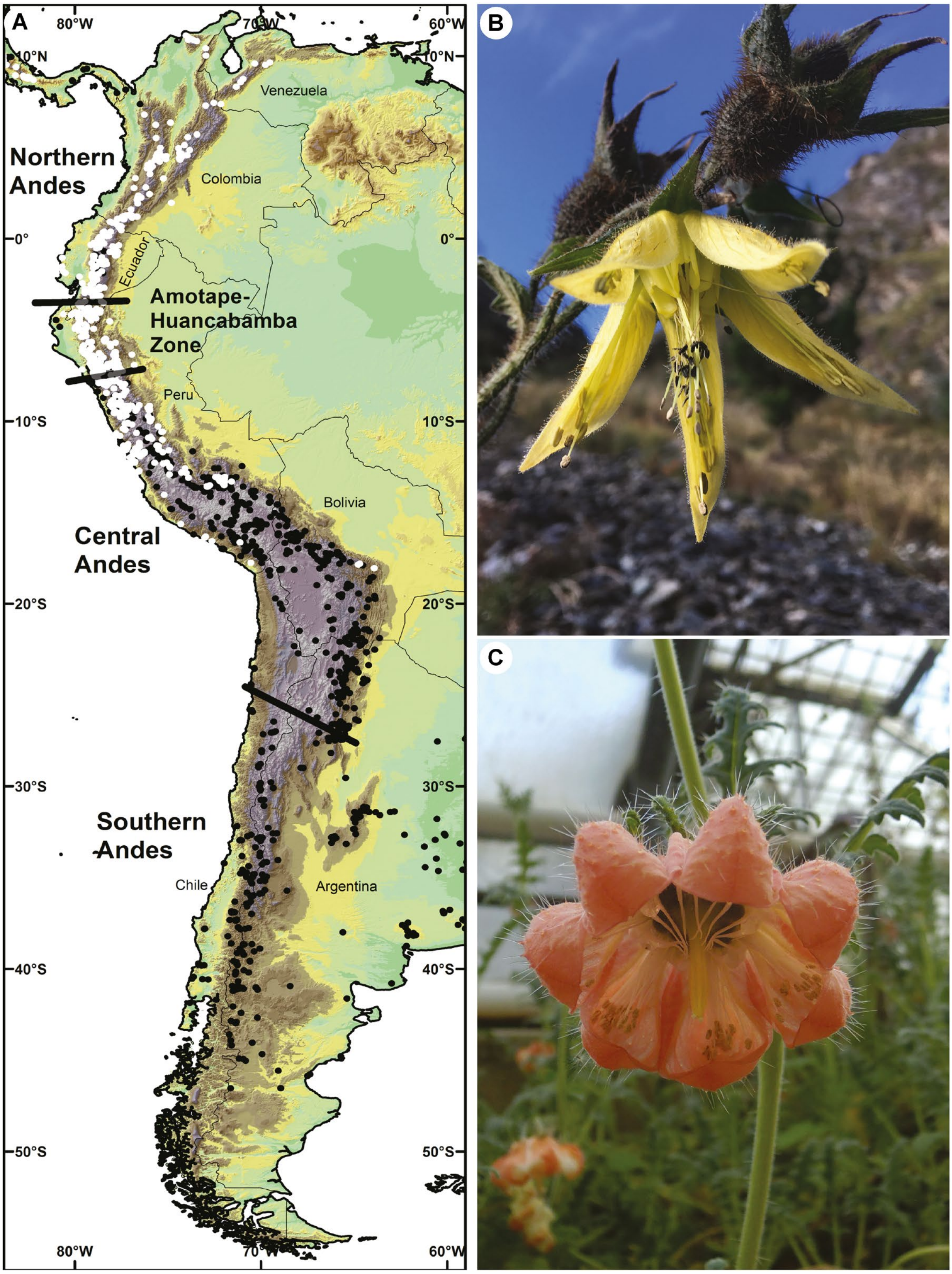

Fig. 1. A. The Andes and its major divisions according to Mutke & Weigend (2017). The dots represent herbarium specimen records of Loasaceae subfam. Loasoideae in the Andes and adjacent regions: White = *Nasa*, black = *Aosa*, *Blumenbachia*, *Caiophora*, *Grausa* and *Presliophytum* as representatives inhabiting other regions of South America. **B.** *Nasa tulipadiaboli* from Pasco, Peru. **C.** *Caiophora deserticola* from southern Peru in cultivation, Bonn Botanical Gardens. Credits: **A.** Relief map by Jens Mutke. **B.** by Tilo Henning.

Andes: tropical) and winter (Southern Andes: temperate) rain regimes (Luebert & Weigend, 2014).

But this orthodox definition may not be suitable for every clade. The Amotape-Huancabamba Zone (AHZ) and the southern limits of what could be considered the Tropical Andes are of particular interest, as different researchers take different stances regarding their limits and definition (Weigend, 2002; Luebert & Weigend, 2014; Mutke & Weigend 2017). Some have opted to consider the AHZ as the limit between the Northern and Central Andes (Antonelli *et al.*, 2009; Rivas Martínez *et al.*, 2011; Pérez-Escobar *et al.*, 2017), but Mutke & Weigend (2017) found no evidence of a major turnover in the generic compositions of the floras that would support treating the Huancabamba Depression as a limit between biotas. Scientists studying the AHZ in detail, have demonstrated that this is an area of high endemism and that many taxa cross into both sides of the Depression (Berry, 1982; Weigend, 2002; Luebert & Weigend, 2014; Mutke *et al.*, 2014). This justifies instead, the recognition of the AHZ as a biogeographic unit on its own (Weigend, 2002, 2004a), at least for some elements of the biota (Fig. 1.). Much further south, the Bolivian Orocline is, geographically, a major feature of the Andes. The recent study by Mutke & Weigend (2017) makes evident that in this area there is an abrupt change in the floristic composition of the Andes at the genus level, with ca. 1000 genera of vascular plants reaching its southernmost limit within few degrees of latitude from it. Hundreds of additional genera reach their southernmost limit to the south of the Orocline into the Yungas areas of S Bolivia and NW Argentina (Mutke & Weigend, 2017), but this shift seems to be much more gradual. The information provided by these studies, suggests that it would be justified to consider the Orocline as an important biogeographic limit between zones of the Andes.

1.3.The family Loasaceae

1.3.1.Morphology and Anatomy

Loasaceae Juss. is a mostly American family of over 300 species found from southwestern Canada (Hufford *et al.*, 2016: *Mentzelia* L.) to southern Chile and Argentina (Weigend *et al.*, 2008: *Blumenbachia* Schrad. sect. *Angulatae* and *Pinnasa* Weigend &

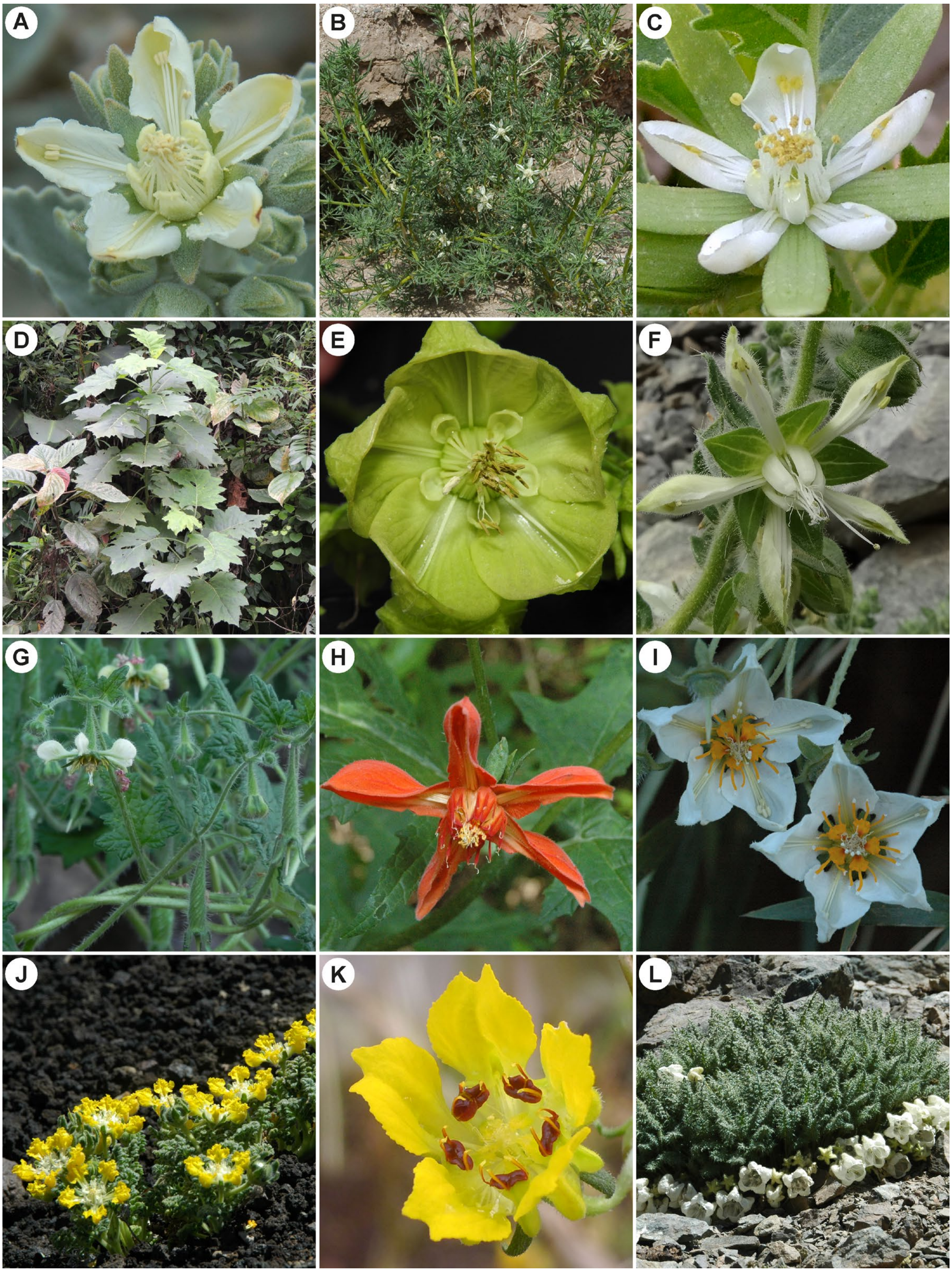

Fig. 2. Examples of Loasaceae subfam. Loasoideae. **A.** *Huidobria fruticosa* a shrub from Antofagasta, Chile. **B.** *Huidobria chilensis* a subshrub from Antofagasta, Chile. **C.** *Kissenia capensis*, a shrub from Namibia, in cultivation, Bonn Botanical Gardens. **D.** *Aosa grandis*, a poorly branched treelet from Cartago, Costa Rica. **E.** *Aosa grandis,* flower. **F.** *Presliophytum sessiliflorum* a subshrub from Antofagasta, Chile. **G.** *Blumenbachia sylvestris* a vine from Biobío, Chile. **H.** *Loasa acanthifolia* a large biennial herb from Biobío, Chile. **I.** *Grausa martini* a vine from Los Ríos, Chile. **J.** *Pinnasa nana*, a rosulate herb from Araucanía, Chile. **K.** *Syphanthus elegans*, a vine from central Chile, cultivated in Bonn Botanical Gardens. **L.** *Caiophora coronata*, a cushion herb from Coquimbo, Chile. Credits: **A.**, **B.**, **F-J.** and **L.** by María Eyzaguirre.

R.H.Acuña) with outliers in southwestern and northeastern Africa, southwestern Arabian Peninsula (Weigend, 2004b: *Kissenia* R.Br. *ex* Endl.) and the Marquesas Archipelago (Weigend, 2004b: *Plakothira* J. Florence). A deep understanding of the morphology and anatomy of Loasaceae has proven to be essential to understand the phylogenetic relationships of the family (Weigend, 1997a, 2004b; Weigend *et al.*, 2004a; Weigend, Gröger, & Ackermann, 2005; Weigend & Gottschling, 2006; Acuña *et al.*, 2017). Most of the major clades show clear sets of characters that allow not only their individual recognition, but also to make robust inferences on their familial relationships, many of which were first suggested by morphology and then confirmed by molecular evidence (Weigend *et al.* 2004a; Weigend & Gottschling, 2006; Acuña et al., 2017). The most significant works dealing in detail with the morphology and anatomy of the family are Payer (1857), Urban (1886, 1892a,b), Gilg (1894, 1925), Urban & Gilg (1900), Brown & Kaul (1981), Carlquist (1984), Weigend (1997a, 2004b) and Mustafa (2018).

Loasaceae are annual, biennial or perennial plants, 5 to 1000 cm tall. Their habit is varied (Urban & Gilg, 1900; Weigend, 1997a, 2004b; Hufford, 2016) and although most species are erect or decumbent herbs, there are also acaulescent plants, vines, subshrubs, shrubs, lianas and even trees (Fig. 2). Stems are usually pithy, rarely solid, typically terete, more rarely quadrangular, grooved or ridged, sometimes covered with white, green or black lenticels and/or calli. Some shrubby species, especially in xeric or strongly seasonal areas, exfoliate their epidermis. Older stems of woody species may have varying degrees of growth of the phellem, with this being especially well developed in *Petalonyx* A.Gray. Stolons have been observed in *Caiophora* C.Presl*, Grausa* Weigend & R.H.Acuña, *Nasa* Weigend and *Pinnasa*, while thickened rhizomes are common in the *Nasa ranunculifolia* (Kunth) Weigend species group and long-lived xylopodia are produced by *Schismocarpus pachypus* Blake and *Xylopodia klaprothioides* Weigend. Most genera that have been studied have a typical root system, but the primary root is evanescent in *Nasa,* being substituted by adventitious roots early in development. Thickened storage roots have been observed in *Blumenbachia*, *Caiophora* and *Nasa*.

Wood anatomy of Loasaceae is quite diverse for a family of its size (Carlquist, 1984) and shows traits that indicate paedomorphosis (herb-like wood anatomy: Carlquist, 1992). Growth rings can be seen in some *Eucnide* Zucc., *Mentzelia* and *Petalonyx* (Carlquist, 1984) as well as in *Xylopodia klaprothioides* (Weigend, 2004b). Vessel element perforation plates are mostly simple in woody taxa (Carlquist, 1984), but scalariform plates have been observed in the primary xylem (Carlquist, 1984, 1987). The lateral vessel pitting is formed by circular to oval pits although occasionally these can be scalariform. Due to its vine habit, *Fuertesia domingensis* Urb. has a very distinctive wood anatomy with strong vessel dimorphism including both very wide and very narrow elements. Diffuse axial parenchyma is found across many genera of the family and vascular rays can be uni or multiseriate or predominantly multiseriate (Carlquist 1984, 1987). Many of the characters found in *Plakothira frutescens* J.Florence, are strongly suggestive of a secondarily woody habit (Carlquist, 1987). Carlquist (1984) notices that the diversity in wood anatomy seems to be result of ecological adaptations more than phylogenetic relatedness, however, the anatomy of several woody taxa (e.g. *Aosa grandis* (Standl.) R.H.Acuña & Weigend, both *Huidobria* Gay species, species of *Nasa* ser. *Alatae*, *N.* ser. *Carunculatae*, *N. grandiflora* species group) still remains to be studied.

Most aerial surfaces of the plants (except when phellem has developed) are covered in trichomes. Trichomes are important from a systematic perspective, because some clades show diagnostic morphologies and these have been used to infer relationships in the family (Dostert & Weigend, 1999; Henning, Rodríguez, & Weigend, 2011). Trichomes can be divided into three main categories (Weigend, 1997a, 2004b; Mustafa, Ensikat, & Weigend, 2017):

1) Unicellular scabrid/glochidiate trichomes are the most characteristic of Loasaceae, being the only ones found universally in the family (Hufford, 1989; Weigend, 1997a) and thus of recognized systematic value for a long time (Barthlott, 1981; Behnke & Barthlott, 1983). Scabrid trichomes have a scabrous or barbed shaft with a straight, sharp tip, while glochidiate tricomes have a smooth, scabrous or barbed shaft with a retrorsely-barbed, climbing-hook shaped tip. In *Cevallia sinuata* Lag. and *Huidobria fruticosa* Phil. the barbs are very long and the trichomes have an arbuscular appearance, a condition not reported in the rest of the family so far (Davis & Thompson, 1967; Poston & Nowicke, 1993; Ensikat, Mustafa, & Weigend, 2017). In some species of the *Nasa triphylla* (Juss.) Weigend species group, medifixed trichomes cover the stems and petioles (Dostert & Weigend, 1999). In *Fuertesia domingensis* a unique and different kind of asymmetrically medifixed scabrid trichomes, called “splinter hairs” by Weigend (1997a, 2004b), is found and could be the main cause of the irritation when this plant is handled (Liogier, 1981). Scabrid/glochidiate trichomes have distinctive topographic biomineralization patterns, with silicates found especially at the barb tips, phosphates mostly restricted to the barbs

and carbonates in most of the entire structure, but deviations from this pattern may occur in different genera, with one or more biominerals sometimes absent (Ensikat, Geisler, & Weigend, 2016; Ensikat *et al.*, 2017; Mustafa *et al.*, 2017; Barthlott *et al.*, 2017). Scabrid trichomes are found in also in Hydrangeaceae Dumort. (Gregory, 1998; Hufford, 2004; Weigend, 2004b; De Smet *et al.*, 2017) the family sister to Loasaceae. The scabrid/glochidiate trichome presence is one of the most reliable traits to differentiate Loasaceae from the externally similar Cucurbitaceae Juss. and Malvaceae Juss. especially when sterile (Weigend, 2009). These trichomes seem to have important ecological functions, first in herbivore defense, both against vertebrates, due to their biomineral content leading to teeth abrasion (apatite, silicates: Ensikat *et al.*, 2017; Barthlott *et al.*, 2017) and, although often not effective, against arthropods, due to their barbed structure (Eisner, Eisner, & Hoebeke, 1998; pers. obs). Second, the capsules of *Aosa plumieri* (Urb.) Weigend, *Blumenbachia* sect. *Blumenbachia*, *Klaprothia mentzelioides* Kunth and several *Mentzelia* have tack shaped glochidiate trichomes that make them sticky and potentially enhance the fruit capacity to attach to fur or feathers (Weigend, 1997a). Third, xeric area taxa such as *Cevallia sinuata*, *Huidobria fruticosa*, *Kissenia, Presliophytum* (Urb. & Gilg) Weigend and several species of *Mentzelia* have dense scabrid/glochidiate trichome indumenta, especially on the stem and the abaxial surface of the leaves (which gives them a greyish, whitish or bluish color). In other pubescent xerophytes, trichomes reduce the evapotranspiration by reflecting excessive radiation and by increasing the boundary layer of the leaves (Evert, 2006) and could facilitate the condensation of atmospheric moisture (Weigend, 1997a).

2) Unicellular stinging trichomes are usually the longest trichomes and have a smooth, tapering shaft, a bulbous tip and a multicellular pedestal (Weigend, 1997a, Ensikat *et al.*, 2016, 2017, Mustafa *et al.* 2017). They are filled with irritating substances of unknown composition. The cells walls are mineralized to various extents in different genera (Ensikat *et al.*, 2017) with silicates (usually the tip), phosphates and carbonates (usually throughout). The coloration of these trichomes is variable, ranging from whitish or yellowish, to red, brown or black (Fig. 1B,C). True stinging trichomes are known with certainty from *Aosa* Weigend, *Blumenbachia*, *Caiophora, Cevallia sinuata*, *Eucnide*, *Gronovia* L., *Loasa* Adans*., Nasa* and *Presliophytum* (Davis & Thompson, 1967, Poston & Nowicke, 1993; Ensikat *et al.*, 2017; Mustafa *et al.*, 2017). They are mostly or entirely absent from the remaining genera (Weigend, 1997a, 2004b). The main function of these

trichomes is to deter vertebrate herbivores but apparently their effect on most invertebrates is minimal (Weigend, 2004c; pers. obs.).

3) Multicellular uniseriate glandular trichomes, are not mineralized and have a terminal gland that exudes substances of unknown composition. These can be found across most of Loasoideae (*Aosa*, *Blumenbachia*, *Caiophora*, *Loasa*, *Nasa*, *Presliophytum*) but are most conspicuous in *Nasa* (especially in *Nasa* ser. *Grandiflorae,* where they could be branched and form a dense layer). Its presence outside Loasoideae has not been confimed, although glandular trichomes seem to be present in *Eucnide* (these appear to be unicellular, though, and externally similar to the short smooth trichomes found in some *Presliophytum*: Mustafa *et al.*, 2017; pers. obs.). The ecological function of these trichomes and their secretions has not been determined.

Across Loasaceae, leaf phyllotaxis is usually opposite in the basalmost leaves and alternate in distal leaves (Weigend, 1997a), however it is opposite throughout in *Aosa uleana* (Urb. & Gilg) Weigend, the three genera of the tribe Klaprothieae (*Klaprothia* Kunth, *Plakothira* and *Xylopodia* Weigend), *Mentzelia arborescens* Urb. & Gilg, most species of *Nasa* ser. *Alatae*, some of *Nasa* ser. *Grandiflorae*. and the 6 genera (*Blumenbachia*, *Caiophora*, *Grausa*, *Loasa*, *Pinnasa* and *Scyphanthus* Sweet) of the "South Andean Loasas" (SAL from now on), however, most *Loasa* ser. *Floribundae* species, and occasionally some *Grausa* specimens, have mostly alternate distal leaves (Urban & Gilg, 1900).

Foliage is usually perennial across the family (Weigend, 1997a, 2004b), but some taxa can be deciduous (some *Mentzelia*, *Nasa* ser. *Carunculatae*, *Nasa urentivelutina* Weigend*,* several taxa of SAL, *Xylopodia klaprothioides*) at least partially (*Kissenia*). Petioles are usually well developed, although several *Mentzelia* have sessile leaves (Hufford *et al.*, 2016) and in linear leaved species (e.g. *Huidobria chilensis* Gay, *Petalonyx linearis* Greene) the petiole becomes indistinct from the lamina. Many species with petiolate leaves, may develop sessile or subsessile upper leaves and bracts. Leaves are exstipulate but a few species, mostly of *Nasa* (Weigend, 2001, Weigend, 2002b; Weigend & Rodríguez, 2003), develop pseudostipules [*Nasa panamensis* Weigend*, Nasa perijensis* (Weigend) Weigend, *Nasa stuebeliana* (Urb. & Gilg) Weigend species group, occasionally in *Aosa grandis*]. The blade texture ranges from membranous to coriaceous and succulent. The most frequent leaf blade outline is ovate to elliptic, however it ranges from linear to subcircular (*Nasa ferox* Weigend, *N. orbicularis* Weigend, *N. tabularis* Weigend,), including lanceolate, hastate, sagittate, and reniform. In most taxa of the family, the leaf blade is simple, even if it can be deeply palma-, pinna- or bipinnatisect [e.g. *L. multifida* Gay, *Nasa urens* (Jacq.) Weigend*, Scyphanthus*]. However it can be truly compound in some *Caiophora*, *Grausa*, the *Nasa triphylla* and *N. venezuelensis* (Steyerm.) Weigend species groups, *Nasa weigendii* E.Rodr. and *Pinnasa*. The lamina base ranges from cuneate to peltate, including truncate and cordate. The margins of the lamina are usually serrulate, serrate, dentate, crenate or lobed (Weigend, 1997a). Only in linear leaved taxa and *Fuertesia domingensis*, the margins are entire (Urban, 1910).

The details of the inflorescence architecture are explained extensively by Weigend (1997a). In Loasaceae each flower is usually subtended by two bracts, but in *Nasa* there is only one bract per flower and these can be completely absent in most *Aosa* and *Klaprothia fasciculata* (C.Presl.) Poston. Recaulescence and concaulescence are widespread in Loasaceae with only some *Menzelia* and most of SAL lacking metatopy (Weigend, 2004b). Thyrsoids with a well-developed terminal dichasium and few additional paracladia are widespread while anthocladial dichasia (e.g. *Loasa*) anthocladial monochasia (e.g. *Caiophora*), early proliferation (e.g. *Blumenbachia prietea*, *Pinnasa*) and different complex synflorescence patterns (e.g. *Aosa*, *Blumenbachia* sects. *Blumenbachia* and *Gripidea*, *Grausa Huidobria*, *Presliophytum*) tend to be restricted to specific clades. Only in *Petalonyx* the inflorescences are racemose.

The flowers are perfect and complete (Brown & Kaul, 1981), usually actinomorfic, rarely weakly zygomorphic (Hufford, 2016; e.g. *Kissenia*, *Petalonyx crenatus* A.Gray ex S.Watson, *Schismocarpus pachypus*), and pentamerous (although tetramerous in Klaprothieae and up to octamerous in some populations of the *Caiophora chuquitensis* species group: Weigend, 1997a; Ackermann & Weigend, 2007; Slanis, Perea, & Grau, 2016). The flowers of the different subfamilies differ considerably from each other and this lead some early researchers (Payer, 1857) to think that *Mentzelia* and *Caiophora* were not even part of the same order. In the non-Loasoids, flowers are held erect to horizontal on the inflorescence, but in most taxa of Loasoideae these are deflexed to pendent, although some deserticolous and acaulescent clades have erect to horizontal flowers. The calyx is persistent and the sepals range from very reduced, inconspicuous and few mm in length to being the longest element of the flower segments (*Kissenia*), reaching >3cm in length (at least in fruit) These can be linear, lanceolate, ovoid, obovoid, or pinnatifid, with entire, serrate, dentate or lobed margins. The corolla can be white, green, yellow, bicolorous (white and yellow, white and green or white and rust), orange, pink or red. The petals are usually free (but basally connate in some *Eucnide*) and can be linear, lanceolate, laciniate, spathulate, olong, ovoid, or subcircular in outline, ranging from few mm (*Klaprothia fasciculata*) to ca. 8 cm [*Eucnide grandiflora* (Groenl.) Rose] long. The margins are usually entire (excluding the tooth between claw and limb found in many loasoids) although they can be serrulate to laciniate in the *Caiophora pterosperma* (Ruiz & Pav. ex G. Don) Urban & Gilg group *Fuertesia domingensis*, *Grausa micrantha*, *Pinnasa* and *Scyphanthus*. In non-Loasoids, petals are flat or slightly concave with a poorly differentiated claw and limb (except e.g. in *Petalonyx*), in Loasoideae these are cymbiform, usually strongly concave and with a well differentiated claw and limb (except in in bird pollinated groups, particularly in several *Nasa* sers. *Alatae* and *Grandiflorae*).

The androecium is formed by 5 (Gronovioideae and Petalonychoideae) to >200 stamens (Weigend, 2004b). Some elements of the androecium are not fertile and either into turn into fliform to petaloid staminodes (*Mentzelia* sect. *Bartonia, Petalonyx crenatus*) or the nectar scale complex (Loasoideae, Fig. 2), early in development (Hufford, 2003). In the past the petaloid staminodes and the nectar scales were often considered as elements of the corolla (Adanson, 1763; Endlicher, 1836-40; Harvey, 1859), but Payer (1857) found them to be modified stamens. The nectar scale complex is formed usually by 5 staminodial primordia. The three outer ones fuse in most of their length and originate the nectar scale that usually has three dorsal threads (absent in *Aosa grandis*, some *Caiophora*, *Kissenia*, most *Nasa* and *Xylopodia klaprothioides*). The two inner staminodes remain free. In both species of *Huidobria*, the number of staminodes forming the complex is always >6 (Grau, 1997). The nectar scales are variable morphologically and have been recognized as having systematic value (Gilg, 1894; Urban & Gilg, 1900; Weigend, 1997a; Acuña *et al.*, 2017), with most genera differing in the morphology and development of nectar sacs, apical wings, dorsal threads, dorsal calli, double arc and neck. The fertile stamens have filiform filaments but they can be flattened or forked in several *Mentzelia*. Anthers are basifixed and tetrasporiangate (Weigend, 1997a, 2004b; Hufford, 2003). Autonomous stamen movement is widespread in Loasoideae (Schlindwein & Wittmann, 1997; Weigend, Ackermann, & Henning, 2010; Henning & Weigend, 2012), with many taxa also showing thigmonasty (stimulus triggered movements). Stamen movement has not been reported for non Loasoids. As Hufford

(1989), Poston & Nowicke (1993), Weigend (1997a, 2004b), García de Albano & Slanis (2006) and Noguera-Savelli, Ruiz, & Jáuregui (2009) indicate, pollen grains are tricolpate to tricolporate, usually spheroidal with the exine spinulose, echinate or rugulose (Gronovioideae), longitudinally striate (the remaining non-Loasoids), reticulate (most Loasoideae), equatorially striate (*Loasa* sers. *Deserticolae* and *Floribundae*), punctated (*Loasa* sers. *Loasa* and *Macrospermae*) or microreticulate (*Aosa grandis*, *Kissenia*, some *Mentzelia*).

The gynoecium has a single style (Weigend 1997a, 2004b), which can be straight (In most of the family) or curved (e.g. *Schsmocarpus pachypus*), although it may twist late in anthesis or early in post-anthesis. The stigma has three to five lobes and shows moderate variation across the family (Urban & Gilg, 1900, Thompson & Ernst, 1967; Hufford, 1989; Weigend 1997a, Moody & Hufford, 2000; Acuña & Weigend, 2017). with it being globular, conic-obtuse, linear, acute or punctiform. The ovary has been interpreted as strictly unilocular (Weigend, 1997a), although, due to extensive protrusion of the placentae septa of some taxa, it may appear bilocular [*Kissenia* (as was interpreted by Urban & Gilg, 1900, and Chapter 4 of this thesis) and *Presliophytum sessiliflorum* (Grau, 1997, as was interpreted by Acuña & Weigend, 2017)] or plurilocular (as in *Blumenbachia* and many *Caiophora,* Weigend, 1997a). In most of the family the, ovary is inferior, but in some taxa it can be semi-superior (e.g. *Aosa grandis, Aosa sigmoidea* Weigend, *Grausa martini* (Phil.) Weigend & R.H.Acuña, *Loasa elongata*, *Nasa longivalvi*s E.Rodr. & Weigend, *Schismocarpus pachypus, Xylopodia klaprothioides*) or, even more rarely, mostly superior (e.g. *Aosa rostrata* (Urb.) Weigend, *Caiophora pulchella* Urb. & Gilg). Petalonychoideae and Gronovioideae have pseudomonomerous gynoecia (Hufford, 2016) and a single, subapical placenta (Weigend, 2004b). In the other groups the placentation is parietal and the number of carpels and placentae per flower is three (the most frequent condition e.g. in most Loasoids and *Mentzelia*) or five (e.g. *Aosa grandis*, *Blumenbachia* sect. *Blumenbachia*, 'pleiomerous' *Caiophora*, *Eucnide*, *Huidobria*, *Schismocarpus pachypus*, occasionally in *Loasa, Nasa* and *Presliophytum* and rarely in *Mentzelia*), but there can be inter- or intraspecific variation in this number (Urban, 1892b; Urban & Gilg, 1900; Grau, 1997; Weigend, 1997a, 2004; Acuña & Weigend, 2017). Much less frequent is the presence of two (e.g. *Kissenia,* apparently fused postgenitally *fide* Urban & Gilg, 1900, *Presliophytum sessiliflorum*) or four ('pleiomerous' *Caiophora*, Klaprothieae, sometimes in *Presliophytum*) placentae. The anatomy of the ovules has been poorly researched, and the number can vary between one (Gronovioideae) to several hundred (*Caiophora*, *Eucnide*, *Huidobria*, *Nasa*, *Presliophytum*) per placenta. Wunderlich (1959), García (1962) and Vijayaraghavan & Prabhakar (1984) agree that the ovules are unitegmic and tenuinucellate with well-developed chalazal and micropylar haustoria (but in Petalonychoideae and Gronovioideae these seem to be crassinucellar and have reduced or absent chalazal and micopylar haustoria, Weigend, 1997a).

The fruits in most species of Loasaceae are dehiscent capsules, except for *Kissenia* (and probably *Presliophytum sessiliflorum*) where they are indehiscent and Gronovioideae and Petalonychoideae that have cypselas. These capsules remain attached to the plant during maturity (Weigend, Aitzetmüller, & Bruehl, 2004b), except in the few cases where the fruit is the actual diaspore. Their shape ranges from cupuliform, globose, urceolate or elliptical, to sigmoid, clavate, cylindrical and narrowly cylindrical. Pedicels undergo post-anthetic elongation in chasmocarpous *Eucnide* and many Loasoids, while in some *Mentzelia* and in some *Scyphathus*, the fruits are sessile. The calyx is usually persistent, and very well developed in *Kissenia* as well as in Gronovioideae and Petalonychoideae. The dehiscing mechanism is made up of 3-5 (rarely more) apical valves (Urban, 1892b), however in *Blumenbachia* and most *Caiophora* species, fruits have coherent apices, and along with *Klaprothia fasciculata* and *Scyphanthus*, dehiscence is mostly through longitudinal slits. In several shrubby species of the *Nasa grandiflora* species group, capsules are dehiscent both with 3-5 apical valves and a single longitudinal slit. In the genera studied by Weigend *et al.*, (2004b), fruits are xerochasious (poorly in some *Loasa* and not at all in *Blumenbachia*).

The number of seeds per fruit varies between one (Gronovioideae) and a few thousand (*Eucnide*, *Huidobria*, peruvian *Presliophytum*). Their size ranges between the dust seeds ca 0.5 mm long seen in peruvian *Presliophytum* and the globose ones > 5mm long of *Loasa* ser. *Macrospermae* (Urban & Gilg, 1900; Weigend *et al.*, 2004b, 2005). There is considerable diversity in seed morphology especially in the SAL clade (Weigend *et al.*, 2005) and *Mentzelia* (Hufford *et al.*, 2016). Ovoid seeds are common across the family, but other morphologies also exist (globose, fusiform, protracted with two long, terminal wings, irregular, flattened and winged or flattened and non-winged). The seed testa sculpturing is equally diverse and can be used to recognize the major generic or subgeneric clades of the family (Hill, 1976; Hufford, 1988, 1989; Weigend *et al.*, 2005), with it being indistinct, longitudinal with simple striations, longitudinal with torulose or transversely banded striations, striate-reticulate with long rectangular anticlinal walls, reticulate with low polygonal anticlinal walls, reticulate with domed periclinal walls, reticulate and deeply grooved transversally, reticulate-tuberculate or reticulate with high anticlinal walls. Weigend (1997a, 2004b) indicates that in general endosperm is copious and oily, however in *Kissenia* the seeds are exalbuminous and the mature seed lacks endosperm.

1.3.2.Distribution and Ecology

Loasaceae has two main centers of diversity: the Andes, especially from Colombia to Central Chile and Argentina (mostly for subfamily Loasoideae, Fig. 1) and southwestern North America (including Mexico, Hufford, 2016). They grow in most habitats available (Fig. 3) from sea level to almost the snowline (Weigend, 2004b; Slanis *et al.*, 2016) but tend to be virtually absent from lowland mesic forests such as in most of North America to the east of the Mississippi and the Amazonian-Guianan region. On the other hand, Loasaceae reaches its highest diversity at 2000-4000 m a.s.l. in the slopes on both sides of the Andes (Fig. 3). Loasaceae can be found in deserts and subdeserts (e.g. *Cevallia sinuata, Eucnide*, *Huidobria*, *Kissenia, Loasa, Petalonyx*, *Presliophytum*, many *Mentzelia*), seasonally dry tropical formations like the Caatinga, dry forest and scrub (e.g. *Aosa*, *Fuertesia*, *Gronovia*, *Mentzelia*, *Xylopodia klaprothioides*), seasonally dry temperate formations like Matorral, Great Plains, Pampas, and the Patagonian steppe (e.g. *Blumenbachia*, *Grausa*, most of *Loasa*, *Mentzelia, Pinnasa, Scyphanthus*), permanently humid tropical montane forests like the Yungas and "Cloud" Forests (e.g. *Caiophora*, many *Nasa*, *Klaprothia*), vegetation above the treeline in both tropical (several *Caiophora* and *Nasa*) and temperate areas (e.g. *Blumenbachia*, *Caiophora*, *Pinnasa*). A few species grow in low elevation broadleaved forests of tropical (*Aosa grandis*), subtropical (*Blumenbachia* sect. *Gripidea*) and temperate (*Grausa*) latitudes Many species inhabit naturally disturbed environments, such as talus and scree slopes, fast-flowing river margins, dry river beds, and forest clearings (Weigend, 2004b; Hufford, 2016; Fig. 2B, C), as well as rocky outcrops and cliffs, with shallow and rocky soils. These plants often act as pioneers on roadsides, pastures and recently deforested areas (Fig. 2D), with some species able to withstand considerable human disturbance and become weedy [*Gronovia scandens* L., *Klaprothia fasciculata*, *Nasa chenopodiifolia* (Desr.) Weigend, *Nasa triphylla* subsp. *rudis* (Benth) Weigend, *Presliophytum incanum*

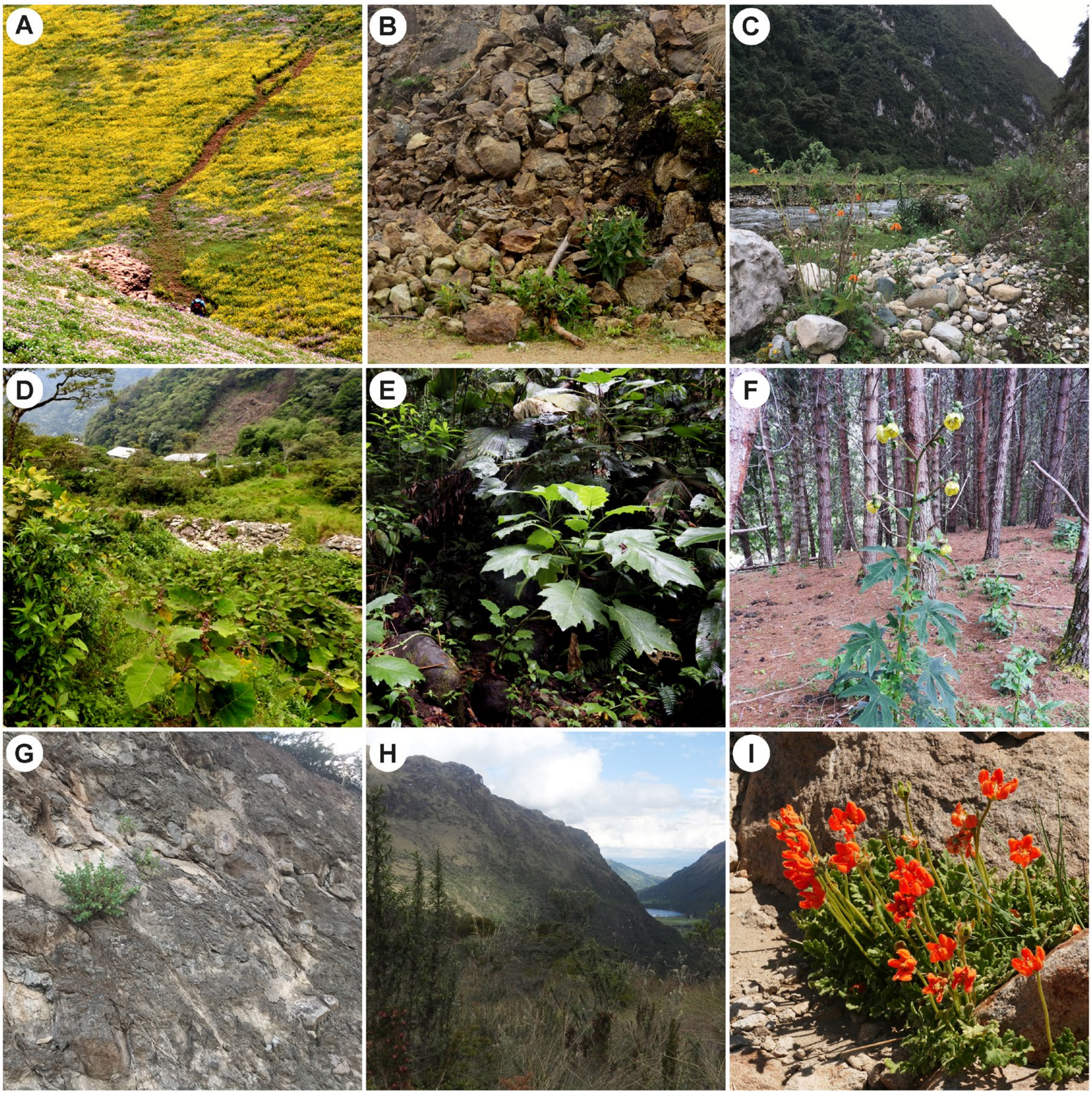

Fig. 3. Some habitats of Loasaceae subfam. Loasoideae. **A.** Lomas of Central Peru with thousands of *Nasa urens*. **B.** Andean scree slope in El Oro, Ecuador with *Nasa connectans*. **C.** Andean riverside in Amazonas, Peru with *Nasa basilica*. **D.** Recently cleared agricultural area in Tungurahua, Ecuador, habitat of *Nasa triphylla* subsp. *papaverifolia*. **E.** Small forested stream, inside premontane wet forest, in Costa Rica with *Aosa grandis*. **F.** Old pine plantation in El Oro, Ecuador with *Nasa profundilobata*. **G.** Vertical rock surface on a roadside in Cajamarca, Peru with *Presliophytum incanum*. **H.** Subparamo area in Azuay, southern Ecuador, habitat of *Caiophora contorta*. **I.** Area above the treeline in Maule, Chile with barren rocky terrain and *Grausa lateritia*. Credits: **A.** by Maximilian Weigend. **C.** and **G.** by Tilo Henning**.** **I.** by Elna von Harpe.

(Graham) Weigend], while others seem to be intolerant to persistent and extensive habitat changes [e.g. *Aosa uleana*, *Nasa ferox* Weigend, *N. hastata* (Killip) Weigend, *N. pumachini* (Weigend) Weigend, *N. rufipila* Weigend, *N. solaria* (J.F.Macbr.) Weigend, *Presliophytum arequipense* Weigend].

The main herbivores that attack Loasaceae are insects, especially homopterans and caterpillars (Eisner *et al.*, 1998; Weigend, Kufer, & Müller, 2000), with the latter being able to cause extensive foliage damage (Weigend 2004c; obs. pers.). While vertebrates mostly seem to avoid consuming Loasaceae species (Weigend *et al.*, 2000), a few include them in their diet (Jennings & Berry, 2015). Undetermined insect larvae have been found inside the capsules of *Nasa tabularis* (Weigend) but it is uncertain if they acted as seed predators (obs. pers.). In cultivation, spider mites (Tetranychidae) and fungi can cause serious damage, even in large plants, but this kind of infestations has not been reported in the wild.

In Loasaceae the flowering period can be extended for long periods of time if the conditions are favourable. Most flowers in the family seem to be adapted for animal pollination (Fig. 4), especially melittophily, but ornithophily has evolved repeatedly, especially in Andean Loasoideae (Harter, Schlindwein, & Wittmann, 1995; Weigend, 1997a, 2004b; Strelin *et al.*, 2016a,b; Fig. 4H-L). Some species are facultatively autogamous (Brown & Kaul, 1981), with some populations even cleistogamous (Weigend, 2004b). Phalaenophily, psychophily, myophily and pollination by rodents have been reported but these syndromes are apparently not widespread (Weigend, 2004b). Nectar and pollen are the main pollinator rewards, although nectar production has not been observed in many *Mentzelia* (Weigend, 1997a). Weak scents are detected in some species (obs. pers.) but their role in pollinator attraction has not been studied.

One of the most interesting aspects in the floral biology of Loasaceae subfam. Loasoideae is the capacity of movement of the stamens, a strategy that may improve the efficiency in pollen presentation and the male fitness of the plant (Weigend *et al.*, 2010; Henning *et al.*, 2018). This allows fresh pollen to become available shortly after a pollinator visit. Autonomous stamen movement was detected already in the 19th century (Urban, 1886), and it is present in most of the genera of Loasoideae. Thigmonastic (touch induced) stamen movement was first studied in detail much more recently and has been subject to extensive research since (Harter *et al.*, 1995; Schlindwein & Wittmann, 1997; Weigend *et al.*, 2010; Henning & Weigend, 2012, 2013; Leite, Nadia, & Machado, 2016; Henning *et al.*, 2018; Siriani-Oliveira, Oliveira, & Schlindwein, 2018). According to Henning & Weigend (2012) the longevity of the flower and its gender phases is affected by the speed at which pollen is depleted which is in turn consequence of pollinator activity intensity.

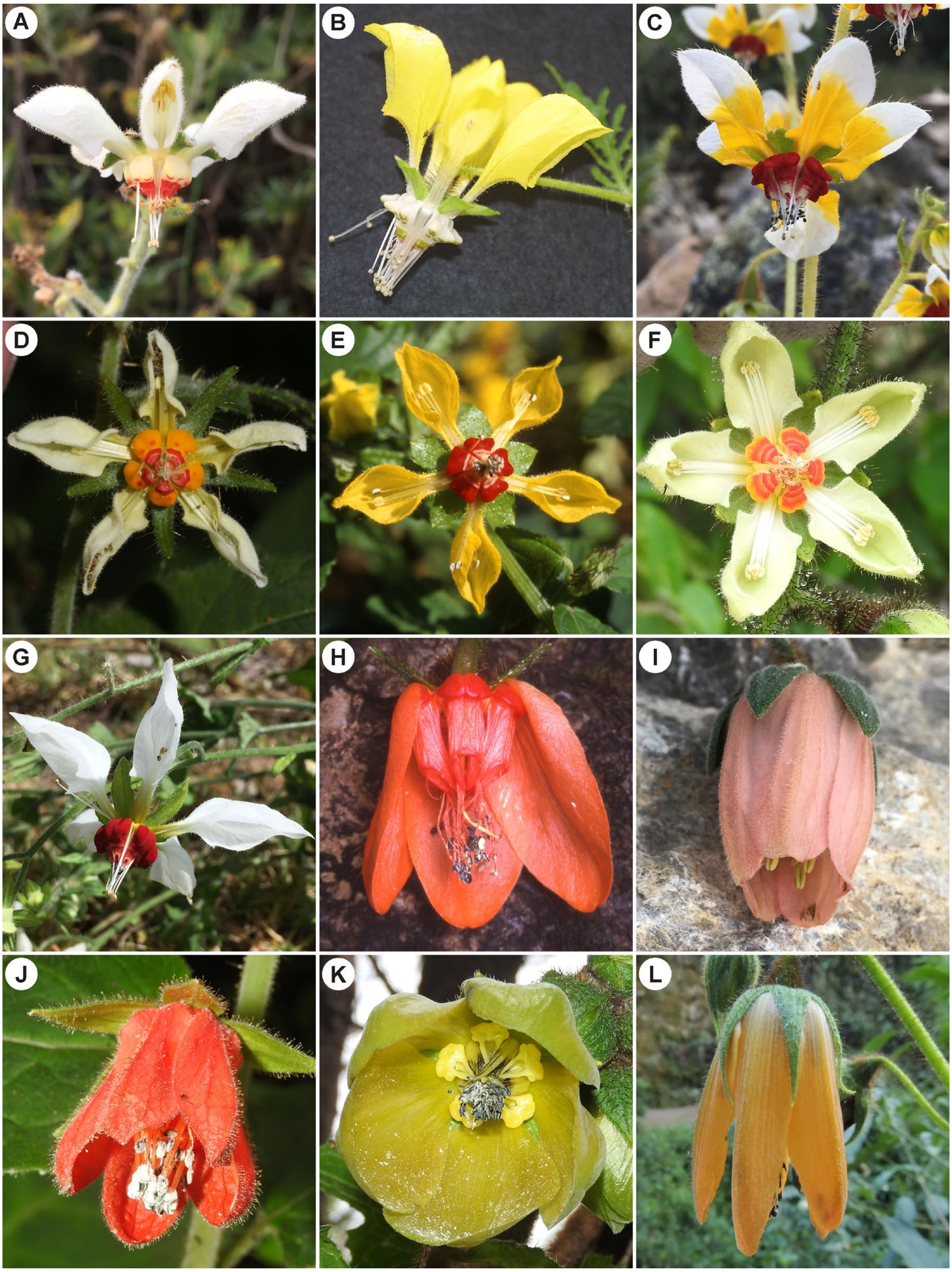

Fig. 4. Floral diversity in *Nasa*. **A.** *Nasa carunculata*, from Áncash, Peru. **B.** *Nasa urens,* from central Peru. **C.** *Nasa picta* subsp. *picta*, from La Libertad, Peru. **D.** *Nasa ramirezii*, from Imbabura, Ecuador. **E.** *Nasa pteridophylla* subsp. *geniculata*, from Cajamarca, Peru. **F.** *Nasa connectans*, from El Oro, Ecuador. **G.** *Nasa formosissima*, from Cajamarca, Peru. **H.** *Nasa limata*, from Apurímac, Peru. **I.** *Nasa urentivelutina*, from Cajamarca, Peru. **J.** *Nasa amaluzensis*, from Loja, Ecuador. **K.** *Nasa profundilobata,* from El Oro, Ecuador. **L.** *Nasa speciosa*, from San José, Costa Rica. Credits: **A.**, **C.**, **G.**, and **I.** by Tilo Henning. **B.**, **E.** and **H.** by Maximilian Weigend. **D.** by Ruth Ripley.

Henning *et al.* (2018) suggest that changes in the quality of thigmonasty could have had an influence in the diversification of Loasoideae.

Seeds are the commonest kind diaspore in Loasaceae. However fruits may act as diaspores in Gronovioideae, Petalonychoideae (Weigend, 2004b; Raimúndez-Urrutia & Varela, 2005) and *Kissenia* (Weigend, 1997a, 2004b). Here the mature fruits are indehiscent and have adaptations for anemochory (winged ribs or long trichomes on the outer fruit wall and/or persistent, large calyx or bracteoles). On the other hand, the capsules of *Aosa plumieri Blumenbachia* sect. *Blumenbachia*, *Klaprothia mentzelioides* and several *Mentzelia* species, seem to be adapted for epizoochory (Weigend, 1997a; Weigend *et al.*, 2004b).

Seeds seem to be either anemochorous or barochorous (Weigend *et al.*, 2004b, 2005). The most obvious adaptations for anemochory appear to be the diminutive and light dust seeds that evolved independently in desert inhabiting taxa (*Eucnide*, *Huidobria*, peruvian *Presliophytum*), and the different winged structures, that also evolved independently, in *Caiophora* (Weigend *et al.* 2005*)* and *Mentzelia* (Hufford *et al.* 2016). Other apparent adaptations for anemochory can be seen in *Blumenbachia*: short longitudinal wings or longated, more or less cylindrical to conical, terminal wings. Some evidence found by Weigend *et al.* (2004b) indicates that *Blumenbachia* sects. *Angulatae*, (and maybe sect. *Gripidea* and *Aosa grandis*; Weigend, 1997a, 2004b), would be facultatively hydrochorous while the large seeds of *Loasa* sects. *Loasa* and *Macrospermae* seem to be mostly barochorous.

1.3.3.Systematics and relationships

Plants of the family Loasaceae are widespread in the New World, and may have been known for several millennia to the native inhabitants of the Americas, as these are still used in traditional medicine by the Amerinds of Western North America and Andean South America (Weigend, 2004b,c). Studies on these plants, were carried out by Europeans decades before Species Plantarum was published (Linnaeus, 1753) as the works of Plumier (1703; Burman, 1756) and Feuillée (1714) make evident. However the formal taxonomic history of this family begun, as with many other plant groups, in Species Plantarum with the descriptions of *Gronovia scandens* L. and *Mentzelia aspera* L. (Linnaeus, 1753), although at first these were not considered closely related (Weigend, 1997a).

The relationships of Loasaceae remained controversial until the last decade of the 20th Century. Weigend (1997a) has summarized the history of the suggested interfamilial relationships and only the main points will be presented here. Since the time of Linnaeus (1753) and Adanson (1763) *Mentzelia* and *Loasa* were considered as closely related, but distant from *Gronovia,* with the former genera considered by Adanson as part of Caprifolia and the latter as part of Onagrae. Jussieu (1789), Lamarck (1789) and Desrousseaux (in Lamarck, 1792; in Desrousseaux, Poiret & Savigny, 1797) considered *Mentzelia* and *Loasa* as part of Onagrae and *Gronovia* as a Cucurbitaceae. A few years later, Jussieu (1804) united the former two genera in Loasaceae and suggested that they would be related to Onagraceae, Myrtaceae, Aizoaceae, Cucurbitaceae and probably Cactaceae. Starting with Bonpland, Humboldt & Kunth (1823), several authors (De Candolle, 1828; Reichenbach, 1837; Endlicher, 1836-40) thought that Turneraceae were among the closest relatives of Loasaceae. Malesherbiaceae, Passifloraceae, Begoniaceae, Cactaceae, Cucurbitaceae, Flacourtiaceae (p.p.) and Fouquieriaceae, were also suggested as probable relatives. Reichenbach (1837) considered *Gronovia* as close to Loasaceae and this was followed by most subsequent authors that either included this genus in Loasaceae or in its own family. By the end of the 19th century, Urban & Gilg (in Gilg, 1894) considered Begoniaceae as the only family that could be considedred closely related to Loasaceae. Hallier (1905) considered Loasaceae close not only to Cucurbitaceae and Begoniaceae but also to Onagraceae and Campanulaceae, as part of a greatly expanded and heterogeneous Passiflorales. Wunderlich (1959) noticed that the tenuinucellate, cellular endosperm of Loasaceae with terminal haustoria and a single integument, differed from that of other Parietales and instead resembled that of families now included in Ericales and the Lamiids. During the 1970 and 1980 two major hypothesis regarding the relationships of Loasaceae were established, one supporting a placement in the Asterids and the other in Parietales/Violales. Takhtajan (1973) considered that Loasaceae was part of the Polemoniales, in Asteridae, close to Boraginaceae and Hydrophyllaceae based on trichome and embryological traits. Dahlgren (1975a) included the family in its own superorder, also in the Asterids, closest to Gentiananae, Cornanae and Lamianae, and based this relationship mostly on embryological (Dahlgren 1975b) and phytochemical (Kooiman, 1974; Jensen *et al.*, 1975) grounds. Afterwards Takhtajan (1997) followed Dahlgren, and suggested a close affinity of this family to the “Sympetalae”. On the other hand and with reserves, Cronquist (1981, 1988) left the family as part of Violales, in Dillenidae, although he expressed that Loasaceae had similarities with his Asteridae.

Hempel *et al.*, (1995) published one of the first phylogenies that included a significant portion (about one third) of the extant genera of the family. These researchers retrieved explicit Cornalean affinities for the family, supporting the interpretations of the embryological and phytochemical evidence. The inclusion of Loasaceae in Cornales was eventually confirmed in every molecular study since (Xiang, Soltis, & Soltis, 1998; Moody *et al.*, 2001; Hufford *et al.*, 2003) with Hydrangeaceae obtained as their closest living relative. This evidence has been accepted by the APG (1998), Weigend (2004b) and APGIV (2016), and remains as the most widely agreed systematic placement for the family nowadays.

The internal relationships of Loasaceae have been subject to less intensive research. One more time the best compilation about previous hypothesis of the intrafamilial relationships is Weigend (1997a) and here will be considered only the insights that have been published since, especially those based on molecular evidence. The molecular studies have relied mostly in information from the plastid markers *atp*B, *mat*K, *ndh*F, *ndh*F-*rpl*32, *psb*A-*trn*H*,* *rbc*L, *rpl*20-*rps*12*,* *rpl*32-*trn*L*,* *rps*16, *rps*16-*trn*K, *trn*L-*trn*F, *trn*H-*trn*K, *trn*S-*trn*G and *trn*S-*trnf*M (Hempel *et al.*, 1995; Moody *et al.*, 2001; Xiang *et al.*, 2002; Xiang, Thomas, & Xiang, 2011; Hufford *et al.*, 2003, 2005; Weigend *et al.*, 2004a; Weigend & Gottschling, 2006; Brokaw & Hufford, 2010a,b; Schenk & Hufford, 2011; Grissom, 2014; Acuña *et al.*, 2017) and less heavily on the nuclear sequences 26S rDNA, ETS, *idh* and ITS (Moody *et al.*, 2001; Weigend & Gottschling, 2006; Brokaw & Hufford 2010a, b; Schenk & Hufford, 2011; Grissom, 2014; Acuña *et al.*, 2017). Hempel *et al.* (1995) and Weigend (1997a) found that Loasaceae in the traditional sense is monophyletic. The deepest difference between Weigend's (1997a) classification system and that suggested by molecular phylogenies is that Gronoviaceae is not sister to Loasaceae but actually well nested within, as sister of *Mentzelia*, and that although Mentzelioideae was retrieved as a monophyletic entity (with low support) in some of the first published phylogenies (Hempel *et al.*, 1995; Xiang *et al.*, 2002), this was found not to be the case in posterior and better sampled studies (Moody *et al.*, 2001; Hufford *et al.*, 2003), with *Schismocarpus* and *Eucnide* as successive sister groups to the rest of the family and *Mentzelia* sister to Gronovioideae-Petalonychoideae. Loasoideae on the other hand has always been retrieved as a clade, and at least since Moody *et al.* (2001), sister to *Mentzelia*-Petalonychoideae-Gronovioideae.

The generic limits proposed by Weigend (1997a, 2004b, 2006) for Loasoideae, represent a major contribution towards a natural system of classification for the subfamily, and although these agree for the most part with clades obtained by molecular phylogenies, there are important deviations. The most obvious of these being: the assessment on the monophyly of *Loasa* s.str. with regards to *Presliophytum*, *Caiophora* and *Scyphanthus,* the placement of *Chichicaste* Weigend and the relationships of *Presliophytum, Aosa* and *Huidobria* (Weigend *et al.*, 2004a; Hufford *et al.*, 2005; Acuña *et al.*, 2017; Strelin *et al.*, 2017; Acuña, Chinchilla, & Weigend, 2018). The most comprehensive phylogenies that have dealt with Loasoideae so far (Hufford *et al.*, 2003, 2005; Weigend *et al.*, 2004; Weigend & Gottschling, 2006; Strelin *et al.*, 2017) have worked with a limited sampling in terms of species or molecular markers and several questions about the generic and infrageneric relationships in the family remain to be solved as is suggested by the disagreement between the different molecular topologies and morphological interpretations.

In synthesis, although the relationships of Loasaceae subfam. Loasoideae with other Cornalean clades appear to be clear nowadays, the internal relationships remain unsatisfactorily resolved. For this reason the systematics of the group as they stood until recently, are in need of a thorough revision as they may not accurately reflect natural relationships within the subfamily.

1.3.4.Biogeography of Loasaceae

The first works dealing with the biogeography of Loasaceae were descriptive in nature. Dandy (1926) considered that northern Mexico (and southwestern United states) was the center of development of both Mentzelioideae and Gronovioideae, while Chile and Argentina were suggested for Loaseae. The distribution of *Kissenia* was explained by means of continental drift. No hypothesis was proposed for Klaprothieae. Weigend (1997a) supported Dandy, however he also provided new and more detailed hypothesis (in particular about Loasaceae subfam. Loasoideae). Weigend (1997a) proposed a “Northern Central American” origin for Loasaceae as far back as the Cretaceous. He considered that the origin of the most derived clades (SAL, *Nasa*) in Loasoideae took place 20 Ma at the latest. Andean uplift in the late Tertiary and associated climatic change, would have been the reason for the formation of the habitats (high mountains, deserts, dissected landscapes) where these clades grow and diversified. Regarding the

SAL, of putative temperate South American origin, Andean uplift was proposed as the cause for the vicariance between *Caiophora* and *Loasa* while the glaciations of the Pleistocene could have influenced the northwards-eastwards expansions of *Blumenbachia* and *Caiophora*. In the case of *Nasa*, northern Peru was suggested as its the center of diversity. Dispersal both to the north and south of this area influenced by Andean uplift and consequential landscape diversification, were proposed as the causes of the geographic and radiation patterns of *Nasa*.

More recently, two works opted for quantitative approaches to explain the patterns of historical biogeography in two distantly related clades of Loasaceae. Schenk (2013) obtained a well resolved phylogeny of *Mentzelia* sect. *Bartonia* and suggested an origin <3.04 Ma, probably in the Colorado plateau. The transitions rates between adjacent areas in western North America, were found to be very different (e.g. Sonoran and Mojave Deserts), but at the same time no directional bias in the transitions between arid and mesic habitats was detected. Strelin *et al.*, (2017) obtained a poorly to moderately resolved phylogeny centered on Argentinean *Caiophora*. These researchers suggested that ancestrally, this genus was found in low to intermediate elevations, partly agreeing with Weigend (1997a). The divergence of this clade from its sister group, overlaps with the uplift of the southern Central Andes. The consequent uplift of progressively more easterly ranges was suggested as a probable cause of the parallel diversification of the *Caiophora* following the same geographic trend.

So far, the work of Strelin *et al.* (2017) remains as the only published study on quantitative historical biogeography for Loasaceae subfam. Loasoideae. The research on historical biogeography of this clade has been hindered by the lack of resolution and sparse taxonomic sampling of the available phylogenetic reconstructions. This topic remains insufficiently researched and will be one of the main focal points of the present contribution.

1.4.Working hypotheses

The unsatisfactory relationships of some clades of Loasaceae subfam. Loasoideae in previous phylogenetic studies, could be the result of sparse sampling at the taxonomic or molecular levels. At the same time, this group diversified most notably along the Andean Cordillera, thus it is expected that the major geologic events in South America during the

Cenozoic may have played a role in the history of this group. Considering these premises: (i) sampling with increased taxonomic and molecular marker representation could clarify the relationships of the South Andean Loasas clade, *Presliophytum*, *Chichicaste grandis* (Standl.) Weigend and *Nasa* and contribute in recognizing and establishing monophyletic groups as genera in the subfamily, (ii) the timing of the major diversification events in Loasaceae subfam. Loasoideae should coincide with major geologic and climatic changes in South America, particularly Andean uplift pulses for low-age nodes of the phylogeny. (iii) The Central Andes and western South America should have been important ancestral areas for crown Loasoideae and *Nasa*, as most clades with putatively plesiomorphic morphologies are mostly or entirely restricted to these areas and (iv) the inability to recognize apomorphies along with morphological convergence in the group has obscured the phylogenetic relationships in the subfamily causing artificial groups to be identified as supraspecific taxa.

1.5 Objectives

1.5.1. Research questions

Based on the working hypotheses the following questions were used as guidelines for the present contribution:

1. What are the major clades in Loasaceae subfam. Loasoideae and how are they related to each other?
2. Are these major clades in agreement with the currently accepted generic taxonomy of Loasaceae subfam. Loasoideae?
3. What taxonomic changes are necessary to render the genera in the subfamily as monophyletic entities?
4. Which of those clades are in need of a modern taxonomic revision?
5. What is the approximate timing of diversification of the major clades of Loasoideae, their areas of origin and biogeographic history?
6. What are the relationships within the largest genus of Loasaceae, *Nasa*? Would traditional molecular techniques allow the recognition of well supported clades?
7. What were the most likely ancestral ecological preferences in *Nasa*? Could morphological data offer insights on the relationships of species of *Nasa* for which no molecular data is available?

8. Are the differences in thigmonastic behavior in Loasaceae subfam. Loasoideae result of phylogenetic relatedness?

1.5.2. Specific objectives

1. Infer the relationships between and within the main clades of Loasaceae subfam. Loasoideae. Traditional molecular phylogenetic analyses employing plastid and nuclear markers have been used in order to attempt fulfilling this objective.

2. Define and delimit the genera in Loasaceae subfam. Loasoideae based on well-supported phylogenetic evidence in order to render the genera as monophyletic entities. Molecular phylogenetic techniques employing plastid and nuclear markers, as well as traditional morphological and taxonomic studies have been used to address this objective.

3. Provide a modern taxonomic revision on the genera *Presliophytum* and *Kissenia* in agreement with the phylogentic evidence accumulated. Traditional morphological and taxonomic techniques have been employed to in order to complete this objective.

5. Estimate the divergence times and historical biogeography of Loasaceae subfam. Loasoideae. For this objective, phylogenetic molecular techniques employing plastid markers and historical biogeography methodologies have been used.

6. Infer the relationships, historical biogeography, ancestral ecology of the genus *Nasa*. For this objective molecular phylogenetics employing plastid markers as well as historical biogeography and ancestral character estimation methodologies have been used. To infer the relationship between phylogeny and morphology, the phylogenetic signal of morphological traits was analyzed and the morphological similarity of the species assessed using cluster analyses.

8. Analyze the effect of phylogeny on the thigmonastic behavior of the stamens of Loasaceae subfam. Loasoideae. To achieve this, a phylogenetic reconstruction using plastid markers was obtained, and statistical analyses on the thigmonastic behavior and their phylogenetic signal were carried out.

1.6 Overview of the Dissertation

The following paragraphs will be dedicated to summarize briefly what is included in each chapter of the present dissertation. Considering that this is a cumulative thesis, each chapter from 2 to 8, corresponds to a different paper that has either been published in a peer-reviewed journal or is in preparation to be submitted to one. Each of the papers has its respective Materials and Methods section, and is structured with a different format, depending on the respective journal's guidelines. References are cited at the end of each chapter, only those of the Introduction and General Conclusions are provided in a separate literature list after the English and German Summaries. Annexes including supplementary data of the articles and shorter notes, are arranged after the literature list.

Chapter 2 deals with the relationships of the main clades of the SAL (South Andean Loasas), which until recently had remained unsolved. The main goal is to assess and evaluate the generic limits of this clade, as previous works were unable to reach a satisfactory solution, and to modify the generic taxonomy of the group if paraphyletic or polyphyletic genera were detected. The phylogenetic analyses were carried out employing four plastid markers (*rps*16, *trn*L-*trn*F, *trn*S-*trn*G, *mat*K) and ITS and were run under Maximum Likelihood and Bayesian Inference approaches. The ITS and plastid trees were mostly congruent. Three major clades were identified in a monophyletic SAL: *Blumenbachia*, *Loasa* and the *Caiophora*-clade, with these sister to *Presliophytum*. The genus *Loasa* as previously defined (Weigend 2004b; Weigend, Grau & Ackermann; 2008), had to be split, with two species transferred into *Presliophytum* and the new genera *Grausa* Weigend & R.H.Acuña and *Pinnasa* Weigend & R.H.Acuña described. The limits of the remaining genera of the clade were also clarified.

Chapter 3 is a revision of the genus *Presliophytum*. This clade lacked a formal revision and it required to be recircumscribed with the inclusion of two species from *Loasa*, according to plastid and nuclear molecular data. The main goal of this contribution is to present a modern revision for the genus. Traditional taxonomy including extensive revision of herbarium specimens and living plants, along with SEM assisted morphological studies, were employed. Five species were found to be part of this clade, with aspects of morphology, ecology and distribution studied. A complete synonym list with explicit type specimen data is provided. This group is of ecological interest as it grows in some of the driest areas of the world that can support vascular plants.

Chapter 4 is a taxonomic account of the genus *Kissenia*. Although, known for almost 200 years, and represented by only 2 extant species, the nomenclatural history of this genus is convolute and needs to be resolved fully. The main goal of this contribution is to present a revision of the taxonomic history and descriptions of the taxa in the genus, clarifying name validity, priority and typification. Traditional taxonomic methodologies, including extensive revision of herbarium specimens and living plants, along with SEM assisted morphological studies were employed. The correct names of the species should be *Kissenia capensis* and *K. spathulata,* each name is provided with explicit type assignment and a full synonym list. Aspects of morphology, distribution and ecology were also compiled.

Chapter 5 tackles the uncertain phylogenetic affinities of the morphologically isolated taxon *Chichicaste grandis*. Although previous research suggested that this species could be part of *Aosa*, its exact position remained uncertain. The phylogenetic analyses were carried out employing four plastid markers (*rps*16, *trn*L-*trn*F, *trn*S-*trn*G, *mat*K) and ITS and were run under Maximum Likelihood and Bayesian Inference approaches. The ITS and plastid trees were mostly congruent. Morphological studies were carried out in virtually every available specimen of the species as well as in living plants, including SEM assisted morphological studies. *C. grandis* was found to be nested in in a highly supported *Aosa*, sister with moderate support of *A. plumieri* with this species pair in turn sister to the sampled Brazilian representatives of *Aosa* ser. *Parviflorae*.

Chapter 6 is focused in the historical Biogeography of Loasaceae subfam. Loasoideae. The main goals of this study are to suggest plausible divergence time estimates for the main clades in Loasoideae, their most probable ancestral areas, and which geological events may have influenced the group's evolution in agreement with their spatio-temporal distribution. In order to make these inferences, dated phylogenetic reconstructions of Loasoideae were obtained employing four plastid markers (*rps*16, *trn*L-*trn*F, *trn*S-*trn*G, *mat*K) and ca. 70% of the described species of the clade. Seven Cornales fossils were used as primary calibration points. The data was analyzed using Dispersal Extinction Cladogenesis (DEC: Ree & Smith, 2008) approaches. Loasoideae diverged from its sister group in the Late Cretaceous-early Paleocene (ca. 72 Ma.), with crown Loasoideae having a Middle Paleocene to Middle Eocene age (ca. 52 Ma.). Most of currently accepted genera crown nodes have Oligocene or Miocene (30-10 Ma) ages. For crown Loasoideae, the most probable ancestral area remains ambiguous, but the Tropical Andes and Pacific deserts were retrieved frequently. The divergence of most genus-level clades predates Andean uplift and only the divergence timing of recent subclades could be associated to uplift events. The Andes appear to have acted mostly as source of new habitats and latitudinal corridors for the subfamily. Long Distance Dispersal across ocean barriers appears to have been involved in the distribution of three small clades.

Chapter 7 approaches the phylogeny, biogeography, ecological evolution and morphology of the genus *Nasa*. The goals of this study include the retrieval of major, well-supported clades in *Nasa*, the identification of probable ancestral distribution areas of these clades, the geological events that may have had historical association with divergence events, and to infer both the probable ancestral ecology and probable close relatives of the species that were not sampled using molecular methods. In order to achieve this, phylogenetic analyses were run under Maximum Likelihood and Bayesian Inference approaches employing on ca. 70% of the described taxa in the genus. Dated phylogenetic reconstructions using seven fossils as primary calibration points were obtained. Dispersal Extinction Cladogenesis (DEC: Ree & Smith, 2008) approaches were used to infer the historical biogeography of the group. Stochastic character mapping on elevational distribution and habitat data was used to infer the ancestral ecology of the genus. Cluster analyses were run in order to assess the similarity of the species based on morphological characters. The main results indicate that *Nasa* is composed by four well supported. *Nasa* diverged from their extant sister group in the Early Eocene (ca. 49 Ma) while its crown group originated in the Oligocene (ca. 29 Ma). The crown ages of three of the four main clades date to the early to mid-Miocene (20-9 Ma). The most probable ancestral area for crown *Nasa* was retrieved as the combination of Central Andes + AHZ. Mid elevation and seasonal scree habitats appear to be the ancestral conditions in the genus. Although most of the species of each major clade share similar morphologies, the morphology of several unsampled species seems to be too ambiguous to infer a reasonable phylogenetic placement. It is hypothesized that reticulate phylogenetic patters and subsequent disruptive and directional selection have had an effect in the discordance between morphology and phylogenetic placement of some taxa.

Chapter 8 deals with the relationship between thigmonasic floral behavior and phylogeny. The main goal of this is to infer the main evolutionary causes and consequences of the thigmonastic behavior in Loasaceae subfam. Loasoideae. For this study, data on stamen movement was collected from 44 species of Loasoideae, either in the field or in greenhouses. Generalized additive mixed model was applied to compare thigmonastic patterns between species with different pollination syndromes. Phylogenetic analyses were carried out for a dataset of four four plastid markers (*rps*16, *trn*L-*trn*F, *trn*S-*trn*G, *mat*K) of the 44 species. A Maximum likelihood reconstruction was used to test the phylogentic signal of the variation of the stamen movement between species. The data presented show that thigmonasty strategies are quite diversified in Loasoideae and that effect of shared ancestry on this behavior, if any, is very weak. At the same time, thigmonasty strategies in species in different clades but with similar pollinators shared more in common than with species of the same clade but with different pollinators.

In Chapter 9, the major conclusions of this thesis are summarized, with special emphasis on the relevant discoveries in phylogeny, generic relationships and biogeographic history of Loasaceae subfam. Loasoideae, as well as the taxonomic changes derived from them. Aspects of the phylogeny, taxonomy and evolution of this group that require further attention, as well as recommendations for topics that have remained unresearched so far, are identified and briefly commented.

1.7 Contribution to chapters

Chapter 2: **Acuña R, Fließwasser S, Ackermann M, Henning T, Luebert F & Weigend M. 2017.** Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae). *Taxon* **66**: 365–378.

Own contribution: Collected plant material (together with all co-authors), extracted and amplified the DNA (together with S. Fließwasser), analyzed the molecular sequences and built the phylogenetic reconstructions (together with F. Luebert), made the necessary taxonomic rearrangements (together with M. Weigend) and wrote the manuscript (with contributions from all co-authors).

Chapter 3: **Acuña R & Weigend M. 2017.** A revision of the western South American genus *Presliophytum* (Loasaceae). *Phytotaxa* **329**: 51–68.

Own contribution: Studied and georeferenced the herbarium specimens and living plants, researched the taxonomic literature, collected plant material (together with M. Weigend), and wrote the manuscript (with contributions from M. Weigend)

Chapter 4: **Acuña R & Holstein N. *In prep.*** Back and forth: Species and names of *Kissenia* R.Br. ex Endl. (Loasaceae). To be submitted to the *South African Journal of Botany*.

Own contribution: Designed the study (together with N. Holstein), studied and georeferenced the herbarium specimens and living plants (together with N. Holstein), researched the taxonomic literature (together with N. Holstein), collected plant material, wrote the manuscript.

Chapter 5: **Acuña R, Chinchilla I & Weigend M. 2018.** An unusual disjunction in Loasaceae: Central American *Chichicaste grandis* is nested in Brazilian *Aosa*. *Phytotaxa* **365**: 273–287.

Own contribution: Designed the study (together with M. Weigend), collected plant material (together with I. Chinchilla), extracted and amplified the DNA, analyzed the molecular sequences and built the phylogenetic reconstructions, studied and georeferenced the herbarium specimens and living plants (together with I. Chinchilla), wrote the manuscript (with contributions from M. Weigend and I. Chinchilla).

Chapter 6: **Acuña R, Luebert F, Henning T, & Weigend M. *In prep.*** Major lineages of Loasaceae subfam. Loasoideae diversified along with the Andean uplift. To be submitted to *Molecular Phylogenetics and Evolution*.

Own contribution: Designed the study (together with F. Luebert and M. Weigend), collected plant material (together all co-authors), extracted and amplified the DNA (together with T. Henning), analyzed the molecular sequences and built the phylogenetic reconstructions, ran the historical biogeography analyses (together with F. Luebert), wrote the manuscript (with contributions from all co-authors).

Chapter 7: **Acuña R, Romoleroux K, Luebert F, Henning T, & Weigend M. *In prep.*** Diversification patterns of *Nasa* (Loasaceae subfam. Loasoideae) in the Neotropics. To be submitted to *Botanical Journal of the Linnean Society*.

Own contribution: Designed the study (together with F. Luebert and M. Weigend), collected plant material (together with M. Weigend, T. Henning and K. Romoleroux), extracted and amplified the DNA, analyzed the molecular sequences and built the phylogenetic reconstructions, ran the historical biogeography analyses, the ancestral character estimations (together with F. Luebert) and morphological analyses, wrote the manuscript (with contributions from all co-authors).

Chapter 8: **Henning T, Mittelbach M, Ismail SA, Acuña R & Weigend, M. 2018.** A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation. *Scientific Reports* **8**: 14018.

Own contribution: Extracted and amplified the DNA, analyzed the molecular sequences and built the phylogenetic reconstructions, provided GenBank and voucher information, wrote portions of the Materials and Methods section, contributed with Figure 1 and to every section of the manuscript.

CHAPTER 2

Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae)[i]

Loasa floribunda, in its habitat,in the Metropolitan Region of Santiago, Chile. *Loasa* reaches its highest diversity in central Chile. Photograph courtesy of María Eyzaguirre.

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Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae)

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Abstract Loasaceae, a mostly American group, is one of the largest families of Cornales. In spite of considerable progress over the last 20 years, the relationships of some clades remain controversial, especially in the "South Andean Loasas" (SAL—*Blumenbachia*, *Caiophora*, *Loasa*, *Scyphanthus*). The present study addresses the phylogenetic relationships in SAL employing four plastid markers (*rps16*, *trnL-trnF*, *trnS-trnG*, *matK*) and ITS and aims at resolving the systematics and evolution of the group. Sequences obtained from a total of 59 SAL species (ca. 70% of the total, representing all lineages in the group) and 25 outgroup taxa were analysed using maximum likelihood and Bayesian inference approaches. ML best and BI strict consensus trees showed no significant differences in their topologies. Our results confirm that two species of *Loasa* ser. *Malesherbioideae* are not part of the SAL clade, but should be included in *Presliophytum*, a result which is here formalized. *Blumenbachia* (including sect. *Angulatae* and sect. *Gripidea*) is confirmed as a monophylum with high support. *Loasa* has to be redefined and restricted to a clade including only ser. *Deserticolae*, ser. *Floribundae*, ser. *Loasa* and ser. *Macrospermae*. *Scyphanthus* and *Caiophora* both are each monophyla and sister groups, but with two clades of *Loasa* as successive sister groups: (((*Caiophora*+*Scyphanthus*)+*Loasa* ser. *Pinnatae*)+(*L.* ser. *Volubiles*+*L.* ser. *Acaules*)) in a very well-supported clade. Accordingly, *Caiophora*, *Loasa* ser. *Pinnatae*, *L.* ser. *Volubiles*, *L.* ser. *Acaules* and *Scyphanthus* could be included into a single genus, with *Scyphanthus* taking priority over *Caiophora*, creating a fairly heterogenous genus of ca. 52 species and requiring 50 new names. Alternatively, the clades *Loasa* ser. *Pinnatae* and *L.* ser. *Volubiles*+ser. *Acaules* can be removed into new segregate genera, which is here argued for and which requires the creation of only 16 new names. The new genus names and some of the new combinations are here formalized.

Keywords *Blumenbachia*; *Caiophora*; morphology; *Loasa*; plastid markers; *Scyphanthus*

Supplementary Material Electronic Supplement (Tables S1 & S2; Fig. S1) and DNA sequence alignments are available in the Supplementary Data section of the online version of this article at http://ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

Loasaceae (ca. 265 spp.) is one of the largest families of Cornales, and is closely related to Hydrangeaceae (Stevens, 2001–; Xiang & al., 2011; APG IV, 2016). Morphologically the most intriguing group is the large subfamily Loasoideae (ca. 200 spp.) with complex floral morphology and function (Brown & Kaul, 1981; Hufford, 2003; Ackermann & Weigend, 2006; Henning & Weigend, 2012; Strelin & al., 2016a, b). Loasoideae is largely South American, with seven species native to Central America and the West Indies and also comprising the only extra-American taxa of Loasaceae, *Kissenia* R.Br. ex Endl. (two species in Africa and the Arabian Peninsula) and *Plakothira* J.Florence (three species in the Marquesas Archipelago).

The last comprehensive systematic revision of Loasoideae was provided by Urban & Gilg (1900) in their monumental "Monographia Loasacearum", recognizing seven genera in the subfamily. Weigend (1997, 2006) revised the genus limits in Loasoideae on the basis of morphology, creating five new genera and transferring two sections from *Caiophora* C.Presl into *Blumenbachia* Schrad. The infrageneric taxa accepted by Urban & Gilg (1900) provided the foundation for re-circumscribing the genera *Caiophora* (ca. 37 spp.), *Blumenbachia* (11 spp.) and *Presliophytum* (Urb. & Gilg) Weigend (3 spp.) and to segregate the new genera *Aosa* Weigend (7 spp.) and *Nasa* Weigend (ca. 100 spp.) from *Loasa* Adans. (Weigend, 1997, 2006). Additionally, Grau (1997) revalidated the genus *Huidobria* Gay (2 spp.), previously treated as a section of *Loasa* by

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Urban & Gilg (1900). Weigend (1997, 2006) described the genera *Chichicaste* Weigend (1 sp.) and *Xylopodia* Weigend (1 sp.), both unknown to science at the time of publication of "Monographia Loasacearum" (Urban & Gilg 1900).

Iridoid phytochemistry underscores the monophyly of *Caiophora* (Weigend & al., 2000), while the specific composition of seed fatty acids emphasizes the coherence of *Nasa* (Weigend & al., 2004a). Likewise, most genus limits are also underscored by seed morphology (Weigend & al., 2004a, 2005). Subsequent molecular studies confirmed most of the generic re-arrangements, but also highlighted residual problems with genus delimitation, especially in a group informally called "South Andean Loasas" (Weigend, 1997; hereafter SAL). The group comprises over 80 species in the genera *Caiophora*, *Blumenbachia*, *Scyphanthus* Sweet and *Loasa* (Weigend & al., 2004b, 2005, 2008; Ackermann, 2011) and—as the name implies—has its centre of diversity in the southern Andes, but representatives extend from Cotopaxi (central Ecuador) and Rio de Janeiro (southeastern Brazil), to Magallanes (Chile) and Santa Cruz (Argentina). SAL share several synapomorphies (Weigend & al., 2004b) and represent a monophyletic group based on molecular data (Moody & al., 2001; Hufford & al., 2003, 2005; Weigend & al., 2004b; Strelin & al., 2017). *Loasa*, originally the largest genus (Urban & Gilg, 1900), had already lost most of its species to the segregate genera (Weigend, 1997, 2004; see above), but molecular data indicated that the redefined *Loasa* s.str. was still non-monophyletic.

The purpose of the present study is to re-examine the phylogeny and systematics of South Andean Loasas based on an extended sampling and using four plastid markers and ITS. According to the phylogenetic analyses, genus limits, especially of *Loasa*, are adjusted and two new genera are formally described.

■ MATERIALS AND METHODS

Plant material and outgroup selection. — The taxon sampling for the molecular data included dry leaf material of 59 species of SAL from the genera *Blumenbachia* (9 of 11 species), *Caiophora* (28 of 37 species), *Loasa* (20 of 34 species) and *Scyphanthus* (both species) (based on Weigend, 1997). These represent ca. 70% of the species diversity in the SAL and include at least one species from each major infrageneric entity. A few species are represented by more than one accession (*Blumenbachia dissecta* (Hook. & Arn.) Weigend & Grau, *B. sylvestris* Poepp., *Caiophora cirsiifolia* C.Presl, *Loasa bergii* Hieron., *L. elongata* Hook. & Arn., *L. micrantha* Poepp.), in these cases each accession represents populations with different morphologies, often assigned to distinct species until recently. Additionally, all known species of *Presliophytum*, five species of *Aosa*, five species from different clades of *Nasa*, *Plakothira parviflora* J.Florence, *Xylopodia klaprothioides* Weigend, *Kissenia capensis* Endl., both species of *Huidobria*, *Gronovia scandens* L., two species from different clades of *Mentzelia* L. and *Eucnide urens* Parry ex Coville were included in the analyses. Finally, three species of Hydrangeaceae, as well as *Actinidia chinensis* Planch. (Actinidiaceae), *Antirrhinum majus* L. (Plantaginaceae) and *Panax ginseng* C.A.Mey. (Araliaceae) were chosen as distant outgroups. Outgroups were selected based on the phylogenetic studies of Weigend & al. (2004b) and Hufford & al. (2005) as well as on the familial and ordinal classification outlined by APG IV (2016). All sampled plant material with its geographic origin, herbarium voucher, and GenBank accession numbers is listed in Appendix 1.

DNA extraction, amplification and sequencing. — DNA was extracted from samples of silica gel-dried leaves or herbarium leaf material with a modified CTAB method (Doyle & Doyle, 1987). The PCR amplifications were performed in 25 µl of a mix containing 0.6 U of *Taq* Polymerase, 5.0 mM $MgCl_2$, 100 µM of each dNTP, 0.2 µM of each primer and about 50 ng of genomic DNA. We sequenced the plastid regions *trnL-trnF*, *matK*, the *trnS-trnG* intergenic spacer, and the *rps16* intron (taxon sampling overlaps for all markers except for *matK* that was not obtained for *Loasa lateritia* Gillies ex Arn. and *Blumenbachia scabra* (Miers) Urb.). The primers "c" and "f" (Taberlet & al., 1991) were used to amplify the *trnL-trnF* region, including the *trnL*(UAA) intron and the spacer between the *trnL*(UAA) 3′exon and *trnF*(GAA), with a 2 min initial denaturation at 94°C, 35 cycles of 94°C, 30 s; 52°C, 1 min; 72°C, 1 min, and a final elongation period of 10 min at 72°C. The *matK* region was amplified in two fragments, one with the primers trnK-3914F and matK-1848R and the other with matK-710F and trnK-2R, all obtained from Johnson & Soltis (1995) and using the cycling conditions described by the same authors. The *trnS-trnG* intergenic spacer was originally amplified with the primers trnS and trnG described by Hamilton (1999) with the following cycling conditions: 5 min initial denaturation at 95°C, 34 cycles of 95°C, 30 s; 50°C, 1 min; 72°C, 1.5 min, and a final elongation period of 4 min at 72°C. However, as amplification was difficult for this region in some cases, the primers $trnS^{GCU}$ and $3'trnG^{UUC}$ and the cycling conditions explained in protocol No. 2 described by Shaw & al. (2005) were used to amplify additional samples for the *trnS-trnG* intergenic spacer. The primers rpsF and rpsR2 (Oxelman & al., 1997) were used to amplify the *rps16* intron with a 5 min initial denaturation at 94°C, 34 cycles of 94°C, 1 min; 55°C, 1 min; 72°C, 1 min, and a final elongation period of 2 min at 72°C. PCR products were cleaned following Werle & al. (1994). The same primers used for the amplification of each marker were used for the sequencing, except for $3'trnG^{UUC}$, which was replaced by 5′trnG2S (Shaw & al., 2005). The ITS region was successfully amplified for a subset of 52 ingroup and 18 outgroup species, representing all the major clades found in the plastid analyses, with the exception of *Blumenbachia* sect. *Gripidea* (Miers) Urb.; amplification of this region was performed using the primers ITS5 and ITS4 and the PCR conditions described in White & al. (1990). In addition to the four plastid markers and ITS, we attempted to obtain sequences of the nuclear regions *DAL1*, ETS, *G3pdh*, *LFY*, *PHYC*, *TIF3H1*, *TOPO6* and *waxy* (Electr. Suppl.: Table S1). However, amplification results were unsatisfactory and it was impossible to obtain a meaningful dataset. Sequencing was performed using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, U.K.) and an ABI 3730xl DNA Analyzer (Applied Biosystems).

The resulting sequences were assembled in Geneious v.8.0.1 (Kearse & al., 2012) using the default De Novo assemble settings. The sequences from *Actinidia chinensis* and *Panax ginseng* were obtained from complete chloroplast genomes and independent ITS accessions available in GenBank. For *Antirrhinum majus* five different GenBank accessions were used (see Appendix 1).

Phylogenetic analysis. — The assembled sequences were aligned in MAFFT v.7 (Katoh & Standley, 2013) using the G-INS-1 option which is a progressive method recommended for medium scale (up to a few hundred sequences) alignments and that shows relatively lower instability than other alignment methods (Boyce & al., 2015), followed by manual adjustments using PhyDE v.0.9971 (Müller & al., 2010).

FindModel (available from http://hcv.lanl.gov/content/sequence/findmodel/ findmodel.html), which implements Posada & Crandall’s (2001) Modeltest, based on the Akaike information criterion, selected GTR+Gamma as the model that best fits all four plastid markers and K80 (Kimura 2-parameter) as the model that best fits ITS. Phylogenetic reconstructions were carried out for maximum likelihood (ML, Felsenstein, 1981), conducted in RAxML v.8 (Stamatakis, 2014) included in RAxMLGUI v.1.3.1 (Silvestro & Michalak, 2012), and Bayesian inference (BI, Mau & al., 1999), conducted in MrBayes v.3.2 (Huelsenbeck & Ronquist 2001). Each marker was at first analyzed separately. In the absence of topological conflict (defined as incongruence in the topologies of nodes with bootstrap support [BS] >80%) the plastid markers were combined. We found significant topological BS conflict between the ITS and the combined plastid dataset at four nodes within *Caiophora* and one in *Loasa* ser. *Pinnatae* Urb. & Gilg. In consequence, the plastid and ITS datasets were not combined. Both ML and BI analyses were then run independently for the combined plastid dataset (partitions unlinked) and for ITS. ML analyses were implemented using the GTRCAT approximation, because it works in an analogous way to GTR+Gamma (K80: Kimura 2-parameter is not available in RAxML) and yields similar results but with less intensive computational costs (Stamatakis, 2014). The statistical support for nodes was assessed by 1000 ML slow bootstrap replicates with 100 runs under the same analysis conditions. The BI were conducted, under the respective best fit model, with two independent runs with one cold and four heated chains; the Markov chain had a length of 4 million generations, sampled every 1000 generations. After convergence was assessed in Tracer v.1.5 (Rambaut & Drummond, 2007), the first million generations were discarded as burn-in.

■ RESULTS

The combined plastid dataset with the four sequences contained 6459 aligned positions (3205 different alignment patterns), representing 65 ingroup and 28 outgroup accessions. The ITS dataset contained 864 aligned positions (656 different alignment patterns), representing 58 ingroup and 18 outgroup accessions.

Both ML and BI strict consensus plastid dataset trees showed no significant differences in their topologies and therefore only the ML tree is shown (Fig. 1). The ITS ML tree has the same major clades as the plastid dataset, with only significant topological differences at four nodes in *Caiophora* and one in *Loasa* ser. *Pinnatae* (compare Electr. Suppl.: Fig. S1). The remaining conflicting nodes, including those along the backbone, have low support in the ITS tree. SAL is a well-supported monophylum in both datasets (plastid: 100 BS, 1.00 PP [posterior probability]; ITS: 74 BS, 1.00 PP), comprising the well-supported genera *Blumenbachia* (plastid: 96 BS, 1.00 PP; ITS: 99 BS, 1.00 PP), *Caiophora* (plastid: 100 BS, 1.00 PP; ITS: 96 BS, 1.00 PP), *Scyphanthus* (plastid: 100 BS, 1.00 PP) and a highly paraphyletic *Loasa* s.str. Two species of *Loasa* corresponding to ser. *Malesherbioideae* Urb. & Gilg, are retrieved outside SAL in a clade with *Presliophytum* (plastid: 100 BS, 1.00 PP; ITS: 91 BS, 1.00 PP).

SAL represents a polytomy of three clades: a *Blumenbachia*-clade (all three sections), a *Loasa*-clade including most of its series (plastid: 68 BS, 0.99 PP) with *Loasa* ser. *Loasa*, ser. *Floribundae* Urb. & Gilg, ser. *Deserticolae* Urb. & Gilg and ser. *Macrospermae* Urb. & Gilg, and a third clade (plastid & ITS: 100 BS, 1.00 PP), with *Loasa* ser. *Volubiles* Urb. & Gilg and ser. *Acaules* Urb. & Gilg (plastid: 100 BS, 1.00 PP; ITS: 80 BS, 0.98 PP; the latter series includes only *Loasa lateritia* Gillies ex. Arn.) as sister to *Loasa* ser. *Pinnatae* Urb. & Gilg+(*Scyphanthus*+*Caiophora*) (plastid: 99 BS, 1.00 PP). *Loasa* ser. *Pinnatae* (plastid & ITS 100 BS, 1.00 PP) is sister to *Scyphanthus*+*Caiophora* (plastid: 78 BS, 0.97 PP; ITS 99 BS, 1.00 PP). *Loasa* ser. *Acaules* and ser. *Volubiles* constitute a well supported monophylum in both datasets, with ser. *Acaules* resolved as sister to *L. gayana* Urb. & Gilg in the plastid dataset (100 BS, 1.00 PP).

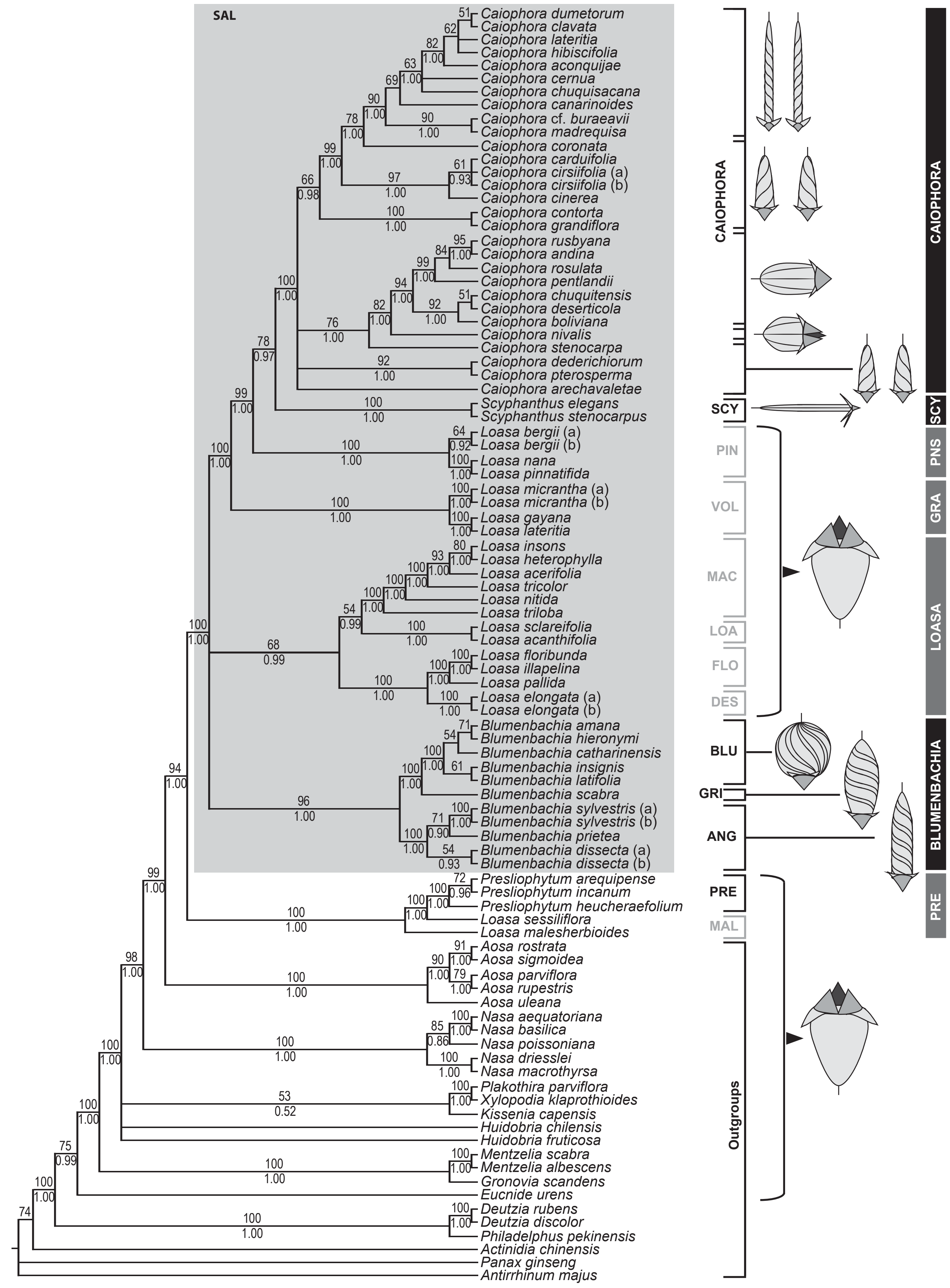
The *Loasa* clade shows a poorly supported sister relationship between *Loasa* ser. *Loasa*+ser. *Macrospermae* (plastid: 54 BS, 0.99 PP) and ser. *Floribundae*+ser. *Deserticolae* (plastid: 100 BS, 1.00 PP; ITS: 84 BS, 0.97 PP). Each of the four series is very well supported (plastid & ITS: 100 BS, 1.00 PP, except ser. *Floribundae* with 94 BS support for ITS). The *Blumenbachia* clade is very well supported, with sect. *Angulatae* Urb. & Gilg (plastid: 100 BS, 1.00 PP; ITS: 68 BS, 1.00 PP) sister to sect. *Gripidea*+sect. *Blumenbachia* (plastid 100 BS, 1.00 PP). Section *Blumenbachia* is also very well-supported (plastid: 100 BS, 1.00 PP; ITS: 99 BS, 1.00 PP).

The SAL-clade itself is sisters to the *Presliophytum*-clade (plastid: 94 BS, 1.00 PP; ITS: 58 BS, 0.99 PP). According to the plastid dataset, *L. sessiliflora* Phil. and *L. malesherbioides* Phil. are consecutive sister to *Presliophytum* (100 BS, 1.00 PP).

The well-supported clades *Aosa* (plastid & ITS: 100 BS, 1.00 PP) and *Nasa* (plastid & ITS: 100 BS, 1.00 PP) are closely related to the SAL–*Presliophytum*-clade and, according to the plastid data, are sequentially retrieved, with *Aosa* sister to *Presliophytum*+SAL and *Nasa* sister to *Aosa*+(*Presliophytum*+SAL).

■ DISCUSSION

Approximately 70% of the currently recognized species in SAL were included in this study, and the results confirm the conclusions obtained in previous molecular studies (Weigend


SAL
Caiophora dumetorum
Caiophora clavata
Caiophora lateritia
Caiophora hibiscifolia
Caiophora aconquijae
Caiophora cernua
Caiophora chuquisacana
Caiophora canarinoides
Caiophora cf. buraeavii
Caiophora madrequisa
Caiophora coronata
Caiophora carduifolia
Caiophora cirsiifolia (a)
Caiophora cirsiifolia (b)
Caiophora cinerea
Caiophora contorta
Caiophora grandiflora
Caiophora rusbyana
Caiophora andina
Caiophora rosulata
Caiophora pentlandii
Caiophora chuquitensis
Caiophora deserticola
Caiophora boliviana
Caiophora nivalis
Caiophora stenocarpa
Caiophora dederichiorum
Caiophora pterosperma
Caiophora arechavaletae
Scyphanthus elegans
Scyphanthus stenocarpus
Loasa bergii (a)
Loasa bergii (b)
Loasa nana
Loasa pinnatifida
Loasa micrantha (a)
Loasa micrantha (b)
Loasa gayana
Loasa lateritia
Loasa insons
Loasa heterophylla
Loasa acerifolia
Loasa tricolor
Loasa nitida
Loasa triloba
Loasa sclareifolia
Loasa acanthifolia
Loasa floribunda
Loasa illapelina
Loasa pallida
Loasa elongata (a)
Loasa elongata (b)
Blumenbachia amana
Blumenbachia hieronymi
Blumenbachia catharinensis
Blumenbachia insignis
Blumenbachia latifolia
Blumenbachia scabra
Blumenbachia sylvestris (a)
Blumenbachia sylvestris (b)
Blumenbachia prietea
Blumenbachia dissecta (a)
Blumenbachia dissecta (b)
Presliophytum arequipense
Presliophytum incanum
Presliophytum heucheraefolium
Loasa sessiliflora
Loasa malesherbioides
Aosa rostrata
Aosa sigmoidea
Aosa parviflora
Aosa rupestris
Aosa uleana
Nasa aequatoriana
Nasa basilica
Nasa poissoniana
Nasa driesslei
Nasa macrothyrsa
Plakothira parviflora
Xylopodia klaprothioides
Kissenia capensis
Huidobria chilensis
Huidobria fruticosa
Mentzelia scabra
Mentzelia albescens
Gronovia scandens
Eucnide urens
Deutzia rubens
Deutzia discolor
Philadelphus pekinensis
Actinidia chinensis
Panax ginseng
Antirrhinum majus
CAIOPHORA
SCY
PIN
VOL
MAC
LOA
FLO
DES
BLU
GRI
ANG
PRE
MAL
Outgroups
CAIOPHORA
SCY
PNS
GRA
LOASA
BLUMENBACHIA
PRE

& al., 2004b; Hufford & al., 2005), while providing increased support and resolution (Fig. 1). The genus *Loasa* is confirmed as paraphyletic with strong evidence from both plastid and nuclear markers, concurring with a large body of published evidence (Weigend, 1997; Weigend & al., 2004a, b, 2005; Hufford & al., 2005; Strelin & al., 2017). Plastid and ITS data support the same major clades. However, the plastid data provide considerably better resolution and support for the backbone of the South Andean Loasas than ITS (Electr. Suppl.: Fig. S1). The same difference between plastid data and ITS has been reported for *Hydrangea* L. of the Hydrangeaceae, the sister family to Loasaceae, by De Smet & al. (2015) and several other plant groups (*Urtica* L.—Grosse-Veldmann & al., 2016, Boraginaceae—Chacón & al., 2016).

Weigend (1997) and Weigend & al. (2004b) argued that *Loasa* ser. *Malesherbioideae* (Fig. 2A) and *Presliophytum* (Figs. 2B, 3D, 4A) share a very complex and essentially identical inflorescence morphology (characterized by pronounced metatopia), alternate phyllotaxis (rare in Loasoideae) and very similar flower (Fig. 2A, B) and leaf morphology. Both species of this series are here included (*L. malesherbioides*, *L. sessiliflora*) and are retrieved as consecutive sisters to *Presliophytum* (with all three species included in the phylogeny). This relationship is in agreement with both morphology and previous molecular data (Hufford & al., 2003, 2005; Weigend & al., 2004b), and the two newly required names under *Presliophytum* are formalized below.

The remaining species of *Loasa*, and the genera *Blumenbachia*, *Scyphanthus* and *Caiophora* are retrieved as a monophyletic SAL-clade. The transfer of sect. *Angulatae* and sect. *Gripidea* from *Caiophora* into *Blumenbachia* (Weigend 1997; Weigend & al., 2008) is clearly supported. *Blumenbachia*, as here resolved, is morphologically homogeneous with fruits always twisted in the same direction within an inflorescence (Fig. 1) and virtually identical floral morphology (Figs. 2C–E, 3E) with a unique combination of characters (petal and floral scale morphology) not found elsewhere in the family (Weigend & al., 2004b). The Brazilian sect. *Gripidea* (Fig. 2D) is sister to the predominantly Brazilian sect. *Blumenbachia* (Figs. 2E, 4B) and these two in turn are sister to the South Andean sect. *Angulatae* (Fig. 2C) as previously argued based on inflorescence, fruit and seed morphology (Weigend, 1997; Weigend & al., 2005; Henning & al., 2015).

Caiophora is resolved as monophyletic in the circumscription of Weigend (1997) and Weigend & Ackermann (2003). Internal resolution of major clades is poor to moderate, but *Caiophora arechavaletae* (Urb.) Urb., *C. dederichiorum* Mark. Ackermann & Weigend, *C. pterosperma* (G.Don) Urb. & Gilg and *C. stenocarpa* Urb. & Gilg, of the sect. *Bicallosae* (Urban & Gilg, 1900) and the "loasoid" *Caiophora* group (Ackermann & Weigend, 2013), are retrieved as paraphyletic at the base of the genus. This finding is in agreement with their "primitive" floral morphology, i.e., flowers showing plesiomorphic characters shared with the sister genus *Scyphanthus* and their consecutive sister, *Loasa* ser. *Pinnatae* (Fig. 3J, K), rather than with the floral morphology of the more derived species of *Caiophora* (Fig. 3L). Two major, moderately supported clades are retrieved in *Caiophora* (Fig. 1), corresponding roughly to a clade with straight fruits (sect. *Orthocarpae*; Urban & Gilg, 1900) and a clade with twisted fruits (sect. *Dolichocarpae*; Urban & Gilg, 1900). However, the markers here employed were unable to provide a well-supported phylogeny for *Caiophora*. A sister relationship of *Caiophora* and *Scyphanthus* found with weak support by Hufford & al. (2003, 2005) and Weigend & al. (2004b) is here confirmed with moderate (plastid) to high (ITS) support. *Loasa* ser. *Pinnatae* is sister to *Caiophora*+*Scyphanthus* and these three groups form a strongly supported clade. The serrate petal margins (Figs. 2K, L, 3A; lost in the most-derived clades of *Caiophora*: Fig. 3B, C), elaborate floral scales, finely dissected leaves (Fig. 4F–I), and deeply pitted seeds with fenestrate anticlinal walls (Weigend & al., 2005) support the molecular data from a morphological perspective. *Scyphanthus* and *Caiophora* share fruits opening with longitudinal dehiscence, whereas *Loasa* ser. *Pinnatae* (Fig. 1) has retained the plesiomorphic condition of fruits opening only apically. The sister group to these three clades is *Loasa* ser. *Volubiles* (including monotypic ser. *Acaules*)—an essentially Chilean group, usually with variously ternate leaves and flowers which are similar to those of the *Caiophora*-clade, but less elaborate in sepal, petal and floral scale morphology (Figs. 2I, J, 3I). They all share open inflorescences (i.e., with indeterminate growth), in the case of ser. *Volubiles* elongated and winding, in the case of ser. *Acaules* axillary flowers arising from the basal rosette.

The remaining series of *Loasa* (ser. *Loasa*, ser. *Macrospermae*, ser. *Floribundae*, ser. *Deserticolae*) form a moderately-supported clade according to the plastid data. They have been consistently considered as closely related in the past (Urban & Gilg, 1900) and have similar flowers and floral scales (Weigend & al., 2004b) (Fig. 3F–H), as well as seeds unique in Loasoideae for their subterminal hilum or hilar scar (Weigend & al. 2005). This clade is resolved into two sister clades, which are clearly consistent with vegetative, flower, fruit, and seed morphology (Urban & Gilg, 1900; Weigend & al., 2004b, 2005): Large-seeded and large-leaved ser. *Loasa* and ser. *Macrospermae* are retrieved in one clade. Series *Loasa* is differentiated by its biennial to perennial habit and pinnatisect

Fig. 1. Maximum likelihood tree based on a plastid combined dataset (*matK*, *rps16*, *trnL-trnF*, *trnS-trnG*). ML bootstrap support values are indicated above branches and Bayesian posterior probabilities are indicated below; only values above 50 and 0.5, respectively, are shown. SAL clade is included in the large grey box. Currently accepted generic and infrageneric names are indicated in the first column next to species names, with those marked in grey referring to *Loasa*. Schematic representations of fruit structure for major clades of SAL and related groups are depicted in the middle column. Pedicels of exclusively pendulous fruits point to the top of the page. Proposed generic and infrageneric delimitations in SAL are shown as bars in the far right column. The dark grey bars represent new delimitations that differ from those currently accepted. Abbreviations: ANG, sect. *Angulatae*; BLU, sect. *Blumenbachia*; DES, ser. *Deserticolae*; FLO, ser. *Floribundae*; GRA, *Grausa* gen. nov.; GRI, sect. *Gripidea*; LOA, ser. *Loasa*; MAC, ser. *Macrospermae*; MAL, ser. *Malesherbioideae*; PIN, ser. *Pinnatae*; PNS, *Pinnasa* gen. nov.; PRE, *Presliophytum*; SCY, *Scyphanthus*; VOL, ser. *Volubiles* and ser. *Acaules*. (a) and (b) indicate different accessions for the respective species.

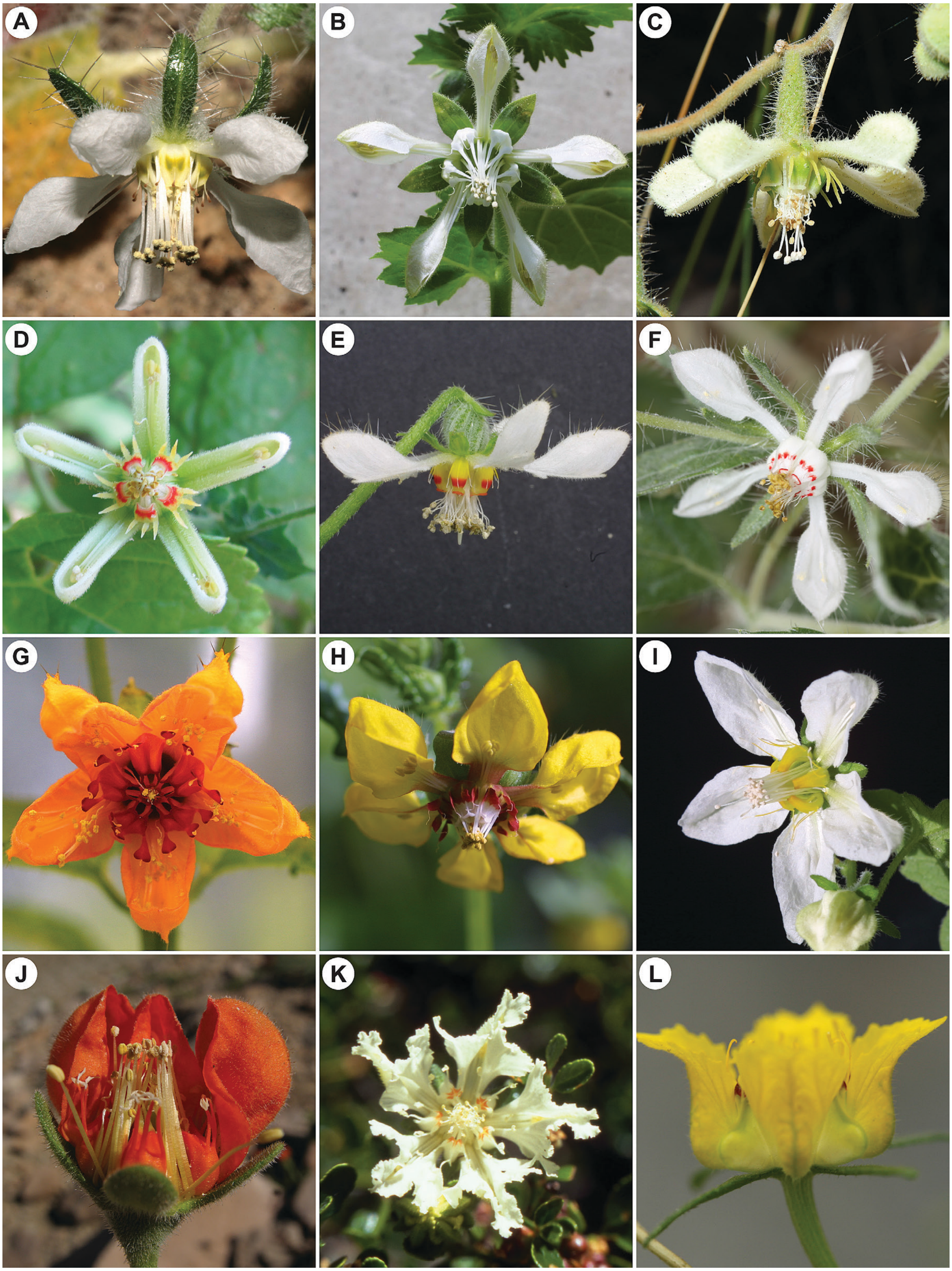


Fig. 2. Flowers of selected species of SAL and related clades. **A,** *Loasa malesherbioides* (ser. *Malesherbioideae*; *Weigend KW 1084*, BONN; photo by H. Hilger); **B,** *Presliophytum heucheraefolium* (*Weigend 5136*, B); **C,** *Blumenbachia sylvestris* (sect. *Angulatae*; not vouchered; photo M. Neumann); **D,** *Blumenbachia exalata* (sect. *Gripidea*; not vouchered; photo J. Durigon); **E,** *Blumenbachia insignis* (sect. *Blumenbachia*; *Weigend 7475*, B); **F,** *Loasa pallida* (ser. *Floribundae*; *Ackermann 1319*, BONN); **G,** *Loasa acanthifolia* (ser. *Loasa*; *Weigend 6925*, BSB); **H,** *Loasa nitida* (ser. *Macrospermae*; *Weigend 7346*, B); **I,** *Loasa gayana* (ser. *Volubiles*; *Weigend 7057*, BSB); **J,** *Loasa lateritia* (ser. *Acaules*; not vouchered; photo M. Belov); **K,** *Loasa pinnatifida* (ser. *Pinnatae*; *Weigend 6880*, BRCO); **L,** *Scyphanthus elegans* (*Weigend 9032*, BSB). — Plants from the wild or cultivated in the botanical gardens of Berlin and Bonn. Photographs M. Ackermann or M. Weigend unless otherwise stated.

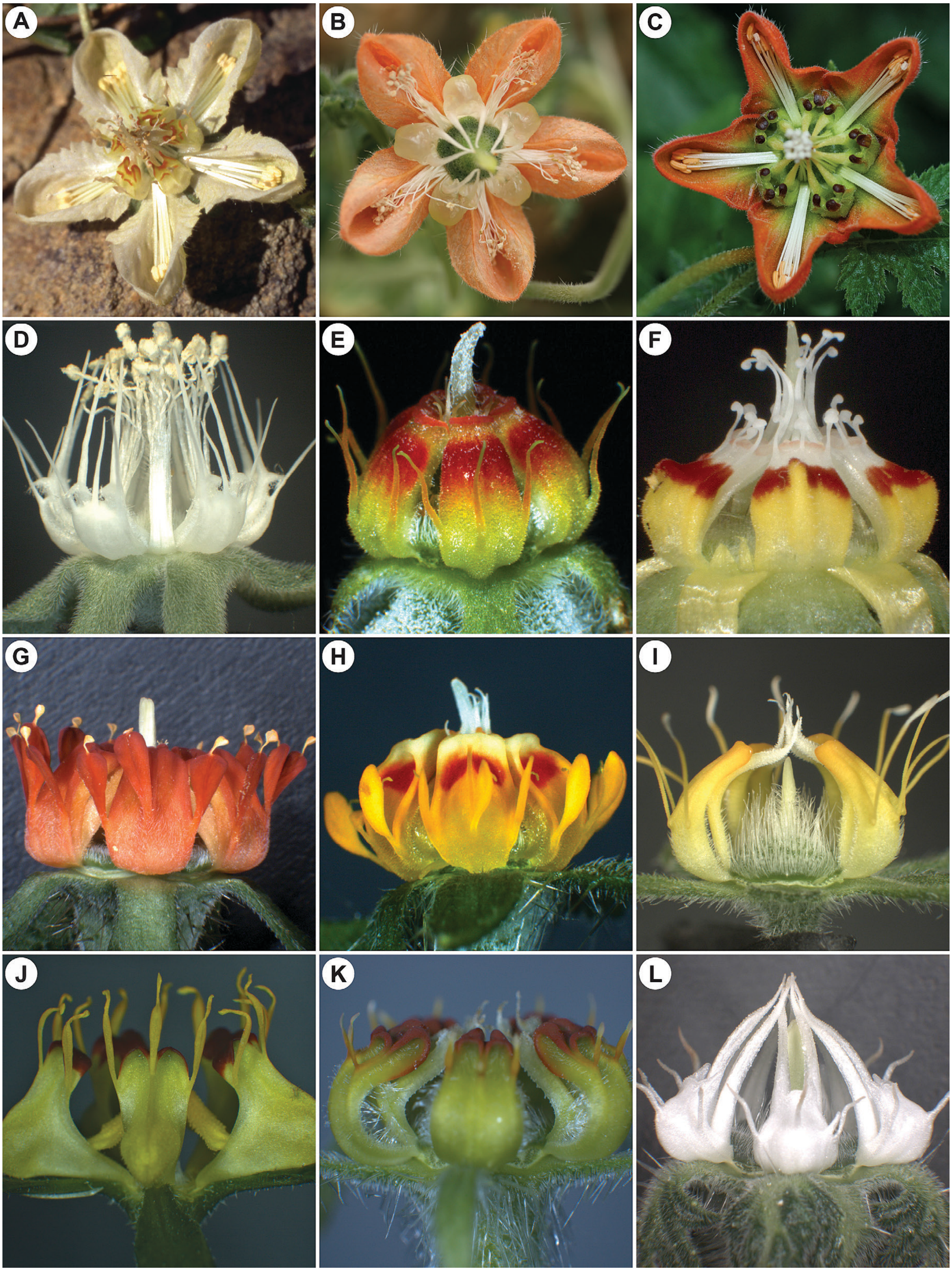


Fig. 3. Flowers of selected species of *Caiophora*. **A,** *Caiophora dederichiorum* (*Weigend 7738*, BSB); **B,** *Caiophora pentlandii* (*Weigend 8771*, BSB); **C,** *Caiophora lateritia* (*Ackermann 1104*, BONN); Floral scales of selected species of SAL and related clades. **D,** *Presliophytum incanum* (*Weigend 8064*, B; photo P. Beckers); **E,** *Blumenbachia latifolia* (sect. *Blumenbachia*; *Weigend 9135*, B); **F,** *Loasa elongata* (ser. *Deserticolae*; *Ackermann s.n.*, BONN); **G,** *Loasa acanthifolia* (ser. *Loasa*; *Weigend 6925*, BSB); **H,** *Loasa triloba* (ser. *Macrospermae*; *Luebert & Bidart 3014*, B); **I,** *Loasa gayana* (ser. *Volubiles*; *Weigend 7057*, BSB); **J,** *Scyphanthus stenocarpus* (*Gardner & Knees 8351*, BSB); **K,** *Caiophora stenocarpa* (*Ackermann 758*, BSB); **L,** *Caiophora deserticola* (*Weigend 7761*, BSB). — Plants from the wild or cultivated in the botanical gardens of Berlin and Bonn. Photographs M. Ackermann or M. Weigend unless otherwise stated.

leaves (Fig. 4D) from ser. *Macrospermae* with an annual habit and palmate to palmatifid leaves (Fig. 4E). Series *Floribundae* (Figs. 2F, 4C) and *Deserticolae* (Fig. 3F) are retrieved in the other clade as sister to each other; they share indeterminate, usually winding inflorescences and primarily differ in details of flower morphology.

Phylogenetic relationships here shown are thus highly consistent with morphological data presented by various authors in the past (e.g., Urban & Gilg, 1900; Weigend, 1997; Weigend & al., 2004b). Accordingly, the required generic re-arrangements should be undertaken in order to adjust the classification of this group to phylogenetic relationships as now understood.

The species of *Loasa* ser. *Malesherbioideae* are transferred to the genus *Presliophytum*, requiring only minor additions to the genus description, mainly referring to habit (*Presliophytum* so far included only shrubs, and now also herbaceous species) and seed morphology. The circumscription of the genus *Loasa* needs to be adjusted to include only ser. *Loasa* (including the type *Loasa acanthifolia* Lam.), ser. *Macrospermae*, ser. *Floribundae* and ser. *Deserticolae.*

The remaining three series of *Loasa*, ser. *Volubiles*, ser. *Acaules* and ser. *Pinnatae* are part of a well-supported clade that also includes *Scyphanthus* and *Caiophora*, providing several taxonomical options: Redefinition as a single genus would require transferring all species of the clade to *Scyphanthus* Sweet 1828, which has priority over *Caiophora* C.Presl 1831. The resulting genus would be morphologically heterogeneous in vegetative, fruit, seed and floral morphology and new combinations would be required for all ca. 37 species of *Caiophora* (which have never previously been placed in *Scyphanthus*) as well as the species of *Loasa* ser. *Volubiles*, ser. *Acaules* and ser. *Pinnatae*. A combination of *Scyphanthus* and *Caiophora* or a combination of *Scyphanthus*, *Caiophora* and *Loasa* ser. *Pinnatae* would be defensible on the basis of morphology, although undesirable from a nomenclatural point of view, since numerous new names would still be required. Overall, the most conservative

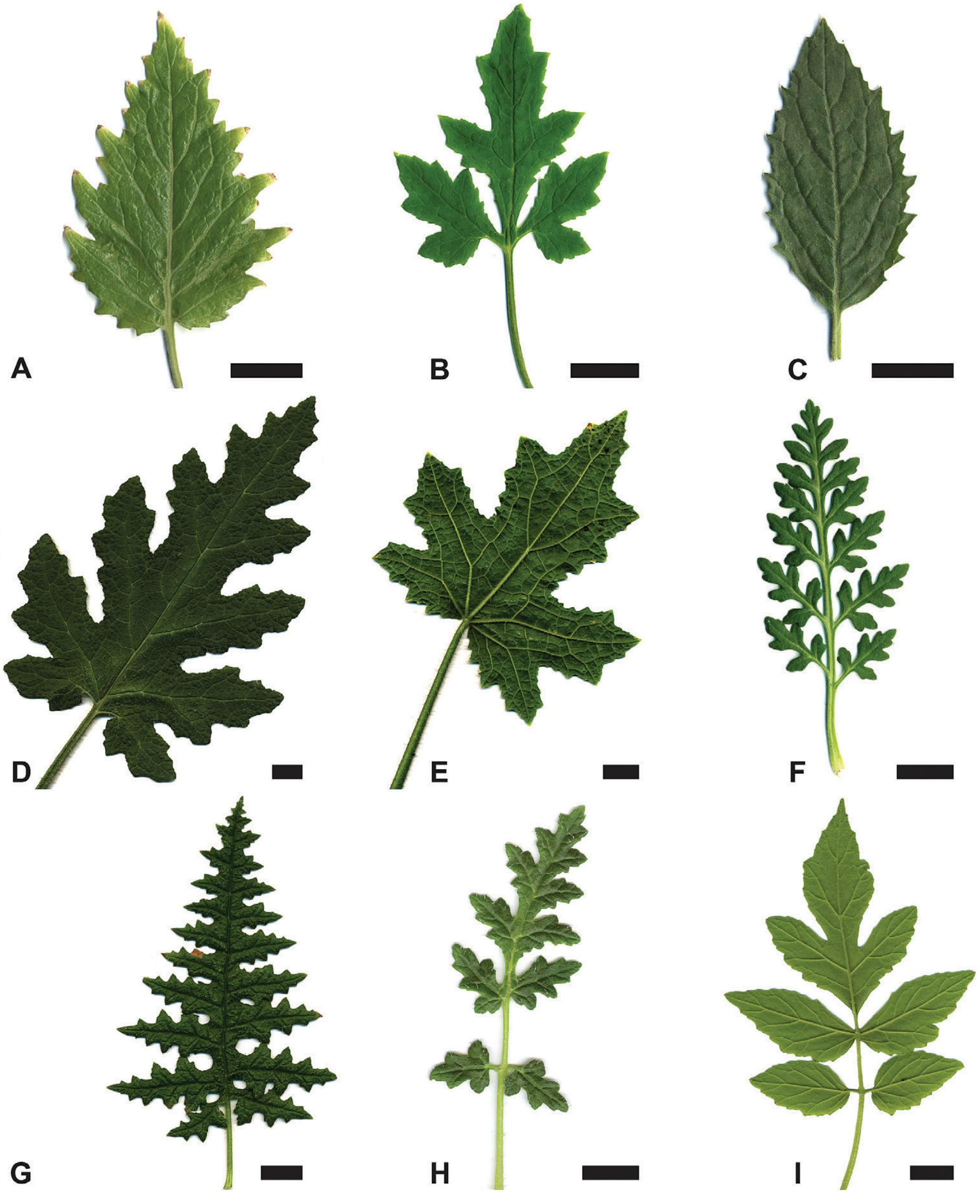


Fig. 4. Leaf morphology of selected species of SAL and related clades. **A,** *Presliophytum incanum* (*Weigend & Hilger 8912*, BONN); **B,** *Blumenbachia latifolia* (sect. *Blumenbachia*; *Weigend 9315*, BONN); **C,** *Loasa pallida* (ser. *Floribundae*; *Joßberger 66*, BONN); **D,** *Loasa acanthifolia* (ser. *Loasa*; *Joßberger s.n.*, BONN); **E,** *Loasa acerifolia* (ser. *Macrospermae*; *Weigend 9142*, BSB); **F,** *Scyphanthus elegans* (*Weigend 9032*, BSB); **G,** *Caiophora stenocarpa* (*Ackermann 758*, BSB); **H,** *Caiophora deserticola* (*Weigend 7761*, BSB); **I,** *Caiophora hibiscifolia* (*Ackermann 1103*, BONN). — All views are adaxial, except E and I. The black bars measure 2 cm. Plants cultivated in the botanical gardens of Berlin and Bonn. Photographs M. Ackermann or R. Acuña.

approach with regards to nomenclature is the segregation of ser. *Volubiles*+*Acaules* (5 spp., Weigend & al., 2008) into one new genus, *Grausa* gen. nov. (requiring 5 new names) and the segregation of ser. *Pinnatae* (ca. 11 spp., Weigend & al., 2008) into another new genus, *Pinnasa* gen. nov. All the genera then recognized are monophyletic, morphologically easily circumscribed and represent both morphologically and ecologically distinct units. A general comparison of the major taxonomic changes between Urban & Gilg (1900), Weigend (1997) and the present study is presented in Electr. Suppl.: Table S2.

■ FORMAL TAXONOMY

New combinations for *Loasa* ser. *Malesherbioideae* in *Presliophytum*

Presliophytum malesherbioides (Phil.) R.H.Acuña & Weigend, **comb. nov.** ≡ *Loasa malesherbioides* Phil. in Linnaea 33: 74. 1864 – Type: [Chile, IV Región de Coquimbo, Prov. Elqui] Baños del Toro, Coquimbo, 1860/61, [*H.*] *Volckmann s.n.* (B† [photo F Neg. No. 10208!]; SGO?).

= *Loasa longiseta* Phil. in Anales Univ. Chile 27: 347. 1865 – **Lectotype (designated here):** [Chile, III Región de Atacama, Prov. Copiapó] Quebrada de Puquios, Des. Atacama, 1865, *F. Geisse s.n.* (SGO barcode SGO000003405 [photo!]; isolectotype: SGO barcode SGO000003404 [photo!]).

Note. – A re-examination of the type specimens led to the conclusion that *L. longiseta* is synonymous with *Presliophytum malesherbioides*, contrary to the conclusion by Weigend & al. (2008), who used it as the correct name for *L. sessiliflora*. In the protologue, Philippi mentions that "[*L. longiseta*] is so similar to my *L. malesherbioides* that at first glance I took it for the latter". The supposed differences in setae (stinging trichome) cover between *L. malesherbioides* and *L. longiseta* stated by Philippi are, in our opinion, inconsistent, merely representing individual variation within the same species, as can be seen when comparing the original material for both names. According to Muñoz-Schick & al. (2012) all the type specimens collected by F. Geisse in 1865 and deposited in SGO come from Quebrada de Puquios in the Atacama Region.

Presliophytum sessiliflorum (Phil.) R.H.Acuña & Weigend, **comb. nov.** ≡ *Loasa sessiliflora* Phil. in Anales Univ. Chile 85: 12. 1893 – Holoype: [Chile, III Región de Atacama, Prov. Chañaral and II Región de Antofagasta, Prov. Taltal] Sierra Esmeralda, Des Atacama, 20 Oct 1883, [*F.*] *S*[*an*] *Roman s.n.* (SGO barcode SGO000003420 [photo!]).

Note. **–** Philippi based this name on a single specimen, making it effectively the holotype for this name. It was collected in Sierra Esmeralda without any more precise locality information. *Presliophytum sessiliflorum* is quite different from *P. malesherbioides* as the first has strongly and regularly lobed leaf margins, shortly pedicellate to subsessile flowers and sepals that are almost as long as wide, while the latter has indistinctly and irregularly lobed to subentire leaf margins, long pedicellate flowers and sepals that are at least three times longer than wide.

New genera segregated from *Loasa*, including new combinations

Pinnasa Weigend & R.H.Acuña, **gen. nov.** – Type: *Pinnasa volubilis* (Dombey ex Juss.) Weigend & R.H.Acuña (≡ *Loasa volubilis* Dombey ex Juss.).

Perennial, rosulate herbs with thick tap-roots, sometimes stoloniferous. Leaves congregated into dense, basal leaf rosette, petiolate, deeply pinnatifid to bipinnate, petiole distinct, much shorter than lamina/rachis. Indumentum mostly of scabrid trichomes, stinging hairs few or absent. Inflorescences usually axillary, arising from the axils of the rosette leaves, one- to many-flowered, often with a winding axis, frondose with opposite, pinnate or pinnatifid bracts. Flowers distinctly pedicellate, ovary mostly inferior, petals yellow and/or white, sometimes with a distinct longitudinal green band in the middle, spreading, basally narrowed, deeply boat-shaped, margins serrate to laciniate in distal half, anthers much shorter than petals. Floral scales distinctly L-shaped in lateral view and deeply cucullate, with a distinct double-arch on the back and three, usually distally flattened dorsal threads in the upper third, usually with distinct winged ridges where attached to the scale, coloured contrastingly to the petals, usually red, dark red or orange. Floral scales enclosing two irregularly thickened, L-shaped staminodes dramatically tapering into a thin filamentose apex above scale apex. Superior part of the ovary broadly conical, style with 3 parallel stigmatic lobes. Placentae 3, ovules numerous, developing into numerous, small, tan or brown, ovoidal to oblong seeds with a deeply pitted testa and without a differentiated hilar cone.

Note. – This new genus comprises the species assigned to *Loasa* ser. *Pinnatae* Urb. & Gilg (in Gilg, 1894). Species limits in this group are still very problematical and we only provide new combinations for four species, the oldest species in the group and those which we believe represent clearly identifiable entities and were included in the molecular phylogeny. Weigend & al. (2008) tentatively recognized a total of 11 species which could be assigned to this new genus.

Pinnasa bergii (Hieron.) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa bergii* Hieron. in Bol. Acad. Nac. Ci. Republ. Argent. 3: 349. 1881 – **Lectotype (designated here):** [Argentina] Cercanías del Río Negro cerca Carmen de Patagones, 17 Nov 1874, *C. Berg 91* (CORD barcode CORD 00003395, [photo!]: isolectotype: B†, photo F Neg. No.10179!).

Note. – Berg collected the type of *Pinnasa bergii* near Carmen de Patagones as stated in the protologue and on the lectotype label. Before 1880 the name "Carmen de Patagones" referred to both what nowadays is Carmen de Patagones (on the northern side of the Río Negro, Partido de Patagones, Province of Buenos Aires) and Viedma (southern side of Río Negro, Department Adolfo Alsina, Province of Río Negro). It is unknown from which precise locality the collection came.

Pinnasa nana (Phil.) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa nana* Phil. in Anales Univ. Chile 85: 14–15. 1893 – **Lectotype (designated here):** [Argentina, Prov. Neuquén, Dept. Lácar] Ad nives perpetuas Huahuin [sic], Jan 1887, *O. Philippi s.n.* (SGO barcode SGO000003412 [photo!]; isolectotypes: B† [photo F Neg. No.10210!], SGO barcode SGO000003411 [photo!]).

Note. – According to Muñoz-Schick & al. (2012) Huahuim [sic] corresponds to Hua-Hum, a locality erroneously cited for Chile. Hua-Hum is located on the Argentinian side of the international border Chile/Argentina in the Province of Neuquén, a region visited by O. Philippi in the summer of 1887.

Pinnasa pinnatifida (Gillies ex Arn.) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa pinnatifida* Gillies ex Arn. in Edinburgh J. Nat. Geogr. Sci. 3: 275. 1831 – **Lectotype (designated here):** [Without locality details] Andes of Chile, [Mar 1826], *J. Gillies s.n.* (E barcode E00158269 [photo!]; possible isolectotypes: B† [photo F Neg. No.10215!], BM barcode BM000756685 [photo!], GH barcode 00076026 [photo!], K barcodes K000372796 [photo!] & K000372794 [photo!]).

Note. – The type locality "Cuesta del Inga" could not be located in current maps, but is likely on the way between Mendoza, Argentina, and Valparaíso, Chile, where Gillies collected in early 1826 (Gibbs, 1951).

Pinnasa volubilis (Dombey ex Juss.) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa volubilis* Dombey ex Juss. in Ann. Mus. Natl. Hist. Nat. 5: 26–27, t. 5, fig. 1. 1804 – **Lectotype (designated here):** [Chile], [1782/83], [*J.*] *Dombey s.n.* (P barcode P02273153 [photo!]; possible isolectotypes: P barcodes P02273154 [photo!] & P02273155 [photo!]).

Possible syntypes. – BM barcodes BM000756683 [photo!] & BM001011552 [photo!] were determined as probable isotypes by J. Grau; MA barcode MA 813487 [photo!] could be also original material of the species collected by Dombey.

Note. – Joseph Dombey visited three South American countries and spent several months in Chile during the years 1782 and 1783, mostly around Concepción (Hamy, 1905). Jussieu stated in the protologue that the type locality of *Pinnasa volubilis* are sandy areas near the city of Concepción. P barcode P02273153 is selected as the lectotype, matching most closely the illustration of the species as it appears in the protologue.

Grausa Weigend & R.H.Acuña, **gen. nov.** – Type: *Grausa micrantha* (Poepp.) Weigend & R.H.Acuña (≡ *Loasa micrantha* Poepp.)

Perennial, rosulate or weakly rhizomatous, or climbing herbs with diffuse root-system. Leaves congregated into dense, basal leaf rosette, petiolate, sagittate, pinnatisect or palmatisect to ternately compound, very rarely pinnate, petiole distinct. Indumentum mostly of scabrid trichomes, stinging hairs few or absent. Inflorescences axillary and one-flowered, or lax, open, winding with apparently axillary flowers, flowering branches few, one- to many-flowered, frondose with opposite, pinnatisect or palmatisect bracts. Flowers distinctly pedicellate, ovary inferior, petals white or red, half-spreading to spreading, basally narrowed, boat-shaped, margin distally entire, distinctly flattened, rarely toothed, anthers much shorter than petals. Floral scales yellow, greenish or red, slightly curved in lateral view and shallowly cucullate, with three indistinct ridges on the back and three, thin, slightly flattened and distally widened dorsal threads attached to the middle, rarely to the bottom, of the scale. Floral scales enclosing two curved, more or less isodiametrical staminodes dramatically tapering into a thin filamentose apex above scale apex. Superior part of the ovary broadly conical, style with 3 parallel stigmatic lobes. Placentae 3, ovules numerous, developing into numerous, small, dark brown, ovoidal to oblong seeds with a reticulate or deeply pitted testa, anticlinal walls sometimes with elaborate fenestrations, hilum often protracted into a distinct hilar cone.

Note. – The new genus *Grausa* includes ser. *Volubiles* and ser. *Acaules*. It is named after the Munich scientist Jürke Grau, who made numerous valuable contributions to our understanding of Chilean Loasaceae. His surname is combined with the second half of the name "*Loasa*" to indicate its relationships. Five species can be assigned to this new genus.

Grausa gayana (Urb. & Gilg) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa gayana* Urb. & Gilg in Engler & Prantl, Nat. Pflanzenfam. 3(6a): 117. 1894 – **Lectotype (designated here):** [Chile, X Región de Los Lagos, Prov. Osorno] Sepusque circa Osorno, [1835], *C. Gay 101* (P barcode P04589503 [photo!]; isolectotype: P barcode P04589505 [photo!]).

Note. – The protologue of this species is a very brief diagnosis of the species in German. According to Urban and Gilg (Gilg, 1894), *Grausa gayana* was one of four species of *Loasa* ser. *Volubiles* and differs from other species of the series (*L. martini* Phil. was not discussed in this publication) by having trilobate leaves (which actually is often, but not always, the case). Crucially, Urban and Gilg made clear that *G. gayana* corresponds to specimens identified as *L. sagittata* by Claude Gay. In Urban & Gilg (1900) they reaffirm this observation and state that Gay's specimens identified as *L. sagittata* (then known only from the type collection) belong to a different species. The P specimen here selected as lectotype bears a handwritten label by Urban & Gilg. The specimen *C. Gay. s.n.* (K barcode K000372849 [photo!]) may be a syntype or even an isolectotype, but has no collection number. According to Muñoz-Pizarro (1944), Gay visited the region of Osorno in February 1835, however the date is missing from the specimens examined.

Grausa lateritia (Gillies ex Arn.) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa lateritia* Gillies ex Arn. in Edinburgh J. Nat. Geogr. Sci. 3: 275. 1831 – **Lectotype (designated here):** [Chile] Andes of Chile, [1827], *Gillies s.n.* (E barcode E00158250 [photo!]).

Syntypes. – [Chile, VII Región del Maule, Prov. Curicó] Los Imposibles, Mar 1827, *Gillies s.n.* (GH barcode 00062357 [photo!], K barcode K000372852 [photo!]); [Chile, VI Región de O'Higgins, Prov. Colchagua] Quebrada de Fray Carlos, Apr 1827, *Gillies s.n.* (BM barcode BM001008731 [photo!]); [Chile] Andes of Chile, [1827], *Gillies s.n.* (E barcode E00158249 [photo!], GH barcode 00062358 [photo!], K barcode K000372851 [photo!]).

Note. – The protologue cites two different collection localities (Los Imposibles and El Valle de Fray Carlos) and dates (March and April 1827 respectively). Although no place known as Los Imposibles can be located in recent maps of Chile, according to the protologue it is near the Planchón Volcano, most likely referring to the Chilean side of the Planchón-Peteroa-Azufre Volcanic complex, located in Curicó Province, Maule Region, meanwhile El Valle [Quebrada or Estero] de Fray Carlos is located to the north, in the Colchagua Province in the O'Higgins Region. An additional specimen of *G. lateritia* collected by Gillies is known (BM barcode BM001008370 [photo!]), however the locality mentioned for it (La Sepultura) is not mentioned in the protologue. E barcode E00158250 was selected as the lectotype because it is part of the GL collections, so it was very likely studied by Arnott himself.

Grausa martini (Phil.) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa martini* Phil. in Anales Univ. Chile 41: 716. 1872 – **Lectotype (designated here):** [Chile, X Región de Los Lagos, Prov. Llanquihue] Puerto Montt, 1870, [*C.*] *Martin s.n.* (SGO barcode SGO000003408 [photo!]; isolectotype: SGO barcode SGO000003407 [photo!]).

Note. – SGO barcode SGO000003408 was selected as the lectotype for this name because it conforms most closely to the description in the protologue.

Grausa micrantha (Poepp.) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa micrantha* Poepp., Fragm. Syn. Pl.: 24. 1833 – **Lectotype (designated here):** [Chile, VIII Region de Bío Bío, Prov. Bío Bío] Chile austral. rarissima in sepibus Antuco, Jan 1829, *E. Poeppig s.n.* (W No. 0017735 [photo!]).

Grausa sagittata (Hook. & Arn.) Weigend & R.H.Acuña, **comb. nov.** ≡ *Loasa sagittata* Hook. & Arn. in Bot. Misc. 3: 328. 1833 – **Lectotype (designated here):** [Chile, Región X de Los Lagos?, Prov. Chiloé?] Chili, [1831?], *H. Cuming 60* (K barcode K000372791 [photo!]); isolectotypes: B†, photo F Neg. No.10217!, E barcodes E00158298 [photo!] & E00158299 [photo!], GH barcode 00076030 [photo!], K barcode K000372790 [photo!]).

Note. – This species was only known from the type collection for 150 years. In recent years only few further collections have been made: *G. Villagrán & I. Meza 922* (SGO No. 112860 [photo!]) and *W.D. Stevens & al. 34062* (BONN!, MO, SGO). Muñoz-Schick (1981) indicated that "Chiloe", cited as the type locality, could be an error. However, in the archives at K one of the plant lists with Cuming's collections cites Chiloé as the locality for *Cuming 60* and the same applies to the isolectotypes B (F Neg. No. 10217), E barcodes E00158298 & E00158299 and GH barcode GH00076030. On the other hand, K barcode K000372790 (and the more recent collection *G. Villagrán & I. Meza 922*) has Valparaíso as the region where the specimen was collected. K barcode K000372791 was selected as the lectotype, because it must have been seen by Hooker as part of his personal herbarium. It also is the most complete type specimen associated with this name.

Generic key to *Presliophytum* and the "South Andean Loasas"

1\. All but the lowermost leaves alternate (including bracts), simple; nectar scales often the same colour as the corolla, if not, then seeds with regular constrictions perpendicular to their main axis; xeric habitats .... ***Presliophytum***

1\. All leaves opposite (including bracts), often compound; nectar scales often of contrasting colour with the corolla; seeds never with regular constrictions perpendicular to the main axis; diverse habitats 2

2\. Fruits all twisted in the same direction within an inflorescence; nectar scales without double arc, dorsal threads dorsoventrally flattened, but never flag-shaped, attached to the nectar scale near its base; seeds winged or with fibrous testa or angular, but never deeply pitted with fenestrate anticlinal walls nor rugulose .... ***Blumenbachia***

2\. Fruits straight or, if twisted, alternating clockwise and anticlockwise in the inflorescence; nectar scales with or without double arc and/or dorsal threads, the latter usually not dorsoventrally flattened, attached to different areas of the scale (if threads dorsoventrally flattened, these flag-shaped or attached to the upper half of the scale); seed testa usually deeply pitted with fenestrate anticlinal walls, rarely irregularly rugulose 3

3\. Capsules straight and opening solely with apical valves . 4

3\. Capsules straight or twisted, opening mostly with longitudinal slits (very rarely solely with apical valves, if so, small rosulate herbs with oblong, cream-coloured petals, and flowers born singly on ebracteose peduncles) 6

4\. Plants with stinging trichomes; leaves never compound (though sometimes deeply dissected); seeds with a lateral or subterminal hilum or hilar scar .... ***Loasa***

4\. Plants without stinging trichomes; leaves usually variously ternate or pinnate, less frequently deeply pinnatifid or sagittate; seeds with a terminal hilum 5

5\. Leaves pinnatifid or pinnate; flowers erect; petals white to yellow, rarely pinkish; seeds without a protracted hilar cone .... ***Pinnasa***

5\. Leaves sagittate, variously ternate or very rarely pinnate; flowers pendent or, if erect, petals brick red; petals white or brick red; seeds usually with a conspicuous hilar cone .... ***Grausa***

6\. Flowers erect; fruits narrowly cylindrical, straight, more than 10× as long as wide, opening with both apical valves and longitudinal slits .... ***Scyphanthus***

6\. Flowers usually pendent; fruits cylindrical, ovoidal or clavate, never more than 7× as long as wide, often twisted, opening with longitudinal slits or very rarely apical valves or both .... ***Caiophora***

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LITERATURE CITED

Ackermann, M. 2011. *Studies on systematics, morphology and taxonomy of Caiophora and reproductive biology of Loasaceae and Mimulus (Phrymaceae).* Dissertation, Freie Universität Berlin, Berlin, Germany.

Ackermann, M. & Weigend, M. 2006. Nectar, floral morphology and pollination syndrome in Loasaceae subfam. Loasoideae (Cornales). *Ann. Bot. (Oxford)* 98: 503–514. https://doi.org/10.1093/aob/mcl136

Ackermann, M. & Weigend, M. 2013. A revision of loasoid *Caiophora* (*Caiophora pterosperma* group, Loasoideae, Loasaceae) from Peru. *Phytotaxa* 110: 17–30. https://doi.org/10.11646/phytotaxa.110.1.2

APG IV 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.* 181: 1–20. https://doi.org/10.1111/boj.12385

Boyce, K., Sievers, F. & Higgins, D. 2015. Instability in progressive multiple sequence alignment algorithms. *Algorithms Molec. Biol.* 10: 26. https://doi.org/10.1186/s13015-015-0057-1

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Kallersjo, M. & Barkhordarian, E. 2002. Phylogenetics of Asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molec. Phylogen. Evol.* 24: 274–301. https://doi.org/10.1016/S1055-7903(02)00240-3

Brown, D.K. & Kaul, R.B. 1981. Floral structure and mechanism in Loasaceae. *Amer. J. Bot.* 68: 361–372. https://doi.org/10.2307/2442772

Chacón, J., Luebert, F., Hilger, H.H., Ovchinnikova, S., Selvi, F., Cecchi, L., Guilliams, C.M., Hasenstab-Lehman, K., Sutorý, K., Simpson, M.G. & Weigend, M. 2016. The borage family (Boraginaceae s.str.): A revised infrafamilial classification based on new phylogenetic evidence, with emphasis on the placement of some enigmatic genera. *Taxon* 65: 523–546. https://doi.org/10.12705/653.6

De Smet, Y., Granados-Mendoza, C., Wanke, S., Goetghebeur, P. & Samain, M.-S. 2015. Molecular phylogenetics and new (infra) generic classification to alleviate polyphyly in tribe Hydrangeeae (Cornales: Hydrangeaceae). *Taxon* 64: 741–753. https://doi.org/10.12705/644.6

Doyle, J.J. & Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull. Bot. Soc. Amer.* 19: 11–15.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Molec. Evol.* 17: 368–376. https://doi.org/10.1007/BF01734359

Gibbs, F.W. 1951. John Gillies, M.D., traveller and botanist, 1792–1834. *Notes & Rec. Roy. Soc. London* 9: 115–136. https://doi.org/10.1098/rsnr.1951.0008

Gilg, E. 1894. Loasaceae. Pp. 100–121 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, 3(6a). Leipzig: Engelmann.

Grau, J. 1997. *Huidobria*, eine isolierte Gattung der Loasaceae aus Chile. *Sendtnera* 4: 77–93.

Grosse-Veldmann, B., Nürk, N.M., Smissen, R., Breitwieser, I., Quandt, D. & Weigend, M. 2016. Pulling the sting out of nettle systematics—A comprehensive phylogeny of the genus *Urtica* L. (Urticaceae). *Molec. Phylogen. Evol.* 102: 9–19. https://doi.org/10.1016/j.ympev.2016.05.019

Hamilton, M. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molec. Ecol.* 8: 521–523.

Hamy, E.T. 1905. *Joseph Dombey, médecin, naturaliste, archéologue, explorateur du Pérou, du Chili et du Brésil (1778–1785): Sa vie, son oeuvre, sa correspondance, avec un choix de pièces relatives à sa mission*. Paris: Eurdin et Cie.

Henning, T. & Weigend, M. 2012. Total control—Pollen presentation and floral longevity in Loasaceae (blazing star family) are modulated by light, temperature and pollinator visitation rates. *PLoS ONE* 7: e41121. https://doi.org/10.1371/journal.pone.0041121

Henning, T., Oliveira, S., Schlindwein, C. & Weigend, M. 2015. A new, narrowly endemic species of *Blumenbachia* (Loasaceae subfam. Loasoideae) from Brazil. *Phytotaxa* 236: 47–93. https://doi.org/10.11646/phytotaxa.236.2.9

Huelsenbeck, P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Hufford, L. 2003. Homology and developmental transformation: Models for the origins of the staminodes of Loasaceae subfamily Loasoideae. *Int. J. Pl. Sci.* 164: S409–S439. https://doi.org/10.1086/376873

Hufford, L., McMahon, M.M., Sherwood, A.M., Reeves. G. & Chase, M.W. 2003. The major clades of Loasaceae: Phylogenetic analysis using the plastid *matK* and *trnL-trnF* regions. *Amer. J. Bot.* 90: 1215–1228. https://doi.org/10.3732/ajb.90.8.1215

Hufford, L., McMahon, M.M., O'Quinn, R. & Poston, M.E. 2005. A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. *Int. J. Pl. Sci.* 166: 289–300. https://doi.org/10.1086/427477

Johnson, L. & Soltis, D. 1995. Phylogenetic inference in Saxifragaceae sensu stricto and *Gilia* (Polemoniaceae) using *matK* sequences. *Ann. Missouri Bot. Gard.* 82: 149–175. https://doi.org/10.2307/2399875

Katoh, K. & Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molec. Biol. Evol.* 30: 772–780. https://doi.org/10.1093/molbev/mst010

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kim, K., Lee, S.C., Lee, J., Lee, H.O., Joh, H.J., Kim, N.H., Park, H.S. & Yang, T.J. 2015. Comprehensive survey of genetic diversity in chloroplast genomes and 45S nrDNAs within *Panax ginseng* Species. *PLoS ONE* 10: e0117159. https://doi.org/10.1371/journal.pone.0117159

Loefstrand, S.D. & Schoenenberger, J. 2015. Molecular phylogenetics and floral evolution in the sarracenioid clade (Actinidiaceae, Roridulaceae and Sarraceniaceae) of Ericales. *Taxon* 64: 1209–1224. https://doi.org/10.12705/646.6

Mau, B., Newton, M. & Larget, B. 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* 55: 1–12. https://doi.org/10.1111/j.0006-341X.1999.00001.x

Moody, M.L., Hufford, L., Soltis, D.E. & Soltis, P.S. 2001. Phylogenetic relationships of Loasaceae subfamily Gronovioideae inferred from *matK* and ITS sequence data. *Amer. J. Bot.* 88: 326–336. https://doi.org/10.2307/2657022

Moore, M.J., Soltis, P.S., Bell, C.D., Burleigh, J.G. & Soltis, D.E. 2010. Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots. *Proc. Natl. Acad. Sci. U.S.A.* 107: 4623–4628. https://doi.org/10.1073/pnas.0907801107

Müller, J., Müller, K., Nienhuis, C. & Quandt, D. 2010. PhyDe: Phylogenetic Data Editor. Available from http://www.phyde.de/

Muñoz-Pizarro, C. 1944. El itinerario de Don Claudio Gay. *Bol. Mus. Nac. Hist. Nat. Santiago de Chile* 22: 27–44.

Muñoz-Schick, M. 1981. Comentario sobre la distribución de *Loasa sagittata* Hook. et Arn. *Not. Mens. Mus. Nac. Hist. Nat.* 297: 14.

Muñoz-Schick, M., Moralez, V. & Moreira-Muñoz, A. 2012. La colección de tipos de plantas vasculares del Herbario Nacional de Chile (SGO). Análisis histórico, temporal y espacial. *Gayana, Bot.* 69: 123–135. https://doi.org/10.4067/S0717-66432012000100012

Oxelman, B., Lidén, M. & Berglund, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* 206: 393–410. https://doi.org/10.1007/BF00987959

Posada, D. & Crandall, K. 2001. Selecting the best-fit model of nucleotide substitution. *Syst. Biol.* 50: 580–601. https://doi.org/10.1080/106351501750435121

Rambaut, A. & Drummond, A. 2007. Tracer, version 1.4. http://beast.bio.ed.ac.uk/Tracer

Shaw, J., Lickey, E., Beck, J., Farmer, S., Liu, W., Miller, J., Siripun, K., Winder, C., Schilling, E. & Small, R. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer. J. Bot.* 92: 142–166. https://doi.org/10.3732/ajb.92.1.142

Silvestro, D. & Michalak, I. 2012. RAxMLGUI: A graphical front-end for RAxML. *Organisms Diversity Evol.* 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stevens, P.F. 2001–. Angiosperm Phylogeny Website, version 14, Apr 2015. http://www.mobot.org/MOBOT/research/APweb/

Strelin, M., Benitez-Vieyra, S., Ackermann, M. & Cocucci, A. 2016a. Flower reshaping in the transition to hummingbird pollination in Loasaceae subfam. Loasoideae despite absence of corolla tubes or spurs. *Evol. Ecol.* 30: 401–417. https://doi:10.1007/s10682-016-9826-7

Strelin, M., Benitez-Vieyra, S., Fornoni, J., Klingenberg, C. & Cocucci, A. 2016b. Exploring the ontogenetic scaling hypothesis during the diversification of pollination syndromes in *Caiophora* (Loasaceae, subfam. Loasoideae). *Ann. Bot. (Oxford)* 117: 937–947. https://doi:10.1093/aob/mcw035

Strelin, M., Arroyo, J., Fließwasser, S. & Ackermann, M. 2017. Diversification of *Caiophora* (Loasaceae subfam. Loasoideae) during the uplift of the Central Andes. *Organisms Diversity Evol.* 17: 29–41. https://doi:10.1007/s13127-016-0312-4

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109. https://doi.org/10.1007/BF00037152

Urban, I. & Gilg, W. 1900. Monographia Loasacearum. *Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur.* 76: 1–384.

Weigend, M. 1997. *Nasa & the conquest of South America*. Dissertation, Ludwig-Maximilians Universität, Munich, Germany.

Weigend, M. 2004. Loasaceae. Pp. 239–254 in: Kubitzki, K. (ed.), *The families and genera of vascular plants*, vol. 6, *Flowering plants: Dicotyledons; Celastrales, Oxalidales, Rosales, Cornales, Ericales*. Berlin: Springer.

Weigend, M. 2006. Validating subfamily, genus and species names in Loasaceae (Cornales). *Taxon* 55: 463–468. https://doi.org/10.2307/25065594

Weigend, M. & Ackermann, M. 2003. Los nombres antiguos en el género *Caiophora* (Loasaceae subfam. Loasoideae) y una clasificación infragenerica preliminar. *Arnaldoa* 10: 75–94.

Weigend, M., Kufer, J. & Müller, A.A. 2000. Phytochemistry and the systematics and ecology of Loasaceae and Gronoviaceae (Loasales). *Amer. J. Bot.* 87: 1202–1210. https://doi.org/10.2307/2656658

Weigend, M., Aitzetmüller, K. & Bruehl, L. 2004a. The seeds of Loasaceae subfam. Loasoideae (Cornales) I: Seed release, seed numbers and fatty acid composition. *Flora* 199: 424–436. https://doi.org/10.1078/0367-2530-00170

Weigend, M., Gottschling, M., Hoot, S. & Ackermann, M. 2004b. A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on *trnL* (UAA) sequence data, with consequences for systematics and historical biogeography. *Organisms Diversity Evol.* 4: 73–90. https://doi.org/10.1016/j.ode.2003.12.001

Weigend, M., Gröger, A. & Ackermann, M. 2005. The seeds of Loasaceae subfam. Loasoideae (Cornales) II: Seed morphology of ‘South Andean Loasas’ (*Loasa*, *Caiophora*, *Scyphanthus* and *Blumenbachia*). *Flora* 200: 569–591. https://doi.org/10.1016/j.flora.2005.06.009

Weigend, M., Grau, J. & Ackermann, M. 2008. Loasaceae. Pp. 2413–2424 in: Zuloaga, F.O., Morrone, O. & Belgrano, M.J. (eds.), *Catálogo de las plantas vasculares del Cono Sur (Argentina, southern Brazil, Chile, Paraguay, and Uruguay)*, vol. 3, *Dicotyledoneae (Fabaceae 2–Polygonaceae)*. St. Louis: Missouri Botanical Garden Press.

Werle, E., Schneider, C., Renner, M., Völker, M. & Fiehn, W. 1994. Convenient single-step, one tube purification of PCR products for direct sequencing. *Nucl. Acids Res.* 22: 4354. https://doi.org/10.1093/nar/22.20.4354

White, T.J., Bruns T., Lee, S. & Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.), *PCR protocols: A guide to methods and applications.* New York: Academic Press. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Wilson, Y. 2009. *Evolutionary relationships and morphological variation between* Antirrhinum *species*. Dissertation, University of Edinburgh, Edinburgh, United Kingdom.

Xiang, Q.Y., Thomas, D.T. & Xiang, Q.P. 2011. Resolving and dating the phylogeny of Cornales—Effects of taxon sampling, data partitions, and fossil calibrations. *Molec. Phylogen. Evol.* 59: 123–138. https://doi.org/10.1016/j.ympev.2011.01.016

Yao, X., Tang, P., Li, Z., Li, D., Liu, Y. & Huang, H. 2015. The first complete chloroplast genome sequences in Actinidiaceae: Genome structure and comparative analysis. *PLoS ONE* 10: e0129347. https://doi.org/10.1371/journal.pone.0129347

Young, N.D., Steiner, K.E. & de Pamphilis, C.W. 1999. The evolution of parasitism in Scrophulariaceae/Orobanchaceae: Plastid gene sequences refute an evolutionary transition series. *Ann. Missouri Bot. Gard.* 82: 149–175. https://doi.org/10.2307/2666173

Appendix 1. List of taxa sampled with their respective voucher specimen, geographic origins and GenBank accession numbers (*trnL-trnF*, *matK*, *trnS-trnG*, *rps16*, ITS, respectively) for the sequences used in the analyses. Dashes (–) indicate missing data. All the accessions without a citation were newly obtained for this study. *Loasa micrantha* Poepp. (b) was misnamed as *Loasa martini* in GenBank.

Actinidia chinensis Planch., Yao & al. (2015): KP297245.1, KP297245.1, KP297245.1, KP297245.1, Loefstrand & Schoenenberger (2015): KR819508.1; ***Antirrhinum majus*** L., Bremer & al. (2002): AJ430929.1, Young & al. (1999): AF051978.1, Wilson (2009): FR690224.1, Moore & al. (2010): GQ997033.1, Braeuchler & al. (unpub.): AY591288.1; ***Aosa parviflora*** (Schrad. ex DC.) Weigend, Brazil, *Grant 4650* (BSB), KY286967, KY286698, KY286877, KY286787, KY286615; ***Aosa rostrata*** (Urb.) Weigend, Brazil, *Salino 3042* (M), KY286948, KY286679, KY286858, KY286768, KY286598; ***Aosa rupestris*** (Gardner) Weigend, Brazil, *Weigend*

Appendix 1. Continued.

7138 (BSB), KY286925, KY286657, KY286835, KY286745, KY286581; ***Aosa sigmoidea*** Weigend, Brazil, *Guedes & al. 10360* (ALCB), KY286995, KY286725, KY286905, KY286815, KY286639; ***Aosa uleana*** (Urb. & Gilg) Weigend, Brazil, *Joßberger 342* (BONN), KY286998, KY286728, KY286908, KY286818, KY286641; ***Blumenbachia amana*** T.Henning & Weigend, Brazil, *Acuña 1204* (BONN), KY287001, KY286731, KY286911, KY286821, KY286643; ***Blumenbachia catharinensis*** Urb. & Gilg, Brazil, *Trevisan 1723* (BONN), KY287006, KY286736, KY286916, KY286826, KY286648; ***Blumenbachia dissecta*** (Hook. & Arn.) Weigend & Grau (a), Chile, *Grau s.n.* (M), KY286968, KY286699, KY286878, KY286788, KY286616; ***Blumenbachia dissecta*** (Hook. & Arn.) Weigend & Grau (b), Argentina, *Weigend & al. 6816* (BRCO), KY286961, KY286692, KY286871, KY286781, KY286611; ***Blumenbachia hieronymi*** Urb., Germany (Cultivated), *Ackermann 601* (BSB), KY286966, KY286697, KY286876, KY286786, KY286614; ***Blumenbachia insignis*** Schrad., Germany (Cultivated), *Weigend 7475* (B), KY286964, KY286695, KY286874, KY286784, KY286613; ***Blumenbachia latifolia*** Cambess., Brazil, *Schwabe s.n.* (B), KY286949, KY286680, KY286859, KY286769, KY286599; ***Blumenbachia prietea*** Gay, Argentina, *Weigend & al. 6823* (BRCO), KY286963, KY286694, KY286873, KY286783, KY286612; ***Blumenbachia scabra*** (Miers) Urb., Brazil, *Hatschbach 41572* (NY), KY286993, –, KY286903, KY286813, –; ***Blumenbachia sylvestris*** Poepp. (a), Argentina, *Coccuci & Sersic 4780* (CORD), KY286969, KY286700, KY286879, KY286789, KY286617; ***Blumenbachia sylvestris*** Poepp. (b), Argentina, *Weigend & al. 6807* (BRCO), KY286919, KY286651, KY286829, KY286739, KY286577; ***Caiophora aconquijae*** Sleumer, Argentina, *Strelin s.n.* (CORD), KY286974, KY286705, KY286884, KY286794, KY286622; ***Caiophora andina*** Urb. & Gilg, Chile, *Moreira & Luebert 2379* (SGO), KY287005, KY286735, KY286915, KY286825, KY286647; ***Caiophora arechavaletae*** (Urb.) Urb., Brazil, *Weigend 9330* (BSB), KY286970, KY286701, KY286880, KY286790, KY286618; ***Caiophora boliviana*** Urb. & Gilg, Bolivia, *Badcock 619* (K), KY286955, KY286686, KY286865, KY286775, KY286605; ***Caiophora canarinoides*** (Lenné & K.Koch) Urb. & Gilg, Peru, *Ackermann 375* (BSB), KY286975, KY286706, KY286885, KY286795, KY286623; ***Caiophora carduifolia*** C.Presl, Peru, *Ackermann 288* (BSB), KY286939, KY286671, KY286849, KY286759, KY286591; ***Caiophora cernua*** (Griseb.) Urb. & Gilg ex Kurtz, Argentina, *Ackermann 1100* (BONN), KY286972, KY286703, KY286882, KY286792, KY286620; ***Caiophora*** **cf.** ***buraeavii***, Peru, *Grant & al. 10-4619* (BSB), KY286981, KY286712, KY286891, KY286801, KY286629; ***Caiophora chuquisacana*** Urb. & Gilg, Bolivia, *Ritter & Wood 1498* (LPB), KY286982, KY286713, KY286892, KY286802, KY286630; ***Caiophora chuquitensis*** (Meyen) Urb. & Gilg, Argentina, *Ackermann 1101* (BONN), KY286983, KY286714, KY286893, KY286803, KY286631; ***Caiophora cinerea*** Urb. & Gilg, Peru, *Weigend & al. 5715* (BSB), KY286953, KY286684, KY286863, KY286773, KY286603; ***Caiophora cirsiifolia*** C.Presl (a), Peru, *Ackermann 610* (BSB), KY286984, KY286715, KY286894, KY286804, KY286632; ***Caiophora cirsiifolia*** C.Presl (b), Peru, *Henning & Brokamp 16* (BSB), KY286971, KY286702, KY286881, KY286791, KY286619; ***Caiophora clavata*** Urb. & Gilg, Argentina, *Ackermann 1102* (BONN), KY287002, KY286732, KY286912, KY286822, KY286644; ***Caiophora contorta*** (Desr. ex Lam.) C.Presl, Ecuador, *Weigend & Brokamp 9110* (BSB), KY286985, KY286716, KY286895, KY286805, –; ***Caiophora coronata*** (Gillies ex Arn.) Hook. & Arn., Argentina, *Coccuci & Sersic 4845* (CORD), KY286973, KY286704, KY286883, KY286793, KY286621; ***Caiophora dederichiorum*** Mark.Ackermann & Weigend, Peru, *Henning & Schulz 19* (BSB), KY286977, KY286708, KY286887, KY286797, KY286625; ***Caiophora deserticola*** Weigend & Mark.Ackermann, Chile, *Muñoz-Schick 4296* (BSB), KY286952, KY286683, KY286862, KY286772, KY286602; ***Caiophora dumetorum*** Urb. & Gilg, Argentina, *Strelin 5481* (CORD), KY286986, KY286717, KY286896, KY286806, KY286633; ***Caiophora grandiflora*** (G.Don) Weigend & Mark.Ackermann, Peru, *Henning & Brokamp 3* (BSB), KY286987, KY286718, KY286897, KY286807, –; ***Caiophora hibiscifolia*** (Griseb.) Urb. & Gilg, Argentina, *Ackermann 1103* (BONN), KY286988, KY286719, KY286898, KY286808, KY286634; ***Caiophora lateritia*** Klotzsch, Argentina, *Ackermann 1104* (BONN), KY286989, KY286720, KY286899, KY286809, KY286635; ***Caiophora madrequisa*** Killip, Peru, *Weigend & Weigend 2000/440* (HUSA), KY286954, KY286685, KY286864, KY286774, KY286604; ***Caiophora nivalis*** Lillo, Argentina, *Coccuci & Sersic 4840* (CORD), KY286976, KY286707, KY286886, KY286796, KY286624; ***Caiophora pentlandii*** (Paxton ex Graham) G.Don ex Loudon., Peru, *Ackermann 360* (BSB), KY286938, KY286670, KY286848, KY286758, KY286590; ***Caiophora pterosperma*** (Ruiz & Pav. ex G.Don) Urb. & Gilg, Peru, *Weigend & al. 5484* (BSB), KY286940, KY286672, KY286850, KY286760, KY286592; ***Caiophora rosulata*** (Wedd.) Urb. & Gilg, Bolivia, *Schlumpberger & Brokamp 675* (BSB), KY287004, KY286734, KY286914, KY286824, KY286646; ***Caiophora rusbyana*** Urb. & Gilg ex Rusby, Bolivia, *Schlumpberger & Brokamp* 627 (BSB), KY287003, KY286733, KY286913, KY286823, KY286645; ***Caiophora stenocarpa*** Urb. & Gilg, Peru, *Ackermann & al.* 758 (BSB), KY286978, KY286709, KY286888, KY286798, KY286626; ***Deutzia discolor*** Hemsl., Germany (Cultivated), *Weigend 5615* (BSB), KY286929, KY286661, KY286839, KY286749, KY286584; ***Deutzia rubens*** Rehder, Germany (Cultivated), *Weigend 5613* (BSB), KY286928, KY286660, KY286838, KY286748, –; ***Ecunide urens*** Parry ex Coville, United States, *Weigend 9153* (BSB), KY286996, KY286726, KY286906, KY286816, –; ***Gronovia scandens*** L., Peru, *Weigend & al. 8522* (BSB), KY286997, KY286727, KY286907, KY286817, KY286640; ***Huidobria chilensis*** Gay, Chile, *Ackermann 490* (BSB), KY286931, KY286663, KY286841, KY286751, –; ***Huidobria fruticosa*** Phil., Chile, *Dillon 8034* (F), KY286932, KY286664, KY286842, KY286752, KY286586; ***Kissenia capensis*** Endl., South Africa, *Greuter 2167* (B), KY286944, KY286675, KY286854, KY286764, KY286596; ***Loasa acanthifolia*** Lam., Argentina, *Weigend & al. 6924* (BRCO), KY286959, KY286690, KY286869, KY286779, KY286609; ***Loasa acerifolia*** Dombey ex Juss., Argentina, *Weigend & al. 6848* (BRCO), KY286937, KY286669, KY286847, KY286757, KY286589; ***Loasa bergii*** Hieron. (a), Argentina, *Weigend 6991* (BSB), KY286960, KY286691, KY286870, KY286780, KY286610; ***Loasa bergii*** Hieron. (b), Argentina, *Weigend 6800* (BRCO), KY286920, KY286652, KY286830, KY286740, KY286578; ***Loasa elongata*** Hook. & Arn. (a), Chile, *Ackermann 491* (BSB), KY286956, KY286687, KY286866, KY286776, KY286606; ***Loasa elongata*** Hook. & Arn. (b), Chile, *Weigend 9333* (BSB), KY287000, KY286730, KY286910, KY286820, KY286642; ***Loasa floribunda*** Hook. & Arn., Chile, *Weigend & al. 5937* (BSB), KY286951, KY286682, KY286861, KY286771, KY286601; ***Loasa gayana*** Urb. & Gilg, Chile, *Weigend & al. 7057* (BSB), KY286962, KY286693, KY286872, KY286782, –; ***Loasa heterophylla*** Hook. & Arn., Chile, *Weigend & al. 5920* (BSB), KY286930, KY286662, KY286840, KY286750, KY286585; ***Loasa illapelina*** Phil., Chile, *Ackermann 519* (BSB), KY286950, KY286681, KY286860, KY286770, KY286600; ***Loasa insons*** Poepp., Chile, *Ackermann 536* (BSB), KY286943, KY286674, KY286853, KY286763, KY286595; ***Loasa lateritia*** Gillies ex Arn., Chile, *Werdermann 1342* (M), KY286941, –, KY286851, KY286761, KY286593; ***Loasa malesherbioides*** Phil., Chile, *Wagenknecht 18509* (M), KY286933, KY286665, KY286843, KY286753, KY286587; ***Loasa micrantha*** Poepp. (a), Chile, *Grau s.n.* (M), KY286957, KY286688, KY286867, KY286777, KY286607; ***Loasa micrantha*** Poepp. (b), Chile, *Heibl 03-097* (not vouchered, only photograph of plant available), KY286990, KY286721, KY286900, KY286810, KY286636; ***Loasa nana*** Phil. Argentina, *Weigend 7080* (BRCO), KY286980, KY286711, KY286890, KY286800, KY286628; ***Loasa nitida*** Lam., Peru, *Weigend & al. 7346* (BSB), KY286936, KY286668, KY286846, KY286756, –; ***Loasa pallida*** Gillies ex Arn., Chile, *Ackermann 1319* (BONN), KY286991, KY286722, KY286901, KY286811, KY286637; ***Loasa pinnatifida*** Gillies ex Arn., Argentina, *Weigend & al. 6880* (BRCO), KY286942, KY286673, KY286852, KY286762, KY286594; ***Loasa sclareifolia*** Juss., Chile, *Weigend 8183* (B), KY286994, KY286724, KY286904, KY286814, KY286638; ***Loasa sessiliflora*** Phil., Chile, *Ehrhardt s.n.* (M), KY286945, KY286676, KY286855, KY286765, KY286597; ***Loasa tricolor*** Ker Gawl., Chile, *Luebert 3021* (B), KY286979, KY286710, KY286889, KY286799, KY286627; ***Loasa triloba*** Dombey ex Juss., Chile, *Luebert & Bidart 3014b* (B), KY286999, KY286729, KY286909, KY286819, –; ***Mentzelia albescens*** (Gillies ex Arn.) Griseb., Argentina, *Weigend & al. 6865* (BRCO), KY286921, KY286653, KY286831, KY286741, KY286579; ***Mentzelia scabra*** Kunth, Peru, *Weigend & al. 98/470* (F), KY286922, KY286654, KY286832, KY286742, –; ***Nasa aequatoriana*** (Urb. & Gilg) Weigend, Ecuador, *Weigend & Jaramillo 3937* (F), KY286947, KY286678, KY286857, KY286767, –; ***Nasa basilica*** T.Henning & Weigend, Peru, *Weigend & al. 97/370* (F), KY286935, KY286667, KY286845, KY286755, –; ***Nasa driesslei*** Weigend, Peru, *Henning & Schneider 243* (BSB), KY286917, KY286649, KY286827, KY286737, –; ***Nasa macrothyrsa*** (Urb. & Gilg) Weigend, Peru, *Weigend & al. 97/s.n.* (M), KY286934, KY286666, KY286844, KY286754, KY286588; ***Nasa poissoniana*** (Urb. & Gilg) Weigend, Peru, *Weigend & Weigend 00/208* (NY), KY286918, KY286650, KY286828, KY286738, KY286576; ***Panax ginseng*** C.A.Mey., Kim & al. (2015): KM088020.1, KM088020.1, KM088020.1, KM088020.1, Daniel & Knoess (unpub.): FJ593178.1; ***Philadelphus pekinensis*** Rupr., Germany (Cultivated), *Weigend 5614* (BSB), KY286927, KY286659, KY286837, KY286747, KY286583; ***Plakothira parviflora*** J. Florence, France (Marquesas Is.), *Weigend s.n.* (BSB), KY286926, KY286658, KY286836, KY286746, KY286582; ***Presliophytum arequipense*** Weigend, Peru, *Ortiz 121* (BONN), KY286965, KY286696, KY286875, KY286785, –; ***Presliophytum heucheraefolium*** (Killip) Weigend, Peru, *Weigend 7368* (BSB), KY286946, KY286677, KY286856, KY286766, –; ***Presliophytum incanum*** (Graham) Weigend, Peru, *Weigend & Förther 97/848* (F), KY286924, KY286656, KY286834, KY286744, KY286580; ***Scyphanthus elegans*** Sweet., Chile, *Grau & Ehrhardt 2-093* (M), KY286958, KY286689, KY286868, KY286778, KY286608; ***Scyphanthus stenocarpus*** (Poepp.) Urb. & Gilg, Chile, *Gardner & Knees 8351 C* (BSB), KY286992, KY286723, KY286902, KY286812, –; ***Xylopodia klaprothioides*** Weigend, Peru, *Weigend & al. 97/450* (M), KY286923, KY286655, KY286833, KY286743, –.

CHAPTER 3

A taxonomic revision of the western South American genus *Presliophytum* (Loasaceae)[i]

Presliophytum arequipense,in cultivation in the Botanical Gardens of Bonn. Notice the proportionally large sepals in relation to the petals. This species was restricted to the area near Mollendo, in Arequipa, Peru, and could be extinct in the wild.

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A taxonomic revision of the western South American genus *Presliophytum* (Loasaceae)

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Abstract

Presliophytum is a small genus of five species endemic to arid western South America, including coastal Peru and the Atacama Desert. The type species, *Presliophytum incanum*, was originally described in *Loasa*, but recognized as highly distinctive and placed into a monotypic section in the late 19th century. Together with *Loasa heucheraefolia* and a newly described species, it was placed into the genus *Presliophytum* in 1997. Subsequent molecular studies confirmed the monophyly of the genus and indicated a close relationship to two Chilean species, traditionally placed in *Loasa* series *Malesherbioideae*, a placement formalized in 2017 by providing the necessary new combinations. However, a detailed revision and description of the taxon has not been provided and the present study aims at filling this gap. We provide data on the morphology and micromorphology, distribution and ecology of the five species, as well as a key for all the species. *Presliphytum incanum* is the most common and widespread species, but also morphologically the most variable. There are differences in leaf and flower morphology between northern and southern populations, but these are difficult to discern in herbarium specimens. The species is therefore here maintained in the broader sense, since at present it seems impossible to clearly differentiate two morphologically discrete entities.

Key words: Cornales, endangered species, endemic species, floral morphology and anatomy, systematic relationships, trichomes

Introduction

Presliophytum (Urb. & Gilg) Weigend (2006: 467) is a small genus composed by five species: *P. arequipense* Weigend (2006: 467), *P. heucheraefolium* (Killip) Weigend (2006: 467), *P. incanum* (Graham) Weigend (2006: 467), *P. malesherbioides* (Phil.) R.H. Acuña & Weigend in Acuña *et al.* (2017: 373) and *P. sessiliflorum* (Phil.) R.H. Acuña & Weigend in Acuña *et al.* (2017: 373). The first three species are shrubs endemic to the Pacific slope of Peru and were placed in *Presliophytum* based on morphological data (Weigend 1997, 2006). Molecular data published over the last two decades clarified the phylogeny of Loasaceae subfamily Loasoideae and confirmed the assumption of Weigend (1997) that two herbaceous northern Chilean/Argentinean species, *Loasa malesherbioides* Philippi (1864:74) and *L. sessiliflora* Philippi (1893:12), belong to the same clade (Weigend 2004, Weigend *et al.* 2004b, Hufford *et al.* 2005, Acuña *et al.* 2017).

Presliophytum incanum, the type species of the genus, was collected by European botanists at least as early as 1778 (Ruiz & Pavón 1959) when the Botanical Expedition to the Viceroyalty of Peru lead by Hipólito Ruiz and José Pavón (accompanied by Joseph Dombey) collected material in the Obrajillo area (Departmento de Lima, Lang 1985). The species was published as *Loasa incana* Graham (1830: 169) several decades later from material of the same area.

During the last decade of the 19th century, *Loasa* section *Presliophytum* Urb. & Gilg in Gilg (1894: 118), including only *Loasa incana*, and *Loasa* section *Euloasa* Urb. & Gilg in Gilg (1894: 115) series *Malesherbioideae* Urb. & Gilg in Gilg (1894: 116, 118), including *Loasa malesherbioides* and *L. longiseta* Philippi (1865: 347), were described. Weigend (1997) investigated the genus limits in Loasaceae subfam. Loasoidaeae and segregated section *Presliophytum* as a separate genus, including three species: *Presliophytum incanum*, newly described *Presliophytum arequipense,*

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and renamed *Presliophytum heucheraefolium* based on *Loasa heucheraefolia* Killip (1928: 90). Weigend (1997) also indicated that series *Malesherbioideae* probably belonged to the same lineage and should be placed in *Presliophytum*, however, this was not formalized by renaming these species. No additional nomenclatural changes were done since then, until Acuña *et al.* (2017) transferred *Loasa malesherbioides* and *L. sessiliflora* into *Presliophytum,* establishing the genus as currently understood.

The relationships of *Presliophytum* to other clades in Loasaceae were obscure until recently. As pointed out by Weigend (1997; 2004), morphologically and ecologically, there are several similarities between *Presliophytum* and the northern Chilean genus *Huidobria* Gay (1847: 438–439). Both genera include deserticolous shrubs with a strong, fleshy tap-root, alternate phyllotaxis, complex asymmetrical dichasial inflorescences with extensive concaulescence and recaulescence, erect flowers, white to pale yellow corollas and floral scales, and thousands of minute seeds per fruit (Weigend 1997, 2004). However, based on the molecular data now available (Acuña *et al.* 2017), all these traits are apparently either plesiomorphic or the result of convergent evolution in similar environments. More detailed morphological analyses reveal a series of profound differences between both of these groups of plants: *Huidobria* lacks the stinging hairs (setae) found in *Presliophytum*. Also, the floral scales in *Presliophytum* are typical of the bulk of Loasoideae, with the staminodial complexes consisting of three outer staminodes fused into a scale and two free, inner staminodes (Urban & Gilg 1900, Grau 1997, Weigend 1997). Conversely, *Huidobria* has staminodial complexes with the scales variably formed by 4–7 incompletely fused staminodia and 3–5 inner free staminodia (Grau 1997, Hufford 2003). The dust seeds of *Huidobria* are oblong with sparse testa reticulations or striations, while those of shrubby *Presliophytum* are ovoid with a densely reticulate-foveate testa (Grau 1997, Weigend 1997).

Molecular data indicate a distant relationship between *Huidobria* and *Presliophytum,* and a paraphyly of *Huidobria* (Moody *et al.* 2001, Weigend *et al.* 2004b, Hufford *et al.* 2005, Acuña *et al.* 2017). Both Hufford *et al.* (2005) and Acuña *et al.* (2017) retrieved *Presliophytum* as sister of the South Andean Loasas clade, whereas *H. fruticosa* Philippi (1855: 219) is retrieved as the basal-most branch of subfamily Loasoideae and *H. chilensis* Gay (1847: 440) as sister of Klaprothieae+*Kissenia* R.Br. ex Endlicher (1842: 76).

According to the latest phylogenetic evidence *Presliophytum malesherbioides*, from Chile and western Argentina is the basal-most branching species of the genus (Acuña *et al.* 2017). This taxon differs from the rest in habit (annual or subperennial herbs vs. perennial subshrubs or shrubs), development of the root system (thin and simple vs thickened and branched), leaf morphology (margins subentire to shallowly lobate vs margins regularly lobate), chromatic contrast between the nectar scales and the corolla (conspicuous vs inconspicuous), and morphology of the nectar scale (dorsal threads short and clavate vs dorsal threads long and filiform; apical wings well developed vs apical wings rudimentary). *Presliophytum sessiliflorum* from northern Chile is sister to *P. arequipense*, *P. heucheraefolium* and *P. incanum* (the Peruvian shrubby species). In habit it is intermediate between *P. malesherbioides* and the Peruvian taxa, being a perennial subshrub with thickened underground structures. The external morphology of the leaves (with regularly lobate blades) and flowers (broad sepals, white nectar scales with filiform dorsal threads and rudimentary apical wings) is closer to the shrubby Peruvian species, while in seed morphology (with conspicuous transversal constrictions and undulate periclinal walls) it appears closer to *P. malesherbioides*. Its small 2-placentae, 4-seeded fruit is very characteristic (Grau 1997). The three shrubby Peruvian taxa are more closely related to each other than to any other extant taxon, and form a well-supported clade based both on molecular evidence (Acuña et al 2017) and morphology: all are shrubs to about 1.5 m tall with woody aboveground and underground structures, having strongly metatopic inflorescences, and almost identical floral scales, globose fruits and diminutive seeds.

The goal of this study is to present a revision of the genus *Presliophytum* as currently understood, based on macro- and micro-morphological studies of living plants both from the wild and cultivation, as well as herbarium specimens.

Materials and methods

Field studies were carried out in Peru between 1997–2014. Specimens were prepared in the field following standard techniques and voucher sets were deposited in Peruvian herbaria (USM, HUSA, HUT), and in Berlin (B), Bonn (BONN) and Munich (M). In addition, specimens or photographs from the following herbaria were revised: BAB, BM, BONN, CONC, E, F, G, HUSA, K, L, MA, P, SGO, SI and US. All of the taxa recognized here, except *Presliophytum sessiliflorum*, have been brought into cultivation in the glasshouses at the Institut für Biologie –Morphologie und Systematik der Pflanzen, Freie Universität Berlin and Botanische Gärten der Universität Bonn, Germany.

Measurements of most structures were taken from herbarium specimens. Diagnostic or distinctive characters were identified and are indicated in bold for each species description. The specimens were georeferenced for mapping, whenever possible. When the geographical coordinates were not included in the specimen label information, the original collectors' published itineraries were studied, when available, and the collection localities searched in the free access GeoNames (http://www.geonames.org) or directly in Google Earth Pro ver. 7.1.7.2606 (Google Inc. 2016). Each specimen was plotted using the maps package (Brownrigg 2017) for the R software (R Core team 2014). The conservation status assessments are based on the Red List Criteria by the IUCN (2001).

To analyze the microstructure details of foliar surfaces and seeds we used fresh material from cultivated plants of five different accessions of *Presliophytum* from Peru. Dry material collected in Chile was employed for the two remaining species. Fresh material was studied with cryo scanning electron microscopy in order to avoid drying artifacts. SEM studies were carried out following the methods of Ensikat & Weigend (2013). Both fresh and dry material (including seeds) were sputter coated lightly with Au or Pd for about 20 seconds in a SCD040 (Balzers Union, Liechtenstein) in order to increase the electrical conductivity.

Results

Morphology

Seedlings**:—**The germination, as in other Loasoids is epigeal (Weigend 1997). The cotyledons are 1–2 mm long, ovate, subcircular or oblong and develop an emarginate apex with a terminal hydatode. Veins are inconspicuous. The second pair of leaves produced shows poorly developed toothed margins. Successive leaves become progressively more deeply dissected. During very early stages of development, the leaf morphology of all the species is quite similar, but clear differentiation is evident after few weeks.

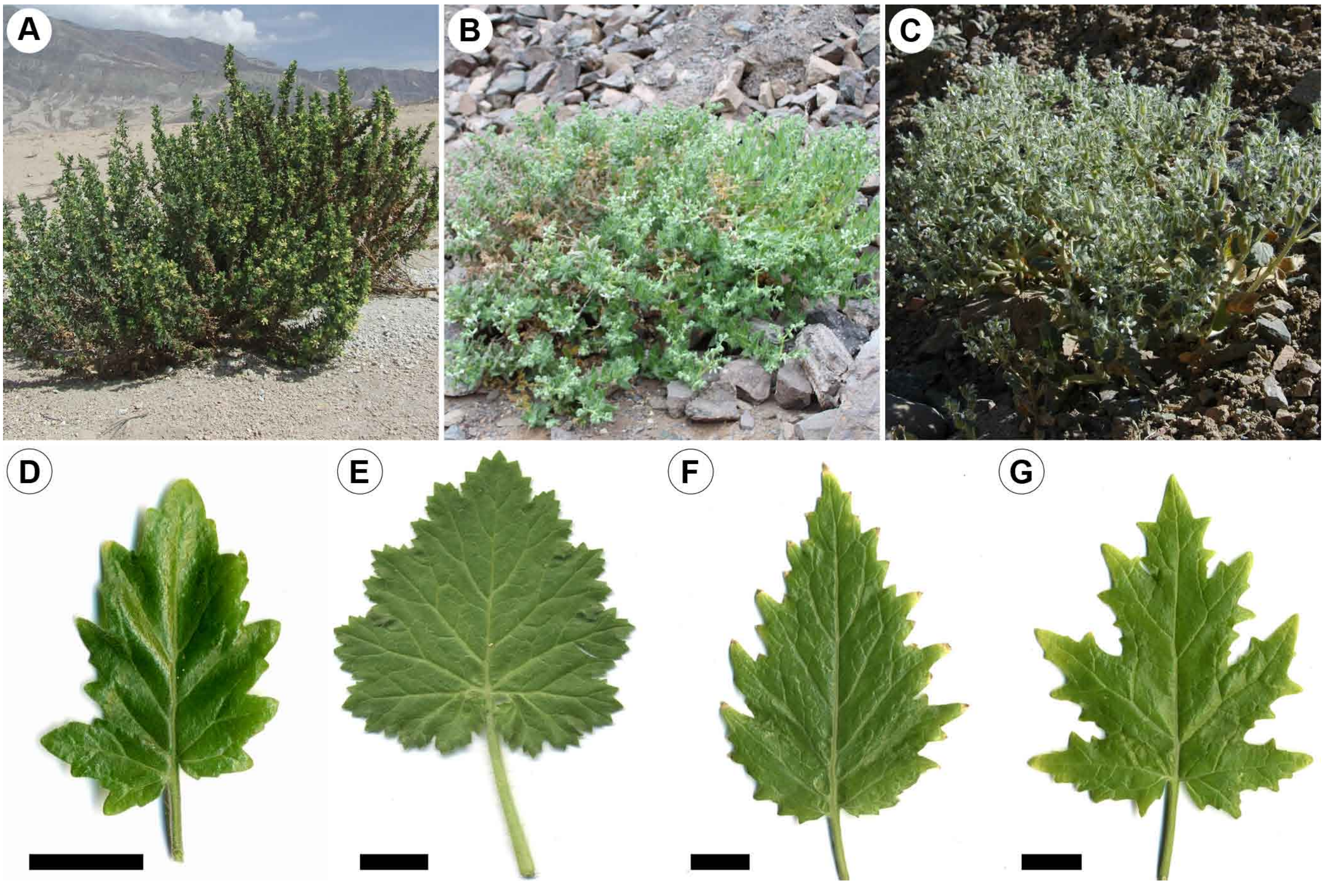

FIGURE 1. Vegetative morphology of *Presliophytum*. **A.** Habit and habitat of shrubby *Presliophytum incanum* (Quinistaquillas, Moquegua, Peru, not vouchered). The three species of Peruvian *Presliophytum* have similar habits. **B.** Habit and habitat of subshrubby *Presliophytum sessiliflorum* (Quebrada La Chimba, Antofagasta, Chile, *Luebert et al. 3405*, BONN; photo courtesy of F. Luebert). **C.** Habit and habitat of herbaceous *Presliophytum malesherbioides* (Juntas del Toro, Coquimbo, Chile, not vouchered; photo courtesy of M. Eyzaguirre). Mature specimens of this species range from unbranched annuals to densely branched subperennial herbs as the one illustrated. **D.** Mature leaf of *Presliophytum arequipense* (cultivated, seeds collected near Mollendo, Arequipa, Peru, *Ortiz et al. 121*, BONN). **E.** Mature leaf of *Presliophytum heucheraefolium,* (cultivated, seeds collected near Río Cacchán, Áncash, Peru, *Weigend et al. 7691C*, BONN). **F.** Mature leaf of *Presliophytum incanum* (cultivated, seeds collected near Ullpan, Áncash, Peru, *Weigend & Hilger 8912C*, BONN). **G.** Mature leaf of *Presliophytum incanum* (cultivated, seeds collected near Omate, Moquegua, Peru, *Ackermann & Cáceres 674*, BONN). Scale bars 15 mm. Photos by R. Acuña and M. Weigend, unless otherwise credited.

Adult habit:—Mature plants are densely branched from the base (Figs. 1A–C). Life form is variable and strongly dependent on moisture availability. Plants range from woody shrubs up to 1.5 m tall (Fig. 1A) to low subshrubs up to 50 cm tall with succulent, persistent underground organs (Fig. 1B) or annual to subperennial herbs less than 30 cm tall (Fig. 1C). Wood anatomy of *Presliophytum incanum* was described by Carlquist (1984) as similar to that of *Kissenia capensis* Endlicher (1842: 76) and *Nasa picta* (Hook.) Molinari (2015: 68), differing from them in the much smaller multiseriate rays and absence of axial parenchyma. All taxa have a dominant taproot.

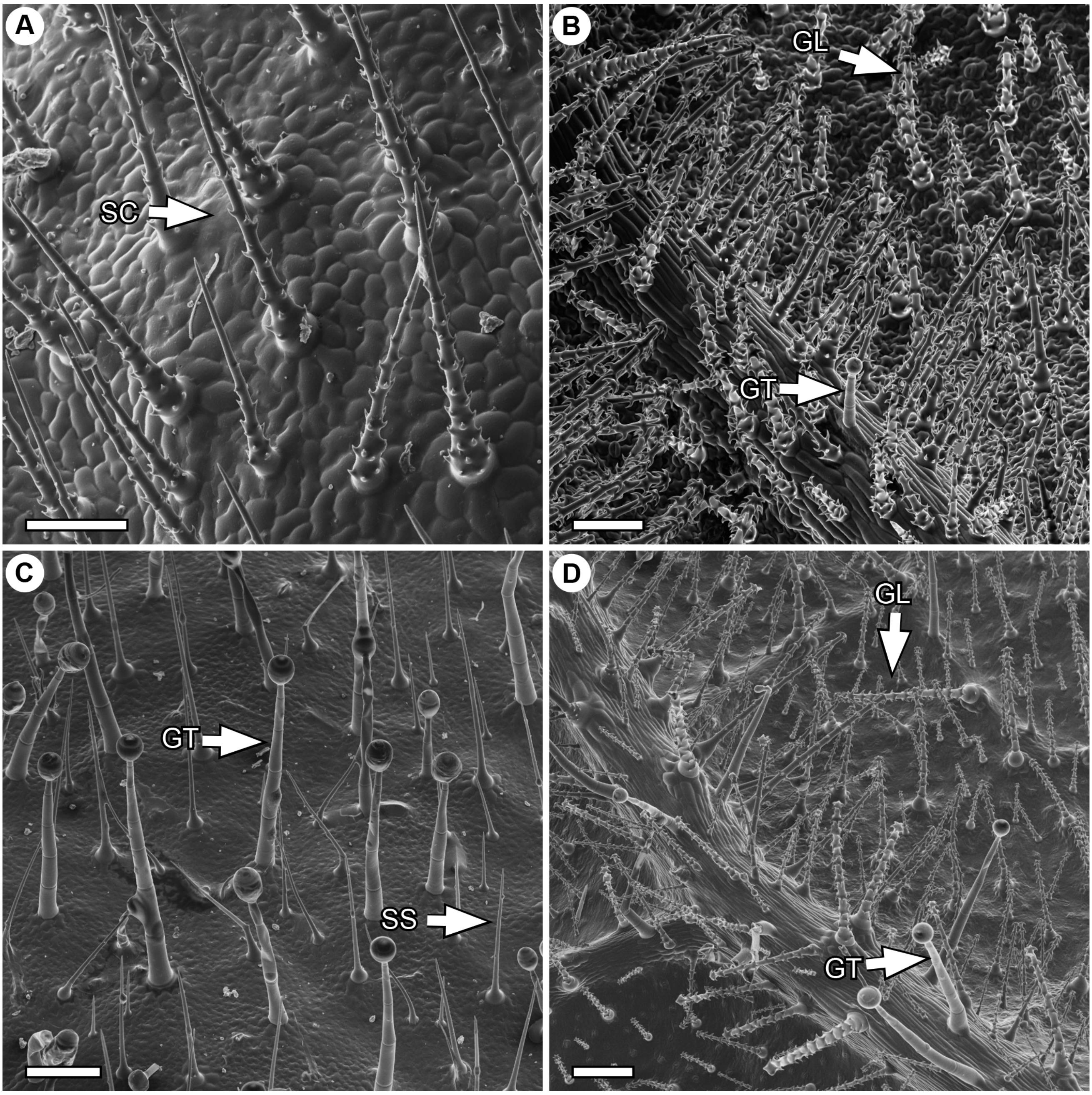

FIGURE 2. Microstructure and trichome morphology of *Presliophytum*. **A.** Adaxial foliar surface of a mature leaf of *Presliophytum arequipense* (*Ortiz et al. 121*, BONN). *Presliophytum incanum* has virtually identical indumentum. **B.** Abaxial foliar surface of a mature leaf of *Presliophytum arequipense* (*Ortiz et al. 121*, BONN). *Presliophytum incanum* may have similar indumentum or mostly made up by scabrid trichomes. **C.** Adaxial foliar surface of a mature leaf of *Presliophytum heucheraefolium* (*Weigend et al. 7691C*, BONN). This species and *Presliophytum sessiliflorum* have abundant and relatively long uniseriate glandular hairs, on leaves and younger stems. *Presliophytum malesherbioides* has similar but much shorter trichomes in some areas of the stem. **D.** Abaxial foliar surface of a mature leaf of *Presliophytum heucheraefolium* (*Weigend et al. 7691C*, BONN). GL: Glochidiate trichome, GT: Uniseriate glandular trichome, SC: Scabrid trichome, SS: Short smooth trichome. Scale bars A, B: 100 µm; C, D 200 µm.

Leaf morphology:—Phyllotaxis is opposite in the lower part and alternate in the upper part of the stem, probably the plesiomorphic condition in Loasaceae (Weigend 1997). Leaves are petiolate (Figs. 1D–G), the frondose prophylls and the leaves just below the inflorescences are often sessile or subsessile. Lamina is (narrowly) ovate in most species, but reniform to subcircular in *Presliophytum heucheraefolium* (Fig. 1E). Leaf bases are cordate to truncate, more rarely cuneate. Leaf margin is usually lobate or lobulated with 3–8 lobes on each side, usually only dentate to subentire in *P. malesherbioides*.

Indumentum**:—**All species have stinging trichomes (setae). These are especially numerous in *Presliophytum malesherbioides* and *P. sessiliflorum,* whereas the shrubby species develop relatively few stinging hairs. Stinging hairs are particularly abundant on the ovary and younger portions of the stem. All the species have a 'hoary' appearance because of the high density of scabrid and glochidiate trichomes on stem and leaves (Figs. 2A, B). Uniseriate glandular trichomes are also common and particularly well developed in the leaves and young stems of *P. heucheraefolium* and *P. sessiliflorum* (Figs. 2C, D). Most *P. malesherboides* plants have short and inconspicuous glandular trichomes on the stems, but the leaves are mostly eglandular. In *P. arequipense and P. incanum* glandular trichomes are absent or extremely rare (Fig. 2B).

Inflorescence morphology**:—**Inflorescences are complex dichasia, with alternate, foliose (sub-) sessile prophylls. In *Presliophytum malesherbioides* and *P. sessiliflorum* the inflorescence branches are symmetrical, but in the shrubby species one of the branches grows much more than the other and is hardly distinguishable from vegetative branches. Each flower is subtended by two prophylls, the alternate phyllotaxis is the result of the conspicuous metatopia product of recaulescence and concaulescence, a trait more developed in the shrubby taxa (whose flowers appear to be solitary: *cf.* Weigend *et al.*, 2004b: Figs. 6A vs 6C). Flowers are erect, less frequently horizontal to deflexed.

Flower morphology**:—**Flowers are epigynous, pentamerous and complete in all taxa (Figs. 3A–F). The sepals range from ovate (Fig. 3A) to narrowly lanceolate or linear (Fig. 3E). Petals are usually spreading and white, sometimes greenish white or cream-coloured (Figs. 3A–C, E–F). Some southern populations of *Presliophytum incanum* are quite different in having half-spreading, yellowish petals (Fig. 3D). Petals are deeply cymbiform with a well-defined claw and limb. Floral scales are usually the same color as the petals, except in *P. malesherbioides*, where they vary from yellow to mostly white, but are always provided with some yellow or green markings (Fig. 3E). Scales in all species have three dorsal threads (Fig. 3G).

There are two staminodes opposite to the interior of each nectar scale. The proximal portion is more or less sigmoid and papillose, the terminal is filiform, extending well beyond the scale. There are 30 to ca. 150 fertile stamens per flower. In most species the ovary is unilocular with 3–5 parietal, non accrescent-placentae with 5–1600 ovules each. *Presliophytum sessiliflorum* differs in a bilocular ovary with two placentae, each with two ovules only.

The flowers of the shrubby taxa are visited by Lepidoptera, Hymenoptera, and Trochilidae. *Presliophytum malesherbioides* has been collected with small Diptera on the stamens and petals (F. Luebert pers. comm.). Henning & Weigend (2011) studied the thigmonastic stamen movement in *P. heucheraefolium* in detail.

Fruit morphology**:—**Fruits are erect, inferior, straight, xerochasious capsules, opening apically with 3–5 valves (Weigend 2004, Weigend *et al.* 2004a). The fruits of *Presliophytum sessiliflorum* may be indehiscent, but detailed observations are missing. Fruit shape varies from globose (almost as long as wide, Fig. 3H) to cylindrical and obconical (longer than wide, Fig. 3I). The smallest fruits (5.0 × 3.0 mm not counting the sepals or trichomes) are found in *P. sessiliflorum* meanwhile the largest (up to 28 × 15 mm) are those of some *P. malesherbioides*.

Seed morphology**:—**The Peruvian taxa have foveate-reticulate dust seeds, ca. 0.5 mm in length (Fig. 3J). *Presliophytum malesherbioides,* especially*,* and *P. sessiliflorum* have conspicuous transversal constrictions (Figs. 3K, L), and very similar seed testa sculpturing, with tetragonal cells and undulate periclinal wall surfaces. The seed set per fruit varies between 4 in *P. sessiliflorum*, to >3000 in the shrubby species (Weigend *et al.* 2004a).

Distribution and habitat

Presliophytum is endemic to arid western South America (Fig. 4). It is mostly restricted to the Pacific slope, from Departamento de Piura in northwestern Peru (*P. incanum*) to Región de Coquimbo in Chile (*P. malesherbioides*), however it crosses the Andes onto the eastern slope in Provincia de San Juan in western Argentina (*P. malesherbioides*). There is an apparent distribution gap in southernmost Peru and northernmost Chile: none of the Peruvian species extend into Chile while the opposite is also true (Weigend 1999, Rodríguez & Weigend 2006, Weigend *et al.* 2008). *Presliophytum* can grow in extremely arid conditions and is commonly found in dry washes and on loose scree slopes, where it may be locally the most common or even the only perennial plant species. It usually grows on sandy or rocky soil and ranges from near sea level up to ca. 3600 m.

All species are allopatric except for *P. incanum*, whose range shows local overlap with *P. heucheraefolium* in Áncash at ca. 1000 m in the lower regions of Cordillera Negra in the Provincia Santa, e.g. along the road from Moro to Pamparomas [(*Weigend & Dostert 97/121* (F, M), *Weigend & Dostert 97/120* (F, M, P)].

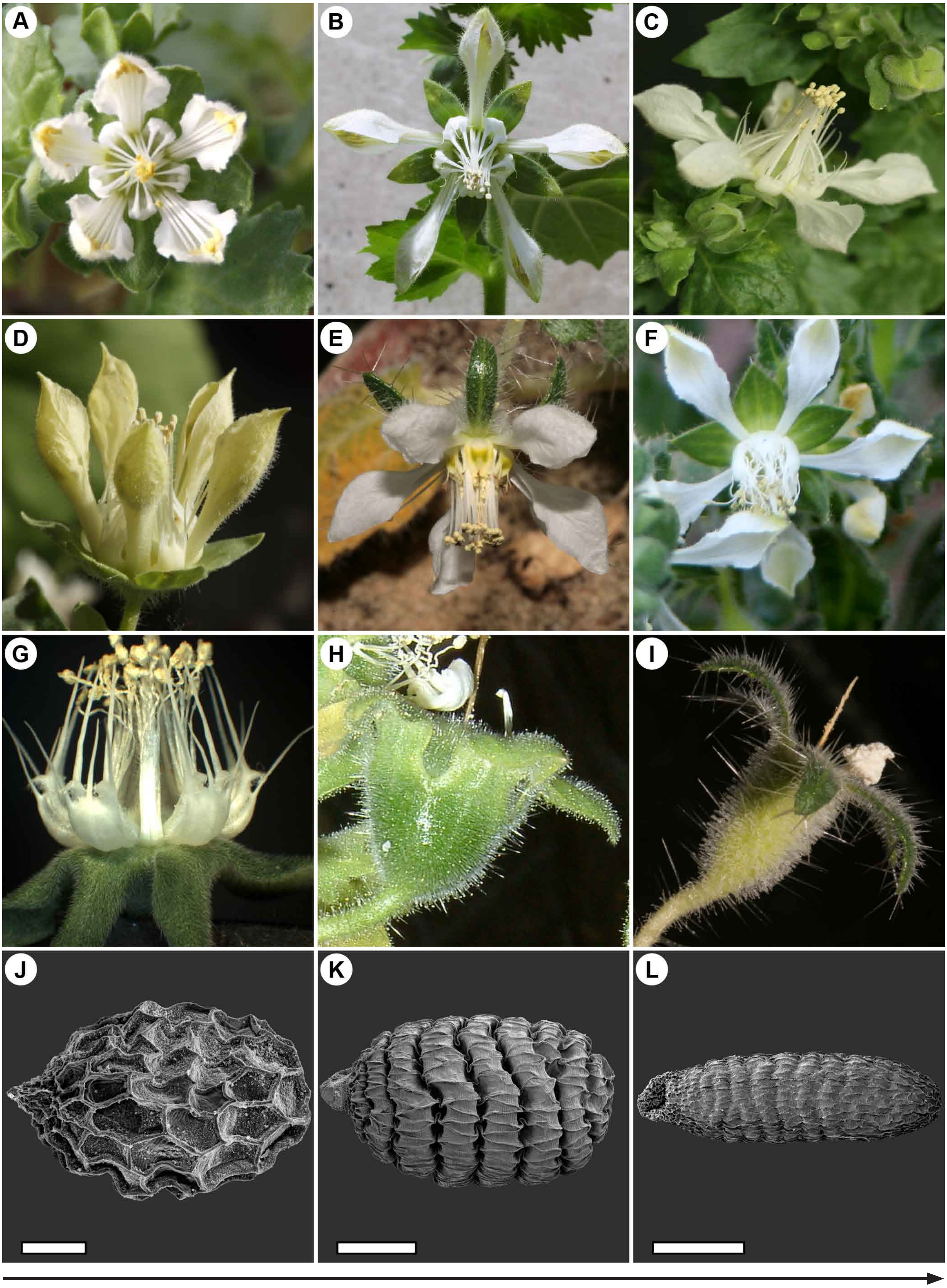

FIGURE 3. Reproductive morphology of *Presliophytum*. **A.** Flower of *Presliophytum arequipense* (cultivated, *Ortiz et al. 121*, BONN; photo courtesy of M. Ackermann). Notice the relatively small corolla compared to the calyx. **B.** Flower of *Presliophytum heucheraefolium* (cultivated, *Weigend et al. 7691C*, BONN; photo courtesy of M. Ackermann). **C.** Flower of *Presliophytum incanum* from Ullpan, Ancash, Peru (*Weigend & Hilger 8912C*, BONN; photo courtesy of M. Ackermann). **D.** Flower of *Presliophytum incanum* from Omate, Moquegua Peru (*Ackermann & Cáceres 674*, BONN; photo courtesy of M. Ackermann). **E.** Flower of *Presliophytum malesherbioides* (cultivated, seeds collected near Chollay, Atacama, Chile (*Weigend KW1024*, BONN, photo by H. Hilger). The nectar scales range from entirely yellow to mostly white but always with some yellow or green present. The range in variation of this trait and the length of the dorsal threads appear to be clinal. **F.** Flower of *Presliophytum sessiliflorum* (*Luebert et al. 3405*, BONN; photo courtesy of F. Luebert). Notice the relatively short and broad sepals, contrasting with those of *Presliophytum malesherbioides*. **G.** Nectar scale complexes of *Presliophytum incanum* (*Weigend 8064*, B; photo by P. Beckers). The nectar scales of the other Peruvian shrubby taxa are very similar. **H.** Fruit of *Presliophytum heucheraefolium* (*Weigend et al. 7691C*, BONN). The other shrubby Peruvian species have similar capsules. **I.** Immature fruit of *Presliophytum malesherbioides* (*Weigend KW1024*, BONN; photo by H. Hilger). This species usually has the largest fruits of the genus. **J.** Mature seed of *Presliophytum heucheraefolium* (*Weigend et al. 7691C*, BONN). The seeds of the Peruvian species of *Presliophytum* are very similar to each other and amongst the smallest in Loasaceae. **K.** Mature seed of *Presliophytum malesherbioides* (Baños del Toro, Coquimbo, Chile; *Luebert et al. 3714*, BONN). **L.** Mature seed of *Presliophytum sessiliflorum* (Quebrada Botija, Antofagasta, Chile; *Hoffmann 187*, CONC). Although differing considerably in proportions, the seeds of the last two species have similar seed testa cell morphology and sculpturing. Scale bars J 100 µm, K 500 µm, L 1000 µm. Photos by R. Acuña and M. Weigend, unless otherwise credited.

Taxonomic treatment

Presliophytum (Urb. & Gilg) Weigend (2006: 467)

≡ *Loasa* section *Presliophytum* Urb. & Gilg in Gilg (1894: 118)

Type species: *Presliophytum incanum* (Graham) Weigend (2006: 467) ≡ *Loasa incana* Graham (1830: 169).

Erect 20–150 cm tall, densely branched herbs to shrubs (rarely poorly branched herbs <10 cm tall) with stinging hairs. Scabrid hairs abundant on most surfaces, especially on abaxial leaf blade and outer ovary, glandular trichomes sometimes present on leaves and younger stems. Taproot present, usually thickened and fleshy, rarely thin and poorly branched. Leaves shallowly lobate, rarely toothed or sub-entire, mostly alternate (except lowermost pairs), petiole 3–100 mm, lamina 12–170 × 5–170 mm, ovate to reniform with crenate margin, rarely weakly toothed, base cuneate to deeply cordate, apices of lobes and blade, acute to rounded. Inflorescences complex dichasia, to ca. 100 cm long, symmetrical (in extra-Peruvian species) or asymmetrical (in shrubby Peruvian species); each flower erect or horizontal to deflexed in anthesis, with 2 foliose prophylls (flowers apparently irregularly alternating with foliage leaves, due to concaulescence and recaulescence) 3.0–60 × 1.0–65 mm, similar to leaves in morphology but smaller and less disected; sepals five, 2.0–12 × 0.5–10 mm green, 3-veined, with entire margins; petals five, 4.0–25 mm long, white (often slightly tinged green or cream) or yellow; nectar scales five, 2–7 mm long, white, yellow or greenish, usually unicolored, rarely bicolored white and yellow or green, with 3 dorsal threads 0.5–7.0 mm long, claviform (shorter than the nectar scale) or filiform (almost as long or longer than the nectar scale). Staminodes 2 per scale, 4–15 mm long. Stamens 30–130. Style 3–15 mm long, straight (twisting after fertilization), ovary inferior, placentae 3 to 5, rarely 2. Fruit a capsule, 5–15 × 3–12 mm, obconical or cylindrical to obovoid or subglobose, opening with 3–5 apical valves (rarely apparently indehiscent); seeds 0.5–4.0 mm × 0.2–1.5 mm, testa dark to tan brown, foveate-reticulate, or with 6–18 transversal constrictions. 2n = 12, 24 (Grau 1988, Weigend 2004). Five species from the xeric regions of western Peru, through northern Chile into western Argentina, frequent on rocky slopes.

Etymology:—'Plant of Presl'. Dedicated to the Czech botanist Carl Presl who made important contributions to the knowledge of Loasaceae in the 19th century.

Similar taxa:—Due to its distribution in xeric regions, alternate phyllotaxis and star-shaped, pale corollas, *Presliophytum* could be confused superficially with the following Loasaceae taxa (characters of *Presliophytum* in parentheses). Both *Huidobria* have stinging trichomes absent (vs. present), nectar scales formed by four or more stamens (vs. always three, as indicated by the number of dorsal threads in each taxon) and seed testa either longitudinally striate or smooth to irregularly wrinkled (vs. foveate-reticulate or transversally constricted). *Nasa* Weigend (2006: 465) species with star-shaped, pale corollas have flowers pendent (vs. usually erect), dorsal threads on nectar scales absent (vs present), and seed testa reticulate (vs. foveate-reticulate or transversally constricted). *Loasa* series *Floribundae* Urb. & Gilg in Gilg (1894: 116, 117) occasionally have alternate phyllotaxis, but have flowers pendent (vs. usually erect), nectar scales with red markings evident (vs. red absent), fruits semisuperior (vs. inferior) and seed testa deeply pitted (vs. foveate-reticulate or transversally constricted).

Key to the species of *Presliophytum*

1. Herbs to sub-shrubs to 50 cm tall. Aerial structures not- to poorly lignified. Fruit with <100 seeds, each seed ca 1–2 mm long, testa transversally constricted. Restricted to northern Chile and western Argentina .... 2.
\- Shrubs to 150 cm tall. Aerial structures lignified. Fruit with >1000 seeds, each seed ca. 0.5 mm long, testa foveate-reticulate. Restricted to Peru .... 3.
2. Plants annual to subperennial herbs. Leaves subentire, toothed to irregularly lobate. Sepals more than 3 × as long as wide. Nectar scales contrasting in color with the corolla. Fruit with >10 seeds. Restricted to the interior (more than 80 Km from the ocean) of northern Chile and western Argentina .... *P. malesherbioides*
\- Plants perennial subshrubs. Leaves always more or less regularly lobate. Sepals less than 2 × as long as wide. Nectar scales the same color as the corolla. Fruit with(3–)4(–6) seeds. Restricted to coastal (less than 20 Km from the ocean) northern Chile .... *P. sessiliflorum*
3. Leaves reniform. Uniseriate glandular hairs abundant on young stems and leaves. Restricted to Departamentos de Áncash and Lima .... *P. heucheraefolium*
\- Leaves ovate. Uniseriate glandular hairs very rare or absent .... 4.
4. Sepals almost as long as wide. Petals subequal in size to sepals. Restricted to coastal Departamento de Arequipa .... *P. arequipense*
\- Sepals ca. 1.5-2 × as long as wide. Petals at least 1.5 × as long as sepals. Widely distributed in the Pacific slope of Peru (Departamentos de Piura to Moquegua) .... *P. incanum*

1. *Presliophytum arequipense* Weigend (2006: 467) (Figs. 1D, 2A, B, 3A)

Type:—PERU, Arequipa [Prov. Islay], Mollendo, ca. 30 m., on rocky cliff, 27 October 1937. *D. Stafford 1017* (holotype: BM barcode BM000021454!; isotype: F No. 1508586 [photo!]).

Coarse, densely branched perennial shrub 50–100 cm tall. Stem epidermis with abundant glochidiate (sometimes obscured) and scabrid trichomes, scattered stinging and short-smooth trichomes. Taproot present, usually thickened and fleshy. Leaves lobate, opposite below, alternate above, petiole 4–20 mm, with glochidiate, scabrid and scattered stinging and short-smooth trichomes, lamina 20–60 × 15–45 mm, ovate with 3–7 lobes on each side, margin crenate, base cuneate to shallowly cordate, often asymmetric, blade and lobe apices obtuse to rounded, upperside with short-smooth, scabrid and sparse stinging trichomes (mostly on veins or blade margins). In older leaves, the trichome apices may fall, leaving behind basal cells, forming scale-like structures, underside densely covered in glochidiate and scabrid trichomes (rarely with glandular and stinging trichomes). Inflorescences densely frondose, complex asymmetrical dichasia, to ca. 50 cm long; each flower erect or horizontal in anthesis with two, sometimes sessile, prophylls (flowers apparently solitary and irregularly alternating with foliage leaves) 3–25 × 5–25 mm, similar to vegetative leaves in morphology and indumentum, but often narrower and weakly lobate; pedicels with glochidiate (sometimes obscured), scabrid, short-smooth and stinging trichomes. **Sepals five, broadly ovate, 5–12 × 5–10 mm** green, 3-veined with entire margins, **almost as long as the petals**, indumentum of each surface similar to that of the respective leaf surface; petals five, full to half spreading, cymbiform, 5–9 mm long, cream to greenish-white, tinged darker greenish on the abaxial surface, with scabrid, glochidiate and scattered, weak, stinging trichomes, margins flat or slightly revolute, finely serrate and clearly distinguishable form the petal central depression; nectar scales five, 2–5 mm long, cream to greenish-white (not contrasting with petal color), unicolored, concave, slightly bulging, with a poorly developed papillae-margined neck and rudimentary apical wings. Filiform dorsal threads, three, 1.5–3(–5) mm long, the central sometimes shorter than the laterals, attached subapically to the scale. Staminodes 2 per scale, 4–10 mm long, the distal 2/3rds filiform, glabrous, the proximal third abruptly expanded, with a flange towards the nectar scale, margins papillose. Stamens 50–75, filaments 5 mm long. Style 5 mm long, straight, twisting after fertilization, ovary inferior, with a densely pubescent roof covered in short-smooth and scabrid trichomes, outer wall with abundant glochidiate and stinging trichomes, sometimes with scabrid and short-smooth trichomes, placentae 3–5. Fruit a capsule 6–9 mm diameter, subglobose, opening with 3–5 apical valves; seeds ca. 1200–3000 per capsule, 0.5 mm × 0.2–0.5 mm, testa dark brown, foveate-reticulate. Seed testa cells polygonal.

Notes:—Although this species was collected for the first time early in the 19th century by D'Orbigny, it was not recognized as a distinct taxon until 1997 (Weigend 2006). Its most distinctive traits are the size, proportions and morphology of the perianth parts, which are markedly different from its close relative *Presliophytum incanum* (Acuña *et al.* 2017).

Etymology:—The epithet alludes Arequipa, the Peruvian department to which this species is endemic.

Illustrations:—Floral scale morphology: Urban & Gilg (1900: Tab. VII. Fig. 11).

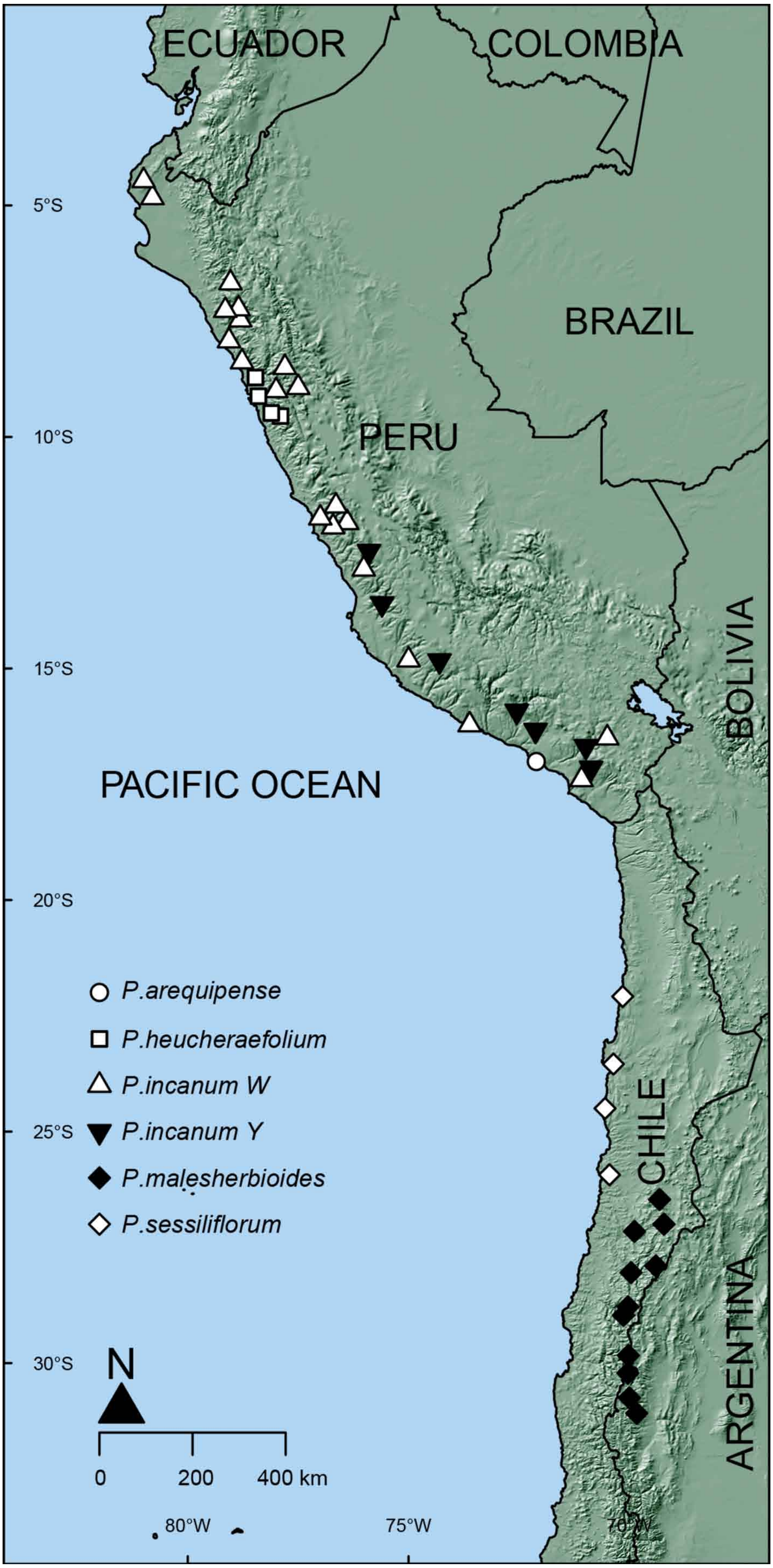

FIGURE 4. Natural distribution of *Presliophytum* based on herbarium specimens. *P. incanum* W = *Presliophytum incanum* with white or indeterminate color corollas. *P. incanum* Y = *Presliophytum incanum* with yellow corollas.

Distribution:—Endemic to southern Peru. So far known from only three localities very close to each other near the coast, at elevations below 100 m in Distrito de Mollendo, Provincia de Islay, Departamento de Arequipa (Fig. 4).

Phenology:—The few known wild collections flowered in October. In cultivation, the plants flower throughout the year.

Ecology:—This plant grows on cliffs, scree slopes and dry river beds and in the Lomas near Mollendo, sometimes sympatrically with cacti. No information about pollinators has been obtained for this species.

Conservation status:—Although not analyzed by Rodríguez & Weigend (2006) this species is known from only two recent collections, both from essentially the same place, which is currently under urban development. Due to its rarity, limited range, and human pressure, we recommend this species to be considered as critically endangered (CR) according to criteria A4bc, B2ab(i,ii,iii,iv,v). Several attempts to find the plant in the wild were unsuccessful and it may be extinct in the wild.

Additional specimens examined:—PERU. **Arequipa:** Prov. Islay, Islay near Arequipa, 1833(?), *D'Orbigny s.n.* (P: P00123875); Antigua trocha desde playa Catarindo hasta la Carretera Panamericana, 32 m, 8 October 2004, *Ortiz et al. 116* (BONN, HUSA); ditto, 42 m, 8 October 2004, *Ortiz et al. 121* (BONN, HUSA).

2. *Presliophytum heucheraefolium* (Killip) Weigend (2006: 467) (Figs. 1E, 2C, D, 3B, H, J)

≡ *Loasa heucheraefolia* Killip (1928: 90)

Type:—PERU [Áncash, Prov. Huaraz], Tambo de Pariacota [*sic*], ca. 1000 m., moist cliff, 8 October 1922, *F. Macbride & [W.] Featherstone 2543* (holotype: F No. 518960/Neg. No. 63414! [photo: US barcode US00115211!]; isotype: G barcode G00368197 [photo!]).

Coarse, densely branched perennial shrub 50–150 cm tall. Stem epidermis with abundant glochidiate, short-smooth and glandular trichomes, sparse scabrid and stinging trichomes. Taproot present, usually thickened and fleshy. Leaves lobate, opposite below, alternate above, petiole 20–100 mm, with glochidiate, short-smooth, glandular and scattered stinging trichomes, **lamina 25–170 × 30–170 mm, reniform,** with 3–8 lobes on each side, margin crenate, **base deeply cordate,** blade and lobe apices obtuse, **upper side with numerous short-smooth, glandular and few stinging trichomes**, very rarely with scabrid trichomes, underside with glochidiate, scabrid and glandular trichomes (rarely with stinging trichomes on veins). Inflorescences densely frondose, complex asymmetrical dichasia, to ca. 100 cm long; each flower erect or horizontal in anthesis, with two, often sessile, prophylls (flowers apparently solitary and irregularly alternating with foliage leaves) 20–60 × 20–65 mm, similar to vegetative leaves in morphology and indumentum; pedicels with glochidiate, scabrid, glandular, short-smooth and scattered stinging trichomes. Sepals five, lanceolate, 10–12 × 3–5 mm green, 3-veined, with entire margins, at least twice as long as wide, indumentum of each surface similar to that of the respective leaf surface; petals five, full spreading, cymbiform, 17–25 mm long, white to lightly tinged greenish, darker greenish on the abaxial surface, with glochidiate, scabrid, short-smooth, glandular and scattered, weak stinging trichomes, margins entire to wavy; nectar scales five, 5–7 mm long, white (not contrasting with petal color), unicolored, concave, slightly bulging, with a poorly developed papillae-margined neck and rudimentary apical wings. Filiform dorsal threads, three, 5–7 mm long, all of about the same length, attached subapically to the scale. Staminodes 2 per scale, 15 mm long, sigmoid, distal 2/3rds filiform and glabrous, proximal third abruptly expanded, with a flange towards the scale, margins papillose. Stamens 100–150, filaments 10–15 mm long. Style 10–15 mm long, straight, but twisting after fertilization, ovary inferior, with a densely pubescent roof, with abundant short-smooth trichomes, outer wall with abundant glochidiate, short-smooth, glandular and stinging trichomes, placentae 3–5. Fruit a capsule 10–12 mm diameter, obovoid to subglobose, opening with 3–5 apical valves; seeds ca. 2000–5000 per capsule, 0.5 mm × 0.2–0.5 mm, testa dark brown, foveate-reticulate. Seed testa cells polygonal.

Notes:—Killip (1928) considered this species to be closely related to *Loasa pallida* , but this is not correct (see Acuña *et al.* 2017). The species is very distinctive due to its reniform leaves with a densely glandular indumentum.

Etymology:—The epithet refers to the similarities in leaf shape between this species and *Heuchera* L. (Saxifragaceae).

Illustrations:—Floral diagram: Grau (1997: Ab. 1). Sepal morphology: Weigend (1997: Fig. 40.1).

Distribution:—Endemic to Peru. Restricted to Departamento de Áncash and the northern part of Departamento de Lima from 400 to 1200 m (Fig. 4). Mainly on the western slope of the Cordillera Negra.

Phenology:—In the wild this species is known to flower in April, May, October and November. In cultivation it flowers all year.

Ecology:—*Presliophytum heucheraefolium* grows on cliffs, dry washes, road banks and scree slopes at low to intermediate elevations, sometimes associated with other xeric habitat plants such as cacti and *Tiquilia* Persoon (1805: 157). Thigmonastic stamen movements were studied by Henning & Weigend (2012).

Conservation status:—This species seems to be rare in nature and because of that, relatively poorly collected. It was considered as endangered (EN), B1ab(iii) by Rodríguez and Weigend (2006).

Additional specimens examined:—PERU. **Áncash:** Prov. Santa, 49 Km above Santa in Río Santa Valley, 400 m, 13 May 2003, *Weigend et al. 7653* (BONN, F); Road from Moro to Pamparomas (Caraz), Cordillera Negra, 900 m, 1997, *Weigend & Dostert 97/120* (F, P); Road from Moro to Pamparomas. Arenal de Moro, 615 m, 25 November 2006, *Ackermann & Albán 615* (BONN); Road from Moro to Pamparomas, 10 October 2002, *Weigend et al. 7368* (BONN); Prov. Casma, Road from Casma to Yaután, 580 m, 10 April 2001, *Weigend et al. 5536* (BONN); Prov. Huaraz, Río Grande/Río Cacchan, 1141 m, 16 May 2003, *Weigend et al. 7691* (BONN, F).

3. ***Presliophytum incanum*** (Graham) Weigend (2006: 467) (Figs. 1A, F, G, 3C, D, G)

≡ *Loasa incana* Graham (1830: 169)

Type:—PERU [Lima, Prov. Canta], Valley of Canta, Yazo [*sic*], 1830. *Cruckshanks s.n.* (holotype: E barcode E00085317!; isotype : BM!, K barcode K000372846!).

= *Loasa atriplicifolia* Presl (1832: 61, Tab. 39). Lectotype (designated in Weigend 1998: 168):—Tab. 39 (Presl 1832).

= *Loasa ruiziana* Don (1834: 64). ≡ *Loasa incana* Ruiz & Pavón (1959: 406, Tab. 441, fig. a). Lectotype (designated in Weigend 1998: 168):—tab. 441, fig. a. (Ruiz & Pavón 1959). Epitype (designated in Weigend 1998: 168):—[PERU, Lima, Prov. Huarochirí] anno? "Loasa sp. nova de Huayaquil" *Pavón. s.n.* (BM!). Possible type:—PERU [Lima, Prov. Canta], ex Obrajillo, 1778, *H. Ruiz et al. s.n.* (MA barcode MA813475 [photo!])

Coarse, densely branched perennial shrub 50–150 cm tall. Stem epidermis with abundant glochidiate and scabrid trichomes, scattered stinging and short-smooth trichomes. Taproot present, usually thickened and fleshy. Leaves lobate (lobes more profound in younger plants), less frequently deeply thoothed, opposite below, alternate above, petiole 7–35 mm, with glochidiate, scabrid and scattered stinging trichomes (rarely with short-smooth trichomes), lamina 40–100 × 25–80 mm, ovate with (0–)3–6 lobes on each side, margin crenate or toothed, base cuneate to shallowly cordate, sometimes asymmetric, blade and lobe apices acute, upperside with scabrid and stinging trichomes (the latter sparse and mostly on veins), rarely with short-smooth trichomes, in older leaves the trichome tips may fall, leaving behind basal cells forming scale-like structures, underside densely covered in glochidiate and scabrid trichomes (rarely with stinging trichomes on veins). Inflorescences densely frondose, complex asymmetrical dichasia, to ca. 100 cm long; each flower erect or horizontal in anthesis with two, petiolate or sessile, prophylls (flowers apparently solitary and irregularly alternating with foliage leaves) 15–45 × 2–25 mm, similar to leaves in morphology and indumentum, but sometimes subentire and very narrowly lanceolate (2–3 mm wide); pedicels with glochidiate, scabrid and scattered stinging trichomes**. Sepals five, lanceolate, 5–12 × 3–6 mm green, 3-veined, margins entire, more than 1.5 × long as wide**, indumentum of each surface similar to that of the respective leaf surface; **petals five, half to full spreading**, cymbiform, **11–20 mm long, white (when full spreading) to beige or yellow (when half spreading)**, sometimes slightly tinged greenish on the abaxial surface, with abundant glochidiate, short-smooth and scattered, weak stinging trichomes, margins entire, vertical to slightly involute, rarely with indistinct, narrow (1 mm wide) flat margins; nectar scales five, 4–5 mm long, white, beige or yellow (not contrasting with petal color), unicolored, concave, slightly bulging, with a poorly developed papillose-margined neck and rudimentary apical wings. Filiform dorsal threads, three, 5–7 mm long, of about the same length, attached subapically to the nectar scale. Staminodes 2 per scale, 10–12 mm, distal 2/3rds filiform, glabrous, proximal third abruptly expanded, with a flange towards the nectar scale, margins papillose. Stamens 50–75, filaments 7–10 mm long. Style 7–10 mm, straight, but twisting after fertilization, ovary inferior, with a densely pubescent roof covered mostly in scabrid trichomes, sometimes with few short-smooth trichomes, outer wall with abundant glochidiate, scabrid and stinging trichomes, placentae 3–5. Fruit a capsule 5–10 mm diameter, subglobose or broadly conical, opening with 3–5 apical valves; seeds ca. 1200–2500 per capsule, 0.5 mm × 0.2–0.5 mm, testa dark brown, foveate-reticulate. Seed testa cells polygonal.

Notes:—Ruiz, Pavón and Dombey were in Obrajillo in 1778 (Lang 1985). Therefore, it seems likely that *H. Ruiz et al. s.n.* (MA barcode MA813475) was collected at that time and possibly is a type of *Loasa incana* Ruiz & Pavón (not *Loasa incana* Graham). This species shows considerable plasticity in leaf morphology. As greenhouse plants age, they produce progressively smaller leaves. The difference in size after two years is quite noticeable. Habitat also seems to account for considerable variation: plants growing in dry river beds and cliffs often have diminutive leaves while plants growing in dry forest edge or scrub have broader leaves. There is morphological variation related to geography (Figs. 1F, G), but this is greatly obscured by the changes resulting of age and habitat conditions. More consistent differences possibly related to geographic origin are evident in floral morphology as most of the the populations from Arequipa and Moquegua have yellow, half spreading corollas (Fig. 3D) that are different from the white, full spreading corollas more common in plants further north (Fig. 3C). The type material studied and cultivated by Graham (1830) had white, spreading corollas. It must be noticed, however, that some plants in Arequipa and Moquegua have been reported as having white flowers, at the same time yellow flowered individuals have been collected as far north as Lima (Fig. 4). In preserved specimens, the color of the corolla turns yellowish, independently of the origin of the plants, and, in the absence of field notes, it is unclear whether white and yellow flowered populations overlap with each other, and how extensive is this overlap, if any. Plants with white flowers tend to have stems predominantly with scabrid trichomes while yellow flowered plants have stems mostly with glochidiate trichomes, but this difference appears to be minor and not always consistent. Preliminary genetic evidence coming from plastid markers suggests that the yellow-flowered populations from the south could be more closely related to *P. arequipense* than to the northern white-flowered populations. But more robust evidence is needed before making a taxonomic decision.

Etymology:—The epithet refers to the hoary appearance of the plants due to their abundant scabrid and glochidiate trichomes.

Illustrations:—Habit and morphology: Presl (1832: Tab. XXXIX), Urban & Gilg (1900: Tab. VII. Fig. 1–10), Ruiz & Pavón (1959: Icon. CDXLI.a). Leaf: Weigend (1997: Fig. 21.8). Inflorescence architecture: Urban (1892a: Taf. XII.6), Weigend (1997: Fig. 29.2), Weigend *et al.* (2004b: Fig. 6C). Floral diagram: Urban (1892b: Taf. XIV.21), Gilg (1894: Fig. 37N), Grau (1997, Ab. 3). Sepal morphology: Weigend (1997: Fig. 40.2). Petal ontogeny: Weigend (1997: Fig. 43.13–14). Nectar scale ontogeny: Weigend (1997: Fig. 53.2 , mislabeled as "*Loasa grandis*"). Fruit: Weigend (1997: Fig. 56.20).

Distribution:—Endemic to Peru. Known from Departmento de Piura to Departmento de Moquegua, from sea level to over 3000 m elevation. This species is very widely distributed on the Pacific slope of Peru, but is largely replaced by *P. heucheraefolium* in coastal Departamento de Áncash (Fig. 4).

Phenology:—This species is known to flower all year round.

Ecology:—This is one of the most widespread taxa of Loasoideae, with a very broad ecological tolerance in dry habitats. Like other species of the genus, it grows on cliffs, in dry washes, on road banks and scree slopes at low to intermediate elevations, sometimes associated with cacti and other dry scrub plants. It can also be found in Andean scrub, coastal lomas and disturbed habitats.

Conservation status:—This species fares well in human disturbed habitats and is one of the most abundant species of Loasaceae in Peru. Due to its abundance and resilience to human disturbance it is considered a LC species (Rodríguez & Weigend 2006).

Additional specimens examined:—PERU. **Dept. Unknown:** San Mateo in the Quebrada, s.a., *coll. Unknown 696* (K: K000372847); s.a., *Martinet s.n.* (P: P04589533); s.a., *Martinet 191* (P: P04589534); San Bartolomé, July 1874, *Martinet 168* (P: P04589536, P04589537); s.a., *Neé s.n.* (F: n.842915); **Piura:** Prov. Unknown, Pariñas Valley, 35 miles east of Cape Pariñas, 4 September 1927, *Haught 186* (F); Prov. Talara, Pariñas valley, 20 miles inland, s.a., *Haught F-111* (F); **Cajamarca:** Prov. Santa Cruz, 35 Km from Santa Cruz on road to Catache, 1 Km after Catache, 4 May 2003, *Weigend et al. 7546* (BONN); Prov. San Miguel, Entre Quindén y Platanal (carretera hacia el Pueblo de Unión Agua Blanca), 800 m, 6 October 2001, *Rodríguez et al. 2423* (F); Prov. Contumazá, Alrededores de Tembladera, 900 m, 23 May 1976, *Sagástegui et al.* 8529 (F); Road from Pacasmayo to Cajamarca, ca. 20 Km from Pacasmayo on rocky roadside, 500 m, 1998, *Dostert 98/165* (F); Road from Chilete to Pacasmayo, 900 m, 1997, *Weigend et al. 97/457* (F); Ca. 30 Km from Chilete on road to Contumazá, 9 May 2003, *Weigend et al. 7585* (BONN); Al N. de Contumazá sobre el camino que conduce a Chilete, y que se desvía de la carretera Contumazá–Chilete, bajando Hoyada Verde, 1600 m, 3 July 1983, *Sánchez et al. 3195* (F); Chilete-Contumazá road, about midway between the villages; 11 April 2003; *Hufford et al. 4018* (F); Alrededores de San Benito, 1300 m, 3 February 1985, *Sagástegui et al. 12461* (BONN, F); El Portachuelo (Ascope - El Algarrobal), 780 m, 20 April 1984, *Sagástegui 11387* (BONN); **La Libertad:** Prov. Gran Chimú, Cascas–Contumazá road, 1.1 Km north of square in Cascas, 1350 m, 10 April 2003, *Hufford et al. 4013* (F); Prov. Ascope–Prov. Trujillo, Cerro Cabezón, 800 m, 3 July 1985, *Mostacero et al. 767* (F); ditto, 250 m, 4 November 1983, *Sagástegui & López 10988* (F); ditto, 500 m, 8 May 1999, *Sagástegui et al. 16142* (BONN, F); Prov. Otuzco, Ruta Simbal–La Cuesta, 1280 m, 2 September 1973, *López & Sagástegui 8008* (F); Prov. Trujillo, Pedregal, 800 m, 1 May 1994, *Sagástegui 15289* (F); Pedregal a Shirán, 300 m, 4 February 1974, *Lourteig & López 2994* (P); Road Trujillo–Otuzco near Shiran, 600 m, 1997, *Weigend et al. 97/198* (F); Alrededores de Shirán, 550 m, 10 June 1993, *Leiva 772* (F); Trujillo–Otuzco road, 31.5 Km east of the PanAmerican Highway in Trujillo and 6.2 Km west of Puente Shirán, 900 m, 15 April 2003, *Hufford et al. 4025* (F); Prov. Virú, Lomas de Virú. Cerro de las Lomas, 350 m, 12 October 2000, *Weigend et al. 2000/695* (BONN, F); **Áncash:** Prov. Corongo, Road from Huallanca to Yanac, near Yanac, 2800 m, 7 March 2001, *Weigend et al. 5013* (BONN); Road Sihuas to Corongo/Mirador (on Río Santa), 3065 m, 26 April 2004, *Weigend & Schwarzer 8039* (BONN, F); Prov. Huaylas, 133 Km from Santa on road to Caraz, 2

Km after Huallanca, 13 May 2003, *Weigend et al. 7655* (BONN, F); Road from Caraz to Huaylas, ca. 2 Km after the turnoff to Huaylas from the Carretera Central, 2614 m, 28 November 2014, *Henning et al. 9719* (BONN); Cordillera Negra, 20.5 Km from Caraz on road to Huaylas, 2278 m, 29 April 2004, *Weigend & Schwarzer 8048* (BONN, F); Surrounding of Pamparomas to Tuteycon, 1950 m, 25 November 2006, *Ackermann & Albán 616* (BONN); Serpentine outside fields Shauintioc and Tuteycon, 15 May 2003, *Weigend et al. 7688* (BONN); Road from Pamparomas to Moro, branch to Ullpan, 2120 m, 14 October 2007, *Weigend & Hilger 8912* (BONN); Prov. Santa, Road from Moro to Pamparomas (Caraz), Cordillera Negra, lower desertic regions, 1400 m, 1997, *Weigend & Dostert 97/121* (F); Road from Moro to Pamparomas, 10 October 2002, *Weigend et al. 7367* (BONN, F); **Lima:** Prov. Unknown, s.a., *Dombey s.n.* (P: P04588969); Road to Puruchuco, s.a., *McLean s.n.* (K: K000372845); Prov. Huaura, Ámbar-Huaura, Laderas de Cerros, 1200 m, 6 August 2003, *coll. Unknown 3267* (BONN); Prov. Canta, Ex Obrajillo, s.a., *Ruiz et al. s.n.* (MA: MA813475); Canta por abajo, 2300 m, 2 April 1953, *Petersen & Ginting 1194* (L); Quives, open rocky slope, 1300 m, 9 June 1925, *Pennell 14309* (F); Prov. Lima, Canta Valley, 7 km NE of Trapiche. On sandy sides of dry wash; 800 m; 4 August 1957, *Hutchinson 1012* (F); Road from Trapiche to Quilca at ± 6 Km from Trapiche. Dry stream bed in *Tillandsia* desert. Granite sand, no cacti, 750 m, 02 January 1971, *Hawkes et al. 4103* (L); Chosica, 800 m, June 1949, *Soukup* 3796 (F); Prov. Huarochirí, Near Huínco, above Chosica, 1900 m, 3 September 2004, *Richter s.n.* (BONN); Carretera Central, just west of Matucana, 2300 m, 1997, *Weigend & Dostert 97/12* (F); Matucana. Steep rocky canyon slope; soil loose, 2500 m, 19 April 1922, *Macbride & Featherstone 257* (F); Prov, Yauyos: Road from Yauyos to Jauja, few Km after Magdalena, 2300 m, 7 October 2002, *Weigend et al. 7233* (BONN); Road from Huancayo to San Vicente de Cañete. 193 Km from Huancayo, 872 m, 22 September 2001, *Weigend & Skrabal 5888* (BONN, F); **Ica:** Prov. Unknown, s.a., *Martinet 47* (P); Prov. Pisco, 1 Km before Puente Huaytará (Km 73 road Pisco-Ayacucho), 1450 m, 29 September 1997, *Weigend & Förther 97/585* (F); Prov. Nazca, Sol de Oro, 840 m, 2 January 2007, *Huamantupa 8432* (BONN); Km 17 on Road Nazca–Puquio, 1140 m, 2 October 1997, *Weigend & Förther 97/642* (F); **Arequipa:** Prov. Caravelí, Quebrada Ático, 50 m, 14 February 1998, *Cátedra Ecología, s.n. (FLSP 1343)* (P: P04574610); Prov. Condesuyos, Road from Aplao (Castilla) to Chuquibamba, S of bridge over Río Arma, Quebrada Huario, ca. 24.5–26 Km from Chuquibamba, 1550 m, 24 July 2010, *Weigend et al. 9374* (BONN); Prov. Camaná, Km 934 Panamericana Sur between Camaná and Tambillo, 1500 m, 5 October 1997, *Weigend & Förther 97/760* (F); **Moquegua:** Prov. General Sánchez Cerro, Omate. Laderas de cerros y bordes de camino, 2400 m, 15 September 2004, *coll. Unknown 3239* (BONN); Anexo de 'Laje'–San Francisco, 2270 m, 8 April 2003, *Cáceres et al. 3014* (BONN); Road from Omate to San Francisco above Omate. Hillsides of Urimalle, 1840 m, 8 December 2006, *Ackermann & Cáceres 674* (BONN, F); From Moquegua to Omate, 2798 m, 14 April 2004, *Weigend & Schwarzer* 7869 (BONN); Prov. Mariscal Nieto, Road Moquegua to Omate, 74 Km from Moquegua, 1 Km before puente over Río Tambo, 14 April 2004, *Weigend & Schwarzer 7862* (BONN, F); Off Moquegua–Torata road, just above Torata, 14 September 2001, *Hufford & McMahon 3835* (F); 20 Km E of Moquegua on road to Torata, 2100 m, 13 October 1997, *Weigend & Förther 97/850* (F); 14 Km E of Moquegua on road to Torata, 1855 m, 13 October 1997, *Weigend & Förther 97/848* (F); Km 1126 Panamericana Sur. 14 Km N of Moquegua turnoff, 1200 m, 14 October 1997, *Weigend & Förther 97/855* (F); Río seco o aluvión, 1200 m, 9 April 1959, *Vargas 12640* (BONN); Cerca a Moquegua. Cauce seco, 800 m, 27 October 1966, *Vargas 17970* (BONN).

4. ***Presliophytum malesherbioides*** (Phil.) R.H. Acuña & Weigend in Acuña *et al.* (2017: 373) (Figs. 1C, 3E, I, K)

≡ *Loasa malesherbioides* Philippi (1864:74)

Type:—[CHILE, Coquimbo, Prov. Elqui] Baños del Toro, 1860/61, [*H.*] *Volckmann s.n.* (SGO?, B [†, photo F Neg. No.10208!]).

= *Loasa longiseta* Philippi (1865: 347). Lectotype (designated in Acuña *et al.*, 2017: 373):—[CHILE, Atacama, Prov. Copiapó] Quebrada de Puquios, Des. Atacama, 1865, *F. Geisse s.n.* (SGO barcode SGO000003405 [photo!]; isolectotype: SGO barcode SGO000003404 [photo!]).

Densely branched annual to subperennial herb to ca. 30 cm tall, less frequently ephemeral, sparsely branched herbs 5–15 cm tall. Stem base sometimes with scarce secondary xylem and large parenchymatous pith, epidermis with abundant glochidiate, scabrid and stinging trichomes, younger portions with glandular trichomes (rarely absent). **Root system poorly branched, taproot long (sometimes as long as the shoot is tall) and thin**. **Leaves subentire to irregularly lobate or deeply thoothed**, usually only the lowermost pairs opposite, alternate above, petiole 3–45 mm, with glochidiate, scabrid, glandular and stinging trichomes, lamina 12–65 × 5–47 mm, ovate with 0–8 lobes/teeth on each side, margin weakly sinuous to deeply toothed, base cuneate, blade and lobe apices obtuse to acute, adaxial surface with scabrid, glochidiate and stinging trichomes (mostly on veins and leaf margins) the amount of stinging trichomes variable, glandular trichomes, when present, restricted to the lamina base, abaxial lamina with abundant glochidiate trichomes, sometimes sparse scabrid and stinging trichomes, glandular trichomes, if present, restricted to the base. Inflorescences frondose dichasia, to 15 cm long (in small plants shorter, with only 1–5 flowers), trichome cover similar to the stem, but glandular trichomes sometimes more abundant; each flower erect or horizontal in anthesis with two, shortly petiolate to subsessile, prophylls, 2 per flower (flowers apparently irregularly alternating with the prophylls), 3–25 × 1–15 mm, similar to vegetative leaves in morphology and indumentum, but diminishing in size towards the terminal part of the inflorescence (sometimes very reduced in size); pedicels 5–20 mm long with glochidiate, scabrid, glandular and stinging trichomes, lengthening considerably (up to 40 mm) after anthesis. **Sepals five, narrowly lanceolate to linear, 2–6 × 0.5–1 mm, more than 3 × as long as wide**, green, only the central vein conspicuous, margins entire, indument of each surface similar to that of the respective leaf surface; petals five, full spreading, cymbiform, 4–7 mm long, white, with abundant glochidiate, scabrid and scattered stinging trichomes on the outer surface, margins entire, often meeting over the midline of the petal; nectar scales five, 2–3 mm long, **entirely yellow to mostly white with green or yellow markings (contrasting with petal color)**, concave, bulging towards the base (specially southern plants: with small round nectar sacs), with a median keel below the neck and apical wings, dorsal threads, three, claviform 0.5–1 mm long, of similar lengths, the tips reaching the scale apex or well beyond it (longer threads in northern plants), attached to the upper part of the scale, below the neck. Staminodes 2 per scale, 5–6 mm, distal 3/4ths filiform, papillose, tips slightly expanded, proximal fourth with long papillae, abruptly expanded, with a flange towards the scale. Stamens ca. 30–60, filaments 2–4 mm long. Style 3–4 mm long, straight, but twisting after fertilization, ovary inferior, with a pubescent roof covered in scabrid and glochidiate trichomes, outer wall with abundant, large glochidiate, and stinging trichomes, placentae 3. **Fruit a cylindrical to slightly ovoid capsule, 5–15(–28) × 3–6(–10) mm, usually >2 × long as wide**, opening with 3–5 apical valves; seeds ca. 14–30(–60) per capsule, 2–3 × 1.0–1.5 mm, testa dark brown, with 6–9 very evident transversal constrictions Seed testa cells tetragonal, periclinal walls with undulate sculpturing.

Notes:—There is considerable diversity in the morphology of this species. As it happens with other short lived taxa in Loasaceae, such as the species of the *Nasa triphylla* group (Dostert & Weigend 1999), the generation time and founder effect may be responsible of the significant morphological variation in this species. The "setae" (stinging trichome) cover in leaves and petioles used by Philippi (1865) to differentiate *L. longiseta* from *L. malesherbioides* show considerable variation within and between populations. Plants in the north (formerly called *longiseta*), tend to have higher density of foliar stinging trichomes, but most plants, independently of their locality, have at least some of these. The floral scale morphology and color vary geographically: plants from the southern part of the range have mostly yellow scales with well-definded, round nectar sacs and dorsal threads that extend to or barely beyond the nectar scale neck. Plants in the northern part of the range, have mostly white scales with green or yellow markings, no distinct nectar sacs, and dorsal threads that extend well beyond the scale neck. The plants from Chollay-Conay and neighboring areas in Provincia de Huasco, Región de Atacama, seem to be intermediate between northern and southern populations, with mostly cream colored scales having yellow-green markings, dorsal threads extending slightly above the scale neck and poorly developed nectar sacs. The specimen *S. Teillier & P. Barahona 6285* (CONC) differs in several regards from the other studied specimens of the species in that the leaves have deep clefts (ca 25% of leaf width), the nectar scales are white with inconspicuous pale green markings near the base, with dorsal threads attaching directly to the lower rim of the neck, and the mature fruits are larger (18–28 × 5–10 mm) and with more seeds (ca. 60) than usual for the species. However, considering the variability of *P. malesherbioides,* we have decided to include these plants in our concept of the species. The dwarf, sexually mature, basically unbranched specimens <10 cm tall are similar in habit to dwarf specimens of *Nasa chenopodiifolia* (Desr.) Weigend in Weigend *et al.* (2006: 73), *N. urens* (Jacq.) Weigend in Weigend *et al.* (2006: 83) and *Aosa rostrata* (Urb.) Weigend (2006: 464) as described by Urban & Gilg (1900) and Henning & Weigend (2009).

Etymology:—The epithet refers to the the similarity of this plant to some species of *Malesherbia* Ruiz & Pavón (1794: 45).

Illustrations:—Habit and morphology: Hoffmann *et al.* (1998: p. 73.1, p. 75.4), Pérez-Moreau & Crespo (2003: Fig. 156). Floral diagram: Grau (1997: Ab. 3). Nectar scale morphology and variability: Urban & Gilg (1900: Tab. VI. Fig. 7–10).

Distribution:—*Presliophytum malesherbioides* is known from Región de Atacama and Región de Coquimbo in Chile as well as from Provincia de San Juan, Argentina (Pérez-Moreau & Crespo 2003). It is found between 1200 and 3600 m, and more than 80 km inland from the Pacific Ocean (Fig. 4).

Phenology:—Known to flower between October and March.

Ecology:—This is the only annual species of the genus. It inhabits very dry areas and it is often one of the few species of vascular plants growing in such localities, where it can be abundant.

Conservation status:—*Presliophytum malesherbioides* is considered either LC or DD by Marticorena *et al.* (2001) and Squeo *et al.* (2008). This species can be frequent in remote areas.

Additional specimens examined:—CHILE. **Atacama**: Prov. Unknown, *coll. unknown* (E: E00158252); February 1888, *Philippi s.n.* (K: K000372859); Prov. Chañaral, Cuesta Pedernales, 3215 m, 28 February 2007, *Letelier & Squeo 1224* (CONC); Prov. Copiapó, Camino al Salar de Maricunga, Km 62, 2250 m, 31 January 1963, *Ricardi et al. 557* (CONC); Quebrada de Paipote. Extremo superior Vegas La Junta, en terreno pedregoso, 2940 m, 06 January 1973, *Marticorena et al. 522* (CONC); Quebrada del Peñón, 3600 m, 9 March 1996, *Brownless et al. 572* (E); Camino a la Quebrada de las Vizcachas, a 37 Km de La Puerta, 2900 m, 1 February 1963, *Ricardi et al. 631* (CONC); Portillo de Acerillos, en la bajada, 3500 m, 30 January 1949, *Krapovickas & Hunziker 5722* (BAB); Río Turbio-vegas, 3170 m, 16 February 2009, *Teillier & Barahona 6285* (CONC); Valle del Río Jorquera, 12 January 1970, *Zöllner 4055* (CONC); Im Jorqueratal an einem sandigen Hang, 1200 m, 12 January 1970, *Zöllner 4259* (L); Estancia Manflas en las faldas de los cerros, entre piedras sueltas, 25 October 1965, *Ricardi et al. 1480* (CONC); Prov. Huasco: Río Laguna Grande, entre Potrero de Toledo y Quebrada Candelilla, 2400–2800 m, 13 February 1981, *Kalin-Arroyo 81545* (CONC); Río Laguna Grande, entre Las Papas y Potrero de Toledo, 2000–2400 m, 19 January 1983, *Marticorena et al. 83342* (CONC); Cajón del Río Conay, 3 Km al interior de Conay, en taludes, 1450 m, 13 October 1983, *Marticorena 9557* (CONC); **Coquimbo:** Prov. Elqui: Baños del Toro, *coll. unknown* (K: K 000372858); Vegas de los Baños del Toro, s.a., *Philippi s.n.* (K: K000372857); Canchas de Sky, 3400 m, 24 February 1988, *F. Squeo 88157* (CONC); Río La Laguna 3.6 Km de central, 2174 m, 19 December 2006, *Rosas 4371* (CONC); Camino Internacional a San Juan entre Juntas y Embalse La Laguna, Km 4, 2100 m, 06 January 1967, *Ricardi et al. 1720* (BAB, CONC); Camino entre Juntas y Embalse La Laguna, 2300–2900 m, 09 January 1981, *Kalin-Arroyo 81149* (CONC); Camino al Embalse de La Laguna a 20 Km de La Junta, 2700 m, 05 February 1963, *Ricardi et al. 716* (CONC); Camino entre Embalse La Laguna y Campamento del Embalse, 2900–3200 m, 08 January 1981, *Kalin-Arroyo 81105* (CONC); Embalse La Laguna, 3050 m, 11 January 1966, *Peña s.n.* (CONC).**—ARGENTINA**. **San Juan:** Dept. Calingasta, Río Melchor, 2800 m, 2 February 1991, *Kiesling et al. 7814* (SI); Río Melchor a Co. Guanaqueros, 2700 m, 6 February 1991, *Kiesling et al. 7823* (SI); Río Manantiales al NW de Calingasta, 3200 m, 15 February 1990, *Kiesling et al. 7489* (BAB, SI).

5. *Presliophytum sessiliflorum* (Phil.) R.H. Acuña & Weigend in Acuña *et al.* (2017: 373) (Figs. 1B, 3F, L)

≡ *Loasa sessiliflora* Philippi (1893:12)

Type:—[CHILE, Antofagasta, Prov. Antofagasta] Sierra Esmeralda, Des Atacama, 20 October 1883, [*F.*] *S*[*an*] *Roman* s.n. (holotype: SGO barcode SGO000003420 [photo!]).

Densely branched subshrub to ca. 50 cm tall. Stem epidermis green, with abundant glochidiate, scabrid and stinging trichomes, glandular trichomes in younger parts. Taproot perennial, fleshy and thick. Leaves opposite below, alternate above, petioles to 20 mm long in basal leaves, similar to the stem in trichome cover, but with more glandular trichomes especially near the leaf blade, **terminal leaves mostly (sub)sessile, lamina 20–60 × 7–35 mm, ovate with 3–8 triangular lobes on each side,** margin toothed, base truncate, sometimes slightly asymmetrical, blade and lobe apices acute, **adaxially with numerous scabrid, short smooth, glandular and stinging trichomes**, rarely with few glochidiate trichomes, abaxially with abundant glochidiate trichomes, stinging trichomes restricted to the larger veins. Inflorescences frondose dichasia, to 10 cm long, the trichome cover similar to the stem, but with more glandular trichomes; flowers horizontal to deflexed in anthesis, with two, sessile prophylls per flower (flowers apparently irregularly alternating with the prophylls), 3–17 × 2–10 mm, diminishing in size towards the terminal portions of the inflorescence, similar to vegetative leaves in trichome cover but usually only slightly serrate to dentate with 3–4 teeth per side, **flowers subsessile or with pedicels, shorter than the petals, to 4(–6) mm,** with glochidiate, glandular and stinging trichomes. **Sepals five, broadly lanceolate to ovate, 2.5–5 × 2.5–4 mm, <1.25 × as long as wide, green, 3-veined, margins entire,** indumentum of each surface similar to that of the respective leaf surface but with less glandular trichomes; petals five, full spreading, cymbiform, 7–14 mm long, white or very light greyish, tinged greenish on the abaxial surface with abundant scabrid, glochidiate, glandular and rarely weak stinging trichomes adaxially, margins slightly undulate; nectar scales five, 3–4 mm long, white (not contrasting with petal color), concave, slightly bulging, **apex weakly bilobate**, neck with a weak rim, rudimentary apical wings . **Dorsal threads, three, filiform, of about the same length, to ca. 3 mm long, attached to the tip of the scale**. Staminodes 2 per scale, to ca. 10 mm long, S-shaped, the distal 2/3rds filiform and glabrous, the proximal third abruptly thickened, flattened and papillose. Stamens 30–50, filaments 5–10 mm long. Style 10 mm long, straight, but twisting after fertilization, persistent in fruit, the basal portion with scabrid trichomes; ovary inferior, with a densely pubescent roof with scabrid trichomes, outer wall with abundant scabrid and stinging trichomes, **placentae 2. Fruit a 2 locule capsule 5–6 × 3 mm (not counting**

the persistent sepals), obovoid, apprently indehiscent; seeds (3–)4(–6), two per locule, 3–4 mm × 1 mm, narrowly ovoid, testa tan brown, darker towards hilar end, with 12–18 transversal constrictions. Seed testa cells tetragonal, periclinal walls with undulate sculpturing.

Notes:—The name *Loasa longiseta* has been often misapplied to this species (Weigend *et al.* 2008). Urban & Gilg (1900) knew about this species and considered it possibly related to *Loasa longiseta*, but they were unable to examine the only specimen known at the time (the holotype). Its taxonomic status has been clarified only recently (Acuña *et al.* 2017). The dimerous gynoecia and two-seeded locules observed in this species are rare in the tribe Loaseae.

Etymology:—The epithet refers to the short-pedicellate to subsessile flowers of this species.

Illustrations:—Inflorescence architecture: Weigend *et al.* (2004b: Fig.6A incorrectly called "*Loasa longiseta*"). Floral diagram: Grau (1997: Ab. 3).

Distribution:—Endemic to Chile. *Presliophytum sessiliflorum* grows only in scattered localities in coastal mountain ranges of the Región de Antofagasta and possibly northernmost Región de Atacama, Chile, at elevations under 1000 masl, and less than 20 km inland from the Pacific Ocean (Fig. 4).

Phenology:—Known to flower between October and April.

Ecology:—This plant seems to be restricted to habitats with oceanic influence. The capsules are apparently indehiscent and thus the fruit and the seeds are expected to be dispersed as a single unit.

Conservation status:—It has been reported by Johnston (1929) and by Jiles, in a specimen label, that this species could be common locally [*Jiles 5343* (CONC)], and may occur in disturbed habitats, e.g., La Chimba NE of Antofagasta [(*Luebert et al. 3405* (BONN)]. Marticorena *et al.* (1998) recorded this species for Región de Antofagasta, but its conservation status was not analyzed. Due to our rudimentary knowledge of its abundance and distribution we consider *Presliophytum sessiliflorum* as DD.

Additional specimens examined:—CHILE. **Antofagasta**: Prov. Tocopilla, La Carmelita, 750 m, 8 November 1969; *Jiles 5343* (CONC); Prov. Antofagasta: Quebrada La Chimba, 340 m*,* 18 October 2016, *Luebert et al. 3405* (BONN); bare rocky canyon, 200 m, 3 April 1925, *Pennell 13026* (F); Quebrada de Botija, al norte de Paposo, 400 m, 29 November 1988, *Hoffmann 187* (CONC); Sierra Esmeralda, along trail between Posada de los Hidalgos and Quebrada Cachina via Portezuelo de Mina Carola. 1.5 Km N, 14 December 1925, *Johnston 5674* (F).

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References

Acuña, R., Fließwasser, S., Ackermann, M., Henning, T., Luebert, F. & Weigend, M. (2017) Generic re-arrangements in "South Andean Loasas" (Loasaceae). *Taxon* 66: 365–378.
https://doi.org/10.12705/662.5

Brownrigg, R. (2017) *maps: Draw Geographical Maps*. R package version 3.2.0 Available from: https://cran.r-project.org/web/packages/maps/index.html (accessed 10 June 2017)

Carlquist, S. (1984) Wood anatomy of Loasaceae with relation to systematics, habit, and ecology. *Aliso* 10: 583–602.
https://doi.org/10.5642/aliso.19841004.07

Don, G. (1834) *A general history of the Dichlamydeous plants Vol 3: Calyciflorae.* Gilbert & Rivington, London, 867 pp.

Dostert, N. & Weigend, M. (1999) A synopsis of the *Nasa triphylla* complex (Loasaceae), including some new species and subspecies. *Harvard Papers in Botany* 4: 439–467.

Endlicher, S. (1842) *Generum Plantarum. Suppl. Secundum*. Fridericum Beck, Vienna, 114 pp.

Ensikat, H.J. & Weigend, M. (2013) Cryo-scanning electron microscopy of plant samples without metal coating, utilizing bulk conductivity. *Microscopy and Analysis* 27: 7–10.

Google Inc. (2016) *Google Earth Pro ver. 7.1.7.2606*. Mountain View, Santa Clara.

Gay, C. (1847) *Historia física y política de Chile. Botánica*. Tomo Segundo. Fain & Thunot Paris, 534 pp.

Graham, R. (1830) Description of several new or rare plants which have lately flowered in the neighbourhood of Edinburgh, and chiefly in the Royal Botanical Garden. *The Edinburgh New Philosophical Journal* 10: 166–172.

Grau, J. (1988) Chromosomenzahlen chilenischer Loasaceae. *Mitteilungen der Botanischen Staatssammlung München* 27: 7–14.

Grau, J. (1997) *Huidobria*, eine isolierte Gattung der Loasaceae aus Chile. *Sendtnera* 4: 77–93.

Gilg, E. (1894) Loasaceae. *In*: Engler, A. & Prantl, K. (Eds.) *Die natürlichen Pflanzenfamilien III: Teil. 6. Abteilung a.* Wilhelm Engelmann, Leipzig, pp. 100–121.

Henning, T. & Weigend, M. (2009) Systematics of the *Nasa poissoniana* group (Loasaceae) from Andean South America. *Botanical Journal of the Linnean Society* 161: 278–301. https://doi.org/10.1111/j.1095-8339.2009.01006.x

Henning, T. & Weigend, M. (2012) Total Control – Pollen presentation and floral longevity in Loasaceae (blazing star family) are modulated by light, temperature and pollinator visitation rates. *PLoS ONE* 7: e41121. https://doi.org/10.1371/journal.pone.0041121

Hoffmann, A., Kalin-Arroyo, M., Liberona, F., Muñoz, M. & Watson, J. (1998) *Plantas altoandinas en la flora silvestre de Chile*. Imprenta Salesianos S.A., Santiago, 281 pp.

Hufford, L. (2003) Homology and developmental transformation: models for the origins of the staminodes of Loasaceae subfamily Loasoideae. *International Journal of Plant Sciences* 164: S409–S439. https://doi.org/10.1086/376873

Hufford, L., McMahon, M.M., O'Quinn, R. & Poston, M.E. (2005) A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. *International Journal of Plant Sciences* 166: 289–300. https://doi.org/10.1086/427477

IUCN. (2001) *IUCN Red List Categories and Criteria. Version 3.1.* Prepared for the IUCN Species Survival Commission. Gland and Cambridge, ii+33 pp.

Johnston, I.T. (1929) Papers on the flora of northern Chile. *Contributions from the Gray Herbarium of Harvard University* 85: 1–172.

Killip, E. (1928) New South American Loasaceae. *Journal of the Washington Academy of Sciences* 18: 89–95.

Lang, C. (1985) Joseph Dombey et l'expédition de Ruiz et Pavon: étude des itinéraires (1778–1784). *Bulletin de la Société Botanique de France. Lettres Botaniques* 132: 259–274.

Marticorena, C., Matthei, O., Rodríguez, R., Kalin-Arroyo, M., Muñoz, M., Squeo, F. & Arancio, G. (1998) Catálogo de la flora vascular de la Segunda Región (Región de Antofagasta), Chile. *Gayana Botánica* 55: 23–83.

Marticorena, C., Squeo, F., Arancio, G. & Muñoz, M. (1998) Catálogo de la flora vascular de la IV Región de Coquimbo. *In*: Squeo, F., Arancio, G. & Gutiérrez, J.R. (Eds.) *Libro rojo de la flora nativa y de los sitios prioritarios para su conservación: Región de Coquimbo.* Ediciones Universidad de La Serena, La Serena, pp. 105–142.

Molinari, E. (2015) Some nomenclatural changes regarding Peruvian endemics. *Polish Botanical Journal* 60: 67–69. https://doi.org/10.1515/pbj-2015-0015

Moody, M.L., Hufford, L., Soltis, D.E. & Soltis, P.S. (2001) Phylogenetic relationships of Loasaceae subfamily Gronovioideae inferred from matK and ITS sequence data. *American Journal of Botany* 88: 326–336. https://doi.org/10.2307/2657022

Pérez-Moreau, R.L. & Crespo, S. 2003. Loasaceae. *In*: Kiesling, R. (Ed*.) Flora de San Juan Vol. II.* Estudios Sigma, Buenos Aires, pp. 152–160.

Persoon, C.H. (1805) *Synopsis Plantarum: seu Enchiridium botanicum, complectens enumerationem systematicam specierum hucusque cognitarum. Vol 1.* Cramerum & Cottam, Paris & Tübingen, 558 pp. https://doi.org/10.5962/bhl.title.638

Philippi, R.A. (1855) Botanica: observaciones sobre la *Huidobria fruticosa*, especie de planta de la familia de las loáseas, por don R. Philippi, leida en la sesion de 23 de mayo. *Anales de la Universidad de Chile* 13: 217–219.

Philippi, R.A. (1864) Plantarum Novarum Chilensium. *Linnaea* 33: 1–308.

Philippi, R.A. (1865) Descripción de algunas plantas de la provincia de Atacama. *Anales de la Universidad de Chile* 27: 339–351.

Philippi, R.A. (1893) Plantas nuevas chilenas de las familias rosáceas, onagráriaceas i demás familias del tomo II de Gay. *Memorias Científicas i Literarias Anales de la Universidad de Chile* 85: 5–18.

Presl, C.B. (1832) *Symbolae Botanicae. Vol 1*. J. Spurny, Prague,76 pp.

R Core Team. (2014) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.

Rodríguez, E. & Weigend, M. (2006) Loasaceae endémicas del Perú. *Revista Peruana de Biología* 13: 391s–402s http://doi.org/10.15381/rpb.v13i2.1872

Ruiz, H. & Pavón, J. (1794) *Florae Peruvianae, et Chilensis Prodromus, sive novorum generum plantrum peruvianum, et chilensium descriptiones et icones.*Imprenta de Sancha, Madrid, 154 pp + XXXVII Tab.

Ruiz, H. & Pavón, J. (1959) Flora Peruviana et Chilensis. V. *Anales del Jardín Botánico AJ Cavanilles* 16: 353–462.

Squeo, F., Kalin-Arroyo, M., Marticorena, A., Arancio, G., Muñoz, M., Negritto, M., Rojas, G., Rosas, M., Rodríguez, R., Humaña, A., Barrera, E. & Marticorena, C. (1998) Catálogo de la flora vascular de la Región de Atacama. *In*: Squeo, F., Arancio, G. & Gutiérrez, J.R. (Eds.) *Libro rojo de la flora nativa y de los sitios prioritarios para su conservación: Región de Atacama.* Ediciones Universidad de La Serena, La Serena, pp. 97–120.

Urban, I & Gilg. E. (1900) *Monographia Loasacearum*. Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curosiorum. Tomus LXXVI. Erhardt Karras, Halle, 384 pp. + VIII Tab.

Urban, I. (1892a) Die Blüthenstände der Loasaceen. *Berichte der Deutschen Botanischen Gesellschaft* 10: 220–225, Taf. XII.

Urban, I. (1892b) Blüthen- und Fruchtbau der Loasaceen. *Berichte der Deutschen Botanischen Gesellschaft* 10: 259–265, Taf. XIV.

Weigend, M. (1997) Nasa *and the conquest of South America: Systematic rearrangements in Loasaceae Juss*. PhD. Dissertation. Lüdwig-Maximilians-Universität, Munich, 271 pp.

Weigend, M. (1998) *Nasa* y *Presliophytum*: Los nombres y sus tipos en los nuevos géneros segregados de *Loasa* Juss. *senso* Urban & Gilg en el Perú. *Arnaldoa* 5: 159–170.

Weigend, M. (2004) Loasaceae. *In*: Kubitzki, K. (Ed.) *The Families and Genera of Vascular Plants VI. Flowering Plants-Dicotyledons: Celastrales, Oxalidales, Rosales, Cornales, Ericales*. Springer Verlag, Berlin, pp. 239–254. https://doi.org/10.1007/978-3-662-07257-8_27

Weigend, M. (2006) Validating subfamily, genus and species names in Loasaceae (Cornales). *Taxon* 55: 463–468. https://doi.org/10.2307/25065594

Weigend, M., Aitzetmüller, K. & Bruehl, L. (2004a) The seeds of Loasaceae subfam. Loasoideae (Cornales) I: Seed release, seed numbers and fatty acid composition. *Flora* 199: 424–436. https://doi.org/10.1078/0367-2530-00170

Weigend, M., Gottschling, M., Hoot, S. & Ackermann, M. (2004b) A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on trnL (UAA) sequence data, with consequences for systematics and historical biogeography. *Organisms Diversity and Evolution* 4: 73–90. https://doi.org/10.1016/j.ode.2003.12.001

Weigend, M., Dostert, N., Henning, T., Schneider, C. & Rodríguez, E. (2006) Valid publication for 101 species and subspecies names of the genera *Nasa* and *Aosa* (Loasaceae: Cornales). *Revista Peruana de Biología* 13: 71–84. https://doi.org/10.15381/rpb.v13i1.1766

Weigend, M., Grau, J. & Ackermann, M. (2008) Loasaceae. *In*: Zuloaga, F.O., Morrone, O. & Belgrano, M.J. (Eds.) *Catálogo de las Plantas Vasculares del Cono Sur (Argentina, Sur de Brasil, Chile, Paraguay y Uruguay) Vol 3. Dicotyledoneae (Fabaceae 2-Polygonaceae)*. Missouri Botanical Garden Press, Saint. Louis, pp. 2413–2424.

CHAPTER 4

Back and forth: Species and names of *Kissenia* R.Br. ex Endl. (Loasaceae)

Kissenia capensis, in its habitat in the Namib-Naukluft Park. *Kissenia* is a deserticolous shrub and differs from all other Loasoids in its very elongated sepals and indehiscent cypselas. Photo courtesy of Ina Dinter.

Back and forth: Species and names of *Kissenia* R.Br. ex Endl. (Loasaceae)

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Abstract

The floristic exchange between South America and Africa after their separation in the Cretaceous, has had deep influences in the floras of both continents. Although in some cases the immigrants at either side of the Atlantic gave rise to very diverse and species rich clades, in other cases only few extant taxa remain as evidence of transoceanic dispersal. *Kissenia* is the only African genus of the otherwise mostly American family Loasaceae. The genus has been known for almost 200 years and comprises only 2 extant species, yet its nomenclature is surprisingly convoluted. The present study aims at clarifying the nomenclatural ambiguities as well as providing the updated formal taxonomy and descriptions. The study is based on the study of extensive herbarium material (over 200 specimens in 16 herbaria) and cultivated plants and provides detailed data on morphology and distribution. We list all published names and provide lectotypifications for *Kissenia* We demonstrate that that the correct species names are *Kissenia capensis* for the South African and *K. spathulata* for the northeast African-southern Arabian species. Although, the leaf lamina morphology changes considerably, even in a single plant, depending on the age of the individual and season of the year, *K. capensis* tends to have narrower, more deeply lobed leaf laminas and lamelliform nectar scale ligulas, while *K. spathulata* has broader leaf laminas and filiform nectar scale

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ligulas. Both species are deserticolous, and can be locally common even in mildly disturbed sites. Considering the interesting morphology, biogeography and ecology of *Kissenia,* we hope that this work could become a useful reference for African botanists working in dry regions, not only for taxonomists but also for physiologists and ecologists interested in the adaptations of the native flora.

Keywords: Africa**,** Arid zones, disjunct distributions, *Kissenia*, lectotypification, Loasaceae, nomenclature.

1. Introduction

Long distance floristic exchange between South America and Africa happened repeatedly during the Tertiary (Barthlott, 1983; Morley, 2003; Pennington and Dick, 2004; Christenhusz and Chase, 2013; Linder, 2014) and may be an important cause for the floristic similarities between African and Neotropical forests detected by Slik et al. (2018). In some cases the dispersal between continents lead to important radiations in the newly colonized areas, e.g., in Angraecinae (Orchidaceae; Pessoa et al., 2018), *Begonia* L. (Moonlight et al., 2016, 2018), Caricaceae (Carvalho and Renner, 2012) and Canellaceae (Müller et al., 2015). However, in other cases the colonization was not followed by extensive radiation: *Maschalocephalus dinklagei* Gilg and K. Schum. (Rapateaceae), *Mayaca baumii* Gürke (Mayacaceae), *Pitcairnia feliciana* (A. Chev.) Harms and Mildbr. (Bromeliaceae), *Rhipsalis baccifera* (Sol.) Stearn. (Cactaceae), *Turnera oculata* Story and *T. thomasii* Story (Turneraceae), *Sacoglottis gabonensis* (Baill.) Urb. (Humiriaceae) and *Voyria primuloides* Baker (Gentianaceae) are apparantely individual or pairs of species present in Africa as the only representatives of much more diverse South American lineages (Barthlott, 1983; Givnish et al., 2004; Thulin et al., 2012).

Loasaceae is a primarily American family with only five species in the Old World (Weigend, 2004). Three of them belong to the genus *Plakothira* Florence, endemic to the Marquesas archipelago (Florence, 1997), and the other two to *Kissenia* R.Br. ex Endl., found in Africa and the southern Arabian Peninsula (Hutchinson, 1946; Weigend, 2004).

The plants of this genus have been known to European researchers since at least the 1830 decade (Drège, 1844) and the interest on them has centered mostly around their unusual biogeographic affinities (Harvey, 1859; Harvey and Sonder, 1862; Gilg, 1894; Dyer, 1975; Kingdon, 1990). Dandy (1966) considered that *Kissenia* is composed by two deserticolous species: *Kissenia arabica* R. Br. ex Chiov., from northeastern Africa and southern Arabian Peninsula and *Kissenia capensis* Endl. from southwestern Africa. This was followed by most subsequent authors and floristic treatments (Roessler, 1968; Codd, 1976; Gilbert, 1993, 2000). Both taxa have been considered very closely related to each other, judging by their almost identical morphology (Urban and Gilg, 1900) and very similar molecular sequences (Acuña et al. in prep.).

The study of vegetative (especially trichomes) and floral (petal shape, disposition and specialization of the androecium) morphology allowed botanists to suggest a relationship with Loasaceae quite early (Fenzl, 1841; Endlicher, 1842), but the distinctive gynoecial and fruit morphology of *Kissenia* set it apart from other genera of Loasaceae. Gilg (1894) and Urban and Gilg (1900) placed this genus in its own tribe (Kissenieae Urb. & Gilg) in the subfamily Loasoideae based on the floral traits (in particular the presence of cymbiform petals and well-developed antesepalous nectar scales). This placement has been confirmed by molecular studies (Moody et al., 2001; Acuña et al., 2017; in prep.). *Kissenia* is retrieved as part of an ‘early diverging’ grade within the subfamily, including groups such as Tribe Klaprothieae, *Huidobria chilensis* Gay and *H. fruticosa* Phil. and diverging before the most recent common ancestor of the species rich *Nasa* and ‘South Andean Loasas’ clades (Weigend et al., 2004; Hufford et al., 2005; Acuña et al., 2017). *Kissenia* is ecologically and morphologically similar to both species of *Huidobria* Gay (Weigend, 2004), all of them representing deserticolous shrubs with well-developed root systems, alternate phyllotaxis and an indument composed almost exclusively of scabrid and glochidiate trichomes. There are, however, important differences between both genera (Weigend, 1997, 2004): the inflorescences of *Huidobria* have frondose bracts that are externally indistinguishable from vegetative leaves, meanwhile the bracts in *Kissenia* are are obviously distinct from the foliage leaves. In *Huidobria* the nectar scales are composed by four or more partially fused androecial elements, while these are formed by the complete fusion of only three staminal primordia in *Kissenia*. And most dramatically, in *Huidobria* each dehiscent capsule holds thousands of diminutive dust seeds (Grau, 1997), however, in *Kissenia* each indehiscent capsule carries only 2–3 large seeds (Weigend, 1997).

The distribution of *Kissenia* is similar to other "Southern Arabia/Eastern Africa – Southern Africa" disjunct xeric elements, discussed extensively in the literature (de Winter, 1971; Beier et al., 2004; Thiv et al., 2011; Thulin et al., 2012; Pokorny et al., 2015; Luebert et al., 2017; Acuña et al., in prep.). The divergence estimates of both *Kissenia*, (7.5–2 Ma, Acuña et al., in prep.), agree broadly with those of similarly distributed elements (Pokorny et al., 2015). These plants may have migrated through East Africa during the Pliocene and Pleistocene (deMenocal, 2004), along a then-extant arid corridor (Bellstedt et al., 2012). Recent evidence suggests that Malawi (and the Rift Valley) may have become wetter since 1.3 Ma (Johnson et al., 2016) and this may have disconnected the xeric floras in NE and SW Africa.

Although each species of *Kissenia* has been featured in regional floras (Roessler, 1968; Dyer, 1975; Codd, 1976; Gilbert, 1993, 2000) there have been no treatments dealing with both species since the brief and mostly nomenclatural, account by Dandy (1966), which left some questions about the typification, name priority and species limits open. The aim of this work is to clarify the status of the names historically associated with *Kissenia* and provide detailed descriptions for the two species.

2. Materials and methods

Over 200 specimens or photographs of specimens (representing over 150 different collections) from the following herbaria were revised: AAU, B, BM, E, FT, HAL, K, L, M, P, S, TCD, TUB, U, WAG, Z and ZT. Additionally, specimens of *Kissenia capensis* Endl. from Namibia were brought into cultivation and studied in the glasshouses at the Botanical Gardens of the University of Bonn, Germany.

Measurements of most structures were taken from herbarium specimens. The distinctive traits of each species are marked in bold in each description. The specimens were georeferenced for mapping, whenever possible. When the geographical coordinates were not included in the specimen label information, the collection localities were searched in the free access database GeoNames (http://www.geonames.org/) or directly in Google Earth Pro ver. 7.1.7.2606 (Google Inc., 2016). Some of the original collectors' published itineraries were studied, when available (Drège, 1844; Révoil, 1882; Clark, 1954; Chelazzi, 2009; Glen and Germishuizen, 2010). Each georeferenced specimen was plotted using the ‘maps’ package (Brownrigg 2017) of the R software (R Core team 2014).

To analyze the microstructure details of foliar surfaces we used fresh material from cultivated plants of *Kissenia capensis*. This was studied with cryo scanning electron microscopy in order to avoid drying artifacts. SEM studies were carried out following the methods of Ensikat and Weigend (2013). Specimens were sputter coated with Au or Pd for about 20 seconds in a SCD040 (Balzers Union, Liechtenstein) in order to increase the electrical conductivity.

3. Results and discusion

3.1. Morphology

3.1.1. Seedlings

The germination in *Kissenia capensis* is epigeal. The cotyledons are oblong, with entire margins, inconspicous venation and are amongst the largest of any Loasaceae. Although the terminal hydatode tooth common in other Loasaceae (Weigend, 1997) was not observed, the base of each cotyledon blade has two small gland-like structures on either side of the petiole insertion. The first few pairs of leaves produced after the cotyledons are opposite, with ovoid to rhomboid, entire blades, irregularly dentate to crenate margins and subpalmate leaf venation(Fig. 1A). By the third pair of leaves produced after the cotyledons, the lamina is lobed. By the fourth pair of leaves, the phyllotaxis switches from opposite to alternate. In these early stages, the leaf blades are generally larger than is common in most adult plants, being almost as long as wide and with a well defined cordate base (Fig. 1B).

3.1.2. Adult habit

Mature plants in the wild tend to be densely branched, sometimes hemispherical shrubs (Figs. 2A, 3A), but their size is variable and probably dependent on the kind of substrate

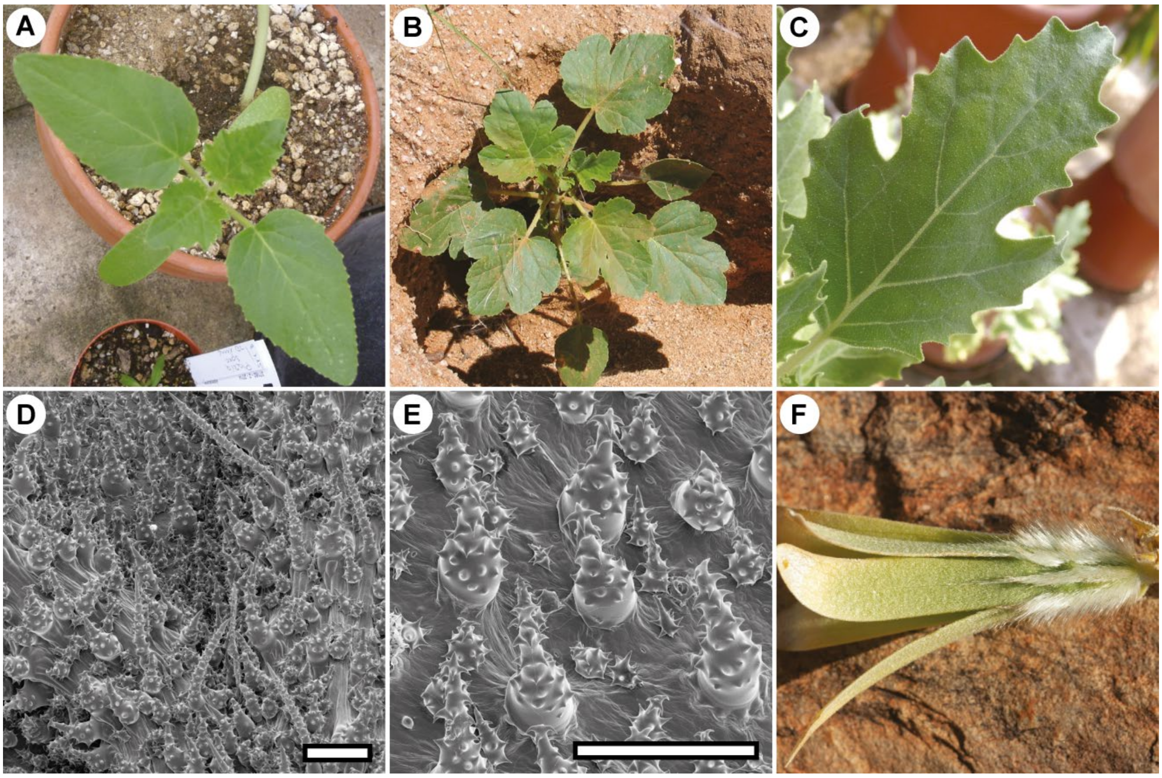

Fig. 1. Distinctive morphological traits of *Kissenia*. **A.** *Kissenia capensis* seedling, about one month old, cultivated at the Botanical Gardens of the University of Bonn. Notice the opposite phyllotaxis, the ovoid leaf laminas and the oblong outline of the relatively large cotyledons under the youngest pair of leaves. **B.** Older young plant of *Kissenia capensis* in its natural habitat in Namibia. The relatively wide pentalobate leaf laminas with a well-defined cordate base are common in plants before their first reproductive cycle. **C.** Ovoid, trilobate leaf, from a fully mature, reproductive *Kissenia capensis* plant, cultivated at the Botanical Gardens of the University of Bonn. **D.** Overview of the abaxial leaf indument of a fully mature leaf of *Kissenia capensis*, obtained from a plant cultivated in the Botanical Gardens of the University of Bonn. Besides their length, there is little variability in trichome morphology. **E.** Detail of an area between the veins from the same sample as (**D**). **F.** Indehischent capsule of *Kissenia capensis* taken from its natural habitat in Namibia. The sepals are persistent and remain attached to the fruit even after it has fallen from the parental plant. Notice the long, smooth tricomes that cover the outer walls of the capsule in row. Scale bars = 300 µm. Credits **B.** by Norbert Jürgens. **F.** by Meredith Cosgrove.

and moisture available in their habitat. Some individuals may start producing flowers at about 30 cm in height, and old plants can be over 1.2 m in height. The wood anatomy of *Kissenia capensis* was studied by Carlquist (1984) who mentions it bears resemblace to *Nasa picta* (Hook.) Molinari and *Presliophytum incanum* (Graham) Weigend, the other loasoids he analyzed. This species has abundant diffuse axial parenchyma and relatively large multiseriate rays. Like other deserticolous loasoids (*Huidobria* Gay, *Presliophytum* (Urb. and Gilg) Weigend), *Kissenia* species exfoliate their epidermis, that turns whitish, as the periderm starts to develop. *Kissenia* also has a well developed taproot. Mature plants seem to be at least partially deciduous in the wild. The plants studied in cultivation became leafless during the winter months for three consecutive years, flushing new leaves each spring.

3.1.3. Leaf morphology

Although the first few pairs of leaves produced by the seedlings are opposite, these are shed in few months. By far, most of the leaves produced in a lifetime are alternate. Mature plant leaves are petiolate, amphistiomatic, with the blades ranging from from narrowly ovate to subcircular, usually tri to pentalobate, with a cuneate, truncate or shallowly cordate base (Fig. 1C). In general the leaves of *K. capensis* are narrower and with deeper incisions in the blade, while the leaves of *K. arabica* are wider with shallower incisions, but there can be significant variation in morphology, even within a single plant, depending on the season, sun exposure, soil moisture and age of the plant. Because of this, differences in leaf blade size and proportion are not always clear cut between both taxa and telling apart some plants without examining locality data or the nectar scales would prove problematic.

3.1.4. Indument

The plants are densely covered with rough, scabrid and glochidiate trichomes (Fig. 1 D,E). These are by far the commonest kind of trichomes that have been observed in *Kissenia*, a similar situation to its potential closest relatives: *Huidobria* and Klaprothieae. This constrasts with the rest of the subfamily, that frequently have abundant stinging and/or glandular trichomes (Weigend, 1997, 2004). The density of the indument causes the foliage to look powdery or greyish (Fig 2 B, 3B). The trichomes on the stem, petioles and over the abaxial veins in mature leaves, measure up to 1 mm long (Fig. 1D) while adaxially and abaxially between the veins, they measure to 500 µm long (Fig. 1E). The differences in trichome structure adaxially and abaxially as well as over and between the veins are minor, not taking into account their length. The ovary and fruit outer walls are covered by much longer (ca. 5 mm), whitish to golden yellowish, smooth trichomes (Fig. 1F), that at least in dry specimens could cause skin irritation, especially near the insertion of the sepals. The ontogeny of these trichomes and their affinities with the trichomes of other Loasaceae have not been studied.

3.1.5. Inflorescence morphology

Inflorescences are cymoids with monochasial branches and alternate bracts. The last are morphologically very different from vegetative leaves, being significantly narrower, sessile, with entire, lanceolate blades and entire to slightly undulate margin. Due to these differences, it is straightforward to distinguish vegetative from reproductive branches. The bracts show concaulescence and recaulescence and of each bract pair (on rare occasions could be more than one pair of bracts per flower), usually one is concaulescent and is attached to the flower pedicel (a trait apparently unique to *Kissenia* in Loasaceae, Weigend, 1997), while the other is recaulescent and inserted near the pedicel of the next flower. Although there is overlap in their sizes, the bracts of *K. capensis* can occasionally grow significantly wider than those of *K. arabica.* The flowers are erect.

3.1.6. Flower morphology

The flowers in *Kissenia* are epigynous, pentamerous, actinomorphic and complete (Figs. 2C, 3C. The sepals are oblong to obovoid, usually slightly wider terminally than proximally, with three conspicuous main veins along their length (two more can be seen often near the base). These are 2–3x longer than the petals, and elongate further after anthesis. The corolla aestivation in *Kissenia* is imbricate, as in *Huidobria* Gay, *Aosa grandis* (Standl.) R.H.Acuña and Weigend and several bird pollinated species of *Nasa* Weigend. The petals are half- to full-spreading, cymbiform, white to cream, with a well-defined claw and limb. The floral scales are antesepalous, lanceolate, formed by three fully fused staminal primordia (Hufford, 2003) and lack dorsal threads. These are mostly the same color as the petals, however the base of the neck is adaxially brighter yellow. The apex of the neck is elongated and transformed into a ligule (Fig. 3 C), which folds over the nectar scale back (both species), and then again distally near the tip (*K. capensis*). The presence of a ligule-like structure is unknown in other loasoids, and it may be lamelliform and have an entire or shallowly lobed to cleft apex (*K. capensis*) or be thread-like, either undivided or deeply divided into 2 (-3) irregularly twisted filiform appendages (*K. arabica*). Adaxially, opposite to the nectar scale there could be two or four free staminodes. The central two staminodes are always present and their base is conspicuously flattened, with a flange directed towards the scale; the area above the flange is filiform with a flattened or clavate tip. If four staminodes develop the lateral pair could have sterile anthers and be intermediate in morphology between the central staminodes and fertile stamens. There are ca. 40–80 fertile stamens per flower, usually arranged in 5 groups opposite to the petals. These have a filiform whitish filament and yellow anthers with semi-circular thecae. The style is ca. 5 mm long and has 3 stigmatic lobes, twisting after anthesis. The ovary is pubescent, asymmetrically bilocular, with two placentae (one on per locule), each with 1–2 anatropous, pendulous ovules (Urban and Gilg, 1900). However, some authors consider the ovary to be actually unilocular, due to the septum apparently not dividing entirely both locules (Weigend, 1997).

3.1.7.Fruit morphology

Fruits are erect, inferior, straight, indehiscent, ligneous capsules, ellipsoid to globose-cylindrical, 5–7 mm in diameter with persistent, post-anthetically elongated, oblong to obovate sepals (Figs. 1F, 2D, 3D). Like the ovaries, the mature capsules are covered by long, yellowish to golden smooth trichomes, ca. 5 mm long. Each capsule usually has 2 seeds. The greatly elongated sepals are probably an adaptation for anemochory (the fruits being the dispersal, not the seeds as in most Loasaceae).

3.1.8. Seed morphology

The seeds are oblong, exalbuminous, yellowish, with a thin, reticulate testa and fill most of the volume of their respective capsule locule. Most of the seed itself is made up of the embryonic cotyledons (Urban and Gilg, 1900; Weigend, 1997).

3.2. Distribution and habitat

Kissenia is a deserticolous genus. Both species are widely disjunct geographically from each other, with one species restricted to southwest Africa (western Namibia and northwest South Africa) and the other to northeast Africa (Djibouti, northeast Ethiopia, northern Somalia) and southern Yemen (Fig. 4). They grow in desertic to semidesertic areas between sea level and 1400 m, on granitic, calcareous, quartzitic or basaltic gravel or sand, often in areas with large exposed rocks, such as mountain slopes, kopjes, dry riverbeds (wadis), alluvial plains, cliffs and gorges. Both species could be common locally, even near settled areas or roads.

3.3. Pollination ecology

The flowers have showy pale corollas and nectar scales that can accumulate enough nectar to overflow them (at least in cultivation: obs. pers.). The latter also have necks that are slightly contrasting in color. These traits suggest some form of entomophily, although pollinators have not been reported so far in the literature. Ants have been observed visiting both species (photo by Ina Dinter: Dressler et al., 2014–Ongoing; Fig. 3B), but whether they are just nectar robbers or actual pollinators has not been assessed.

4. Taxonomic treatment

Kissenia (=Fissenia) R.Br. ex Endl. in Gen. Pl. [Endlicher] Suppl. 2: 76. 1842; *nom. cons.* ≡ ***Cnidone*** E.Mey. ex Fenzl in Denkschr. Königl.-Baier. Bot. Ges. Regensburg 3: 199. 1841; *nom. rej.* – Type: *Kissenia capensis* Endl. ex Harv.

Coarse shrubs 30–130 cm tall with a thick taproot, covered with abundant scabrid and glochidiate hairs on most surfaces. *Leaves* exstipulate, alternate, usually lobate, petiolate to ca. 50 mm in younger plants, lamina to 90 × 80 mm, but usually smaller, green to greyish green, narrowly ovate to suborbicular, usually with 1–4 lobes on each side, margin crenate or irregularly dentate, base cuneate to shallowly cordate. *Inflorescences* bracteose dichasia or thyrsoids, with branches up to to ca. 20 cm long; each flower erect in anthesis with two (rarely more), sessile, narrowly ovoid to lanceolate bracts, to ca. 25 × 10 mm, with entire or weakly undulate margins, one (rarely more more) on the flower pedicel, another near the base of the next flower pedicel; pedicels to 7 mm long, not lengthening significantly post anthetically. *Sepals* five, oblong to obovoid, to 50 × 10 mm (specially post-anthesis), green, 3-veined with entire margins, ca. 2–3x longer than the petals, enlarging after anthesis. *Petals* five, full- to half-spreading, cymbiform, short clawed to ca. 15 mm long, white to cream, with an indistinct central keel, margins vertical to slightly involute, irregularly serrulate, apex truncate to rounded, aestivation imbricate. *Nectar scales* five, to ca. 7 mm long and 2 mm wide, white to cream, with the neck base yellow, back slightly bulging, weakly concave, without dorsal threads or nectar sacks, the margins shortly pubescent to papillose, neck apex elongated into a lamelliform and entire, to thread-like, and deeply divided ligule (about 1/3 of the nectar scale lenght), folded over the scale back only or over the scale back and the tip. *Staminodes* 2–4 per scale, to ca. 7 mm long, the base conspicuously dorsoventrally flattened, with a flange towards the nectar scale, margins with a dense, soft trichome cover, the apical region above the flange filiform with the base papillose, the terminal portion smooth and with a flattened to clavate tip. When four staminodes develop, the lateral ones usually with anthers and intermediate in morphology between the sterile staminodes and the remaining fertile stamens. *Stamens* up to ca. 80, filaments to 8 mm long, anthers yellow, 1 mm long. *Style* to ca. 6 mm long, twisting after anthesis. *Ovary* inferior, bilocular, outer walls hirsute, covered by smooth, long trichomes, up to 5 mm long, creamy to golden brown, alternating with much shorter scabrid and glochidiate trichomes, placentae 2, ovules white, irregularly ovoid to oblong. *Fruit* an indehiscent capsule, to 6 mm in diameter, ovoid, with persistent, post-anthetically elongated, oblong to narrowly obovoid sepals. *Seeds* exoalbuminous, ca. 1–2 (rarely more) per capsule, testa poorly developed, weakly reticulate. n = 12 (Poston and Thompson, 1977).

Notes

The taxonomic history of *Kissenia* is unexpectedly complex for such a small genus. *Cnidone* E.Mey. has traditionally been considered a *nomen nudum* (Endlicher, 1842; Urban and Gilg, 1900; Harvey, 1926; Weigend, 1997). Although Holstein et al. (2018) have reached the conclusion that *Cnidone* description is valid, preceding that of *Kissenia*, the former name has not been used after almost 150 years and thus the same authors proposed to conserve the much more widely used *Kissenia* and reject *Cnidone*. We also follow Holstein et al. (2018) regarding the correct orthography of the genus. *Kissenia* was validly published by Endlicher, however the lines after the description of the embryo ("Herbae capenses et arabicae […] Fissenia R. Brown msc. Cnidone E.Meyer msc. Fenzl Not. msc. (character e sola F. capensi. Cnidone mentzelioide E. Mey.") are ambiguous regarding the number of taxa to be included in the genus and do not explicitly refer to or describe a binomial.

Since before its valid publication (Arnott, 1841) and until Dandy (1926), it was customary to consider *Kissenia* as monospecific [Presl, 1844; Anderson, 1860; Brongniart, 1860; Harvey in Harvey and Sonder, 1862; Masters, 1871; Baillon, 1885; Britten, 1894; Gilg, 1894; Urban and Gilg, 1900]. Only Harvey (1859) and Urban and Gilg (1900) expressed doubts regarding this, but made no change in the taxonomy. As far as we know, Dandy (1926) was the first to find more or less consistent differences between the plants of SW Africa and NE Africa/SW Arabian Peninsula, and provide diagnosis differentiating both species. Altough his assessment seems mostly correct, leaf morphology as a differentiating trait between both taxa could be unreliable in some specimens.

Etymology

The name *Kissenia* is apparently derived from the name of the area where one of the first specimens of the genus was collected: Qishn, in what is now Al Mahra, Yemen (Dandy and Exell, 1932).

Similar looking plants

When fertile, the combination of alternate, exstipulate leaves, covered with barbed (scabrid and glochidate) trichomes (as do most of the plant surfaces), sepals ca. 2–3 x longer than the petals, presence of antesepalous nectar scales in the flowers and indehiscent capsules with persistent sepals render *Kissenia* unmistakable. When sterile it could resemble some Malvaceae species, however *Kissenia* lacks mucilage, has barbed trichomes and exstipulate leaves.

4.1. Key to the species of Kissenia*:*

1. Leaves usually narrowly ovate to ovate, the deepest incisions in the lamina ca. 30–50% of the lamina width towards the midvein. Nectar scales with a lamelliform ligula folded over the scale and then again over itself, distally, with entire or shallowly cleft apex. Only in southwestern Africa … ***Kissenia capensis***

1a. Leaves usually broadly elliptic to broadly ovate or suborbicular , the deepest incisions in the lamina usually up to ca. 25% of the lamina width towards the midvein. Nectar scales with a thread-like ligula folded only over the scale back, sometimes deeply divided into 2 (-3) irregularly twisted filiform appendages. Only in northeastern Africa and the southern Arabian peninsula … ***Kissenia spathulata***

1. *Kissenia capensis* Endl ex Harv. in Thes. Cap. 1: 61–62, Pl. XCVIII. 1859. ≡ *Fissenia mentzelioides* Harv. in Thes. Cap. 1: Pl. XCVIII. 1859; *nom. nud*. Type: [South Africa, Northern Cape Province,] Namaqualand, [1858?], *A. Wyley 67* (TCD, lecto. - image!, here designated). Other original material: [South Africa, Northern] Cape [between Verleptpram and the mouth of the 'Gari[e]p' [Orange River]), <300 m, Sept. 1826–1834], *J.F. Drège s.n.* (TCD - image!, syn.; E barcode E00814842 - image!, HAL barcode HAL0121356 - image!, K barcodes K000310976!, K000310977!, L barcodes L.2449564 - image!, L.2449566 - image!, P barcodes P04650630 - image!, P04650633 - image!, P04650634 - image!, P06136484 - image!, S No. 10-7370 - image!, S No. 10-7373 - image!, TUB barcode TUB-002841 - image!, W No. 0077259 - image!, W No. Reichenbach f. 1889-21341 - image!, W No. Reichenbach f. 1889-312844 - image!, probable isosyn.). [South Africa, Northern Cape Province,] From sandy flats near 'Au'Aaf [?] River', Namaqualand, [1854-55?], *W. Atherstone 10* (K!, syn.).

Kissenia spathulata p.p. R.Br. ex T.Anderson in J. Proc. Linn. Soc., Bot. 5 (Suppl. 1): 43. 1860; *nom. illeg.* Types: [Yemen, Al Mahra Governorate,] 'Keschin [Qishn] Bay", s.d., *Coll. unknown* (BM barcode BM000944628!, syn.); [Yemen,] Aden, 1860, *A. Courbon 339* (K!, P barcodes P04588754 - image!, P04588751 - image!, syn.).

Cnidone mentzelioides E.Mey; *nom. nud*.

Fissenia arabica p.p. Arn. in J. Bot. (Hooker) 3: 259. 1841; *nom. nud.*

Coarse, densely branched, sometimes partly deciduous, shrub 30–130 cm tall with a thick taproot. Stem epidermis with abundant rigid glochidiate and some scabrid trichomes to 1 mm long, stinging trichomes absent, epidermis exfoliating and turning white in older plants. *Leaves* exstipulate, alternate, lobate, petiole 5–30 mm, with scabrid (and glochidiate) trichomes to 1 mm long, lamina 20–90 × 10–80 mm, green to greyish green, **usually ovate to narrowly ovate** (rarely broadly ovate) with 1–4 lobes on each side, the inferior leaves usually deeply tri(-penta)lobate, almost as long as wide, the superior with 2–4 shallow lobes per side, the **incisions between median lobes of at least some leaves, deep, ca. 30–50% the lamina width towards the midvein** (some leaves however can be entire, even in a single plant), margin crenate or irregularly dentate, base truncate to cuneate or shallowly cordate (especially younger plants), sub-symmetric, blade and lobe apices acute to rounded, both adaxial and abaxial surfaces covered with whitish dense scabrid and glochidiate trichomes to 0.5 mm long adaxially and to 0.75 mm long abaxially. *Inflorescences* bracteose dichasia or thyrsoids, with branches up to to ca. 20 cm long; each flower erect in anthesis with two (rarely more), sessile, narrowly ovoid to lanceolate bracts, 10–25 × 3–10 mm, with entire or weakly undulate margins, covered in dense acabrid and glochidiate trichomes, one (or more) on the flower pedicel, the second near the base of the next flower pedicel; pedicels 2–7 mm long, not lengthening considerably post anthetically, with scabrid trichomes. *Sepals* five, oblong to obovoid, 20–50 × 5–10 mm green, 3-veined with entire margins, ca. 2–3x longer than the petals, lengthening further after anthesis, covered mostly only with scabrid trichomes on both surfaces.*Petals* five, full to half spreading, cymbiform, short clawed, 10–15 mm long, white to cream, with an indistinct central keel with short trichomes, margins vertical to slightly involute, irregularly serrulate, apex truncate to rounded, aestivation imbricate. *Nectar scales* five, 7 mm long and **over 1 mm (usually to ca. 1.5–2 mm) wide**, white to cream, with the neck base yellow, back slightly bulging, weakly concave, without dorsal threads or nectar sacks, the margins shortly pubescent to papillose, neck apex elongated into a **lamelliform, entire or shallowly cleft to lobed ligule** (about 1/3 of the nectar scale lenght), **folded over the scale back and then again over the tip**. *Staminodes* 2–4 per scale, to ca. 7 mm long, the base conspicuously dorsoventrally flattened, with a flange towards the nectar scale, margins with a dense trichome cover, the apical region above the flange filiform with the base papillose, the terminal portion smooth and with a flattened to clavate tip. When four staminodes are present, the lateral ones are intermediate in morphology between the usually sterile (without anthers) central staminodes and the normal, fertile stamens, and usually have anthers. *Stamens* 40–80, filaments to 8 mm long, anthers round, yellow, 1 mm long. *Style* 6 mm long, twisting after anthesis, the base with scabrid trichomes. *Ovary* inferior, bilocular, with the roof covered with glochidiate and scabrid trichomes, outer walls hirsute, covered by 10 multiseriate, vertical rows of smooth, long trichomes, up to 5 mm long, creamish to golden brown, alternating with 10 multiseriate, vertical rows of much shorter scabrid and glochidiate trichomes, placentae 2, ovules white, irregularly ovoid to oblong. *Fruit* an indehiscent capsule, 5–6 mm diameter, ovoid, with persistent, post-anthetically expanded sepals, usually sligthly recurved (appearing flat in pressed material). *Seeds* exoalbuminous, ca. 1–2 (rarely more) per capsule, testa poorly developed, weakly reticulate.

Notes

The name *Kissenia capensis* was attributed to R. Brown by Harvey (1859), but we consider that it was actually Endlicher who used the name first, although he did not publish a proper diagnosis of it validly (see *Kissenia* section Notes above). Harvey however, was the first to publish a valid protologue for this taxon. Although Harvey was not sure if all the populations of *Kissenia* belonged to the same species, he only cited South African material in the protologue. *A. Wyley 67* (TCD) was selected as the lectotype of *Kissenia capensis* for being the most complete specimen examined by Harvey (1859) and probably the main reference for Pl. XCVIII ("*Fissenia mentzelioides*") of the same work. Drège's material of *Kissenia* appears to have been colected on Sept. 1830, when he visited the area between the mouth of the Gariep/Orange River and the locality he called "Verleptpram" (Glen and Germishuizen, 2010). Due to the large number of specimens (probably duplicates, most of them annotated by E. Meyer), this material is widespread in many European herbaria and those outside TCD could are probable isosyntypes. Anderson (1860) considered *Kissenia* monotypic and the name "*Fissenia*" *capensis* as illegitimate (Dandy, 1966). He clearly intended that the name *Kissenia spathulata* should replace the former. Athough in the protologue of the name he explicitly cites only two specimens: the one where Robert Brown wrote the upublished description of *Kissenia* (BM barcode BM000944628) from Qishn and *Courbon 339* (P barcodes P04588751, P04588754) from Aden, he states that he also examined specimens from Southern Africa but does not cite any. We agree with Dandy (1966) in considering *Kissenia spathulata* R.Br. ex T.Anderson as illegitimate, and a partial synonym of *Kissenia capensis*.

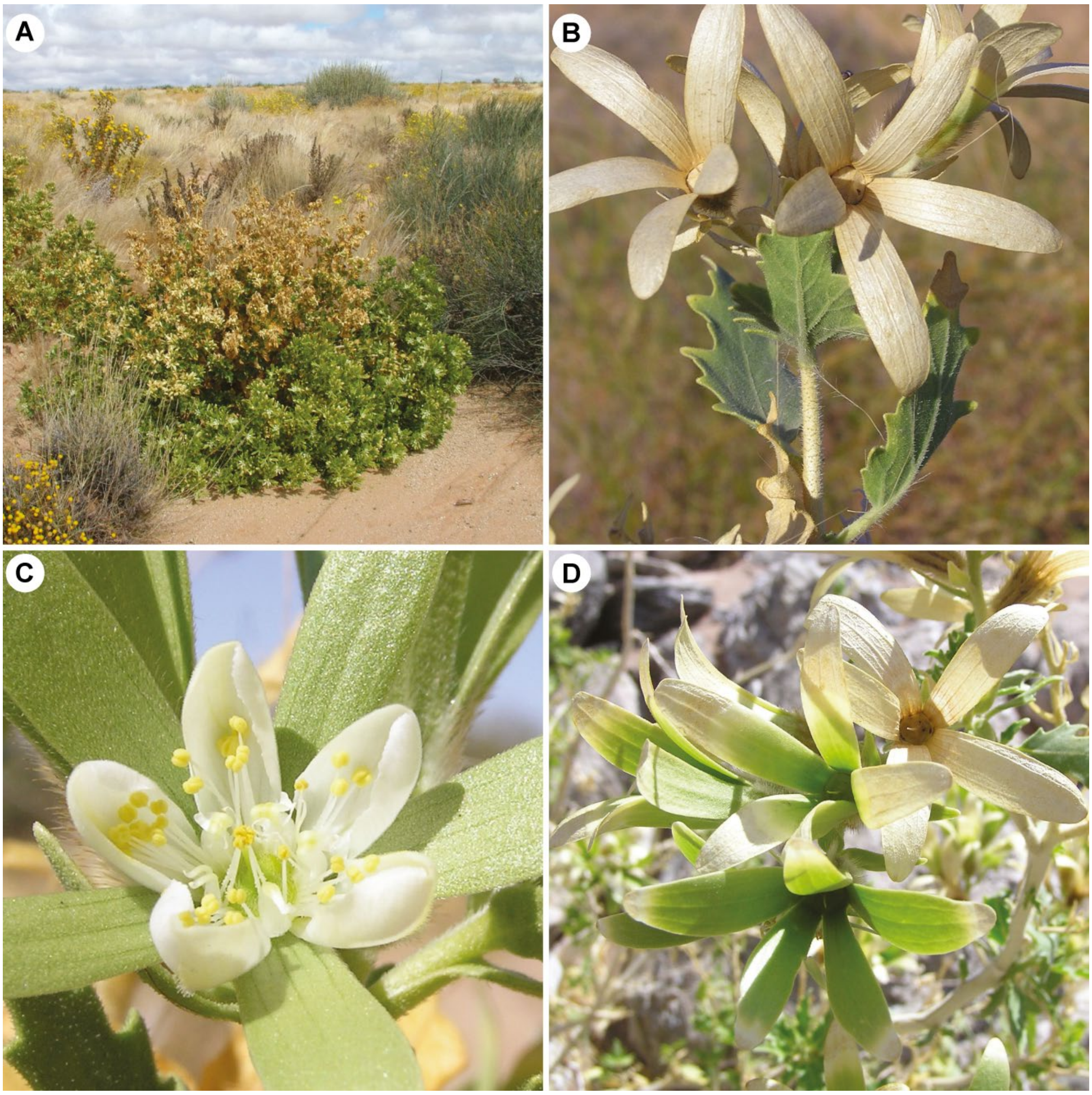

Fig. 2. Morphology of *Kissenia capensis* from several localities of Namibia. **A.** Mature shrub, growing alongside others of the same species. **B.** Terminal portion of a branch of a plant from the Spitzkoppe, including a short inflorescence/infrutescence and fully mature, narrowly ovate, greyish-green leaves. These are in general narrower than those of fully mature *K. spathulata* plants. **C.** Anthetic flower beginning its male phase in the Spitzkoppe. Notice the sepals, much longer than the petals, the yellow neck of the nectar scales and the apical ligulae folded twice. **D.** Infrutescence of a plant from the Fish River Canyon that has already lost some of its leaves. Notice the exfoliating, pale, dead epidermis of the branches in the background. Credits: **A.** by Norbert Jürgens. **B-D.** by Max Antheunisse.

Etymology

The epithet alludes the “Cape” of Southern Africa.

Illustrations

Harvey (1859: Plate XCVIII), Urban and Gilg (in Gilg 1894: Fig. 41, 1900: Tab. IV. Fig. 1–13), Hutchinson (1946: p. 164 Fig.), Codd (1976: Fig. 43), this work (Figs. 1–2).

Distribution

This species is widespread in (mostly western) Namibia (from Kunene to ǁKaras, records from Omaheke uncertain and were not cited by Roessler, 1968) and northwestern South Africa (Northern Cape: Codd, 1976), from ca. 300 to 1400 m elevation (Fig. 4A).

Phenology

In the wild, this species has been collected with flowers and fruits most months of the year. In cultivation however it seems its flowering time is more intense in late spring-early summer after new leaves have flushed during spring. The fruits can remain attached to the plant for several months (Fig. 2B, 2D).

Ecology

This species can grow in dry watercourses, cliffs, roadsides, alluvial plains, rocky (kopjes) or sandy areas on basaltic, quartzitic or calcareous soils (Fig. 2A). Ants have been observed visiting the flowers.

Conservation status

This species can be locally common in adequate habitats in both Namibia and South Africa. It was considered LC (IUCN 2001) by Fodden and Potter (2005) although it was not assessed by Loots (2005) nor IUCN (2018).

Specimens examined

Namibia. REGION UNCERTAIN: [1861-63], *J. Chapman & J. T. Baines s.n.* (K); Wasserstelle Dams, 20 Oct 1938, *O.Volk 796b* (M). KUNENE: Kaokoveld, Orupembe waterhole, Kunene. Extensive flats. Soil coarse gravel of basalt, quartz and limestone, 6 May 1957, *B. de Winter & O. Leistner 5742* (B, K, M); 1812 DD Sanitatas, Purros, growing in riverbeds, 27 Feb 1973, *J. Malan & G. Owen Smith 370* (M); 1812 CB Sanitatas. Kruid, 30 myl van Rocky point in droë revierbedding, 8 May 1962, *T. Kotze 115* (K, M); Outjo, Farm Mooilaagte 322, 41 miles W of Outjo on road linking Fransfontein and Kamanjab roads, 5 Apr 1955, *B. de Winter 3062A* (M, P). KUNENE(?): 1812 DD Sanitatas, Munitum valley, 1 km E. of border of SCP, in dry watercourse, 26 Apr 1982, *M. Müller & B. Loutit 2232* (M). ERONGO: Zisabschlucht, 3 Oct 1929, *R. Wettstein & F. Wettstein 416* (M); Bordering Namib desert, near Brandberg Mt. In dry river bed, 7 Dec 1947, *R. Rodin 2747* (K); District Omaruru, Brandberg, Nebenschlucht des Zisab, 16 Feb 1958, *H. Merxmüller 1649* (M); Omaruru District: Brandberg, Tsisab Valley mouth, black, stony kopjes, 3 May 1963, *B. Nordenstam 2473* (M); An der Pad nach Swakopmund (Omaruru-mündung) häufig in der Namib, Uis Mine, VIII-IX.1963, *H. Wiss 1924* (AAU); Omaruru District: Uis, Uis River E of village. Common in dry riverbed, 800 m, 3 Dec 2005, *H. Kolberg & T. Tholkes 1774* (K); Zwischen Felsen beim Lager, Tal zw Großer Spitzkoppe u. Pontokbergen, mittl. Südwest-Afrika, 1000 m, 1 May 1954, *R. Kräusel 650* (M); Spitzkopje, Namib Desert, amongst rocks, ca. 1100 m, 4 Mar 1950, *E.C. Macdonald 564* (BM); Kleine Spizkoppe, Rivier, 18 Mar 1956, *O. Volk 11844* (M); Farm Nudis, Karibib, Gamikaub River, 12 Feb 1953, *H. Walter & E. Walter 1257* (B, M); Usakos, Jan 1927, *E. Tworeck s.n.* (M), 1938–39, *O. Volk 116* (M); 85 km NE Swakopmund (gegen Usakos), Randbereich der Namib, Wüste, 850 m, 12 Oct 1985, *W. Greuter 20296* (B); Innere Namib, Swakopmund, Station Trekkopje, 600 m, 1 Mar 1958, *R. Seydel 1468* (L, M); Damaraland, 1879, *T.G. Een s.n.* (BM); Welwitsch, 22 Jan 1907, E. *Galpin & H.Pearson 7631* (K); ibid., 2 Oct 1929, *R. Wettstein & F. Wettstein 363* (M); ibid., Damaraland, rocky desert, Apr 1910, *W. Worsdell s.n.* (K); Khan-Tal, Sandiger Kies, 3 Apr 1913, *A. Engler 6064* (K); Damaraland, Khangebirge, 1 Set 1913, *A. Peter 47302* (B); Kuiseb Pass, Namib Naukluft Park. Dry open rocky hillside, 750 m, 14 Apr 1987, *D. Long & D. Rae 777* (E, K); Namib-Naukluft Park, ca. 35 km E Gobabeb, am Inselberg Mirabib, auf schuppigem Fels und in kleinen Rinnen, 800 m, 8 Nov 1986, *S. Breckle 9738* (M). OMAHEKE(?): Hereroland, 1886, *H. Schinz 539* (Z); ibid., 1887, *A. Lüderitz 201* (Z); ibid., 6 Aug 1892, *M. Rautamen 471* (Z). KHOMAS: Rehoboth, Farm Ubib (REH 396), Spreetshoogte, auf halber Höhe, 31 Aug 1972, *H. Merxmüller & W.*

Giess 28116 (M). HARDAP: Buellenhopf-summit Buellspoort., 10 Apr 1947, *R. Strey 2150* (K, P); Buelspoort, Naukluft. Granitic outcrops, 12 Mar 1965, *D. Hardy 1974* (K); Haribes, 10 Apr 1956, *O.H. Volk 12305* (M); Gibeon, Farm Haribes, Rote Kuppe, 27 May 1963, *H. Leippert 4700* (FT); Häufig au Rivieren u. omuramba …(?) Nudis, Namibrand, 650 m, 17 Feb 1954, *R. Seydel 221* (B, K, WAG). HARDAP(?): 40 Km SW of Zaris, 9 Apr 1977, *J. Lavranos & G. Barad 15594* (E). ǁKARAS: Rocky slope near dam farm Helmeringhausen, 20 Apr 1949, *H. Kinges 2179* (M); Great Namaqualand, Klein Karas-Aiais, 7 Aug 1931, *Jan Örtendahl 90* (L); Along dry riverbed, farm Weissenborn, District Lüderitz, 6 Jul 1949, *H. Kinges 2422* (M); District Lüderitz Süd, Namibfläche zwischen Neisip & Eureka, 16 Aug 1963, *H. Merxmüller & W. Giess 2882* (M); Namaland, Keetmanshoop, Dec 1884, *H. Schinz 540* (L, Z, ZT); Keetmanshoop, 1400 m, 31 May 1922, *K. Dinter 3556* (B, BM, K, Z); ibid., Dec 1924, *F. Rogers 29645* (Z); ibid., ca. 1000 m, 14 Oct 1925, *F. Dunham 14* (K); ibid., 1929, *A.K. Hobart-Hampden 13* (BM); Namaland, Jobas bei Keetmanshoop, 14 Oct 1913, *A. Peter 47303* (B); 11 km SW of Keetmanshoop on road to Seeheim, stony hillside facing south with grass tussocks and short scattered shrubs, 6 May 1976, *Oliver et al. 6321* (K); Keetmanshoop, 20.9 miles from Aroab on road to Rietfontein, 4 May 1955, *B. de Winter 3420* (K, M, P); Farm Chanüs-Süd, Bethanien, 25 Mar 1953, *H. Walter & E. Walter 2169* (B); Bethanien, Goageb, steinige Fläche, 11 Dec 1974, *H. Friedrich 560* (M); Seeheim, Felsen, 28 Apr 1913, *A. Engler 6606* (K); Seeheim, Keetmanshoop District, 17 Set 1958, *B. de Winter & W. Giess 6421a* (M); Kuchenas, Great Karas foothills, ca. 1250 m, 16 Nov 1938, *H. Lynes 1908* (BM); 2719 AD Tranental, Farm Tsaraxaibis, WAR 275, auf Gesteinsfläche unterhalb Berghang, 30 May 1970, *W. Giess & M. Wolf 10949* (M); Bezirk Keetmanshoop, Strasse Seeheim-Grünau, bei Strassenrand, 12 Nov 1983, *B. Leuenberger et al. 3298* (B); Grünau, Jul 1937, *F. van der Merwe 1421* (K); East foothills of Obib Mountains; south of Rosh Pinah, s.d., *J. Lavranos & Jan Pehlemann 21647* (E, WAG); 8 miles west of Ariamsvlei, 10 Apr 1956, *G. Theron 1953* (B, K, L); Great Namaqualand, in rocks in kopje near Gabis, 31 Jan 1909, *H. Pearson 4323* (BM, K); Vioolsdrif, 73 Km from Noordoewer, Inland from the Orange River. In dry wash., common next to large boulders or below cliffs, 19 Jul 1984, *P. Goldblatt 7156* (WAG); 78 Km from Noordoewer on main road to Günau, road verge, 28 Oct 1987, *H. Kolberg & G. Maggs 277* (WAG); Warmbad, amongst rocks on banks of dam, 11 Jul 1937, *E. Galpin 14142* (K); Warmbad, 10–15 miles N of Vioolsdrift, 2 Nov 1963, *K. Rechinger A-4831* (M); 2819 CA Ariamsvlei, Farm Vaaldorn: WAR91. Granit-Quatzkuppe, 26 May 1972, *W.*

Giess & M. Müller 12200 (K, M, WAG); Keimas, stony hillside, 26 Jun 1950, *G. Theron 824* (K); Warmbad, Ramansdrift, growing in plains near to “asbosse”, 5 Jul 1960, Jan *Kruger 32* (K); Zwischen Seeheim und Konkiep. Roter Schiefer, 16 Dec 1961, *H. Ihlenfeldt 1846* (M). ǁKARAS(?): Beim Karub, 16 Aug 1948, *S. Rehm 972* (M).

Namibia / South Africa(?). ǁKARAS / NORTHERN CAPE (?): Namaqualand, sandy valley leading down to Bethany [Sendeling’s] drift, 29 Dec 1910, *H. Pearson 6951* (K).

South Africa. NORTHERN CAPE: [No locality], 22 Oct 1928, Jan *Pole-Evans 2269* (K); Zwischen Verleptpram und der Mündung des Gari[e]p [Orange River], <300 m, Set 1830, *J.F. Drège s.n.* (E, HAL, K, L, P, S, TCD, TUB, W); Hay Div. On rocky slopes of mountains part of Langeberge, common on south facing slope, 8 Jul 1936, *J. Acocks 495* (K); Augrabies National Park, S. side along Rooipad, Namaqualand, Broken Veld, 700 m, 7 May 1969, *M. Werger EW333* (AAU, U); Sides of Khusies River, near Viols Drift, Little Namaqualand, Set 1931, *N. Pillans 6390* (K); In Trocknem Flußbett, Wüste, Vioolsdrift, 15 Jul 1992, *M. Weigend s.n.* (M); Kenhardt Distr., Kakamas, Letterkop Bot. Reserve, Bergkamp. Stony hill, summit amongst large rocks, 12 Jul 1946, *E. Wasserfall 1071* (K); Namaqualand, 10 m south of Goodhouse, 30 May 1961, *H. Schlieben 9088* (BM, K, M); 2 km. N of Pella at the S. entrance of Canyon pass. Sandy alluvial plain, 350 m, 11 Oct 1989, *W. Greuter 21627* (B); Bushmanland, broken ground west of Pella, 8 Jan 1909, *H. Pearson 3544* (BM, K); Pella, on Pofadder Road. Eroded sandy banks along roadside, 22 Set 1961, *D. Hardy 761* (K, M); Namaqualand, Pofadder at Pella on sandstone formations, , 22 Set 1961, *Van Breda 1388* (K); Rietfontein, Prieska Div. Black rocky hills near the Orange on the Draghoender–Koegas Rd., Oct 1936, *J. Acocks & A. Halstrom 1163* (K); Prieska, 13 mi. E of Draghoender, Orange R.Valley, scrub on rocky hillside, 15 May 1946, *L. Codd 1228* (K); Prieska, 12 mi. From Draghoender on Koegas Rd., rocky kopje with *Aloe dichotoma*, ca. 900 m, 15 May 1946, *R. Story 1168* (K); Hay C.P., Kameelfontein, steep rocky side of hill, ca. 900 m, 19 Jul 1920, *E. Bryant 114* (K); Hay Div. Top of lava-capped, jasper hill at Kameelfontein. Locally common, 13 Mar 1937, *J. Acocks 2025* (K); Prieska, May 1932, *E. Bryant 547*[?] (K); Namaqualand, [1858?], *A. Wyley 67* (TCD); From sandy flats near Au'Aaf [?] River, Namaqualand, [1854-55?], *W. Atherstone 10* (K); Anisfontein, Little Namaqualand, Oct 1926, *N. Pillans 5291* (K); Galheud, grows on sandy hills, Dec 1929, *E. Bryant 547*[?] (K); Wolveton in collibus, 27 Set 1897, *R. Schlechter 11439* (B, BM, E, K, L, P, WAG).

2. *Kissenia spathulata* R.Br. ex Dandy in Bull. Misc. Inform. Kew. 1926 (4): 176. 1926. Type: [Yemen, Al Mahra Governorate,] 'Keschin [Qishn] Bay', s.d., *Coll. unknown s.n.* (BM barcode BM000944628, holo.!). Other original material: [Yemen,] Aden, Wadi Hâalla, 25 Nov 1888, *G. Schweinfurth 27* (BM!, K!, P barcode P04588752 - image!, para.); [Yemen,] 'Peninsula Aden, cirque de Schamsân, ad radices boreales montis Schamscham', 2 Dec 1889, *A. Deflers 2* (B barcode B131/96-17!, K!, P barcodes P04588757 - image!, P04588758 - image!, para.); [Yemen,] Aden, Northern slopes, *Perry* (Herb.?, para.); [Yemen, Aden,] 'Schugra' [Little Aden?], 19 Mar 1881, *G. Schweinfurth 129* (K!, para.); [Yemen,] Aden, *Hunter* (Herb.?, para.); [Yemen,] Aden, 1860, *A. Courbon 339* (K!, P barcodes P04588751 - image!, P04588754 - image!, para.); [Yemen,] Aden, 11 Apr 1861, *Thomson s.n.* (K!, para.); [Yemen,] Aden, Aug 1880, *coll. unknown, comm. B. Balfour* (K!, para.); [Yemen,] Hadhramaut, Alrail, ca. 600 m, 28 Dec 1893, *W. Lunt 127* (BM!, K!, P barcodes P04588749 - image!, P04588750 - image!, para.); [Yemen,] Hadhramaut, Goldnore Valley, *Lunt 313* (Herb.?, para.); [Yemen,] Hadhramaut, Wadi Bayren, *Hirsch 37* (Herb.?, para.); Somalia, [Sanaag,] 'Vallée du Gueldora (Ouarsanguelis [Warsangali Domains])', 1881, *G. Révoil 50* (P barcode P00346117 - image!, para.).

Kissenia arabica Arn. ex Chiov. Fl. Somala 1: 174–175. 1929. Type: Somalia, [Bari Region,] 'Regione Carin [Karin], nel letto del torrente Dindim [riverbed of Dindim stream]', 5 Jul. 1924, *N. Puccioni & J. Stefanini 1022[1127]* (FT barcode FT0004969, lecto. - image!, here designated; FT barcode FT0004970 - image!, isolecto.).

Kissenia spathulata p.p. R.Br. ex T.Anderson in J. Proc. Linn. Soc., Bot. 5 (Suppl. 1): 43. 1860; *nom. illeg.* Types: [Yemen, Al Mahra Governorate,] 'Keschin [Qishn] Bay", s.d., *Coll. unknown* (BM barcode BM000944628!, syn.); [Yemen,] Aden, 1860, *A. Courbon 339* (K!, P barcodes P04588754 - image!, P04588751 - image!, syn.).

Fissenia arabica p.p. Arn. in J. Bot. (Hooker) 3: 259. 1841 *Nom. nud.*

Coarse, densely branched, shrub 30–150 cm tall with a thick taproot. Stem epidermis with abundant rigid glochidiate and some scabrid trichomes to 1 mm long, stinging trichomes absent, epidermis exfoliating and turning white in older plants. *Leaves* exstipulate, alternate, lobate, petiole 15–30 mm (sometimes up to 50 mm in young plants), with scabrid and glochidiate trichomes to 1 mm long, lamina 20–60 × 20–60 mm, green to greyish green, broadly ovate to ovate, with 0–3 lobes on each side, the inferior leaves usually pentalobate, almost as long as wide, **broadly elliptic, broadly ovate or suborbicular in outline**, the superior with 0–3 shallow lobes per side, ovate, the deepest incisions between median lobes usually shallow, **ca. 25% of the lamina width towards the midvein**, margin irregularly crenate to dentate, base cuneate but truncate to shallowly cordate, less frequently cuneate, sub-symmetric, blade and lobe apices obtuse to rounded, both blade surfaces covered with whitish glochidiate and scabrid trichomes to 1 mm abaxially and to 0.5 mm adaxially. *Inflorescences* bracteose dichasia or thyrsoids, with branches up to to ca. 17 cm long; each flower erect in anthesis with two (rarely more), sessile, narrowly ovoid to lanceolate bracts, 8–20 × 2–4 mm, with entire or weakly undulate margins, similar to vegetative leaves in indumentum in each surface, one on the flower pedicel, the second near the base of the next flower pedicel; pedicels 1–5 mm long, with scabrid trichomes, not lengthening considerably post anthetically. *Sepals* five, oblong to obovoid, 15–40 × 4–8 mm green, 3-veined with entire margins, ca. 2–3x longer than the petals, lengthening further after anthesis, indumentum of each surface similar to that of the respective leaf surface. *Petals* five, full to half spreading, cymbiform, short clawed, 7–10 mm long, white to cream, with an indistinct central keel with short trichomes, margins vertical to slightly involute, irregularly undulate to serrulate, apex truncate to rounded, aestivation imbricate. *Nectar scales* five, 7 mm long, **less than 1 mm wide**, white to cream, neck base yellow to buff, back weakly concave, without dorsal threads or nectar sacks, margins shortly pubescent, apex elongated into a **thread-like, irregularly twisted ligule** (about 1/3 of the nectar scale lenght), **undivided, or divided into 2(-3) filiform appendages, each 0.1–1 mm long, only folded over the scale back**. *Staminodes* usually 2 per scale, to ca. 8 mm long. *Stamens* ca. 60, filaments to 8 mm long, anthers round, yellow. *Style* 5 mm long, twisting after anthesis. *Ovary* inferior, bilocular, with the roof covered in glochidiate and scabrid trichomes, outer walls hirsute, covered with long, up to 5 mm long, smooth, creamish to golden brown trichomes arranged in 10 multiseriate rows of longer and 10 multiseriate rows of shorter trichomes, placentae 2. *Fruit* an indehiscent capsule, ca. 10 mm long (not counting the sepals), 5–7 mm wide, ovoid, with persistent, post-anthetically expanded sepals, usually slightly recurved in (appearing flat in pressed material). *Seeds* exoalbuminous, ca. 1–2 (rarely more) per capsule, testa wrinkly, poorly developed, weakly reticulate with low anticlinal walls.

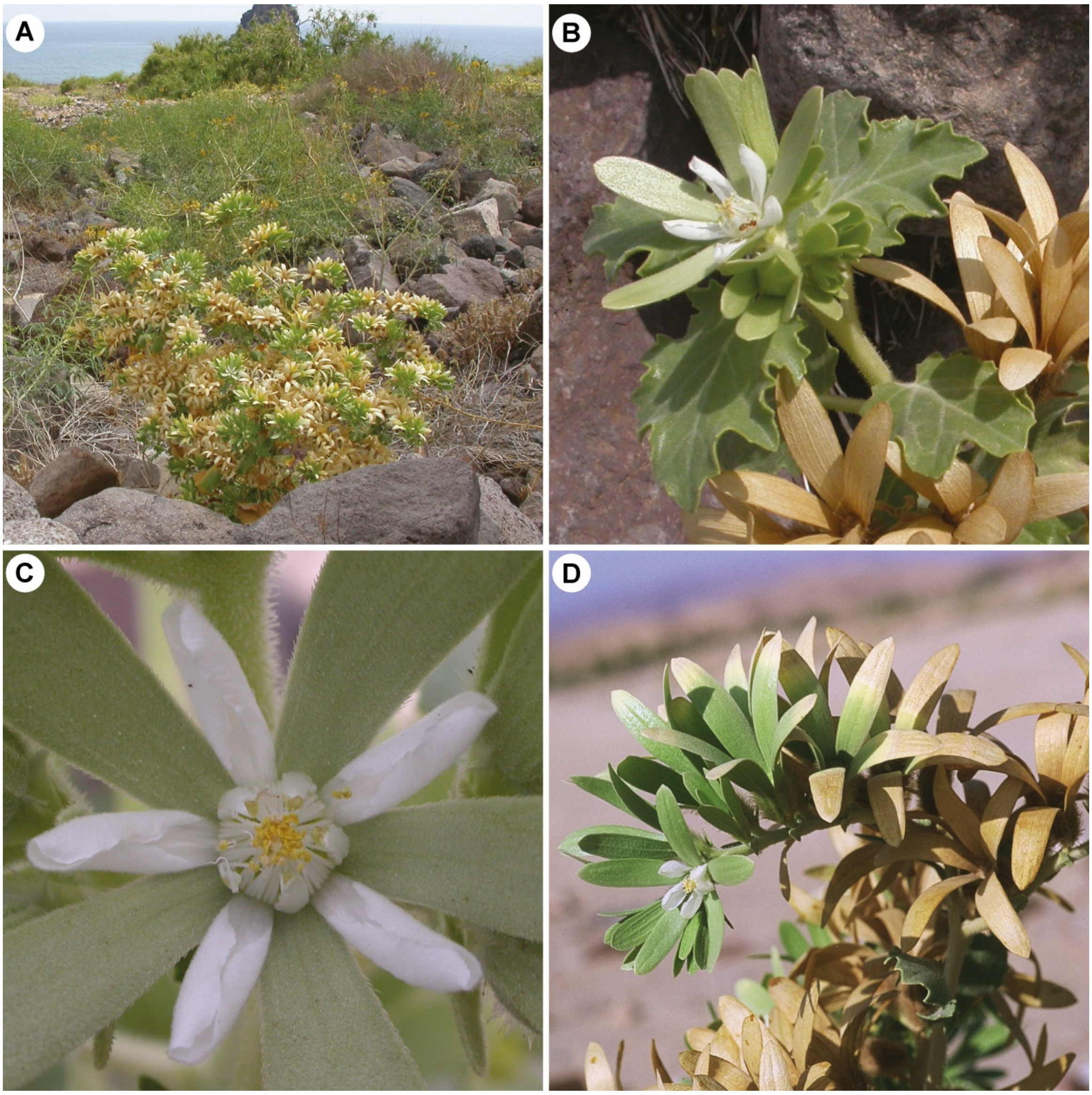

Fig. 3. Morphology of *Kissenia spathulata* from Hadhramaut, Yemen. **A.** Mature shrub, growing in the coastal plain near Mukalla. **B.** Terminal portion of a branch of a plant from Mukalla including a young inflorescence and fully mature, widely ovate leaves. These are usually wider and with shallower lobes than those of fully mature *K. capensis*. **C.** Anthetic flower during its female phase, from near Mukalla. The ligulae in this species have a long, filiform, irregular shaped, apical filaments instead of the lamellar, wider apices of *K. capensis*. **D.** Well-developed inflorescence/infrutescence of a plant growing near Ghayl Ba Wazir. Credits: **A-C.** by Tony Miller. **D.** by Norbert Kilian.

Notes

Dandy (1926: 176) was the first to apply the name *Kissenia spathulata* exclusively to the Arabian-northeast African plants, and we consider that publication to be the valid protologue for this taxon. In this work he designed the specimen BM000944628 from Qishn, and annotated by R. Brown, as the type for this name.

The name *Kissenia arabica* has been attributed to R. Brown (Arnott, 1841; Presl, 1844; Chiovenda, 1929), but we instead found annotations by R. Brown (in BM000944628 and B.65 24 399 both in BM) with the name *Kissenia spathulata*. As far as we know, Arnott (1841) was the first to publish the name "*Fissenia*"*arabica.* This is a *nomen nudum* and as Arnott considered all the populations of the genus as belonging to a single species, we consider the name only a partial synonym of both species of *Kissenia*. Puccioni and Stefanini explored Mijurtinia (Majeerteen Sultanate, which included what is nowadays the Bari Region), close to the border with British Somaliland, during their expedition in 1924 (Clark, 1954; Chelazzi, 2009). Their collection (1022) is the only one mentioned by Chiovenda (1929) in his protologue of *K. arabica* making it the only type material that should be assigned to this name.

Etymology

The epithet alludes to the spathulate, persistent sepals of the species.

Illustrations

Gilbert (1993: Fig. 20; 2000 Fig. 18.1), this work (Fig. 3).

Distribution

This species is found in Yemen (from Aden and Lahij to Al Mahra) and from northeastern Ethiopia (Afar: Gilbert 2000) and northern Djibouti (Obock, Tadjourah) to Northern Somalia (Bari, Sanaag: Chiovenda 1929, Gilbert 1993), at elevations from near sea level to ca. 1100 m (Fig. 4B).

Phenology

This species has been collected with flowers and fruits most months of the year.

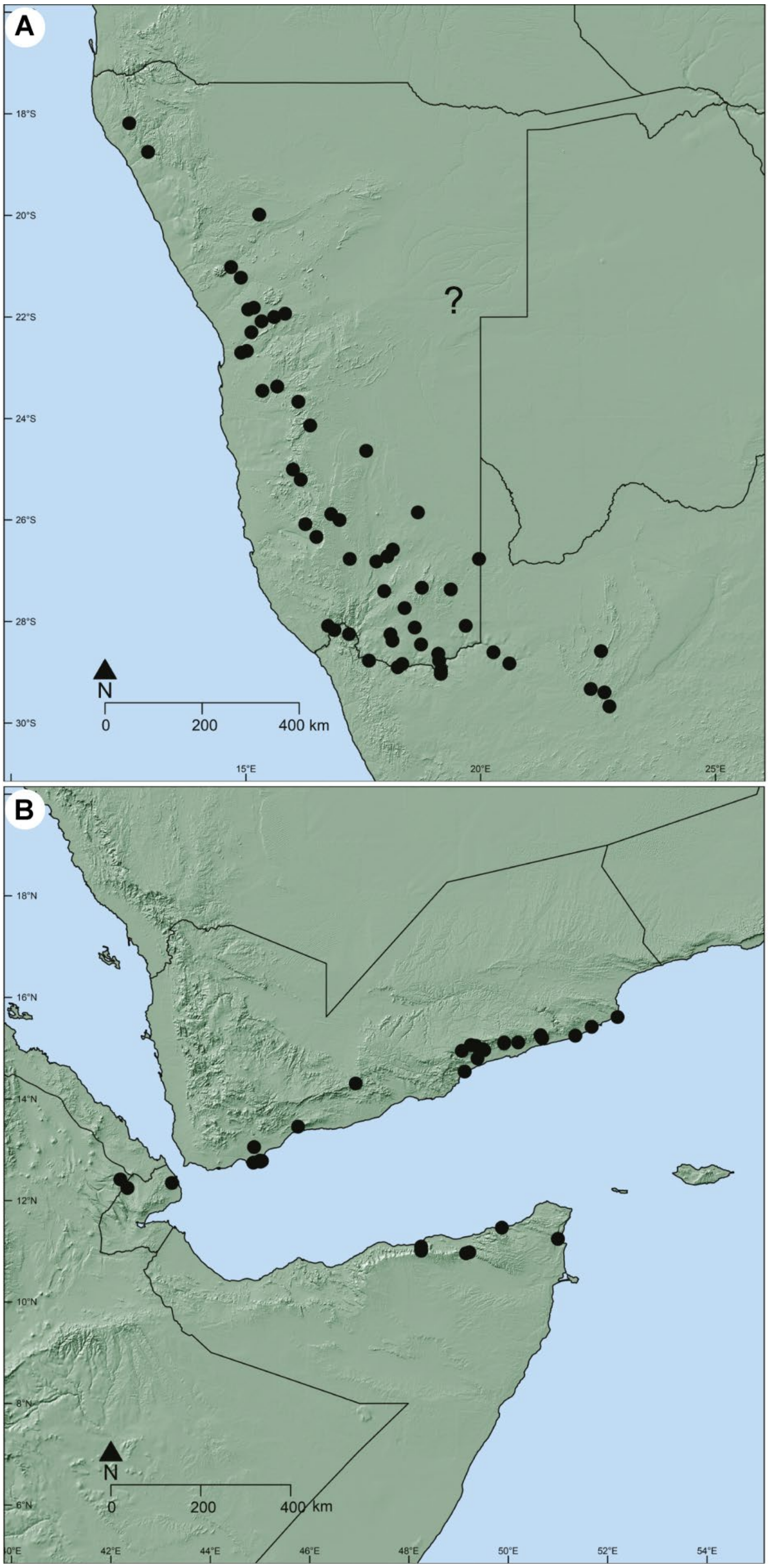

Fig. 4. Distribution maps of *Kissenia* based on the specimens examined for this study. **A.** *Kissenia capensis*. The question mark indicates the uncertain old Hereroland [Omaheke] records, seemingly disjunct from the rest of the range of the species. **B.** *Kissenia spathulata*.

Ecology

The plants of this species grow on a variety of exposed habitats such as cliffs, roadsides, rocky terrain (Fig. 3A) and wadi beds, in regions with high annual average temperatures. It inhabits limestone, granitic and volcanic ash soils sometimes in sympatry with *Tamarix* L. Ants have been observed visiting the flowers (Fig. 3B).

Conservation status

This species seems to be locally frequent in Yemen, even near densely populated areas (e.g. Aden). It appears to have been less frequently collected in the Horn of Africa although it is reported as "very common" in one locality in Ethiopia (cf. *C.F. Hemming 1242* BM, K). This species was not assessed by Al Khulaidi and Miller (2010) nor by the IUCN (2018), due to gaps of our knowledge of this species, specially in the Horn of Africa we consider this species as DD (IUCN 2001).

Specimens examined:

Djibouti**.** OBOCK: Gontoy, 300 m, Feb 1956, *E. Chedeville 1372* (FT).

Djibouti / Ethiopia(?). TADJOURAH / AFAR(?): Inakir, sur cône des scories volcaniques, 800 m, 5 Jan 1957, *E. Chedeville 1746* (FT, P).

Ethiopia. AFAR: Low lava hills, 62 miles from Assab on Dessie Rd, ca. 600 m, 15 May 1957, *C.F. Hemming 1242* (BM, K).

Somalia**.** SANAAG: Vallée du Gueldora (Ouarsanguelis [Warsangali Domains]), 1881, *G. Révoil 50* (P); 10 km S of Las Kiorei [Laasqoray?], stony limestone hill in open scrub, 14 Jan 1973, *P. Bally & R. Melville 15896* (K). BARI: 11 km from Galgallo on road to Bosaso and Karin, in broad wadi bed on sand and limestone pebbles and on hillside, much eroded, 12 Jan 1973, *P. Bally & R. Melville 15878* (K); Karin, nel letto del torrente Dindim, 5 Jul 1924, *N. Puccioni & J. Stefanini 1022[1127]* (FT); Kandala, limestone cliff, beside day tug, ca. 15 m, 2 Set 1957, *J. Newbould 1036* (K); Mountain pass west of

Bargal. Limestone slope, 100–210 m, 25 Nov 1985, *M. Thulin & A.M. Warfa 5513* (E, FT, K).

Yemen. AL MAHRA: Southernmost plateau of the Ras Fartaq, E of Qadifut, expos. E calcareous soil, limestone plateau and slopes, 450 m, 16 Nov 2000, *J. Lavranos 1668* (B); Keschin [Qishn] Bay, s.d., *coll. unknown s.n.* (BM000944628); Jabal Rakhwayt, at Ras W of Itab, S facing rocky slope, 200 m, 17 Nov 2000, *N. Kilian et al. NK6903* (B); 10 km E of Sayhut, on the Jabal Rakhwayt (near the radio station), on rocky embankment, 200 m, 17 Nov 2000, *P. Hein 8277* (B). HADHRAMAUT: Upper Wadi Skoui (Shchawi), on dry slopes, above wadi bed, 320 m, 16 Aug 2002, *N. Kilian et al. YP2767* (B); lower Wadi Skoui (Shchawi), between 15°13'07.7"N, 50°40'56.7"E and 15°11'24.8"N, 50°40'52.8"E. Slopes above wadi bed, 120–170 m, 16 Aug 2000, *N. Kilian et al. YP2715* (B); Lower Wadi Azid al Jabal, c. 25 km N of the town Qusayr (Kosair), gravel in wadi bed, 180 m, 20 Nov 2000, *N. Kilian et al. NK6933* (B); N of Ras Sharma, c. 25 km N of the Al Ghaydah junction, lower part of the village Bidish, common weed in the village, along road, the hedges and the wadi bed, 300 m, 19 Nov 2000, *P. Hein 8286* (B); Middle Wadi Azid al Jabal, c. 25 km N of town Qwasyr (Kosair). Gravelly plain at a gorge-like narrow part of the wadi, 180 m, 20 Nov 2000, *P. Hein 8311* (B); N of Ras Sharma, upper Wadi Bidisch, below the village Howrid, edge of wadi bed on gravel and between rocks, 450 m, 19 Nov 2000, *N. Kilian et al. NK6912* (B); Upper Wadi Araf, immediately below the ascent of the pipeline rd out of the wadi up to the Jol Plateau, narrow valley with running water, sandy places between boulders, 450 m, 18 Set 2001, *N. Kilian et al. YP229* (B); Under Aqabat Arsha, 25 Apr 1947, *W. Thesiger s.n.* (BM); 25 km NE of Ghayl Ba Wazir, middle Wadi Arf 5 km N of beginning of the gorge, on the gravel of the broad wadi, 350 m, 21 Set 1998, *P. Hein 4839* (B); 25 km NE of Ghayl Ba Wazir, middle Wadi Arf 1 km N of the oasis and entrance into the gorge, gravelly wadi bed, 250 m, 21 Set 1998, *N. Kilian & P. Hein NK5023* (B); Gharib, Ba Wazir, Mukalla, 26 Jan 1978, *M. Monod 17487* (P); Granite á Mukalla, 24 Dec 1977, *M. Monod 16518* (P); ditto, 25 Dec 1977, *M. Monod 16565* (P); Ghayl Ba Wazir, somewhat smaller 'Hauma', now almost without water and ground covered with *Tamarix*, 130 m, 6 Mar 2012, *N. Kilian et al. YP1325* (B, P); Common amongst rocks near Mukalla, Jun 1950, *K. Guichard KG/HAD/363* (BM); Mukalla, Jan 1997, *J. Lavranos & Al-Gifri 30774* (WAG); Alrail, ca. 600 m, 28 Dec 1893, *W. Lunt 127* (BM, K, P). SHABWAH: Wadi Yashbum, tributary of Wadi Al-Sa'eid, 25 km SW 'Ataq, large boulders, 1100 m, 26 Jan 1988, *A. Rowaished et al. 2802* (K). SHABWAH (?): Schistes E de Maifah, 17 Jan 1978, *M. Monod 17254* (P). ABYAN:

Jabal Urays, middle Wadi Lobob, w facing slope into the wadi, 300–500 m, 19 Mar 2002, *N. Kilian & C. Oberprieler YP2008* (B). LAHIJ: Lahaj [Lahij], Oct 1927, *R. Moreau 5647* (K). ADEN: s.d. *coll. unknown s.n.* (K); May 1868, *coll. Illegible* (P04588755); Aug 1880, *coll. unknown, comm. B. Balfour* (K); s.d., *P. Sacleur 24* (P); s.d., *E. Krause 15216* (B); 1860, *A. Courbon 339* (K, P); 11 Apr 1861, *Thomson s.n.* (K); 1867, *M. Baudouin* (P); 25 Apr 1865, *O. Beccari s.n.* (FT); Mar 1870, *O. Beccari s.n.* (FT); Dec 1906, *C. D'alleizette* 2568 (L); Basalto, 24 Apr 1876, *C. de Marchesetti s.n.* (FT, K); Lava, ca. 150 m, 22 Mar 1933, *J. Gillett 5516* (K); Aden, on barren rocks near main pass, 12 Jul 1954, *H. Lam 7329* (L); Presqu'île d' Aden, plains d. Mâla [Al Maʻallā?], 5 Mar 1885, *A. Deflers s.n.* (P); Vallée de Kûsaf [Khusaf], 23 Mar 1886, *A. Deflers s.n.* (P); Khusaf valley, ca. 15 m, 1958, *J. Waring 59* (K); Aden Peninsula, Upper Crater, plateau SW of the Tower of Silence, rocky slopes, entrance of grotto, basalt & lava., 120–170 m, 3 Mar 1996, *P. Hein 96-30* (B); ibid., 22 Mar 1997, *P. Hein 3505* (B); Aden Peninsula, Upper Crater, plateau SW of the Tower of Silence, at the foot of a wall of a pit, 120–170 m, 22 Mar 1997, *N. Kilian et al. NK4494* (B); Wadi Goldmohur, igneous rocks, lava and large boulders in the wadi bed, 20–60 m, 6 Jun 1987, *L. Boulos et al. 16525* (BM, K); Gold Muhir [sic] mountains just N of Aden-slope Jebl Shamsan, rocky slope near road, 52 m, 22 Jan 1996, *M. van Slageren & A. Al-Gifri MSAA219* (K); Peninsula Aden, cirque de Schamsân, ad radices boreales montis Schamscham, 2 Dec 1889, *A. Deflers 2* (B, K, P); Gomhor Valley, 22 Apr 1876, *coll. unknown* (P04606349); Wadi Hâalla, 25 Nov 1888, *G. Schweinfurth 27* (BM, K, P); Schugra [Little Aden?], 19 Mar 1881, *G. Schweinfurth 129* (K).

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References

Acuña, R., Fließwasser, S., Ackermann, M., Henning, T., Weigend, M., 2017. Generic re-arrangements in "South Andean Loasas" (Loasaceae). Taxon 66, 365–378.

Al Khulaidi, A.W., Miller, T., 2010. Red List data of Yemen endangered plants. Available from http://ye.chm-cbd.net/implementation/documents/red-list-data-of-yemen-endangered-plants.pdf. Accessed on 17-Aug-2018.

Anderson, T., 1860. Florula Adenensis. Journal of the Proceedings of the Linnean Society of London, Botany 5 (Suppl. 1), 1–43 + 5 pl.

Arnott, G.A.W., 1841. XIX._On some South African Plants. In: Hooker, W.J. (Ed.), The Journal of Botany Vol. 3. Longman, Orme & Co., London, pp. 251–271.

Baillon, H., 1885. Histoire des Plantes Vol. 8ème. Hachette et Cie., Paris, 515 pp.

Barthlott, W., 1983. Biogeography and evolution in Neo- and Paleotropical Rhipsalineae. Sonderbände des Naturwissenschaftlichen Vereins in Hamburg 7, 241–248.

Beier, B.A., Nylander, J.A.A., Chase, M.W., Thulin, M., 2004. Phylogenetic relationships and biogeography of the desert plant genus *Fagonia* (Zygophyllaceae), inferred by parsimony and Bayesian model averaging. Molecular Phylogenetics and Evolution 33, 91–108.

Bellstedt, D., Galley, C., Pirie, M.D., Linder, H.P., 2012. The Migration of the Palaeotropical Arid Flora: Zygophylloideae as an Example. Systematic Botany 37, 951–959.

Bobe, R., 2006. The evolution of arid ecosystems in eastern Africa. Journal of Arid Environments 66, 564–584.

Britten, J., 1894. *Kissenia spathulata*. The Journal of Botany 32, 344.

Brongniart, A., 1860. Sur les resultats relatifs a la botanique obtenus par M. le docteur Alfred Courbon, pendant le cours de une explotration de la Mer Rouge exécutée en 1859-60. Bulletin de la Société Botanique de France 7, 898–906.

Brownrigg, R., 2017. maps: Draw Geographical Maps. R package version 3.2.0 . https://cran.r-project.org/web/packages/maps/index.html

Carlquist, S., 1984. Wood anatomy of Loasaceae with relation to systematics, habit, and ecology. Aliso 10, 583–602.

Carvalho, F.A., Renner, S.S., 2013. A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history. Molecular Phylogenetics and Evolution 65, 46–63.

Chelazzi, L., 2009. Il Museo e le richerche fiorentine in Somalia. In: Barsanti, G., Chelazzi, G. (Eds.), Il Museo di Storia Naturale dell'Università degli Studi di Firenze Volume I Le Colezioni della specola: Zoologia e cere anatomiche. Università degli Studi di Firenze, Florence, pp. 215–252.

Chiovenda, E., 1929. Loasaceae. In: Chiovenda E. (Ed.), Flora Somala 1. Sindacato Italiano Arti Grafiche, Roma, pp. 174–175.

Christenhusz, M.J.M., Chase, M.W., 2012. Biogeographical patterns of plants in the Neotropics – dispersal rather than plate tectonics is most explanatory. Botanical Journal of the Linnean Society 171, 277–286.

Clark, J.D., 1954. The prehistoric cultures of the Horn of Africa. Cambridge University Press, Cambridge, 387 pp.

Codd, L.E., 1976. Loasaceae. In: Ross, J.H. (Ed.), Flora of Southern Africa Vol. 22. Department of Agricultural Technical Services of South Africa, pp. 134–136.

Dandy, J.E., 1926. Notes on *Kissenia* and the geographical distribution of the Loasaceae. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) 1926(4), 176–180.

Dandy, JE., 1966. Further notes on *Kissenia* R. Br. Ex Endl. (Loasaceae). Kew Bulletin 20, 451–453.

Dandy, J.E., Exell, A.W., 1932. Kissen: a mythical traveller in Arabia. The Journal of Botany 70, 198–199.

de Winter, B., 1971. Floristic relationships between the northern and southern arid areas of Africa. Mitteilungen der Botanischen Staatssammlung München 10, 424–437.

deMenocal, P.B., 2004. African climate change and faunal evolution during the Pliocene–Pleistocene. Earth and Planetary Sciences Letters 220, 3–24.

Drège, J.F., 1844. Zwei pflanzengeographische Documente. Leipzig, 230 pp.

Dressler, S., Schmidt, M., Zizka, G., 2014–ongoing. African Plants - A Photo Guide. www.africanplants.senckenberg.de. Forschungsinstitut Senckenberg, Frankfurt/Main, Germany. Accessed Aug-2018.

Dyer, R.A., 1975. The genera of southern African flowering plants Vol. 1 Dicotyledons. Department of Agricultural Technical Services of South Africa, 756 p.

Endlicher, S., 1842. Mantissa Botanica sistens Generum Plantarum Supl. 2. A.F. Beck, Vienna, 114 pp.

Ensikat, H.J., Weigend, M., 2013. Cryo-scanning electron microscopy of plant samples without metal coating, utilizing bulk conductivity. Microscopy and Analysis 27, 7–10.

Fenzl, E., 1841. Darstellung und Erläuterung vier minder bekannter, ihrer Stellung im natürlichen Systeme nach bisher zweifelhaft gebliebener, Pflanzen-Gattungen; gefolgt von einer Abhandlung über die Placentation der ächten und einer Critik der zweifelhaften Bignoniaceen. Denkschriften der Königlich-Bayerischen Botanischen Gesellschaft zu Regensburg 3, 153–270.

Florence, J., 1997. New species of *Plakothira* (Loasaceae), *Melicope* (Rutaceae) and *Apetahia* (Campanulaceae) from the Marquesas Islands. Allertonia 7, 238–253.

Foden, W., Potter, L., 2005. *Kissenia capensis* Endl. National Assessment: Red List of South African Plants version 2017.1. http://redlist.sanbi.org/species.php?species=2033-1 Accessed on 17-Aug-2018.

Glen, H.F., Germishuizen, G., 2010. Botanical exploration of southern Africa. 2nd ed. Strelitzia 26. South African National Biodiversity Institute. Pretoria, 489 pp.

Gilbert, M.G., 1993. 23. Loasaceae. In: Thulin, M. (Ed.), Flora of Somalia Vol. 1 Pteridophyta; Gymnospermae;Angiospermae (Annonaceae-Fabaceae). Royal Botanical Gardens, Kew, p. 37.

Gilbert, M.G., 2000. Loasaceae. In: Edwards, S., Tadesse, M., Demissew, S., Hedberg, I. (Eds.), Flora of Ethiopia and Eritrea Vol. 2 Part 1. Magnoliaceae to Flacourtiaceae. National Herbarium, Addis Ababa, p. 73.

Gilg, E., 1894. Loasaceae. In: Engler, A., Prantl, K. (Eds.), Die natürlichen Pflanzenfamilien III: Teil. 6. Abteilung a. Wilhelm Engelmann, Leipzig, pp. 100–121.

Givnish, T.J., Millam, K.C., Evans, T.M., Hall, J.C., Pires, J.C., Berry, P.E., Sytsma, K.J., 2004. Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American-African disjunctions in Rapateaceae and Bromeliaceae. International Journal of Plant Sciences 165, S35–S54.

Google Inc., 2016. Google Earth Pro ver. 7.1.7.2606. Mountain View. Santa Clara California.

Grau, J., 1997. *Huidobria*, eine isolierte Gattung der Loasaceae aus Chile. Sendtnera 4, 77–93.

Harvey, W.H., 1859. Thesaurus capensis: or illustrations of the South African flora. Vol. I. Hodges, Smith & Co., Dublin, 68 pp + 100 pl.

Harvey, W.H., Sonder, O.W., 1862. Flora capensis. Vol. 2. Hodges, Smith & Co., Dublin, ix + 621 pp.

Hufford, L., 2003. Homology and developmental transformation: models for the origins of the staminodes of Loasaceae subfamily Loasoideae. International Journal of Plant Sciences 164, S409–S439.

Hufford, L., McMahon, M., Sherwood, A., Reeves, G., Chase, M., 2003. The major clades of Loasaceae: phylogenetic analysis using the plastid *mat*K and *trn*L-*trn*F regions. American Journal of Botany 90, 1215–1228.

Hufford, L., McMahon, M.M., O’Quinn, R., Poston, M.E., 2005. A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. International Journal of Plant Sciences 166, 289–300.

Hutchinson, J., 1946. A Botanist in Southern Africa. P.R.Gawthorn Ltd., London, 686 pp.

IUCN., 2001. IUCN Red List Categories and Criteria. Version 3.1. Prepared for the IUCN Species Survival Commission. Gland, Switzerland and Cambridge, United Kingdom, ii + 33 pp.

IUCN., 2018. The IUCN Red List of Threatened Species 2018-1. . http:// http://www.iucnredlist.org/. Accessed on 17-Aug-2018.

Johnson, T.C., Werne, J.P., Brown, E.T., Abbott, A., Berke, M., Steinman, B.A, Halbur, J., Contreras, S., Grosshuesch, S., Deino, A., Scholz, C.A., Lyons, R.P., Schouten, S., Damsté, J.S., 2016. A progressively wetter climate in southern East Africa over the past 1.3 million years. Nature 537, 220–224.

Kingdon, J., 1990. Island Africa: the evolution of Africa's rare animals and plants. Collins, London, 287 pp.

Linder, H.P., 2014. The evolution of African plant diversity. Frontiers in Ecology and Evolution 2, 38.

Loots, S., 2005. Red Data Book of Namibian plants. Southern African Botanical Diversity Network Report No. 38. SABONET, Pretoria and Windhoek, 124 pp. + 24 pl.

Luebert, F., Couvreur, T.L.P., Gottschling, M., Hilger, H.H., Miller, J.S., Weigend, M., 2017. Historical biogeography of Boraginales: West Gondwanan vicariance followed by long-distance dispersal?. Journal of Biogeography 44, 158–169.

Masters, M.T., 1871. Order LXI. Loaseae. In: Oliver, D. (Ed.), Flora of Tropical Africa Vol. II Leguminosae to Ficoideae. L: Reeve & Co., London, pp. 500–501.

Moody, M.L., Hufford, L., Soltis, D.E., Soltis, P.S., 2001. Phylogenetic relationships of Loasaceae subfamily Gronovioideae inferred from *mat*K and ITS sequence data. American Journal of Botany 88, 326–336.

Moonlight. P.W., Richardson, J.E., Tebbit, M.C., Thomas, D.C., Hollands, R., Peng, C.-I., Hughes, M.. 2015. Continental-scale diversification patterns in a megadiverse genus: the biogeography of Neotropical *Begonia*. Journal of Biogeography 42, 1137–1149.

Moonlight, P.W., Ardi, W.H., Arroyo-Padilla, L., Chung, K.-F., Fuller, D., Girmansyah, D., Hollands, R., Jara-Muñoz, A., Kiew, R., Leong, W.-C., Liu, Y., Mahardika, A., Marasinghe, L. D.K., O'Connor, M., Peng, C.-I., Pérez, Á. J., Phutthai, T., Pullan, M., Rajbhandary, S., Reynel, C., Rubite, R. R., Sang, J., Scherberich, D., Shui, Y.-M., Tebbitt, M. C., Thomas, D. C., Wilson, H., Zaini, N. H., Hughes, M., 2018. Dividing and conquering the fastest-growing genus: Towards a natural sectional classification of the mega-diverse genus *Begonia* (Begoniaceae). Taxon 67, 267–323.

Morley, R.J., 2003. Interplate dispersals for megathermal angiosperms. Perspectives in Plant Ecology Evolution and Systematics 6, 5–20.

Müller, S., Salomo, K., Salazar, J., Naumann, J., Jaramillo, M.A., Neinhuis, C., Feild, T.S., Wanke, S., 2015. Intercontinental long-distance dispersal of Canellaceae from the New to the Old World revealed by a nuclear single copy gene and chloroplast loci. Molecular Phylogenetics and Evolution 84, 205–219.

Pennington, R.T., Dick, C.W., 2004. The role of immigrants in the assembly of the South American rainforest tree flora. Philosophical Transactions of the Royal Society B Biological Sciences 359, 1611–1622.

Pessoa, E.M., Viruel, J., Alves, M., Bogarín, D., Whitten, W.M., Chase, M.W., 2018. Evolutionary history and systematics of *Campylocentrum* (Orchidaceae: Vandeae: Angraecinae): a phylogenetic and biogeographical approach. Botanical Journal of the Linnean Society 186, 158–178.

Pokorny, L., Riina, R., Mairal, M., Meseguer, A.S., Culshaw, V., Cendoya, J., Serrano, M., Carbajal, R., Ortiz, S., Heuertz, M., Sanmartín, I., 2015. Living on the edge: timing of Rand Flora disjunctions congruent with ongoing aridification in Africa. Frontiers in Genetics 6, 154.

Poston, M.E., Thompson, H.J., 1977. Cytotaxonomic observations in Loasaceae subfamily Loasoideae. Systematic Botany 2: 28–35.

Presl, C.B., 1844. Botanische Bemerkungen. Gottlieb Haase Söhne, Prague, 154 pp.

R Core Team., 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Révoil, G., 1882. La vallée du Darror: voyage aux pays çomalis (Afrique orientale). Challamel Ainé, Libraire-Éditeur, Paris, 388 pp.

Roessler, H., 1968. 93. Loasaceae. In: Merxmüller, H. (Ed.), Prodromus einer Flora von Südwestafrika Fam. 68–122.J. J. Cramer, Lehre.

Slik, J.W.F., Franklin, J., Arroyo-Rodríguez, V., Field, R., Aguilar, S., Aguirre, N.,..(+ 181 additional authors), Zang, R. 2018. Phylogenetic classification of the world's tropical forests: Proceedings of the National Academy of Sciences 115, E3067.

Thiv, M., van der Niet, T., Rutschmann, F., Thulin, M., Brune, T., Linder, H.P. 2011. Old-New World and trans-African disjunctions of *Thamnosma* (Rutaceae): intercontinental long-distance dispersal and local differentiation in the succulent biome. American Journal of Botany 98, 76–87.

Thulin, M., Razafimandimbison, S.G., Chafe, P., Heidari, N., Kool, A., Shore, J.S., 2012. Phylogeny of the Turneraceae clade (Passifloraceae s.l.): Trans-Atlantic disjunctions and two new genera in Africa. Taxon 61, 308–323.

Urban, I., Gilg, W., 1900. Monographia Loasacearum. Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum. 76: 1–384.

Weigend, M., 1997. *Nasa* and the conquest of South America: Systematic rearrangements in Loasaceae Juss. PhD Thesis, Ludwig-Maximilians-Universität München, Munich.

Weigend, M., 2004. Loasaceae. In: Kubitzki, K. (Ed.), The Families and Genera of Vascular Plants VI. Flowering Plants-Dicotyledons: Celastrales, Oxalidales, Rosales, Cornales, Ericales. Springer. Berlin, pp. 239–254.

Weigend, M., Gottschling, M., Hoot, S., Ackermann, M., 2004. A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on trnL (UAA) sequence data, with consequences for systematics and historical biogeography. Organisms Diversity and Evolution 4, 73–90.

CHAPTER 5

An unusual disjunction in Loasaceae: Central American *Chichicaste grandis* is nested in Brazilian *Aosa*[i]

Inflorescence branch of *Aosa grandis*. Plants of this species could be amongst the tallest of all loasoids, and one of the few that inhabits low elevation permanently humid habitats.

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An unusual disjunction in Loasaceae: Central American *Chichicaste grandis* is nested in Brazilian *Aosa*

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Abstract

The highly distinctive genus *Chichicaste* is restricted to the Costa Rica-Chocó Biogeographic Hotspsot from Costa Rica to northwestern Colombia and comprises a single species, *C. grandis*. Relationships of this taxon have been doubtful in the absence of critical morphological analyses (particularly of living plants) and convincing molecular data. The present study aims at identifying the phylogenetic relationships of *C. grandis* using molecular and morphological data to establish its relationships. Our molecular data set includes four plastid markers (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) and the nuclear marker ITS for 38 in-group taxa, including all of the currently recognized genera of the Loasoideae clade, and six out-group taxa from non-Loasoideae Loasaceae and Hydrangeaceae. The dataset was analyzed using Maximum Likelihood and Bayesian Inference approaches. The plastid and nuclear trees were mostly congruent, with their respective ML best and BI strict consensus trees showing no significant differences in their topologies. *Chichicaste* is nested in *Aosa* series *Parviflorae* and sister to *A. plumieri* from Hispaniola, thus representing northern and western outliers of this otherwise strictly Brazilian genus. A critical morphological re-examination indicates that considering *C. grandis* as part of *Aosa* is plausible, in spite of the ecological and morphological differences between the two taxa. Based on these results the genus *Chichicaste* is synonymized with *Aosa* and the required new combination is provided. An amended key for an expanded *Aosa,* is also presented.

Key words: Brazil, Central America, Cornales, floral morphology, Hispaniola, *Nasa panamensis*, new combination, primary wet forest, systematic relationships, trichomes

Introduction

Loasaceae is a primarily New World family of plants with most of its species belonging to the mostly South American subfamily Loasoideae (Weigend 1997, 2004), which is particularly species rich in the Andean region (Weigend 1997, Acuña *et al.* 2017). Phylogenetic studies have been able to identify the major clades in the group (Hufford *et al.* 2003, 2005, Weigend *et al.* 2004) and certain open questions about the relationships of some major clades have been clarified recently (Acuña *et al.* 2017). Southern Central America has only six species of Loasoideae (Weigend 2002, 2011, Morales 2007). One of the most enigmatic and morphologically distinctive species is *Chichicaste grandis* (Standl.) Weigend (2006: 465), representing one of the tallest plants in the entire family and the only one in the region whose primary habitat is the undergrowth of low-elevation, primary, wet to rain forest (mostly <1000 m; Weigend 1997, 2001). This taxon was originally described as *Loasa grandis* Standley (1927: 12) and was included in the, originally broadly defined, genus *Loasa* Adanson (1763: 501), that has since been shown to be paraphyletic with respect to *Blumenbachia* Schrader (1805: 1706), *Scyphanthus* Sweet (1828: tab. 238) and *Caiophora* Presl (1831: 41, tab. 56) as Weigend (1997) and Hufford *et al.* (2005) point out. *Loasa grandis* was later placed into a monotypic genus, *Chichicaste* Weigend (2006: 464–465), based on its distinctive morphology and ecology. Early molecular data could not satisfactorily resolve its position in Loasaceae (Weigend *et al.* 2004). Hufford *et al.* (2005) retrieved *Chichicaste grandis* in a highly supported, but internally poorly resolved clade including some species of *Aosa* Weigend (2006: 464). *Aosa* was segregated from *Loasa* based on morphology (Weigend 1997, 2006), but has been poorly represented in published molecular studies. The present study aims at clarifying the affinities of *Chichicaste* in tandem with an

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improved phylogenetic resolution of the genus *Aosa* using molecular data and morphological observations on living plants. Additionally, we provide evidence of morphological traits that help distinguish sterile material of *C. grandis* from vegetatively similar *Nasa panamensis* Weigend in Weigend *et al.* (2006: 78).

Material and methods

The sampling for the molecular phylogenetic studies includes one accession each for all accepted species of *Aosa* except *Aosa gilgiana* (Urb.) Weigend (2006: 464) and two accessions from different populations of *Chichicaste grandis* from Costa Rica. Based on Weigend *et al.* (2004), Hufford *et al.* (2005) and Acuña *et al.* (2017), *Deutzia discolor* Hemsl. in Forbes & Hemsley (1887: 275) *Eucnide urens* Parry ex Coville (1893: 109), *Gronovia scandens* Linnaeus (1753: 202), two species of *Mentzelia* Linnaeus (1753: 516) and *Philadelphus pekinensis* Rupr. in Maack & Ruprecht (1857: 365) were chosen as outgroups, while all Loasoideae taxa were considered as the ingroup. This sampling includes all currently recognized genera and most major infrageneric clades of Loasaceae subfamily Loasoideae. All vouchers, including GenBank accession numbers, are listed in Table 1.

DNA was extracted from samples of silica gel dried leaves (Chase & Hills 1991) or herbarium leaf material with a modified CTAB method (Doyle & Doyle 1987). We sequenced the plastid regions *trn*L-*trn*F, *mat*K, the *trn*S-*trn*G intergenic spacers, and the *rps*16 intron as well as the ITS region of the nuclear genome. Taxon sampling overlaps for all markers except for ITS, not obtained from *Blumenbachia scabra* (Miers) Urban (1889: 219), *Eucnide urens*, *Huidobria chilensis* Gay (1847: 440) and *Xylopodia klaprothioides* Weigend (2006: 467). The amplification and sequencing protocols for the molecular markers employed are explained in Acuña *et al.* (2017). The *trn*L-*trn*F sequence of *Aosa plumieri* (Urb.) Weigend (2006: 464) was obtained from GenBank (Hufford *et al.* 2005).

Sequences were assembled in Geneious v. 8.0.1 (Kearse *et al.* 2012) using the default De Novo assemble settings. The assembled sequences were aligned in Mafft v. 7 (Katoh & Standley 2013). For the plastid sequences we used the G-INS-1 option, recommended for medium scale (up to a few hundred sequences) alignments and with relatively lower instability than other alignment methods (Boyce *et al*. 2015). For the ITS sequences we used the Q-INS-I option, which takes into account the secondary structure of the rRNA and is recommended by Chomicki & Renner (2015). Manual adjustments were then done using PhyDE v. 0.9971 (Müller *et al.* 2010). Alignment files are available from the corresponding author on request. Phylogenetic reconstructions were carried out employing Maximum Likelihood (ML; Felsenstein 1981) in RAxML v. 8.1.X (Stamatakis 2014a), included in RAxMLGUI v. 1.5b1 (Silvestro & Michalak 2012), and Bayesian Inference (BI; Mau *et al.* 1999), conducted in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001) implemented in the CIPRES Science Gateway (Miller *et al.* 2010). FindModel (available from http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html), which implements Posada & Crandall's (2001) Modeltest, based on the Akaike information criterion, selected GTR+Gamma as the substitution model that best fits all four plastid markers and HKY+Gamma as the model that best fits ITS. However, we consistently used GTR+Gamma for all datasets and analyses for two reasons: first, RAxML v.8.1.X (Stamatakis 2014b) only implements GTR-based models for nucleotide substitution; second, the HKY model is identical to GTR in its base frequencies at equilibrium, differing in having a fixed transition/transversion ratio, while GTR-based models have a specific ratio for each kind of base substitution (Posada & Crandall 2001). In addition, Stamatakis (2014b) argues that the danger of over-parameterizing large datasets employing GTR-based, instead of simpler models, is comparatively low. Each marker was at first analyzed separately. In the absence of significant topological conflict [defined as incongruence of topologies in nodes with bootstrap support (BS) ≥75] the plastid markers were combined. We found significant topological conflict between the ITS and the combined plastid trees in four nodes [two nodes in *Caiophora* and two nodes in *Presliophytum* (Urb. & Gilg) Weigend (2006:467)] so, the datasets were not combined. Both ML and BI analyses were then run independently for the combined plastid dataset (partitions unlinked) and for ITS. The statistical support for the nodes was assessed by 1000 ML thorough bootstrap replicates in 100 runs. The BI were conducted with four independent runs with one cold and three heated chains; the Markov chain had a length of 10 million generations, sampled every 1000 generations. After convergence was assessed in Tracer 1.5 (Rambaut & Drummond 2007), the first 2.5 million generations were discarded as burn-in. Support was assessed as follows: Low = BS: <75, Posterior Probability (PP): <0.90; Moderate = BS: ≥75–<90, PP: ≥0.90–<0.95; High = BS: ≥90, PP: ≥0.95.

TABLE 1. List of taxa sampled for the molecular analyses of this study with their respective voucher specimen (herbaria acronyms in parentheses), geographic origins and GenBank accession numbers. Dashes (-) indicate missing data.

Taxon	Voucher	Country of Origin	GenBank Codes				
			*trn*L-*trn*F	*mat*K	*trnS*-*trn*G	*rps*16	ITS
Aosa parviflora (Schrad. ex DC.) Weigend	*Grant 4650* (BSB)	Brazil	KY286967	KY286698	KY286877	KY286787	KY286615
Aosa plumieri (Urb.) Weigend	*Acevedo-Rodríguez et al. 13065* (US)	Dominican Republic	-	MF972102	MF972131	MF972112	MF972092
Aosa plumieri (Urb.) Weigend	*Moody et al. 47* (JBSD)	Dominican Republic	AY254225	-	-	-	-
Aosa rostrata (Urb.) Weigend	*Salino 3042* (M)	Brazil	KY286948	KY286679	KY286858	KY286768	KY286598
Aosa rupestris (Gardner) Weigend	*Weigend 7138* (BSB)	Brazil	KY286925	KY286657	KY286835	KY286745	KY286581
Aosa sigmoidea Weigend	*Guedes & al. 10360* (ALCB)	Brazil	KY286995	KY286725	KY286905	KY286815	KY286639
Aosa uleana (Urb. & Gilg) Weigend	*Joßberger 342* (BONN)	Brazil	KY286998	KY286728	KY286908	KY286818	KY286641
Blumenbachia catharinensis Urb. & Gilg	*Trevisan 1723* (BONN)	Brazil	KY287006	KY286736	KY286916	KY286826	KY286648
Blumenbachia scabra (Miers) Urb.	*Lombardi & Saka 8631* (UPCB)	Brazil	MF972121	MF972101	MF972130	MF972111	-
Blumenbachia sylvestris Poepp.	*Weigend & al. 6807* (BSB)	Argentina	KY286919	KY286651	KY286829	KY286739	KY286577
Caiophora arechavaletae (Urb.) Urb.	*Weigend 9330* (BSB)	Brazil	KY286970	KY286701	KY286880	KY286790	KY286618
Caiophora cirsiifolia C.Presl	*Ackermann 610* (BSB)	Peru	KY286984	KY286715	KY286894	KY286804	KY286632
Caiophora hibiscifolia (Griseb.) Urb. & Gilg	*Ackermann 1103* (BONN)	Argentina	KY286988	KY286719	KY286898	KY286808	KY286634
Caiophora pterosperma (Ruiz & Pav. ex G.Don) Urb. & Gilg	*Weigend & al. 5484* (BSB)	Peru	KY286940	KY286672	KY286850	KY286760	KY286592
Caiophora rusbyana Urb. & Gilg ex Rusby	*Schlumpberger & Brokamp 627* (BSB)	Bolivia	KY287003	KY286733	KY286913	KY286823	KY286645
Chichicaste grandis a (Standl.) Weigend	*Acuña et al. 1223* (USJ)	Costa Rica	MF972118	MF972098	MF972127	MF972108	MF972089
Chichicaste grandis b (Standl.) Weigend	*Acuña et al. 1264* (USJ)	Costa Rica	MF972120	MF972100	MF972129	MF972110	MF972091
Deutzia discolor Hemsl.	*Weigend 5615* (BSB)	Germany (cultivated)	KY286929	KY286661	KY286839	KY286749	KY286584
Eucnide urens Parry ex Coville	*Weigend 9153* (BSB)	United States	KY286996	KY286726	KY286906	KY286816	-
Grausa micrantha (Poepp.) Weigend & R.H.Acuña	*Grau s.n.* (M)	Chile	KY286957	KY286688	KY286867	KY286777	KY286607
Gronovia scandens L.	*Weigend et al. 8522* (BSB)	Peru	KY286997	KY286727	KY286907	KY286817	KY286640
Huidobria chilensis Gay	*Ackermann 490* (BSB)	Chile	KY286931	KY286663	KY286841	KY286751	-
Huidobria fruticosa Phil.	*Dillon 8034* (F)	Chile	KY286932	KY286664	KY286842	KY286752	KY286586

...continued on next page

TABLE 1. (Continued)

Taxon	Voucher	County of origin	GenBank Codes				
			*trn*L-*trn*F	*mat*K	*trn*S-*trn*G	*rps*16	ITS
Kissenia capensis Endl.	*Greuter 21627* (B)	South Africa	KY286944	KY286675	KY286854	KY286764	KY286596
Klaprothia fasciculata (C.Presl) Poston	*Weigend et al. 5362* (BSB)	Peru	MF972115	MF972095	MF972124	MF972105	MF972086
Loasa acanthifolia Desr.	*Weigend et al. 6924* (M)	Argentina	KY286959	KY286690	KY286869	KY286779	KY286609
Loasa acerifolia Dombey ex Juss.	*Weigend et al. 6848* (M)	Argentina	KY286937	KY286669	KY286847	KY286757	KY286589
Loasa elongata Hook. & Arn.	*Weigend 9333* (BSB)	Chile	KY287000	KY286730	KY286910	KY286820	KY286642
Loasa floribunda Hook. & Arn.	*Weigend et al. 5937* (BSB)	Chile	KY286951	KY286682	KY286861	KY286771	KY286601
Loasa heterophylla Hook. & Arn.	*Weigend et al. 5920* (BSB)	Chile	KY286930	KY286662	KY286840	KY286750	KY286585
Mentzelia albescens (Gillies ex Arn.) Benth. & Hook.f. ex Griseb.	*Weigend et al. 6865* (BSB)	Argentina	KY286921	KY286653	KY286831	KY286741	KY286579
Mentzelia aspera L.	*Weigend et al. 8421* (B)	Peru	MF972116	MF972096	MF972125	MF972106	MF972087
Nasa macrothyrsa (Urb. & Gilg) Weigend	*Weigend et al. 97/s.n.* (M)	Peru	KY286934	KY286666	KY286844	KY286754	KY286588
Nasa pongalamesa Weigend	*Weigend et al. 2000/752* (BSB)	Peru	MF972114	MF972094	MF972123	MF972104	MF972085
Nasa raimondii (Standl. & F.A.Barkley) Weigend	*Weigend & Weigend 2000/289* (HUSA)	Peru	MF972113	MF972093	MF972122	MF972103	MF972084
Nasa speciosa (Donn. Sm.) Weigend	*Acuña et al. 1261* (USJ)	Costa Rica	MF972119	MF972099	MF972128	MF972109	MF972090
Nasa triphylla subsp. *rudis* (Benth.) Weigend	*Acuña et al. 1211* (BONN)	Costa Rica	MF972117	MF972097	MF972126	MF972107	MF972088
Philadelphus pekinensis Rupr.	*Weigend 5614* (BSB)	Germany (cultivated)	KY286927	KY286659	KY286837	KY286747	KY286583
Pinnasa bergii (Hieron.) Weigend & R.H.Acuña	*Weigend 6991* (BSB)	Argentina	KY286960	KY286691	KY286870	KY286780	KY286610
Pinnasa pinnatifida (Gillies ex Arn.) Weigend & R.H.Acuña	*Weigend et al. 6880* (BSB)	Argentina	KY286942	KY286673	KY286852	KY286762	KY286594
Plakothira parviflora J.Florence	*Weigend s.n.* (BSB)	France (Marquesas Islands)	KY286926	KY286658	KY286836	KY286746	KY286582
Presliophytum incanum (Graham) Weigend	*Weigend & Förther 97/848* (F)	Peru	KY286924	KY286656	KY286834	KY286744	KY286580
Presliophytum malesherbioides (Phil.) R.H.Acuña & Weigend	*Wagenknecht 18509* (M)	Chile	KY286933	KY286665	KY286843	KY286753	KY286587
Presliophytum sessiliflorum (Phil.) R.H.Acuña & Weigend	*Ehrhardt s.n.* (M)	Chile	KY286945	KY286676	KY286855	KY286765	KY286597
Scyphanthus elegans Sweet	*Grau & Ehrhardt 2-093* (M)	Chile	KY286958	KY286689	KY286868	KY286778	KY286608
Xylopodia klaprothioides Weigend	*Weigend & al. 97/450* (M)	Peru	KY286923	KY286655	KY286833	KY286743	-

Traditional morphological studies including the use of stereomicroscopes, were carried out on, to our knowledge, every known preserved specimen of *Chichicaste grandis* and *Nasa panamensis* including the types of both species. The material is deposited in BONN, CR, F, MEDEL, MO, P, PMA, U, US and USJ. Observations of living plants in the field were done in Turrialba and Quepos, Costa Rica in December 2015, January 2016 and March 2017.

To analyze the microstructure details of fully mature and expanded foliar surfaces we used herbarium material of *Chichicaste grandis* from *R. Acuña et al. 1223* (BONN) and fresh material from cultivated plants of *Aosa uleana* (Urb. & Gilg) Weigend (2006: 464), *T. Joßberger 343* (BONN). *Nasa panamensis* was also studied and compared to *C. grandis*, using preserved material from *T. Antonio 2885* (MO). Seed micromorphology was studied for *C. grandis*, *A. parviflora* (Schrad. ex DC.) Weigend (2006: 464) and *A. uleana* using samples from *R. Acuña et al. 1747* (USJ), *T. Joßberger 1213* (BONN) and *T. Joßberger 343* (BONN), respectively. All samples were sputter coated lightly with Pd for about 20 seconds in a SCD040 (Balzers Union, Liechtenstein) in order to increase the electrical conductivity. Cryo scanning electron microscopy was used in fresh leaf material in order to avoid drying artifacts; the equipment and techniques employed are described by Ensikat & Weigend (2013).

Results

Our analyses of both plastid and ITS datasets, reveal no significant topological conflict in the *Aosa*-*Chichicaste* clade (Fig. 1). *Chichicaste grandis* is nested in a highly supported *Aosa* (plastid BS: 100, PP: 1.00; ITS BS: 98, PP: 1.00). Plastid markers retrieve *Aosa uleana* as sister to the remaining species (Fig. 1), while the ITS tree shows low support (BS: 66, PP: 0.85) for a relationship as sister to *A. rostrata* (Urb.) Weigend (2006: 464) + *A. sigmoidea* Weigend in Weigend *et al*. (2006: 71) The latter two species are retrieved in a well-supported clade (plastid BS: 92, PP: 1.00; ITS BS: 100, PP: 1.00) corresponding to *Aosa* series *Pusillae* (Urb. & Gilg) Weigend (2006: 464). The rest of the species of the genus, are included in an expanded *Aosa* series *Parviflorae* (Urb. & Gilg) Weigend (2006: 464), with *C. grandis* nested within. This clade has rather low BS in the plastid tree (68) but high PP support (0.99) as well as high support in the ITS tree overall (BS: 99, PP: 1.00). Interestingly when *A. plumieri* is excluded from the plastid ML analyses, the BS for *Aosa* series *Parviflorae* (including *C. grandis*) increases significantly (BS: 95, data not shown). The plastid marker analyses show that series *Pusillae* and expanded *Parviflorae* are sister to each other with high support (BS: 93, PP: 1.00), unlike the ITS tree that does not retrieve such a relationship. Our results show that *C. grandis* is sister to *A. plumieri* with varying support values in different analyses, ranging from low to moderate in the plastid tree (BS: 70, PP: 0.94) and moderate to high in the ITS tree (BS: 81, PP: 0.99). These two species are sister to a generally well supported *A. parviflora* + *A. rupestris* (Gardner) Weigend (2006: 464) clade (plastid BS: 78, PP: 1.00; ITS BS: 94, PP: 100). In both trees, *Aosa* is sister to *Presliophytum* + "South Andean Loasas" [sensu Acuña *et al.* (2017)], with the entire clade being highly supported by plastid (BS: 98, PP: 1.00) and ITS (BS: 100. PP: 1.00) evidence. *Aosa* + *Presliophytum* + "South Andean Loasas" are sister to *Nasa* Weigend (2006: 465) all together constituting a generally highly supported clade (plastid BS: 98, PP: 1.00; ITS BS: 67, PP: 0.96)

Morphologically *Chichicaste grandis* is among the largest species of Loasaceae, both in terms of absolute plant size and in the size of its organs. In the wild, plants have stems up to ca. 4 m long and 7 cm in diameter (the largest stems sometimes held almost horizontal for most of their length, with adventitious roots often developing from the nodes). The species inhabits the understory of evergreen wet forests, close to forested streams or more rarely, roadsides (Fig. 2A). The leaves are lobed as in *Aosa* and other Loasoideae (Figs. 2D, G), usually very large, reaching lengths up to ca. 50 cm, as are the bracteate inflorescences that can grow to more than 1 m long. Flowers are pendent with a bowl-shaped, green corolla of almost the same color as the calyx (Fig. 2B) and the nectar scales accumulate nectar as droplets, easily visible to the naked eye at least during the afternoon. Meanwhile, *Aosa* species have mostly white corollas (Figs. 2E, H) and produce tiny amounts of nectar, often invisible to the naked eye. In the wild, we saw flowers being visited by unidentified short tongued Hymenoptera. Contrasting with the large size of the plants, the seeds are just 1 mm long and thus smaller than those of *Aosa uleana* or *A. parviflora* (Figs. 2C, F, I). The seeds are reticulate and etuberculate, similar to those of *A. uleana*, while *A. parviflora* (like most *Aosa* species) has tuberculate seeds.

All specimens examined have hypostomatic leaves, so distinguishing foliar surfaces is straightforward. The trichome cover on the adaxial, intercostal surface of the leaves of *Chichicaste grandis* is dominated by short, pustule-shaped scabrid trichomes in adult plants (accompanied by longer, conic or falcate scabrid trichomes) and comparatively few (relative to other Loasaceae) stinging, uniseriate-glandular and glochidiate trichomes, mostly or entirely restricted to the veins (Fig. 3A). The glandular trichomes are small, 2–4 cells long, collapsing and becoming often unrecognizable in dry material. The abaxial foliar surface has relatively few glochidiate and scabrid trichomes in fully mature leaves, and these are mostly restricted to the veins (Fig. 3B). In *Aosa uleana* the trichomes on each leaf surface are similar to those of the respective leaf surfaces of *C. grandis* (Figs. 3E, F), however, no short, pustule-shaped scabrid trichomes have been observed in the former (Fig. 3E). The vegetatively similar *Nasa panamensis* differs notably from *C. grandis* by the presence of numerous smooth-shafted glochidiate trichomes on the intercostal areas of the adaxial foliar surface, at the same time lacking the short, pustule-shaped scabrid trichomes of *C. grandis* (Fig. 3C). The vein areas are covered by what appear to be collapsed, uniseriate-glandular trichomes as well as relatively few, large scabrid and stinging trichomes. The abaxial foliar surface trichomes are similar to both *C. grandis* and *A. uleana* (Fig. 3D).

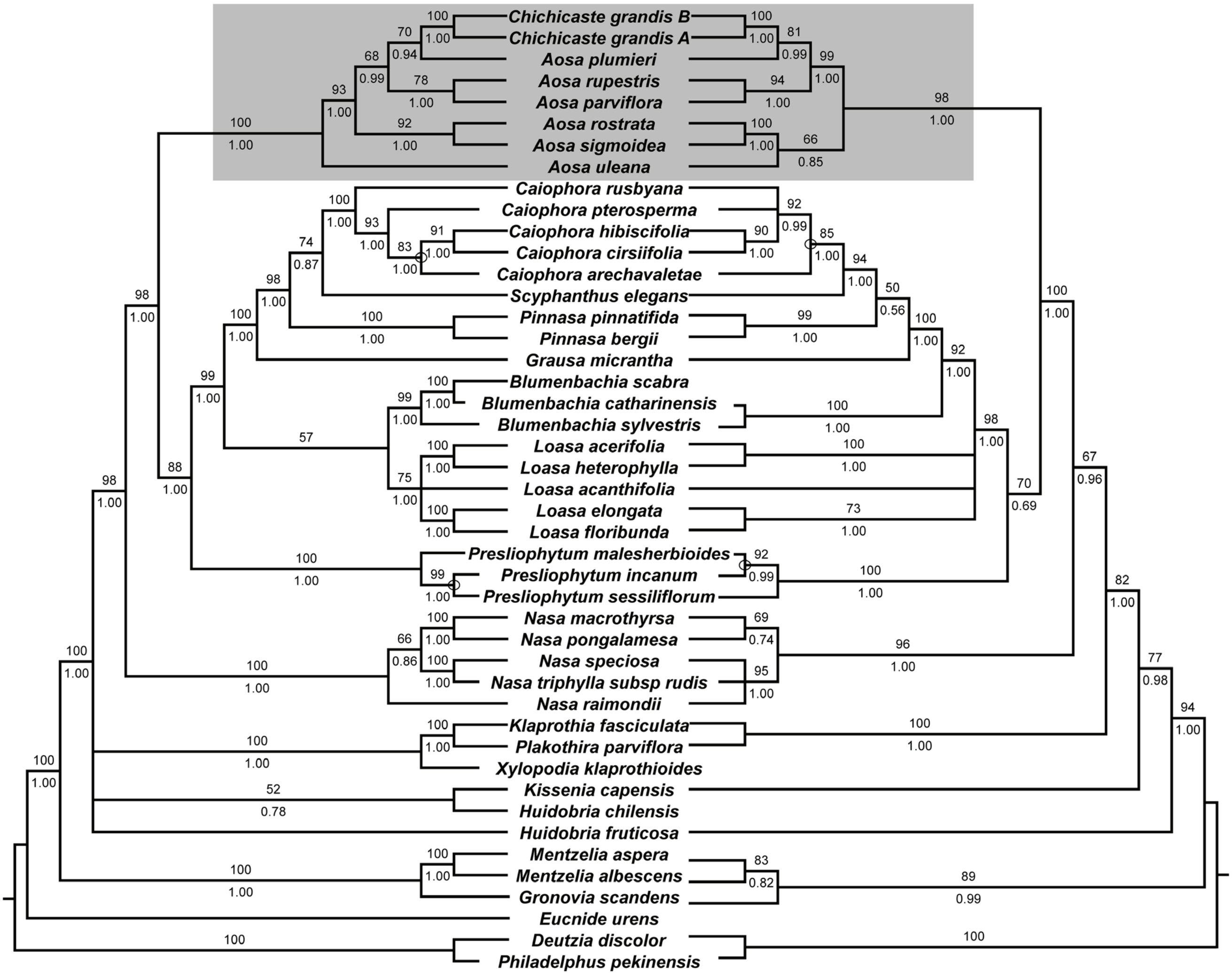

FIGURE 1.Maximum likelihood trees focusing on *Aosa* and related taxa. Left: Concatenated plastid marker dataset tree (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G, *rps*16). Right: ITS dataset tree. The values for Bootstrap support under Maximum Likelihood and Bayesian Posterior Probabilities are above and below the respective branch (only values above 50 and 0.5, respectively, are shown). The grey rectangle marks the *Aosa-Chichicaste* clade. The small circles mark the nodes with significant topological conflict between trees. Branch length is irrelevant in this figure.

Discussion

Our molecular data unequivocally retrieve *Chichicaste grandis* nested in *Aosa* as currently defined. Hufford *et al.* (2005) were the first to show evidence of this relationship, obtaining *Aosa rostrata* as sister to *C. grandis* with moderate support. Our analyses, with an expanded sampling, and including, critically, the type species of the genus *A. parviflora*, indicate that *Chichicaste* is nested in *Aosa* series *Parviflorae*, which does not include *A. rostrata*. *Aosa* series *Parviflorae* is composed of two clades with two species each: Brazilian A. *parviflora* and *A. rupestris* on one hand and Caribbean-Central American taxa *A plumieri* and *C. grandis* on the other. *Aosa plumieri* has been considered allied to series *Parviflorae* since its description (Urban 1910), although it has striking differences in its fruit and floral scale morphology (Weigend 1997, 1999). In general, our molecular data results are in agreement with the subgeneric division of *Aosa* based on morphology (Weigend 1997, 1999).

According to Weigend (1997, 2004) *Chichicaste. grandis* differs from *Aosa* most notably by the bracteate (vs. ebracteate) inflorescences, erect (vs. pendent) flowers, large bowl shaped (vs. small, star shaped) corollas, nectar scales not contrasting (vs. contrasting) in color with the corolla, nectar scales without (vs. with) dorsal filaments, five (vs. three) placentae in the ovary, pendent (vs. erect) capsules and etuberculate (vs. tuberculate) seed testas (Fig. 2).

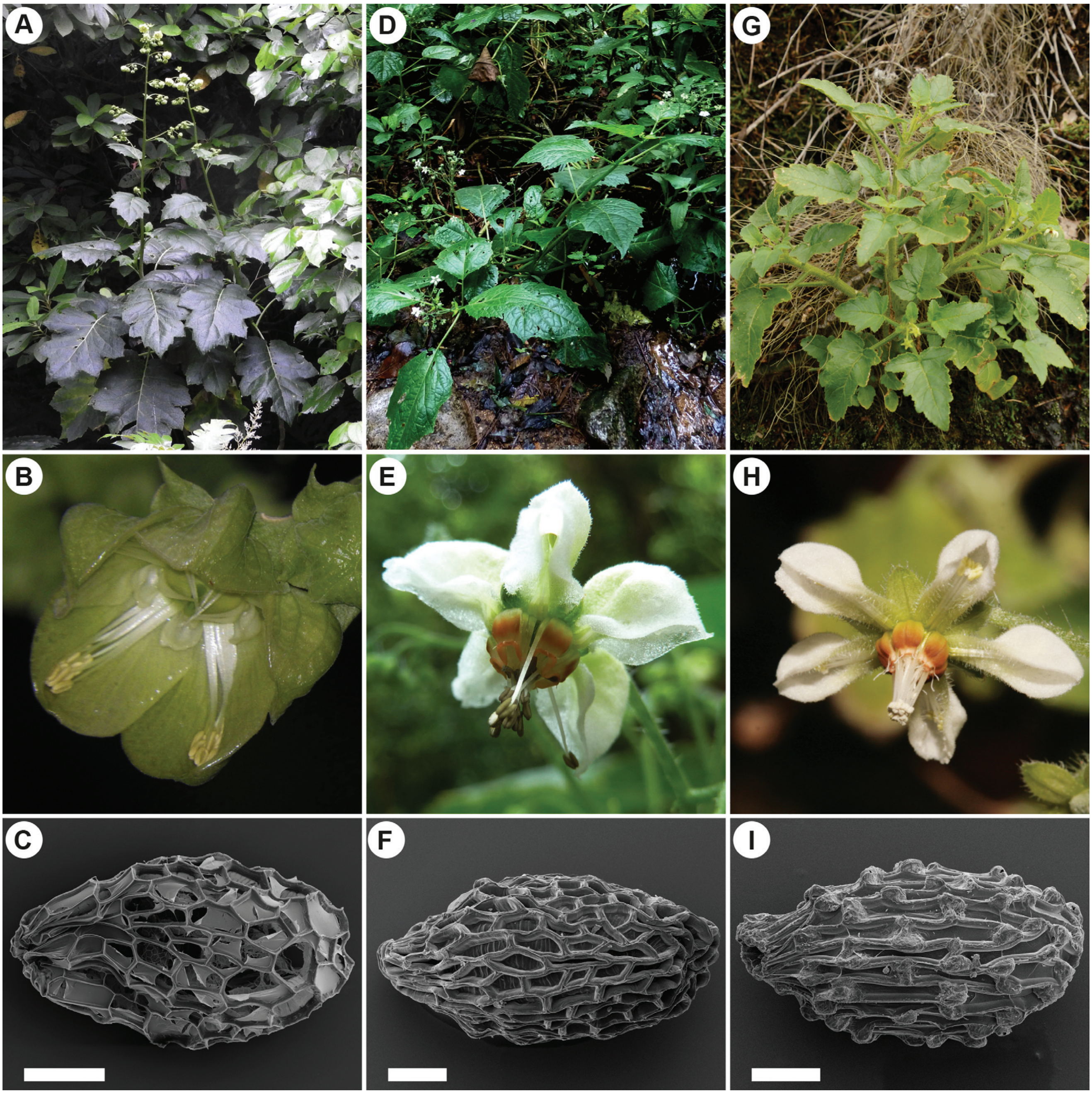

FIGURE 2. Morphological features of *Chichicaste* and *Aosa*. A–C: *Chichicaste grandis* from Turrialba, Cartago, Costa Rica (*Acuña et al. 1747*, USJ): A, Plant habit (flowers are pendent, some plants develop stems to 4 m long and over 3 m tall); B, Mature flower in early male stage (nectar droplets inside the nectar scales evident to the naked eye in vivo); C, Mature seed. D–F: *Aosa uleana* from Parque Nacional. do Itatiaia, Rio de Janeiro, Brazil (*Joßberger 342*, BONN): D, Plant habit; E, Mature flower in middle male stage (nectar inside nectar scales not visible to the naked eye); F, Mature seed. G–I: *Aosa parviflora*: G, Plant habit (Minas Gerais, Brazil, not vouchered; photo C. Schlindwein); H, Mature flower in middle male stage (*Grant 4650*, B; photo H. Hilger); I, Mature seed (plant cultivated in Botanischer Gärten der Universität Bonn, *Joßberger 1213*, BONN). Scale bar: 200 µm. Photographs by T. Joßberger or R. Acuña, unless otherwise credited.

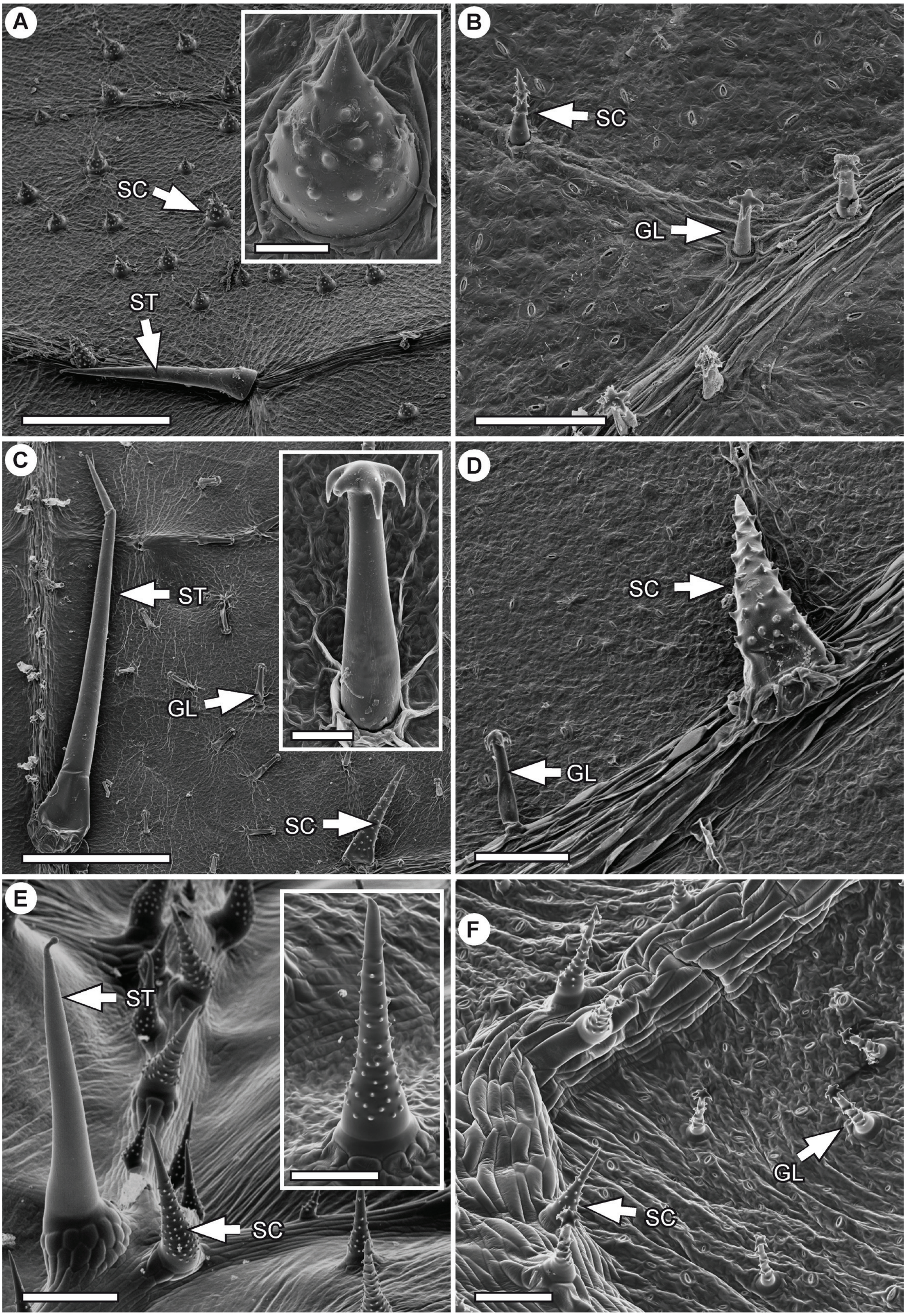

FIGURE 3. Ultrastructure of mature leaf surfaces. *Chichicaste grandis* from Cartago, Costa Rica (*Acuña et al. 1223* USJ, BONN): A, Overview of adaxial surface, inset: detail of a short, pustule shaped scabrid trichome; B, Overview of abaxial surface; *Nasa panamensis* from Chiriquí, Panama (*Antonio 2885*, MO): C, Overview of adaxial surface, inset: detail of a smooth-shafted glochidiate trichome; D, Overview of abaxial surface; *Aosa uleana* from Rio de Janeiro, Brazil (*Joßberger 342*, BONN): E, Overview of adaxial surface, inset: detail of a long scabrid trichome; F, Overview of abaxial surface. Scale bars: A, C: 500 µm, insets: 30 µm; B, D, F: 100 µm; E: 200 µm, inset: 100 µm. GL: Gloquidiate trichome; SC: Scabrid trichome; ST: Stinging trichome.

The etuberculate seeds and bracteose inflorescences of *Chichicaste* are indeed atypical for *Aosa*, but studies of *A. uleana* revealed that, small, foliose or filiform bracts in the basal portions of the inflorescences are often present and its seeds are also etuberculate (Fig. 2F; also: Bovini & Giordano 2005, Henning *et al.* 2017). The petals of this species are shortly clawed and have a wide, boat-shaped limb (Fig. 2E) more similar to those of *Chichicaste* (Fig. 2B) than those of other species of *Aosa* (Fig. 2H). Moreover, study of living *C. grandis* showed that the flowers are not erect, as interpreted by Weigend (1997), but pendent as in *Aosa*. The remaining differences between *Aosa* and *C. grandis* in corolla shape, nectar scale color and morphology and placenta number hold up with re-examination, but are in the range of variability in other, albeit much larger genera of Loasoideae such as *Caiophora* or *Nasa* (Weigend 1997, 2000, 2001, 2004; Acuña *et al.* 2017). The deflexed, semisuperior capsules are likely an adaptation to permanently humid habitats as Dostert & Weigend (1999) previously argued for *Nasa aequatoriana* (Urb. & Gilg) Weigend in Weigend *et al.* (2006: 71). Seed release in erect capsules is severely limited when the seed mass fails to dry out in wet environments, while deflexed and semisuperior capsules are able to release the seeds even under humid conditions.

Although clearly part of the *Aosa* clade, *Chichicaste grandis* is very distinctive morphologically and unlikely to be confused with most of other Loasaceae. Mainly due to distribution and similar gross morphology, rare *Nasa panamensis* had been confused with *C. grandis*, until described in Weigend (2002) and Weigend *et al.* (2006). The gross leaf morphology of *N panamensis* closely resembles that of *C. grandis* and although most populations of *C. grandis* (including those at and near the type locality of this species) lack the amplexicaul bracts and pseudostipules of *N. panamensis*, *C. grandis* specimens from central Panama do develop these structures. Taking this into account and after thorough study of every known specimen of both *C. grandis* and *N. panamensis*, the main vegetative differences between these species can be summarized as follows. In *N. panamensis* the intercostal adaxial foliar surfaces are covered mostly by smooth-shafted glochidiate trichomes. In *C. grandis* these are covered by short, pustule-shaped scabrid trichomes (Figs. 3A, C). The condition of *N. panamensis* is unusual in Loasoideae, because in most species (including *C. grandis* and *Aosa uleana*, Fig. 3E) glochidiate trichomes are usually absent in the intercostal parts of the adaxial lamina (Mustafa *et al.* 2017). In flower, *Nasa panamensis* has yellow corollas (erroneously described as orange red in Weigend 2002), nectar scales with long (ca. 0.5 times as long as the floral scale), well differentiated dorsal threads and ovaries covered with numerous scabrid trichomes, while *C. grandis* has green or whitish corollas, nectar scales without dorsal threads or merely indistinct neck lobes and ovaries without (or with scarce) scabrid trichomes. It is also possible that the inflorescence architecture in these species may differ from each other, but the only two known specimens of *N. panamensis* [*T. Croat 48815* (MO No.2772722!), *T. Antonio 2885* (MO No. 5160005!)] seem not to be mature enough to unequivocally assess this character.

Ecology seems to link *Chichicaste grandis* to *Aosa*, as both taxa are commonly restricted to tropical ecosystems up to ca. 1000 m elevation. These habitats are poor in Loasaceae diversity and only a few weedy species such as *Mentzelia aspera* Linnaeus (1753: 516), *Klaprothia fasciculata* (C.Presl) Poston in Poston & Nowicke (1990: 677) and *Gronovia scandens* commonly grow in these conditions (Weigend 2001, Morales 2007). Most *Aosa* species are endemic to seasonally dry habitats in Brazil (Fig. 4), often growing in very shallow soils on rock outcrops or inselbergs, in the Caatinga and Cerrado biomes, or in the Mata Atlântica restingas (Bovini & Giordano 2005, Henning *et al.* 2017). *Aosa uleana, A. plumieri* and *C. grandis* on the other hand, inhabit mesic, moist forest undergrowth often near watercourses. Both *A. uleana* and *A. plumieri* are restricted to higher elevations (>1000 meters) than is common for other *Aosa*. At least *A. parviflora*, *A. plumieri* and *A. rupestris* are locally common and able to withstand considerable human intervention, but *A. uleana* and *C. grandis* seem to be rarer and more sensitive to anthropic pressure.

The broad range of morphological characters separating the larger groups within Loasoideae has been discussed elsewhere (Weigend 2004) and the morphologically based generic re-alignments in the subfamily only required minor adjustments based on more comprehensive molecular data. In a recent study, the long-standing confusion with regards to the so-called South Andean Loasas was sorted out by redefining the genus *Loasa* and segregating some highly disparate lineages into new genera (Acuña *et al.* 2017). The present study addresses another open question, concerning the affinities of the enigmatic Central American *Chichicaste* and resolving it as nested in *Aosa*. The most conservative taxonomic approach is making a new combination under that later genus name, which is here provided, together with an expanded key to the entire genus.

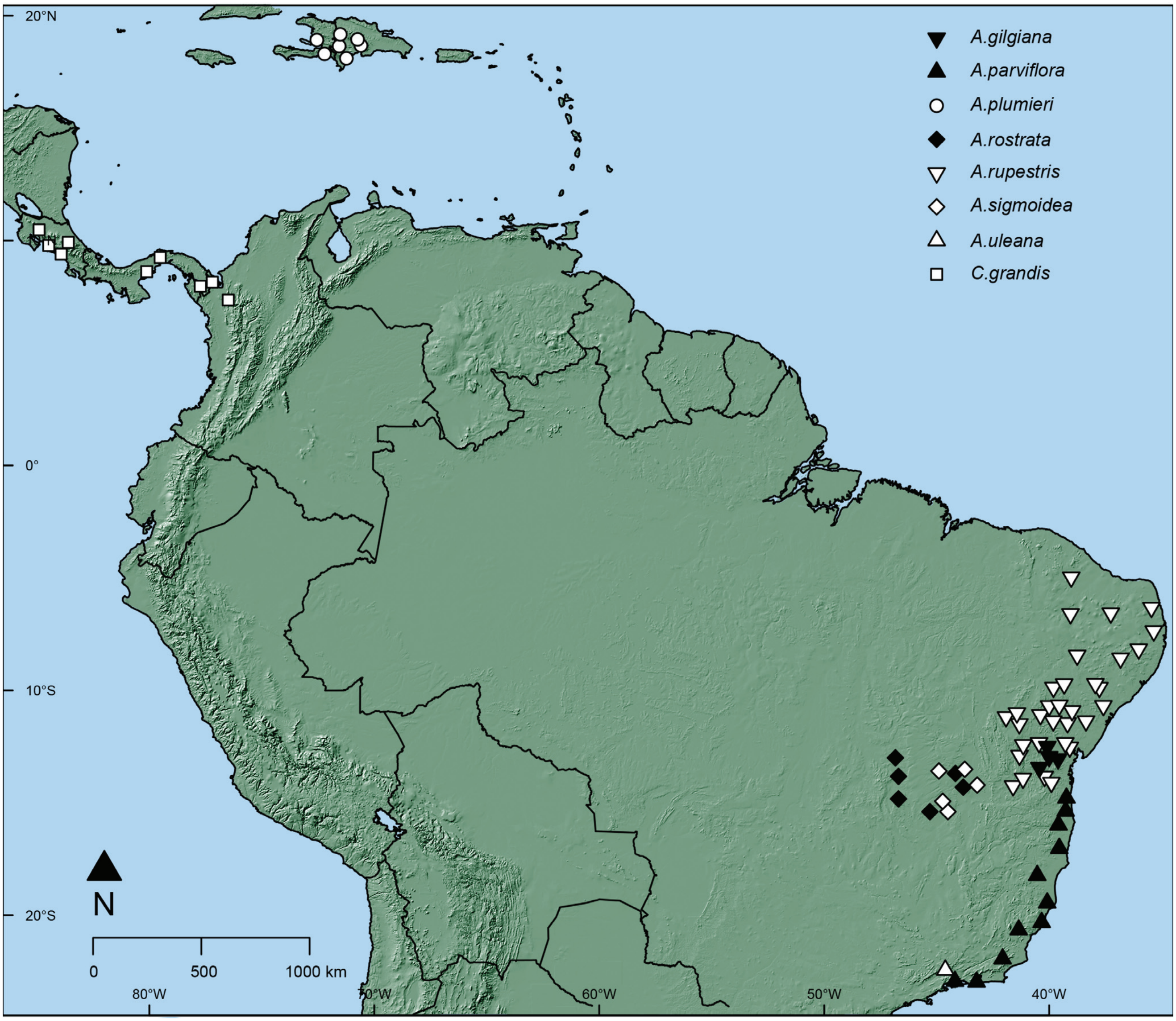

FIGURE 4. Distribution of *Aosa* and *Chichicaste* based on representative herbarium specimens: A.:*Aosa*, C.: *Chichicaste*.

Taxonomic treatment

Aosa Weigend (2006: 464)

Type species:—*Aosa parviflora* (Schrad. ex DC.) Weigend (2006: 464). Basionym: *Loasa parviflora* Schrad. ex De Candolle (1828: 342)

Heterotypic synonym:—*Chichicaste* Weigend (2006: 464–465). Type species: *Chichicaste grandis* (Standl.) Weigend (2006: 465). Basionym: *Loasa grandis* Standley (1927: 12)

Aosa grandis (Standl.) R.H.Acuña & Weigend, *comb. nov.* Basionym: *Loasa grandis* Standley (1927: 12). Homotypic synonym: *Chichicaste grandis* (Standl.) Weigend (2006: 465).

Type:—COSTA RICA, Guanacaste: Vicinity of Tilarán [on the road to El Silencio], 500–650 m, 10–31 January 1926, *P. Standley & J. Valerio 44558* (holotype: US barcode US00115209!, isotype: US barcode US00603972!).

Notes:—There is some variation in vestiture between populations: those in the eastern part of the range, especially in Darién, have more abundant, longer, conical to falcate scabrid trichomes on the adaxial lamina, interspersed between the shorter, pustule-shaped trichomes. The ovaries in plants from Arenal, Chagres and Darién are densely covered in stinging trichomes, while those from San José, Puntarenas and Antioquia have fewer stinging trichomes. Specimens from the Chagres Basin differ from other populations by the presence of pseudostipules and decurrent bracts, but their size and development seem variable (cf. the much larger structures in *C. Dodge & P. Allen 17336* with those of *W. Lewis et. al 3491*). Despite this, the trichome cover and floral morphology of these specimens show no significant differences from other *Aosa grandis* populations. Confirmation if these populations deserve taxonomic recognition will require further study, especially in living plants. Irrespective of their origin and morphological variability, the presence of short-pustulose scabrid trichomes and the absence of smooth-shafted glochidiate trichomes on intercostal areas of the adaxial lamina, is constant in all material studied (Fig. 3).

Distribution:—This species is endemic to the Costa Rica-Chocó biogeographic hotspot in Costa Rica, Panama and Colombia (Fig. 5). According to Morales (2007) and our own observations, populations in Costa Rica are mainly found in three areas: the Arenal Basin (Tilarán, Guanacaste, and adjacent Alajuela), eastern Cartago, and the Wet Premontane Central Pacific Region (southern San José and adjacent Puntarenas). In Panama, populations are known from the Antón Valley area (Coclé), the Chagres Basin (Panamá) and eastern Darién. In Colombia it is known from northwestern Antioquia.

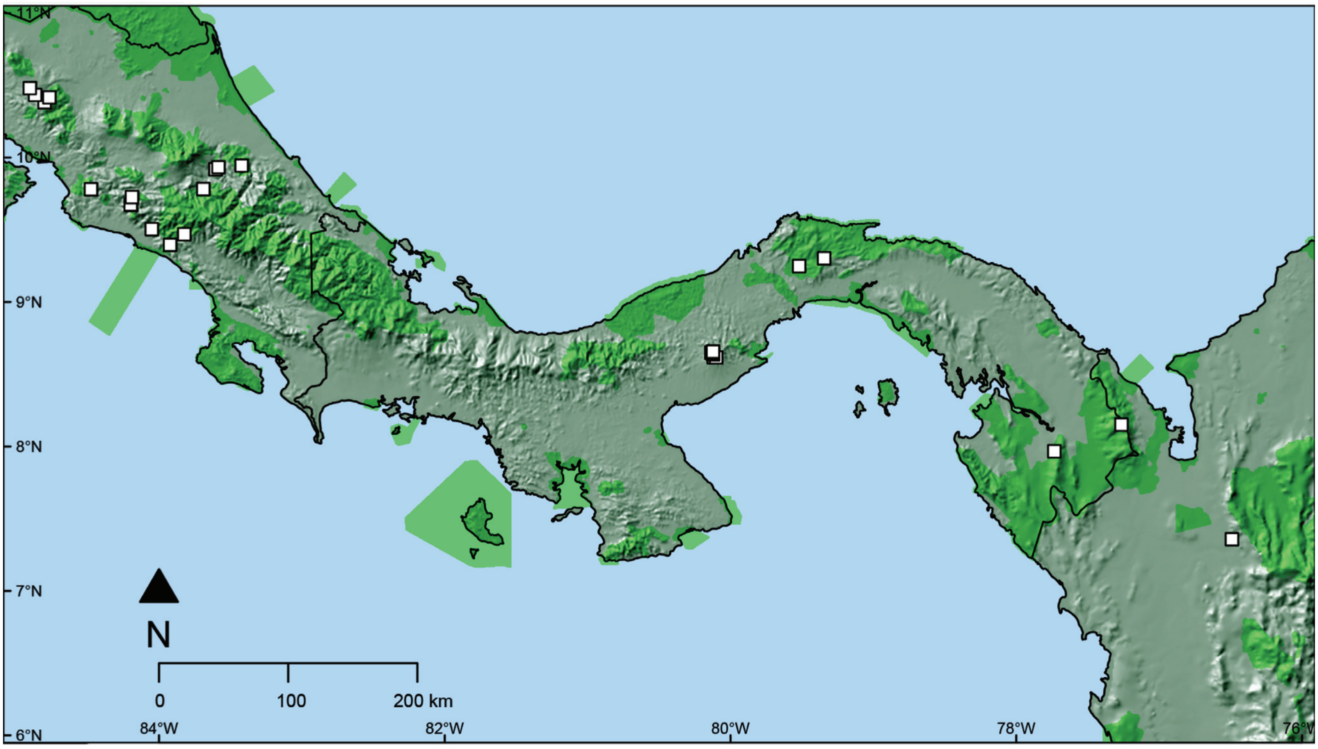

FIGURE 5. Known distribution of *Chichicaste grandis* relative to the most important protected areas (bright green) of Southern Central America and Northwestern Colombia.

Phenology:—This species appears to flower more profusely between December and April, while fruiting seems to take place mostly from March to May.

Habitat and ecology:—*Aosa grandis* is found in wet to rain forests, 70–1300 m elevation, being most abundant between 600–1000 m. It is uncommon and local throughout much of its range (Standley 1927, Weigend 2001). It has been collected mostly near forested river or streams banks, on shallow, but humus rich soils, often on steep slopes. Short-tongued hymenopterans visit the flowers in Costa Rica. From our own observations of plants in Quepos in March 2017, most of the flowers studied (> 80%) set fruit.

Etymology:—*Aosa* is a partial anagram of *Loasa*, the genus from which it was segregated, while *grandis* refers to the large size of this species.

Conservation status:—*Aosa grandis* grows in low densities in extensively forested areas, but it can withstand some habitat fragmentation, as long as some forest remains unaltered e.g. on steep slopes flanking watercourses. In Costa Rica, most known specimens have been collected outside protected areas, but some come from within the current limits of PN (Parque Nacional) Volcán Arenal, PN Barbilla, Refugio de vida silvestre La Marta and PN Los Quetzales. On the other hand, in Panama most collections come from inside or close to currently protected areas, most notably Monumento Natural Cerro Gaital, PN Chagres and PN Darién. Previous records from the Reserva Forestal de Fortuna, Chiriquí, belong to the Critically Endangered *Nasa panamensis*. There is only one collection known from Colombia (*F. López & M. Sánchez 44*). The other specimen reported for the country by Weigend (2001), *S. Espinal 4678* (MEDEL!), is actually *Nasa triphylla* (Juss.) Weigend in Weigend *et al.* (2006: 82) subsp. *papaverifolia* (Kunth) Weigend in Weigend *et al.* (2006: 82). Villa Arteaga is close to (but outside) the current limits of PNN (Parque Nacional Natural) Paramillo (Ministerio de Ambiente y Desarrollo Sostenible de Colombia 2002-Ongoing). This area still remains poorly explored by botanists as the armed conflict precluded scientific research for ca. five decades (M. Vélez, pers. comm.). It is possible that this species could still be found elsewhere on the western slopes of the northwerstern end of the Andes close to PNN Paramillo as well as in PNN Los Katíos (the species is known from adjacent PN Darién close to the international border) or in Serranía del Baudó. Although not officially assessed by the IUCN, we recommend this species to be treated as VU C2a(i); E following the red list criteria (IUCN 2001) due to its low population density, small inferred population size and apparent low tolerance to human disturbance.

Additional specimens examined:—COSTA RICA. Guanacaste: Can. Tilarán, Río Chiquito, bajos de San Pedro. Bosque muy húmedo tropical/premontano, 650–700 m, 15 January 1987, *W. Haber & E. Bello 6594* (MO); Río Negro ford on south side of Lake Arenal; slope and ridges; 10 km NNE of Santa Elena. In premontane wet forest, 600–800 m, 9 May 1986, *W. Haber et al. 4902* (MO); **Alajuela:** [Can. San Ramón?] San Gerardo, Río Caño Negro, Finca de Chavarría, 800 m, 11 January 1989, *E. Bello 646* (CR, MO); **Cartago:** Can. Turrialba, Margen izquierda de Quebrada Jesús. Afluente innominado. Camino a Cerro Tigre, 800 m, 22 March 1996, *G. Herrera & G. Valverde 8844* (CR, F, MO, USJ; Prov. Limón is apparently erroneous); A 16 km SE de Turrialba camino a Puerto Limón. Selva alta perennifolia en cañada, 768 m, 21 February 1982, *O. Téllez et al. 5310* (CR); 13 km E of Turrialba on the Hwy to Limón. Canyon of Río Chitaría, 750–800 m, 10 May 1983, *R. Liesner et al. 15441* (CR, MO); Río Chitaría, on road Turrialba-Limón, forest on steep slope, 700 m, 17 February 1991, *P. Maas 7982* (CR, F, MO, U); Route 10, road between Turrialba and Siquirres, Río Chitaría Canyon. On the steep slopes on the side of the road and river, 768 m, 15 December 2015, *R. Acuña et al. 1223* (BONN, USJ); ditto, 05 March 2017, *R. Acuña et al. 1747* (USJ); Can. Jiménez, Pejibaye, Centro histórico RVS La Marta, cuenca del Reventazón, 781 m, 05 January 2010, *R. Kriebel & D. Santamaría 5447* (CR); **San José:** Can. Puriscal, Cuenca del Tulín. 2 km antes de San Rafael, a orillas del río, 1274 m, 09 December 2004, *A. Soto et al. 438* (CR); Can. Aserrí, Cuenca del Pirrís-Damas, Fila Aguabuena, Quebrada Laja, 1100-1200 m, 23 January 2003, *J. Morales & B. Hammel 9055* (CR); Cuenca del Pirrís-Damas. A lo largo de Quebrada Laja, Ca. 2.5 km al noreste de altos. El Aguacate, 800–900 m, 24 January 2003, *B. Hammel et al. 22686* (CR); Cuenca del Pirrís-Damas, Fila Bustamante, Fila Aguabuena, entre Quebrada Chilamate y Quebrada Pilas, camino, 1300 m, 12 December 1996, *J. Morales 5923* (CR; Can. Acosta is apparently erroneous); Can. Tarrazú, Cuenca del Naranjo y Paquita. San Isidro, Quebrada Seca, 2 Km antes de San Isidro, viniendo de Nara, 800 m, 05 March 2008, *J. Morales 15960* (CR); Can. Pérez Zeledón, R.F. Los Santos. California. Camino entre California y Zaragoza, 1000 m, 06 March 2001, *A. Estrada et al. 2810* (CR); **Puntarenas:** Can. Quepos, Distrito Savegre, Dos Bocas, propiedad privada. Bosque muy húmedo tropical, en claros de bosque ripario, a la orilla de la quebrada, 570 m, 02 March 2015, *I. Chinchilla & O. Chinchilla 2455* (USJ); ditto, 15 April 2017, *I. Chinchilla & O. Chinchilla 3138* (USJ), Dos Bocas, Rio Hatillo Basin creek, 584 m, 08 January 2016, *R. Acuña et al. 1264* (BONN, USJ).**—PANAMA. Coclé:** North rim of El Valle de Antón, 600–1000 m, 12 February 1939, *P. Allen 1658* (MO); La Mesa, near El Valle, 800 m, 18 January 1968, *J. Dwyer & J. Duke 8250* (MO); 3.5 miles NE of El Valle near Los Llanos along deep forested draw, Atlantic slope-headwaters of the Rio Indio, 800 m, 25 April 1979, *B. Hammel 7168* (MO, PMA, US); 2.5 miles from El Valle on road to La Mesa, 11 February 1971, *T. Croat 13381* (MO); La Mesa region N of Cerro Gaital vicinity of El Valle. Roadside and disturbed forest, 800 m, 02 July 1978, *B. Hammel 3867* (MO); [**Panamá?**]: Forest along banks of Quebrada La Palma and cañón of R. Chagres, 70–80 m, 09 January 1935, *C. Dodge & P. Allen 17336* (MO, P, U); **Panamá:** Tributary of Rio Chagres, 5 miles SW of Cerro Brewster. Sandy and rocky river banks, 300 m, 14 December 1967, *W. Lewis et. al 3491* (MO); **Darién:** Río Pucuro base camp, Río Pucuro between Cerro Mali and Cerro Tacarcuna, 650 m, 24 January 1975, *A. Gentry & S. Mori 13871* (MO); Banks of river below Rancho Frío (upper), 400 m, March 1985, *W. D'Arcy & G. McPherson 16217* (MO).**—COLOMBIA. Antioquia:** Mun. Mutatá, Selva Pluvial, carretera al mar, cerca de Villa Arteaga, 150 m, 06 December 1948, *F. López & M. Sánchez 44* (MEDEL, US).

Key to the species of *Aosa* (Modified from Weigend 1999 and Henning *et al.* 2017)

1. Leaves strictly opposite and equal.......... *A. uleana*
-. Leaves alternate, rarely in very unequal pairs (one leaf 3–10 times larger than the other, *A. rostrata* only).......... 2
2. Inflorescences alternating with two leaves on stem.......... 3
- Inflorescences terminal, or in axil of leaf.......... 4
3. Fruit practically indehiscent and densely covered with tack-shaped glochidiate trichomes (burr). Dorsal filaments of floral scales and the two staminodia of each complex with expanded apices.......... *A. plumieri*
-. Fruit opening with well developed apical valves and covered with scabrid trichomes. Dorsal filaments of floral scales and the two

staminodia in each complex with filiform apices .... *A. parviflora*

4. Mature leaves often exceeding 30 cm in length. Corolla bowl shaped, unicolored, greenish. Petals overlapping in anthesis, with poorly differentiated claw and limb .... *A. grandis*

-. Mature leaves rarely exceeding 20 cm in length. Corolla star shaped, often bicolored, predominantly white, rarely greenish, petals not overlapping in anthesis, claw and limb clearly differentiated 5

5. Inflorescence foliose, without long leafless stalk. Capsule with long terminal beak 6

-. Inflorescence leafless, with long, leafless stalk. Capsule without long terminal beak 7

6. Plants usually < 0.8 m tall, setose. Capsule over 75% superior .... *A. rostrata*

-. Plants usually > 1 m tall, virtually esetose. Capsule with inferior portion making up to 50% the total length .... *A. sigmoidea*

7. Inflorescence stalk (in dry material) not thickened at base. Secondary leaf veins in ≥7 pairs, parallel, all ending in teeth, margin shallowly and regularly dentate .... *A. gilgiana*

-. Inflorescence stalk (in dry material) abruptly thickened at base. Secondary leaf veins in 2–4 pairs, divergent, upper ones not ending in teeth, margin irregularly serrate and lobate .... *A. rupestris*

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References

Acuña, R., Fließwasser, S., Ackermann, M., Henning, T., Luebert, F. & Weigend, M. (2017) Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae). *Taxon* 66: 365–378.
https://doi.org/10.12705/662.5

Adanson, M. (1763) *Familles des plantes. II Partie*. Chez Vincent, Paris, 640 pp.
https://doi.org/10.5962/bhl.title.271

Bovini, M. & Giordano, L. (2005) Loasaceae Lindl. no Estado do Rio de Janeiro, Brasil. *Acta Botanica Brasilica* 19: 265–271.
https://doi.org/10.1590/S0102-33062005000200008

Boyce, K., Sievers, F. & Higgins, D. (2015) Instability in progressive multiple sequence alignment algorithms. *Algorithms for Molecular Biology* 10: 26.
https://doi.org/10.1186/s13015-015-0057-1

Chase, M.W. & Hills, H.G. (1991) Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.
https://doi.org/10.2307/1222975

Chomicki, G. & Renner, S.S. (2015) Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. *New Phytologist* 205: 526–532.
https://doi.org/10.1111/nph.13163

Coville, F.V. (1893) Botany of the Death Valley expedition. *Contributions of the United States National Herbarium* 4: 1–363.

De Candolle, A.P. (1828) *Prodromus systematis naturalis Regni vegetabilis. Pars III*. Paris Treuttel & Würtz, 494 pp.

Dostert, N. & Weigend, M. (1999) A synopsis of the *Nasa triphylla* complex (Loasaceae) including some new species and subspecies. *Harvard Papers in Botany* 4: 439–467.

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin of the Botanical Society of America* 19: 11–15.

Ensikat, H.-J. & Weigend, M. (2013) Cryo-scanning electron microscopy of plant samples without metal coating, utilizing bulk conductivity. *Microscopy and Analysis* 27 (6): 7–10.

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* 17: 368–376.
https://doi.org/10.1007/BF01734359

Forbes, F.B. & Hemsley, W.B. (1887) An enumeration of all the plants known from China proper, Formosa, Hainan, Corea, the Luchu Archipelago and the Island of Hongkong, together withe their distribution and synonymy. *Journal of the Linnean Society* 23: 1–489.
https://doi.org/10.1111/j.1095-8339.1887.tb00533.x

Gay, C. (1847) *Historia física y política de Chile. Botánica. Tomo Segundo*. Fain & Thunot, Paris, 534 pp.

Henning, T., Acuña, R., Weigend, M. & Mello-Silva, R. (2017) Loasaceae. *In*: Jardim Botânico do Rio de Janeiro (Ed.) *Flora do Brasil 2020* em construção. Available from: http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB150 (accessed 3 May 2017)

Huelsenbeck, P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
https://doi.org/10.1093/bioinformatics/17.8.754

Hufford, L., McMahon, M., Sherwood, A., Reeves. G. & Chase, M. (2003) The major clades of Loasaceae: phylogenetic analysis using the plastid *matK* and *trnL-trnF* regions. *American Journal of Botany* 90: 1215–1228.
https://doi.org/10.3732/ajb.90.8.1215

Hufford, L., McMahon, M., O'Quinn, R. & Poston, M. (2005) A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. *International Journal of Plant Sciences* 166: 289–300.
https://doi.org/10.1086/427477

IUCN (2001) *IUCN Red List Categories and Criteria*. Version 3.1. Prepared for the IUCN Species Survival Commission. Gland, Switzerland and Cambridge, United Kingdom, ii+33 pp.

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
https://doi.org/10.1093/molbev/mst010

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.
https://doi.org/10.1093/bioinformatics/bts199

Lamarck, J.B. (1792) *Encyclopédie méthodique. Botanique. Tome 3eme*. Chez Panckoucke & Chez Plomteux, Paris & Liége, 759 pp.

Linnaeus, C. (1753) *Species Plantarum. Tomus I.* Laurentius Salvius, Stockholm, 560 pp.

Maack, R. & Ruprecht, F.J. (1857) Die ersten botanischen Nachrichten über das Amurland. Zweite Abteilung: Bäume und Sträucher. *Bulletin de la Classe Physico-Mathématique de l'Académie Impériale des Sciences de Saint-Pétersbourg* 15: 353–383.

Mau, B., Newton, M. & Larget, B. (1999) Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* 55: 1–12.
https://doi.org/10.1111/j.0006-341X.1999.00001.x

Miller, M., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In*: *Proceedings of the Gateway Computing Environments Workshop (GCE),* New Orleans, Louisiana, pp 1–8.
https://doi.org/10.1109/GCE.2010.5676129

Ministerio de Ambiente y Desarrollo Sostenible de Colombia. (2002–Onwards) *Parque Nacional Natural Paramillo*. Available from: http://www.parquesnacionales.gov.co/portal/es/parques-nacionales/parque-nacional-natural-paramillo/ (accessed 25 September 2017)

Morales, J. (2007) Loasaceae. Manual de Plantas de Costa Rica. Vol. VI. *Monographs in Systematic Botany from the Missouri Botanical. Garden* 111: 202–206.

Müller, J., Müller, K., Nienhuis, C. & Quandt, D. (2010) *PhyDe: Phylogenetic Data Editor*. Available from: http://www.phyde.de/ (accessed 19 August 2018)

Mustafa, A., Ensikat, H.-J. & Weigend, M. (2017) Ontogeny and the process of biomineralization in the trichomes of Loasaceae. *American Journal of Botany* 104: 1–12.
https://doi.org/10.3732/ajb.1600417

Posada, D. & Crandall, K. (2001) Selecting the best-fit model of nucleotide substitution. *Systematic Biology* 50: 580–601.
https://doi.org/10.1080/106351501750435121

Poston, M.S. & Nowicke, J.W. (1990) A Reevaluation of *Klaprothia* and *Sclerothrix* (Loasaceae: Klaprothieae). *Systematic Botany* 15: 671–678.
https://doi.org/10.2307/2419163

Presl, C. (1831) *Reliquiae Haenkeanae. Vol.2, Fasc. 2*. J.G. Calve, Prague, 152 pp.+23 Tab.

Rambaut, A. & Drummond, A. (2007) *Tracer v1.4*. Available from: http://beast.bio.ed.ac.uk/Tracer (accessed 19 August 2018)

Schrader, H.A. (1825) 171. Stuck. *Göttingische gelehrte Anzeigen unter der Aufsicht der Königlichen Gesellschaft der Wissenschaften* 1825 (3): 1705–1712.

Silvestro, D. & Michalak, I. (2012) RAxMLGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* 12: 335–337.
https://doi.org/10.1007/s13127-011-0056-0

Stamatakis, A. (2014a) RAxML version 8: a tool for phylogenetic analysis and post analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033

Stamatakis, A. (2014b) *The RAxML v8.X.X Manual*. Available from: https://github.com/stamatak/standard-RAxML/tree/master/manual (accessed 19 August 2018)

Standley, P. (1927) New plants from Central America–IV. *Journal of the Washington Academy of Sciences* 17: 7–16.

Sweet, R. (1828) *The British Flower Garden. Vol. III.* W. Simpkin & R. Marshall, London, 300 plates.

Urban, I. (1889) Loasaceae. *In*: Martius, C.F.P. & Eichler, A.G. (Eds.) *Flora Brasiliensis Vol. XIII, Pars. III*. R. Oldenbourg, Munich & Leipzig, pp. 197–224.

Urban, I. (1910) Zwei neue Loasaceen von Sto. Domingo. *Berichte der Deutschen Botanischen Gesellschaft* 28: 515–523.

Urban, I & Gilg. E. (1900) *Monographia Loasacearum*. Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curosiorum. Tomus LXXVI. Erhardt Karras, Halle, 384 pp. + VIII Tab.

Weigend, M. (1997) Nasa *and the conquest of South America: Systematic rearrangements in Loasaceae Juss*. PhD. Dissertation. Lüdwig-Maximilians-Universität, Munich, 271 pp.

Weigend, M. (1999) *Aosa sigmoidea*, a new species of the genus *Aosa* (Loasaceae) from Brazil. *Nordic Journal of Botany* 19: 213–216.
https://doi.org/10.1111/j.1756-1051.1999.tb00666.x

Weigend, M. (2000) A revision of the Peruvian species of *Nasa* ser. *Alatae* (*Loasaceae*). *Nordic Journal of Botany* 20: 15–31.
https://doi.org/10.1111/j.1756-1051.2000.tb00727.x

Weigend, M. (2001) 22. Loasaceae. *In*: Bernal, R. & Forero, E. (Eds.) *Flora de Colombia*. Universidad Nacional de Colombia, Bogotá, 100 pp .

Weigend, M. (2002) *Nasa panamensis*, a new species of *Nasa* (Loasaceae) from Central America. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*. 124: 211–216.
https://doi.org/10.1127/0006-8152/2002/0124-0211

Weigend, M. (2004) Loasaceae. *In*: Kubitzki, K. (Ed.) *The Families and Genera of Vascular Plants VI. Flowering Plants-Dicotyledons: Celastrales, Oxalidales, Rosales, Cornales, Ericales.* Springer Verlag, Berlin, pp. 239–254.
https://doi.org/10.1007/978-3-662-07257-8_27

Weigend, M. (2006) Validating subfamily, genus and species names in Loasaceae (Cornales). *Taxon* 55: 463–468.
https://doi.org/10.2307/25065594

Weigend, M. (2011) 173A. Loasaceae In: *Flora Mesoamericana website*. Missouri Botanical Garden. Pp 19 Available from: http://www.tropicos.org/docs/meso/loasaceae.pdf (accessed 3 May 2017)

Weigend, M., Gottschling, M., Hoot, S. & Ackermann, M. (2004) A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on trnL (UAA) sequence data, with consequences for systematics and historical biogeography. *Organisms Diversity and Evolution* 4: 73–90.
https://doi.org/10.1016/j.ode.2003.12.001

Weigend, M., Dostert, N., Henning, T., Schneider, C. & Rodríguez, E. (2006) Valid publication for 101 species and subspecies names of the genera *Nasa* and *Aosa* (Loasaceae: Cornales). *Revista Peruana de Biología* 13: 71–84.
https://doi.org/10.15381/rpb.v13i1.1766

CHAPTER 6

Major lineages of Loasaceae subfam. Loasoideae diversified along with the Andean uplift

Flower of *Plakothira parviflora* cultivated in the Botanical Garden of Berlin. This genus is endemic to the Marquesas archipelago and diverged from its closest living relative (*Klaprothia*) before the Marquesas emerged above the sea level. Photo courtesy of Maximilian Weigend.

Major lineages of Loasaceae subfam. Loasoideae diversified along with the Andean uplift

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ABSTRACT

Predominantly Neotropical-montane Loasaceae subfam. Loasoideae has its centre of diversity in the Andes, but is also widespread elsewhere in South and Central America. The present study aims at resolving the historical biogeography of the group and its relation to what is known about the history of the corresponding biomes, with a special emphasis on Andean orogeny. We employed plastid markers from 170 accessions (134 ingroup taxa) to infer a phylogeny of Loasoideae. Chronograms using three, six or seven fossils as primary calibration points were generated, and DEC analyses were conducted to reconstruct the ancestral ranges of the subfamily. Our results show that stem Loasoideae diverged from its sister group in the Late Cretaceous-early Paleocene (95% HPD: 83–62 Ma). The crown group was retrieved to have a Middle Paleocene to Middle Eocene (95% HPD: 60–45 Ma) age. Although the crown groups of most of currently accepted genera appear to have originated in the Oligocene to Miocene (median ages: 28–10 Ma), their stems had diverged diverged in the Eocene, prior to most of the Andean orogeny. This

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roughly coincides with the Paleocene-Eocene Thermal Maximum. Divergence of some extant lineages appear to has been more or less parallel to Andean uplift pulses and was likely facilitated by orogeny and the concomitant establishment of new habitats, and latitudinal corridors. For crown Loasoideae, we retrieved different area combinations as the most probable ancestral areas, but the tropical Andes and Pacific deserts were obtained most frequently. Most of the extant clades have remained basically restricted to their ancestral areas according to our analyses. Transoceanic Long Distance Dispersal appears to have been involved in the arrival of Loasoid ancestors to South America, and in the distribution of the small clades *Kissenia* in Africa and *Plakothira* on the Marquesas Archipelago.

Keywords: Ancestral range, Andes, biogeography, divergence time, Loasaceae, Loasoideae

1. Introduction

South America is one of the most phytodiverse regions on Earth, with three of the five most diverse centres of plant diversity in its territory (Barthlott et al., 2007). Its flora has been shaped by events at both the continental and intercontinental scales. Since the Cretaceous, the biotic exchange either through direct land connections or long distance dispersal (LDD) between South America and Africa, North America, Australia and Antarctica, has had profound effects in the flora and fauna of these landmasses, as the fossil record (Wilf et al., 2013) and molecular phylogenetic studies (Luebert and Weigend, 2014) demonstrate. At the continental scale, Andean uplift, and the environmental changes associated with it, such as the change in drainage and rain patterns in the Amazon Basin, or the aridification of the Atacama desert, have been identified as major forces, shaping the distribution and diversification of many plant groups, both in the Andes and in adjacent regions (Amazonia, Atacama, Central America) especially after the Oligocene (Luebert and Weigend, 2014). The spatio-temporal geodiversity of this mountain range is considered as an essential driver for the elevated biodiversity that currently inhabits the region (Mutke and Weigend, 2017). Although there is still controversy concerning the details of Andean uplift (Barnes and Ehlers, 2009; Richardson et al., 2018), major trends, such as the general progression of the orogeny from South to North and from West to East (Graham, 2009; Gianni et al., 2016), are generally accepted and the historical biogeography of many plant groups seems to reflect this pattern (Hughes et al., 2013; Luebert and Weigend, 2014).

Cornales represent one of the earliest unequivocally documented radiations of any extant clade of eudicots (89 Ma; Atkinson et al., 2018) and are sister to the remaining Asterids, themselves representing one of the major radiations of land organisms (Soltis et al., 2018). Extant Cornales are subcosmopolitan in distribution, with considerable diversity in the subtropical to temperate zones of the Northern Hemisphere (Christenhusz et al., 2017; Soltis et al., 2018). Loasaceae, the largest family of the order is believed to have diverged from its sister, Hydrangeaceae, during the Late Cretaceous (Xiang et al., 2011). In Loasaceae, Mentzelioideae (apparently a grade), Gronovioideae and Petalonychoideae have centres of phyletic diversity in southwestern North America (including Mexico), which is inferred as the likely ancestral area of Loasaceae (Weigend, 1997, 2004; Hufford, 2004; Schenk et al., 2017).

However, Loasaceae subfam. Loasoideae, the largest clade of the family, is most diverse in the Andes (Weigend, 2004; Mutke et al., 2014) and with a minor extra-Andean centre of diversity in E Brazil, like other mostly-Andean groups. Loasaceae subfam. Loasoideae thus presents an opportunity to study the diversification of a clade across much of montane South America.

Historical biogeography of Loasoideae is still very incompletely understood, in spite of some published studies on their phylogeny (Weigend et al., 2004; Hufford et al., 2005; Acuña et al., 2017). The most recent common ancestor (MRCA) of Loasoideae and its sister group has been hypothesized to have been native to Mexico and/or adjacent areas in the Late Cretaceous-Early Paleogene (Weigend, 1997). Few dating estimates exist for this clade: Schenk and Hufford (2010) estimated the age of the crown group of Loasoideae around the Eocene-Oligocene (ca. 56–23 Ma), while Strelin et al. (2017), focusing on the Argentinean *Caiophora* C.Presl., dated the MRCA of the living *Caiophora* to the Miocene-Pliocene (17.64–4.37 Ma).

None of these studies addresses the historical biogeography of the subfamily Loasoideae as such and this is a gap that we are trying to fill here based on a molecular phylogeny and explicit geographic data. We address three questions in the present study: 1-When did the main clades of Loasoideae diverge from their closest extant relatives and how old are the crown groups of the accepted genera?. 2-Where did these clades originate and spread afterwards?. 3-What important geologic events have a spatio-temporal correspondence with the diversification of the main clades of Loasoideae? Do other elements of the flora with similar ecology and distributions, share similar crown ages? In order to address these questions we generated a dated phylogeny of Loasoideae and estimated the ancestral ranges of the clade.

2. Materials and methods

2.1. Taxon sampling

We sampled a total of 170 species of Cornales: 36 outgroup [non-Loasoid Loasaceae, Hydrangeaceae, Nyssaceae, Cornaceae and *Curtisia dentata* (Burm.f.) C.A.Sm.] and 134 ingroup species, representing ca. 65% of the currently accepted species of Loasoideae. Detailed voucher specimen information, is presented in Table D.1 in Appendix D.

2.2. DNA Amplification, sequencing and alignment

DNA was extracted using the CTAB method (Doyle and Doyle, 1987). Four plastid regions were amplified: *trn*L–*trn*F, *mat*K, the *trn*S–*trn*G intergenic spacers, and the *rps*16 intron. Although many sequences were newly generated for this study, we also included the majority of the plastid molecular datasets generated by Acuña et al., (2017, 2018) and Henning et al., (2018). The *trn*L–*trn*F sequence of *Aosa plumieri* (Urb.) Weigend was obtained directly from GenBank (Hufford et al., 2005). GenBank accession numbers of every sequence are presented in Table A.1. The amplification, sequencing and alignment protocols, including the software used, are explained in Acuña et al., (2017). Individual marker phylogenic reconstructions were inspected visually in order to find significant topological (>75 Bootstrap support) incongruences. Two nodes showed incongruent topologies between the different markers, these being *Scyphanthus* as sister to *Pinnasa* or *Caiophora* in *mat*K and *rps*16 respectively and *Nasa laxa* (J.F.Macbr.) in a polytomy with *N. macrothyrsa* (Urb. & Gilg) Weigend and *N. usquiliensis* Weigend, T.Henning & C.Schneid. or as sister to *N. trianae* (Urb. & Gilg) Weigend in *trn*L–*trn*F and *trn*S–*trn*G. The incongruent nodes had bootstrap support values of 75 and 88 respectively. The four plastid marker sequences were combined in a single matrix, divided in four partitions (one per marker).

2.3 Molecular dating

The entire molecular dataset was prepared in BEAUti v1.8.4 (Drummond et al., 2012). FindModel (http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html), which implements Posada and Crandall's (2001) Modeltest, suggested GTR+Γ as the best fit model for all partitions. For each partition we chose an uncorrelated relaxed clock in order to allow clock rates to vary across the tree. Yule Process was chosen as tree prior, which assumes a constant birth rate. We placed the following seven fossil calibration points for Cornales, using absolute ages (Ogg et al., 2016) corresponding to the youngest boundary of the geologic age to which the fossils have been assigned:

1. The crown node of *Cornus* was set to a minimum age of 72 Ma (end of the Campanian) based on recently described fossil fruits of *Cornus* cf. *piggae* from the Late Campanian (~73 Ma) of Vancouver Island, British Columbia, which have been confidently assigned to the extant Cornelian Cherry clade (Atkinson et al, 2016).

2. The node that marks the divergence between *Davidia* Baill. and *Camptotheca* Decne.+ *Nyssa* L. was set to a minimum age of 56 Ma (end of the Thanetian), based on fruits and leaves of *Davidia antiqua* (Newberry) Manchester, from the Late Paleocene (57-55.5 Ma) of Dakota, Montana and Wyoming, USA (Manchester, 2002), displaying many diagnostic traits of the extant genus. Although older fossils of *Davidia* (fruits, dating as far back as the late Campanian ca. 72 Ma) have been reported (Serbet et al., 2004; Manchester et al., 2015), a recent morphological analysis by Atkinson (2017, 2018) revealed that these (along with those assigned to *Nyssa* from the same formation and age) cannot be unequivocally assigned to the crown group of Nyssaceae and so we have decided not to include them in our analyses.

3. The stem node of Nyssaceae (sensu APG IV, 2016) was set to a minimum age of 86 Ma (end of the Coniacian) based on the fossil fruits of *Obamacarpa edenensis* Atkinson, Stockey & Rothwell (Atkinson et al., 2018), from the Early Coniacian (89 Ma) of Vancouver Island, British Columbia. Although these cannot be assigned with certainty to any extant group Cornales, as they show a mosaic of traits that seem to be basal to

Cornales, morphological analyses place this genus as more closely related to Nyssaceae than to any other extant group (Atkinson, 2017, 2018).

4. The crown node of Jamesioideae (Hydrangeaceae) was set to a minimum age of 23 Ma (end of the Chattian) based on fossil leaves of *Jamesia caplani* Axelrod from the Late Oligocene (26.5 ± 0.3 Ma) of Colorado (Axelrod, 1987). Although it is not known if every specimen assigned to this name belonged to the same species, at least some show close resemblance to extant *Jamesia americana* Torr. & A.Gray leaves, indicating that by this time the extant genera of the subfamily had already diverged.

5. The crown node of *Hydrangea* was set to a minimum age of 41 Ma (end of Lutetian) based on *Hydrangea knowltonii* Manchester from the Late Eocene (43-45 Ma) of Oregon (Manchester, 1994; Manchester et al., 2015). This species has dorsoventrally flattened seeds, a morphological trait shared only with *H. anomala* D.Don from Asia among extant Hydrangeaceae (Hufford, 1995). Although not included in our sampling, *H. anomala* was retrieved as sister to *Hydrangea* Sect. *Cornidia* by De Smet et al. (2015) which is represented in our study by *H. oerstedii* Briq. Potentially older (possibly Paleocene) fossils of *Hydrangea* from Washington have been described by Mustoe (2002), but their actual age remains uncertain.

6. The stem node of *Philadelphus*+*Carpenteria* (the latter was found to be nested in the former by Guo et al., 2013) was set to a minimum age of 23 Ma (end of the Chattian) based on leaf fossils of *Philadelphus creedensis* Axelrod, from the Late Oligocene (26.5 ± 0.3 Ma) of Colorado (Axelrod, 1987). According to Axelrod (1987), leaf morphology resembles that of the extant *Philadelphus microphyllus* A.Gray.

7. The crown node of Klaprothieae was set to an age of 28 Ma (end of Rupelian) based on amber preserved structures of *Klaprothiopsis dyscrita* Poinar, Weigend & T.Henning, from Dominican Republic amber. The Dominican amber fossils have not been dated precisely and could have ages between 45 and 15 Ma (Poinar et al., 2015). Although *K. dyscrita* lacks many traits found in extant Loasoideae, it bears a closer resemblance to extant *Plakothira* Florence and *Klaprothia* Kunth than to their closest living relative, *Xylopodia* Weigend.

We did not include *Tylerianthus crossmanensis* Gandolfo, Nixon & Crepet because although it has been cited as representing one of the oldest Cornalean taxa (e.g. Manchester et al., 2015; Soltis et al., 2018) due to its Turonian-Coniacian age (88.5–90.4 Ma) and putative hydrangeaceous affinities (Gandolfo et al., 1998), its age and phylogenetic assignment remain equivocal (Friis et al., 2011; Atkinson et al., 2018).

A single secondary calibration point was placed at the crown node of Cornales based on the result of Magallón et al., (2015). We therefore set a normal distribution with mean 104.6 and standard deviation of 5.45.

The partitioned dataset was run in BEAST v1.8.4 (Drummond et al., 2012) on the CIPRES Science Gateway 3.3 (Miller et al., 2010). The Markov Chain Monte Carlo was set to 200 million generations sampling every 10,000th generation. We discarded 10% of the trees as burn-in. The effective size sample (ESS) and plot likelihoods were examined in Tracer 1.6 (Rambaut and Drummond, 2014) [ESS was >200 for all parameters, except the prior (ESS=130), most recent common ancestor (*Philadelphus creedensis*, ESS=159), matk.ucld.mean (ESS=186), matk.meanRate (ESS=170) and speciation (ESS=123)]. TreeAnnotator 1.8.4 (Drummond et al., 2012) was used to obtain a maximum clade credibility tree from the 18,000 trees of the posterior distribution.

In order to assess the influence that alternative calibrations could have in our analysis, we explored three calibration schemes: (a) including all calibration points; (b) excluding the dubious in-group fossil *Klaprothiopsis dyscrita*; and (c) including only the single secondary calibration and the fossils *Cornus* cf. *piggae*, *Davidia antiqua* and *Hydrangea knowltonii*, whose affinities are considered unequivocal. Unless otherwise stated, the age ranges obtained in our analyses and cited in the discussion correspond to 95% Highest posterior density intervals (HPDI). The chronograms provided in the Appendix D (Fig. D.1.) were prepared using the package ′phyloch’ (Heibl, 2013).

2.4 Ancestral area reconstruction

Ancestral areas were reconstructed for Loasoideae. The distribution data came from specimens or images of specimens, deposited in herbaria in the Americas and Europe (see Acknowledgements), with additional data from the literature (Sleumer, 1955; Crespo and Pérez-Moreau, 1988; Noguera-Savelli, 2012; Slanis et al., 2016), and GBIF (2017, with doubtfully identified specimens excluded). The ancestral area reconstruction was performed using the Dispersal Extinction Cladogenesis (DEC) approach described by Ree and Smith (2008) as implemented in the R-package 'BioGeoBEARS' 0.2.1 (Matzke, 2013). We defined eight geographic areas: A) Central America and the Caribbean (including tropical Mexico and the lowlands of extreme northwestern Colombia), B) tropical Andes (Andes north of the Bolivian Orocline), C) Pacific deserts (deserts of western Peru and northern Chile), D) Altiplano (Central Andes between the Bolivian Orocline and ca. 30°S), E) eastern South America (Eastern Brazil, Pampas and Chaco, including the Sierras de Córdoba), F) southern South America (South of the Atacama desert and the Pampas), G) Africa (including the Arabian Peninsula) and H) Marquesas Islands.

We ran two alternative biogeographic scenarios using the chronogram obtained from calibration scheme (a). First, a scenario with no dispersal constraints between areas and a maximum of three areas per node [the number of areas occupied by the most widely distributed extant species of Loasoideae]. Second, a dispersal constrained scenario where the adjacency matrix was modified manually so dispersal was allowed only between adjacent areas, even when separated by the sea (i.e., including combinations AE, AG, AH, BH, CH, EG, FG and FH). In order to allow the possibility of ancestral reconstructions under these dispersal constraints, the maximum number of areas per node was set to four. R scripts are available on request.

3. Results

Divergence times for the major nodes retrieved are shown in Table 1. All calibration schemes yielded the same topologies in all moderately to well-supported clades (Posterior Probability > 0.9) (Fig. 1, Fig. B.1). Divergence time estimates for major nodes in Loasoideae were similar, independent of the specific calibration scheme (Table 1, Fig. B.1.), although the ages obtained with calibration scheme (c) tended to be higher. Median ages for the stem node of Loasoideae ranged between 72–77 Ma, placing it around the late Campanian, while the crown node of Loasoideae was dated to a median age between 52–55 Ma (Ypresian). Crown node ages of most genera fell into the Oligocene to Miocene (31–10 Ma). Only the crown node of *Loasa* was retrieved as older (37–38 Ma, Eocene).

Table 1. Comparison between the divergence time estimates in Millions of years before present (Ma) for the major nodes of the Loasaceae subfam. Loasoideae phylogeny under different calibration schemes with seven (a), six (b) and three (c) primary calibration points and estimates from other studies. Numbers in parentheses refer to 95% highest posterior density intervals, except for Schenk and Hufford (2010) who reported 95% Confidence Intervals. See the Material and Methods sections for the details on each calibration scheme.

	Calibration schemes					
Node	(a)	(b)	(c)	Schenk and Hufford (2010)	Xiang et al., 2011)	Strelin et al., 2017)
Stem Loasoideae	72.24 (62.11–83.29)	72.48 (62.10–83.42)	76.71 (67.73–86.35)	ca. 65 (44–75)	46.69–47.35	_
Crown Loasoideae	52.08 (44.78–59.73)	52.29 (45.38–59.90)	54.73 (48.26–61.89)	ca. 44 (24–65)	19.98–23.39	_
Crown Klaprothieae	27.81 (21.72–34.73)	28.49 (22.01–35.74)	29.60 (23.01–36.80)	ca. 20 (9–30)	_	_
Crown 'Core Loaseae'	47.82 (41.30–54.78)	47.89 (41.78–54.90)	50.09 (44.25–56.49)	ca. 40 (19–49)	19.98–23.39	_
Crown *Nasa*	27.67 (23.12–32.49)	27.84 (23.14–32.48)	28.94 (24.60–33.53)	_	_	_
Crown *Aosa*	20.59 (16.10–25.93)	20.61 (15.65–25.63)	21.38 (16.84–26.58)	ca. 10.5 (2–18)	_	_
Crown *Presliophytum*	30.01 (22.23–38.27)	30.15 (22.20–38.32)	31.48 (23.48–39.50)	ca. 21 (9–30)	_	_
Crown SAL	38.42 (33.49–44.19)	38.38 (33.68–44.11)	40.14 (35.43–45.24)	ca. 28 (13–40)	_	26.97 (13.74–41.00)
Crown *Blumenbachia*	27.48 (20.72–34.22)	27.43 (20.82–34.35)	28.78 (22.07–35.69)	_	_	11.83 (3.29–22.18)
Crown *Loasa*	36.69 (31.34–41.84)	36.66 (31.91–42.08)	38.33 (33.58–43.30)	_	_	20.76 (9.01–35.39)
Crown '*Caiophora* clade'	31.42 (26.52–36.46)	31.35 (26.54–36.28)	32.74 (28.14–37.59)	ca. 22.5 (10–35)	_	20.09 (8.27–31.97)

Crown *Grausa*	23.89 (18.91–29.05)	23.88 (19.06–29.14)	24.90 (19.82–29.99)	_	_	_
Crown *Pinnasa*	11.02 (7.00–15.55)	11.06 (6.92–15.47)	11.48 (7.30–16.07)	_	_	4.81 (0.59–10.71)
Scyphanthus-*Caiophora* divergence	26.31 (21.48–31.32)	26.17 (21.60–31.19)	27.41 (22.67–33.36)	_	_	_
Crown *Caiophora*	10.18 (7.98–12.66)	10.20 (7.97–12.59)	10.67 (8.40–13.23)	_	_	10.43 (4.37–17.64)

The unconstrained DEC analysis had a LnL = -209.78 (Fig. D.2. in Appendix D), while the constrained analysis had a LnL= -200.54 (Fig. 1, Table 2) The results of both analysis differ mostly in the deeper nodes of the phylogenetic reconstruction (Table 2) and due to the difference in the LnL values, we will focus on the dispersal-constrained analysis. The two most probable ancestral areas for the crown node of Loasoideae included the combinations of the tropical Andes + Pacific deserts + Africa and either Central America or eastern South America (Fig. 1). The most probable ancestral area for crown Klaprothieae includes all the areas where the clade is currently distributed: Central America + tropical Andes + eastern South America + Marquesas, while tropical Andes + eastern South America was retrieved as the most probable ancestral area for crown 'Core Loaseae'. The most probable ancestral area of the crown group *Nasa* corresponds to the tropical Andes, with two dispersal events into Central America and one into the Pacific deserts. On the other hand, the remaining 'Core Loaseae' showed Pacific deserts + eastern South America + southern South America as most probable ancestral area. For crown *Aosa,* eastern South America is the most probable ancestral area with a dispersal event into Central America, while for *Presliophytum* it is the Pacific deserts. Crown South Andean Loasas (SAL) has two area combinations as most probable: southern South America and eastern + southern South America. The most probable ancestral area for

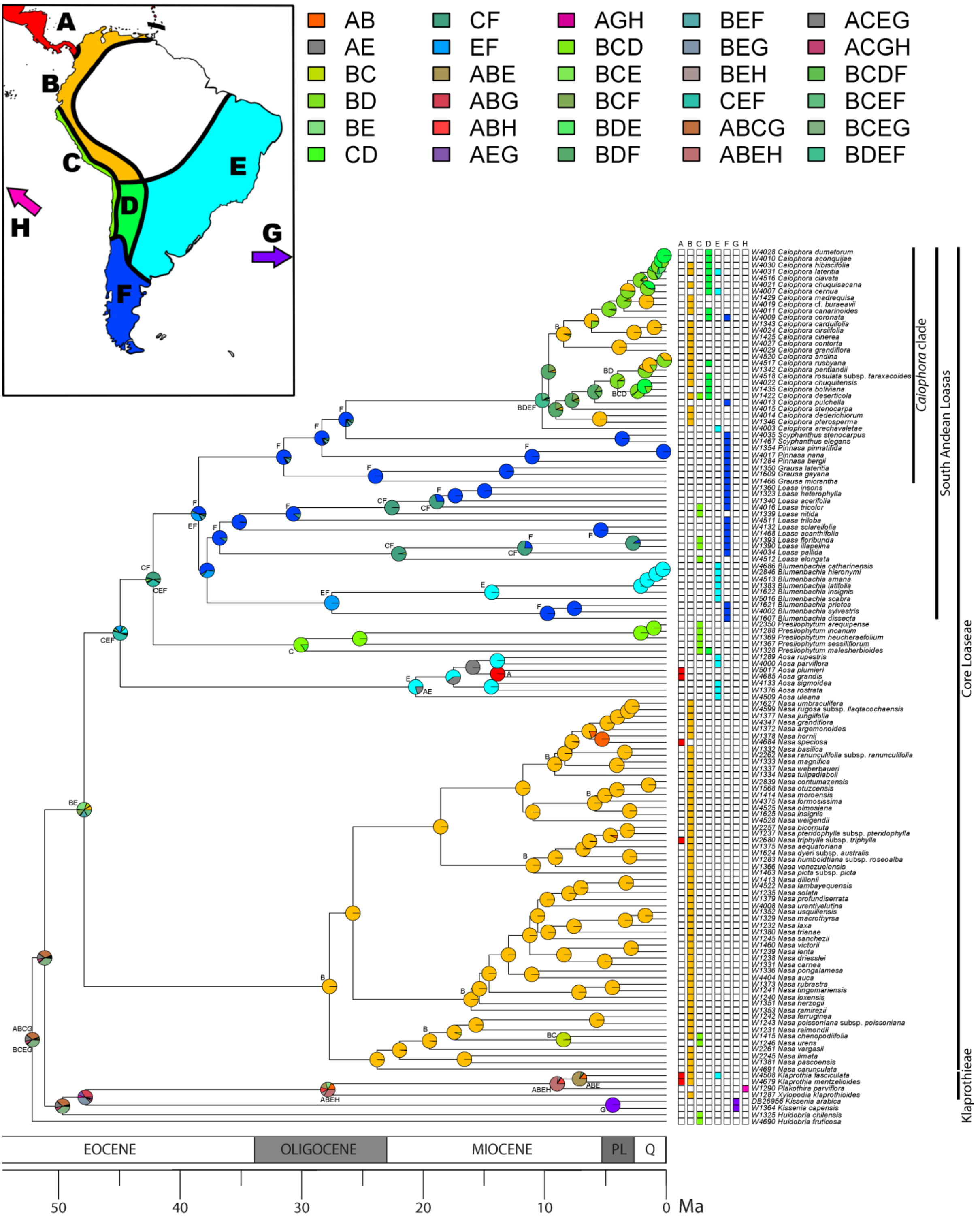

Fig. 1. Divergence time estimates for the phylogeny of Loasoideae using calibration scheme (a) for the dispersal-constrained DEC analysis, with a maximum of 4 areas per node. Coloured grids to the right of the tips indicate the distribution assigned to the species. Colour codes correspond to the areas indicated on the maps and legends. The Pie charts at the nodes depict relative probabilities of areas as estimated from the DEC analyses. The letters next to the pies indicate areas with relative probabilities > 0.2. Only area combinations with relative probabilities > 0.05 are included in the legend. Scale bars in millions of years before present (Ma). PL= Pliocene, Q = Quaternary. Map outline based on Rivas-Martínez et al. (2011).

Blumenbachia is eastern + southern South America, while southern South America is the area obtained for crown *Loasa* (with two identified dispersal events into the Pacific deserts) and the MRCAs of *Caiophora* clade as well as *Grausa*, *Pinnasa* and *Scyphanthus*. Finally, the most likely ancestral area combination for *Caiophora* is tropical Andes + Altiplano + eastern + southern South America, coinciding with the all the areas where this taxon is distributed nowadays.

4. Discussion

The ages for the major clades obtained in our analyses are consistently higher than those reported by other researchers (compare Table 1 with Schenk and Hufford, 2010; Xiang et al., 2011; Strelin et al., 2017). Only the estimates of the crown node age of *Caiophora* by Strelin et al., (2017) are largely congruent with our results although our 95% HPDI is considerably narrower (Table 1).

Several factors may have played a role in our higher age estimations. Although most fossils used as calibration points were employed in previous studies (Schenk and Hufford, 2010; Xiang et al., 2011), we also included calibration points based on recently described, unequivocal fossils from early Cornales that were not available in previous studies (*Cornus* cf. *piggae*, and *Obamacarpa edenensis* Atkinson et al., 2016, 2018). Taxonomic sampling density and dissimilar evolutionary rates can bias molecular clock results (Linder et al., 2005; Soares and Schrago, 2015), while herbs tend to have higher rates of molecular evolution than woody species (Smith and Donoghue, 2008). In the present study the sampling of Loasoideae is considerably expanded (134 species) compared to Schenk and Hufford (2010: 19 taxa); Xiang et al., 2011: five taxa) and Strelin et al., (2017: 31 taxa), at the same time the first two studies included a higher proportion of woody species than ours.

The fossil record suggests that there was considerable biotic exchange between North and South America during late Cretaceous and Paleocene (Wilf et al., 2013). This is underscored by the fossil record of Icacinaceae, Menispermaceae, Araceae, Malvaceae, Myrtaceae and Ulmaceae (Stull et al., 2012) as well as dinosaurs and Therian mammals (Wilf et al., 2013). Divergence times of stem Loasoideae (Table 1), a clade whose sister group is primarily North American, coincide well with this time frame. In the early Paleogene, South America was covered extensively by mesic tropical to subtropical forests (Wilf et al., 2013), biomes with very low extant Loasoideae species diversity

Table 2. Results of the Dispersal Extinction Cladogenesis (DEC) analyses for the major nodes of the Loasaceae subfam. Loasoideae phylogeny. The log-likelihood of each analysis is indicated in the table header. The capital letters correspond to the ancestral areas at the respective node (as defined in Materials and methods) arranged in decreasing order, with their relative probabilities in parenthesis. Only those areas with relative probabilities ≥ 0.05 are shown. A) Central America and the Caribbean, B) tropical Andes, C) Pacific deserts, D) Altiplano, E) eastern South America, F) southern South America, G) Africa, H) Marquesas Islands.

Node	Dispersal unconstrained, three areas maximum LnL = -209.78	Dispersal constrained, four areas maximum LnL= -200.54
Crown Loasoideae (135)	BCG (0.47); BCE (0.20); BC (0.08); BCF (0.07)	BCEG (0.36); ABCG (0.33); ACEG (0.13); ACGH (0.07)
Crown Klaprothieae (140)	B (0.57); BH (0.15); ABH (0.10); AB (0.08)	ABEH (0.34); AB (0.16); ABE (0.15); BE (0.13); ABH (0.12); BEH (0.08)
Crown 'Core Loaseae' (143)	BCE (0.31); BE (0.19); BC (0.15); BCF (0.10)	BE (0.30); BEF (0.16); BCEF (0.16); BCF (0.14); B (0.09); BC (0.09);
Crown *Nasa* (144)	B (0.98)	B (0.99)
Crown *Aosa* (201)	E (0.69); AE (0.29)	E (0.79); AE (0.21)
Crown *Presliophytum* (208)	C (0.89); CD (0.08)	C (0.85); CD (0.15)
Crown SAL (212)	EF (0.39); F (0.35); CF (0.18)	F (0.43); EF (0.34); BEF (0.09); CF (0.05)
Crown *Blumenbachia* (214)	EF (0.97)	EF (1.00)
Crown *Loasa* (222)	F (0.77); CF (0.22)	F (0.91); CF (0.08)
Crown '*Caiophora* clade' (233)	F (0.94)	F (0.82); BEF (0.08)

Crown *Grausa* (234)	F (1.00)	F (1.00)
Crown *Pinnasa* (237)	F (1.00)	F (1.00)
Crown *Scyphanthus* (240)	F (1.00)	F (1.00)
Crown *Caiophora* (241)	BEF (0.88); BE (0.07)	BDEF (0.73); BCEF (0.19)

(Weigend, 2004). The Andean chain may had only reached a fraction of its current height (Graham, 2009), but drier habitats could have existed along the western margin of the continent (Hartley et al., 2005). Around the Paleocene-Eocene Thermal Maximum (PETM: 55 Ma, Zachos et al., 2008) and corresponding with a well-documented rapid increase in floristic diversity (Jaramillo et al., 2010), the main clades of crown Loasoideae started to diverge. The deepest nodes – i.e. the divergence of the Atacama taxa *Huidobria chilensis* and *H. fruticosa*, African *Kissenia* and both stem Klaprothieae and 'Core Loaseae'- date to ca. 57–40 Ma (mostly early Eocene).

Some of the earliest diverging clades of Loasoideae are deserticolous, e.g., *Huidobria fruticosa, H. chilensis* and *Presliophytum* (a later diverging clade). These diverged >35 Ma, suggesting the presence of arid environments in South America as early as the Late Eocene: Hartley et al., (2005) suggest, the Atacama area may have been semi-arid at least since the Mesozoic (150 Ma). Other radiations in the Pacific deserts such as those in *Heliotropium* L. sect *Cochranea* (9.2–27.6 Ma: Luebert et al., 2011), *Oxalis* L. lineages *Carnosae* + *Giganteae* (7.81–19.19 Ma: Heibl and Renner, 2012), the clade of *Mathewsia* Hook. & Arn.+ *Schizopetalon* Sims (3.34–7.26 Ma: Salariato et al., 2016) or *Nolana* L.f., ca. Early Pleistocene-Late Miocene: Dillon et al., 2009) are considerably more recent, and coincide more clearly with the Neogene onset of hyper-arid conditions, partly caused by andean uplift and Central American Seaway closure (Rech et al., 2006). The diversification of the three closely allied Peruvian species of *Presliophytum*, also appears to be a very recent phenomenon (Pliocene to Pleistocene: 3.83–0.78 Ma). The single dispersal event of *Nasa* into the Pacific deserts (5.23–12.05 Ma), overlaps marginally with the inferred age of origin of the Lomas formations in the Early Pliocene (Eichler and Londoño, 2013).

Deserticolous, African *Kissenia* diverged from its closest living relatives 40.07–55.31 Ma, so, LDD must be invoked for the intercontinental dispersal, as previously documented for *Fagonia* L., *Thamnosma* Torr. & Frem. and *Turnera* L., Beier et al., 2004; Thiv et al., 2011; Thulin et al., 2012). Our divergence age estimates between both *Kissenia* (1.96–7.48 Ma: Late Miocene-Pliocene), agree broadly with those of African elements showing similar distributions (Pokorny et al., 2015) and assumed to have dispersed via an arid corridor across East Africa during Pliocene-Pleistocene (Bellstedt et al., 2012).

The MRCA of extant 'Core Loaseae', lived during mid-Eocene (41.30–54.78 Ma), preceding both rapid (ca.10 Ma: Gregory-Wodzicki, 2000), and slow (ca. 40 Ma: Barnes and Ehlers, 2009) estimates of Andean uplift. The historical biogeography of this clade, retrieves two distinct geographical "clusters", with *Nasa* having diversified largely in the tropical Andes, while its sister clade did so mostly in southern and eastern South America (Fig. 1). The topography of South America during that time was dramatically different from current topography: The "Incaic II" deformation would have had an effect in the Western Cordillera by late Eocene (Taylor, 1991; Gregory-Wodzicki, 2000), but apparently, the highlands were not extensive or continuous, as indicated by western Amazonia still draining into the Pacific Ocean (Hoorn et al., 2010) and the presence of low-elevation paleofloras in regions that today lie thousands of meters above sea level (Graham, 2009).

Early *Nasa* lineages could have inhabited the moderate relief areas resulting from the Incaic II phase, that preceded the crown age of this genus (23.12–32.49 Ma). This is broadly contemporary with ages of crown American *Hedyosmum* Sw.*,* ca. Late Oligocene-Early Eocene: Zhang et al., 2011) and Rubiaceae tribe Cinchoneae (22.9–35.1 Ma: Antonelli et al., 2009). These groups reach their highest diversity in mid-elevations (1000–3000 m) of the Andes (Todzia, 1988; Andersson, 1995, Mutke et al., 2014). However, the crown ages of other mid-elevation Andean radiations (*Centropogon* C.Presl and allies, *Ceroxylon* Bonpl., *Fuchsia* L. sect. *Fuchsia*, Gesneriaceae tribe Episcieae, *Vasconcellea* A.St.-Hil.) are generally lower (ca. 4–23 Ma, Luebert and Weigend, 2014;

Lagomarsino et al., 2016; Sanín et al., 2016) and accordingly, it has been suggested that the radiations of these clades were influenced by more recent uplift pulses in this area (Berry et al. 2004; Carvalho and Renner, 2012; Lagomarsino et al., 2016; Sanín et al., 2016). The strikingly gradual diversification of this genus throughout the last ca. 25 Ma may have been in response to increasing topographic (Antonelli et al. 2009) and climatic (Poulsen et al., 2010) complexity, not at the time of its origin, but afterwards, and arguably influenced by orogenic pulses in the tropical Andes that peaked ca. 23 Ma and then again ca. 12 Ma. (Hoorn et al., 2010).

According to our analyses, the remaining genera of 'Core Loaseae', were ancestrally present in the Pacific deserts (*Presliophytum*, see above), eastern and southern South America (Fig. 1), with early splits between these areas. *Aosa* diversified on eastern South America, its crown age (16.10–25.93 Ma) agrees broadly with the crown age of other clades of eastern South American origin: Gesneriaceae tribe Sinningieae (15.0–28.1 Ma: Perret et al., 2013), *Syagrus* Mart.(14.99–24.95 Ma: Meerow et al., 2014), *Ficus* L. sect. *Pharmacosycea* (13.9–27.0 Ma: Machado et al., 2018) and *Attalea* Kunth (ca. Early Miocene-Late Oligocene: Freitas et al., 2016) and with an overall increase of the aridity in South America linked to the radiation of some of these clades (Perret et al., 2013; Machado et al., 2018). We hypothesize that the MRCA of Central American-Caribbean *Aosa grandis* (Standl.) R.H.Acuña & Weigend and *A. plumieri* (Urb.) Weigend arrived via LDD from eastern South America: our age estimates for their divergence from their sister group (12.13–19.78 Ma) post-date the submersion of the hypothetical GAARlandia ca. 33 Ma (Iturralde-Vinent and MacPhee, 1999), and could have pre-dated the closure of the Central American Seaway ca. 15 Ma (Montes et al., 2012).

By the end of the Eocene, the dense forests that covered southern South America (Patagonia) started being replaced by more open habitats (Dunn, et al., 2015) probably allowing Loasaceae subfam. Loasoideae to expand into this area. Crown *Loasa* is ancient (31.34–41.84 Ma), and reaches its highest diversity in Mediterranean Chile. Heibl and Renner (2012) consider this area as a refuge for lineages like *Oxalis*, but for *Loasa* it apparently acts as a source (i.e. area of origin of new lineages, by expansion into new habitats and regions): two independent dispersals into the Pacific deserts (Fig. 1) happened roughly in the same time interval (15.8–28.7 Ma) preceding for the most part estimations of the onset of the hyperaridity (Mid-Miocene: Houston and Hartley, 2003). Other groups with closely related species in Mediterranean Chile and the Pacific deserts (e.g., Southern Tecophilaeaceae, *Tropaeolum* L. sect. *Chilensia*, and *Chaetanthera* Ruiz & Pav.; Luebert, 2011) also indicate floristic exchanges between these adjacent areas.

Blumenbachia includes both Andean and extra-Andean clades. Its crown age overlaps broadly with that of Calyceraceae (22–36.1 Ma: Denham et al., 2016), a group with similar distribution. The divergence between eastern and western lineages of *Blumenbachia* (20.72–34.22 Ma) appears to be the result of vicariance (Fig. 1), but is here retrieved as preceding other similar disjunctions such as those of *Butia* Becc. and *Jubaea* Kunth (8.87–21.39 Ma: Meerow et al., 2014), *Myrceugenia* O. Berg (8.86–21.67 Ma: Murillo et al., 2016) and *Fuchsia* sect. *Quelusia* (ca. 13 Ma, Berry et al., 2004). For these clades, it has been suggested that the major orogenic events in southern South America (22–8 Ma: Giambiagi et al., 2016; Gianni et al., 2016), the establishment of the rainshadow effect in the region (ca. 16 Ma: Gianni et al., 2016) and the first Paranense Marine Transgression (15–13 Ma: Hernández et al., 2005) may have influenced vicariance (Murillo et al., 2016). The last two events correspond with the divergence time (95% HPDI 9.53–19.96 Ma) and ranges of the two extra-Andean sections of *Blumenbachia*.

Andean orogeny pulses, starting ca. 22 Ma, as well as the late Miocene global climate cooling trend (Ogg et al., 2016), could have played a role in the establishment of new habitats, coinciding with the crown ages of the high Andean/Patagonian clades *Blumenbachia* sect. *Angulatae* (6.11–14.11 Ma), *Caiophora* (7.98–12.66 Ma), *Pinnasa* (7.00–15.55 Ma) and the divergence of *Grausa lateritia* (Gillies ex Arn.) Weigend & R.H.Acuña (7.83–18.49 Ma). Other South American groups with similar crown ages and that may have followed the South Andean orogenesis are the subclades of Calyceraceae (ca. 12-15 Ma: Denham et al., 2016) and *Puya* Molina (10 Ma: Givnish et al., 2011). Although the same has not been suggested for *Azorella* Lam. sect *Laretia* (7.49–18.35 Ma: Nicolas and Plunkett, 2014), the Austral Clade of Brassicaeae tribe Eudemeae (6.07–12.89 Ma: Salariato et al., 2016) and *Oxalis* lineage *Palmatifoliae* (5.91–19.6 Ma: Heibl and Renner, 2012) the divergence age estimates in previous studies do agree with the same time frame. Additionally, the uplift pulse in the Central Andes that started ca. 13-10 Ma (Gregory-Wodzicki, 2000; Graham, 2009) may have caused the establishment of an effective South-to-North high elevation corridor (Luebert and Weigend, 2014), facilitating the northward expansion of many clades of temperate origin, including *Caiophora*.

Analyzing the biogeographic history of Loasoideae as a whole, it is apparent that abiotic processes potentially affecting the distribution of the family were different previous to the Oligocene and afterwards. In pre-Oligocene times, when the Andes had yet to reach high elevations, the early cladogenetic events of the subfamily were contemporary with the PETM and potentially arid conditions in western South America. The Pacific deserts were colonized by "Core Loaseae" at least four times in three different time periods (Fig. 1) with only one invasion (by *Nasa* into the Lomas formations) after the onset of hyperaridity (Houston and Hartley, 2003). Since the Oligocene, major Andean uplift pulses took place roughly contemporarily with major radiations concerning the tropical Andean and southern South American groups. As the Andes reached higher elevations they would have become sources of new habitats and a latitudinal corridor (Luebert and Weigend, 2014) for Loasoideae. Most major lineages have dispersed usually only onto adjacent areas, but LDD across an ocean was apparently involved in the arrival of the ancestors of Loasoideae to South America, as well as in the dispersal of *Kissenia,* Klaprothieae and *Aosa*.

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Supplementary material

Supplementary data associated with this work can be found in Appendix D.

References

Acuña, R., Chinchilla, I., Weigend, M., 2018. An unusual disjunction in Loasaceae: Central American *Chichicaste grandis* is nested in Brazilian *Aosa*. Phytotaxa 365, 273–287.

Acuña, R., Fließwasser, S., Ackermann, M., Henning, T., Luebert, F., Weigend, M., 2017. Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae). Taxon 66, 365–378.

Andersson, L., 1995. The tribes and genera of the Cinchoneae complex (Rubiaceae). Ann. Mo. Bot. Gard. 82, 409–427.

Antonelli, A., Nylander, J.A.A., Persson, C., Sanmartín, I., 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. Proc. Natl. Acad. Sci. 106, 9749–9754.

APG IV, 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot J. Linn. Soc. 181, 1–20.

Atkinson, B.A., 2017. Unearthing the Cretaceous Diversification of Cornales. PhD Thesis, Oregon State University, Corvallis, Oregon.

Atkinson, B.A., 2018. The critical role of fossils in inferring deep-node phylogenetic relationships and macroevolutionary patterns in Cornales. Am J. Bot. 105, 1401–1411.

Atkinson, B.A., Stockey, R.A., Rothwell, G.W., 2016. Cretaceous origin of dogwoods: an anatomically preserved Cornus fruit from the Campanian of Vancouver Island. PeerJ 4, e2808.

Atkinson, B.A., Stockey, R.A., Rothwell, G.W., 2018. Tracking the Initial Diversification of Asterids: Anatomically Preserved Cornalean Fruits from the Early Coniacian (Late Cretaceous) of Western North America. Int. J. Plant Sci. 179, 21–35.

Axelrod, D.I., 1987. The Late Oligocene Creede Flora, Colorado. Berkeley, University of California Press. USA.

Barnes, J.B., Ehlers, T.A., 2009. End member models for Andean Plateau uplift. Earth Sci. Rev. 97, 105–132.

Barthlott, W., Hostert, A., Kier, G., Küper, W., Kreft, H., Mutke, J., Rafiqpoor, M.D., Sommer, J.H., 2007. Geographic patterns of vascular plant diversity at continental to global scales. Erdkd. 61, 305–315.

Beier, B.-A., Nylander, J.A.A., Chase, M.W., Thulin, M., 2004. Phylogenetic relationships and biogeography of the desert plant genus *Fagonia* (Zygophyllaceae), inferred by parsimony and Bayesian model averaging. Mol. Phylogenet. Evol. 33, 91–108.

Bellstedt, D.U., Galley, C., Pirie, M.D., Linder, H. P., 2012. The Migration of the Palaeotropical Arid Flora: Zygophylloideae as an Example. Syst. Bot. 37, 951–959.

Berry, P.E., Hahn, W.J., Sytsma, K.J., Hall, J.C., Mast, A., 2004. Phylogenetic relationships and biogeography of *Fuchsia* (Onagraceae) based on noncoding nuclear and chloroplast DNA data. Am. J. Bot., 91, 601–614.

Carvalho, F.A., Renner, S.S., 2013. A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history. Mol. Phylogenet. Evol. 65, 46–63.

Christenhusz, M.J.M., Fay, M.F., Chase, M.W., 2017. An illustrated encyclopedia of vascular plants. Richmond (Surrey) and Chicago. Kew Royal Botanical Gardens and University of Chicago Press.

Crespo, S., Pérez-Moreau, R. L., 1988. Loasaceae. In: Correa, M.N. (Ed.), Flora Patagónica. Vol. 8 (5). Buenos Aires, Colección Científica del Instituto Nacional de Tecnología Agropecuaria, pp. 199–217.

De Smet, Y., Granados-Mendoza, C., Wanke, S., Goetghebeur, P., Samain, M.-S., 2015. Molecular phylogenetics and new (infra)generic classification to alleviate polyphyly in tribe Hydrangeeae (Cornales: Hydrangeaceae). Taxon 64, 741–753.

Denham, S.S., Zavala-Gallo, L., Johnson, L.A., Pozner, R.E., 2016. Insights into the phylogeny and evolutionary history of Calyceraceae. Taxon, 65, 1328–1344

Dillon, M.O., Tu, T., Xie, L., Quipuscoa Silvestre, V., Wen, J., 2009. Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. J. Syst. Evol. 47, 457–476.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Dunn, R.E., Strömberg, C.A.E., Madden, R.H., Kohn, M.J., Carlini, A.A., 2015. Linked canopy, climate, and faunal change in the Cenozoic of Patagonia. Sci. 347, 258–261.

Eichler, T.P., Londoño, A.C., 2013. ENSO Impacts on Lomas Formation in South Coastal Peru: Implications for the Pliocene?. Adv. Meteorol. 2013, Article ID 175947.

Freitas, C., Meerow, A.W., Pintaud, J.-C., Henderson, A., Noblick, L., Costa, F.R.C., Barbosa, C.E., Barrington, D., 2016. Phylogenetic analysis of Attalea (Arecaceae): insights into the historical biogeography of a recently diversified Neotropical plant group. Bot J. Linn. Soc. 182, 287–302.

Friis, E.M., Crane, P. R., Pedersen, K.R., 2011. Early Flowers and Angiosperm Evolution . Cambridge, Cambridge University Press.

Gandolfo M., Nixon, K., Crepet W., 1998. *Tylerianthus crossmanensis* gen. et sp. nov., aff. Hydrangeaceae) from the Upper Cretaceous of New Jersey. Am. J. Bot. 85, 376–376.

GBIF.org, 2017. GBIF Home Page. Available from: https://www.gbif.org

Giambiagi, L., Mescua, J., Bechis, F., Hoke, G., Suriano, J., Spagnotto, S., Moreiras, S.M, Lossada, A., Mazzitelli, M., Toural-Dapoza, R., Folguera, A., Mardonez, D., Pagano, D.S., 2016. Cenozoic Orogenic Evolution of the Southern Central Andes (32–

36°S). In: Folguera, A., Naipauer, M., Sagripanti, L., Ghiglione, M.C., Ort, D.L., Giambiagi L. (Eds.), Growth of the Southern Andes. Cham, Heidelberg, New York, Dordrecht and London, Springer, pp. 63–98.

Gianni, G., Folguera, A., Navarrete, C., Encinas, A., Echaurren, A., 2016. The North Patagonian Orogen: Meso-Cenozoic Evolution from the Andes to the Foreland Area. In: Folguera, A., Naipauer, M., Sagripanti, L., Ghiglione, M.C., Ort, D.L., Giambiagi L. (Eds.), Growth of the Southern Andes. Cham, Heidelberg, New York, Dordrecht and London, Springer, pp. 173–200.

Givnish, T.J., Barfuss, M.H.J., VanEe, B., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., Crayn, D.M., Smith, A.C., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K., Luther, H., Till, W., Zizka, G., Berry, P.E., Sytsma, K.J., 2011. Adaptive radiation and diversification in Bromeliaceae: insights from a eight-locus plastid phylogeny. Am. J. Bot. 98, 872–895.

Graham, A., 2009. The Andes: a geological overview from a biological perspective. Ann. Mo. Bot. Gard. 96, 371–385

Gregory-Wodzicki, K. M., 2000. Uplift history of the Central and Northern Andes: a review. Geol. Soc. Am. Bull. 112, 1091–1105.

Guo, Y.-L., Pais, A., Weakley, A.S., Xiang, Q.-Y., 2013. Molecular phylogenetic analysis suggests paraphyly and early diversification of *Philadelphus* (Hydrangeaceae) in western North America: new insights into affinity with *Carpenteria*. J. Syst. Evol. 51, 545–563.

Hartley, A.J, Chong, G., Houston, J., Mather, A.E., 2005. 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile. J. Geol. Soc. London 162, 421–424.

Heibl, C., 2013) ‘phyloch’: interfaces and graphic tools for phylogenetic data in R. Available: http://www.christophheibl.de/Rpackages.html.

Heibl, C., Renner, S.S., 2012. Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. Syst. Biol. 61, 823–834.

Henning, T., Mittelbach, M., Ismail, S.A., Acuña, R., Weigend, M., 2018. A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation. Sci. Rep. 8, 14018.

Hernández, R.M., Jordan, T.E., Dalenz Farjat, A., Echavarría, L., Idleman, B.D., Reynolds, J.H., 2005. Age, distribution, tectonics, and eustatic controls of the Paranense and Caribbean marine transgressions in southern Bolivia and Argentina. Journal of South American Earth Sci. 19, 495–512.

Hoorn, C., Wesselingh, F.P., ter Steege, H., Bermúdez, M.A., Mora, A., Sevink, J., Sanmartín, I., Sánchez-Meseguer, A., Anderson, C.L., Figuereido, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Särkiinen, T., Antonelli, A., 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Sci. 330, 927–931

Houston, J., Hartley, A.J., 2003. The Central Andean West-slope rainshadow and its potential contribution to the origin of hyper-aridity in the Atacama Desert. Int. J. Climatol. 23, 1453–1464.

Hufford, L., 1995. Seed morphology of Hydrangeaceae and its phylogenetic implications. Int. J. Plant Sci. 156, 555–580.

Hufford, L., 2004. Hydrangeaceae. In: Kubitzki, K. (Ed.), The families and genera of vascular plants Vol. 6. Berlin, Spinger Verlag, pp. 202–215.

Hufford, L., McMahon, M., O'Quinn, R., Poston, M., 2005. A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. Int. J. Plant Sci., 166, 289–300.

Hughes, C.E., Pennington, R.T., Antonelli, A., 2013. Neotropical Plant Evolution: Assembling the Big Picture. Bot. J. Linn. Soc., 171, 1–18.

Iturralde-Vinent, M., MacPhee, R.D., 1999. Paleogeography of the Caribbean region: Implications for Cenozoic biogeography. Bull. Am. Mus. Nat. Hist. 238, 1–95.

Jaramillo, C., Ochoa, D., Contreras, L., Pagani, M., Carvajal-Ortiz, H., Pratt, L.M., Krishnan, S., Cardona, A., Romero, M., Quiroz, L., Rodríguez, G., Rueda, M.J., de la Parra, F., Morón, S., Green, W., Bayona, G., Montes, C., Quintero, O., Ramírez, R., Mora, G., Schouten, S., Bermúdez, H., Navarrete, R., Parra, F., Alvarán M., Osorno, J., Crowley, J.L., Valencia, V., Vervoort, J., 2010. Effects of Rapid Global Warming at the Paleocene-Eocene Boundary on Neotropical Vegetation. Sci. 330, 957–961

Lagomarsino, L.P., Condamine, F.L., Antonelli, A., Mulch, A., Davis, C.C., 2016. The abiotic and biotic drivers of rapid diversification in Andean bellflowers (Campanulaceae). New Phytol. 210, 1430–1442.

Linder, H.P., Hardy, C.R., Rutschmann, F., 2005. Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. Mol. Phylogenet. Evol. 35, 569–582.

Luebert, F., 2011. Hacia una fitogeografía histórica del Desierto de Atacama. Rev. Geogr. Norte Grande 50, 105–133.

Luebert, F., Hilger, H.H., Weigend, M., 2011. Diversification in the Andes: age and origins of South American *Heliotropium* lineages (Heliotropiaceae, Boraginales). Mol. Phylogenet. Evol. 61, 90–102.

Luebert, F., Weigend, M., 2014. Phylogenetic insights into Andean plant diversification. Front. Ecol. Evol. 2, 27.

Machado, A.F.P., Rønsted, N., Bruun-Lund, S., Pereira, R:A.S., de Queiroz, L.P., 2018. Atlantic forests to the all Americas: Biogeographical history and divergence times of Neotropical Ficus (Moraceae). Mol. Phylogenet. Evol. 122, 46–58.

Magallón, S., Gómez-Acevedo, S., Sánchez-Reyes, L.L., Hernández-Hernández, T., 2015. A metacalibrated timetree documents the early rise of flowering plant phylogenetic diversity. New Phytol. 207, 437–453.

Manchester, S.R., 1994. Fruits and seeds of the middle Eocene nut beds flora, Clarno Formation, Oregon. Palaeontogr. Am. 58, 1–205.

Manchester, S.R., 2002. Leaves and fruits of *Davidia* (Cornales) from the Paleocene of North America. Syst. Bot. 27, 368–382.

Manchester, S.R., Grímsson, F., Zetter, R., 2015. Assessing the fossil record of asterids in the context of our current phylogenetic framework. Ann. Mo. Bot. Gard. 100, 329–363.

Matzke, N.J., 2013. Probabilistic historical biogeography: new models for founder–event speciation, imperfect detection, and fossils allow improved accuracy and model–testing. Front. Biogeogr. 5, 4.

Meerow. A.W., Noblick, L., Salas-Leiva, D.E., Sanchez, V., Francisco-Ortega, J., Jestrow, B., Nakamura, K., 2014. Phylogeny and historical biogeography of the cocosoid palms (Arecaceae, Arecoideae, Cocoseae) inferred from sequences of six WRKY gene family loci. Cladistics, 31, 1–26.

Miller, M. A., Pfeiffer, W., Schwartz, T., 2010.. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, pp. 1–8.

Montes, C., Bayona, G., Cardona, A., Buchs, D.M., Silva, C.A., Morón, S., Hoyos, N., Ramírez, D.A., Jaramillo, C.A., Valencia, V., 2012. Arc-continent collision and orocline formation: Closing of the Central American seaway. J. Geophys. Res. 117, B04105.

Murillo, J.C., Stuessy, T.E., Ruiz, E., 2016. Explaining disjunct distributions in the flora of southern South America: evolutionary history and biogeography of *Myrceugenia* (Myrtaceae). J. Biogeogr. 43, 979–990.

Mustoe, G. E., 2002. *Hydrangea* fossils from the early Tertiary Chuckanut Formation. Wash. Geol. 30, 17–20.

Mutke, J., Jacobs, R., Meyers, K., Henning, T., Weigend, M., 2014. Diversity patterns of selected Andean plant groups correspond to topography and habitat dynamics, not orogeny. Front. Genet. 5, 351.

Mutke, J., Weigend M., 2017. Mesoscale patterns of plant diversity in Andean South America based on combined checklist and GBIF data. Ber. Reinhold-Tüxen Ges. 29, 83–97.

Nicolas, A.N., Plunkett, G.M., 2014. Diversification times and biogeographic patterns in Apiales. Bot. Rev. 80, 30–58.

Noguera-Savelli, E., 2012. Revisión taxonómica de Loasaceae en Venezuela. Caldasia 34, 43–67.

Ogg, J.G., Ogg, G.M., Gradstein, F.M., 2016. A Concise Geologic Time Scale 2016. Amsterdam, Elsevier.

Perret, M., Chautems, A., Onofre de Araujo, A., Salamin, N., 2013. Temporal and spatial origin of Gesneriaceae in the New World inferred from plastid DNA sequences. Bot. J. Linn. Soc. 171, 61–79.

Poinar, G.O., Weigend, M., Henning, T., 2015. *Klaprothiopsis dyscrita* gen. et sp. nov., Loasaceae) in mid-tertiary Dominican amber. J. Bot. Res. Inst. Tex. 9, 369–379.

Pokorny, L., Riina, R., Mairal, M., Meseguer, A.S., Culshaw, V., Cendoya, J., Serrano, M., Carbajal, R., Ortiz, S., Heuertz, M., Sanmartín, I., 2015. Living on the edge: timing of Rand Flora disjunctions congruent with ongoing aridification in Africa. Front. Genet. 6, 154.

Posada, D., Crandall, K.A., 1998. MODELTEST, testing the model of DNA substitution. Bioinformatics, 14, 817–818.

Poulsen, C.J., Ehlers, T.A., Insel , N., 2010. Onset of convective rainfall during gradual Late Miocene rise of the Central Andes. Sci. 328, 490–493.

Rambaut, A., Drummond, A.J., 2014. Tracer v.1.6. Available at: http://tree.bio.ed.ac.uk/software/tracer/.

Rech, J.A., Currie, B.S., Michalski, G., Cowan, A.M., 2006. Neogene climate change and uplift in the Atacama Desert, Chile. Geol. 34, 761–764.

Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4–14.

Richardson, J.E., Madriñán, S., Gómez-Gutiérrez, M.C., Valderrama, E., Luna, J., Banda., K., Serrano, J., Torres, M.F., Jara, O.A., Aldana, A.M., Cortés, R., Sánchez, D., Montes, C., 2018, Using dated molecular phylogenies to help reconstruct geological, climatic, and biological history: Examples from Colombia. Geol. J. 2018, 1–9.

Rivas-Martínez, S., Navarro, G., Penas, A., Costa, M., 2011. Biogeographic map of South America. A preliminary survey. Int. J. Geobot. Res. 1, 21–40 + Map.

Salariato, D.L., Zuloaga, F.O., Franzke, A., Mummenhoff, K., Al-Shehbaz, I., 2016. Diversification patterns in the CES clade (Brassicaceae tribes Cremolobeae, Eudemeae, Schizopetaleae) in Andean South America. Bot. J. Linn. Soc. 181, 543–566.

Sanín, M.J., Kissling, W.D., Bacon, C.D., Borschenius, F., Galeano, G., Svenning, J.-C., Olivera, J., Ramírez, R., Trénel, P., Pintaud, J.-C., 2016. The Neogene rise of the tropical Andes facilitated diversification of wax palms (*Ceroxylon*: Arecaceae) through geographical colonization and climatic niche separation. Bot. J. Linn. Soc. 182, 303–317.

Schenk, J., Hufford, L., 2010. Effects of Substitution Models on Divergence Time Estimates: Simulations and an Empirical Study of Model Uncertainty Using Cornales. Syst. Bot. 35, 578–592

Schenk, J., Jacobs, S., Hufford, L., 2017. Comparative Diversification Analyses of Hydrangeaceae and Loasaceae: The Role of Continental Dispersal in Generating Species Diversity. Abstract. Botany2017 Conference. Available at: http://2017.botanyconference.org/engine/search/index.php?func=detail&aid=82

Serbet, R., Manchester, S.R., Aulenbach K., Braman, D., 2004. Nyssaceae among the dinosaurs: Anatomically preserved fruits from the Upper Cretaceous Horseshoe Canyon Formation, Drumheller, Alberta, Canada. Abstract. Botany 2004 Conference. Available at: http://2004.botanyconference.org/engine/search/index.php?func=detail&aid=485

Slanis, A.C., Perea, M.C., Grau, A., 2016. Revisión taxonómica del género *Caiophora* (Loasaceae) para Argentina: C. sleumerii una nueva especie. Darwiniana, nueva ser. 4, 138–191.

Sleumer, H., 1955. Die Loasaceen Argentiniens. Bot. Jahrb. Syst, Pflanzengesch. Pflanzengeogr. 76, 411–462.

Smith, S.A., Donoghue, M.J., 2008. Rates of molecular evolution are linked to life history in flowering plants. Sci. 322, 86–89.

Soares, A.E., Schrago, C.G., 2015. The influence of taxon sampling on Bayesian divergence time inference under scenarios of rate heterogeneity among lineages. J. Theor. Biol. 364, 31–39.

Soltis, D., Soltis, P., Endress, P., Chase, M., Manchester, S., Judd, W., Majure, L., Mavrodiev, E., 2018. Phylogeny and Evolution of the Angiosperms: Revised and Updated Edition. Chicago, University of Chicago Press.

Strelin, M., Arroyo, J., Fließwasser, S., Ackermann, M., 2017. Diversification of *Caiophora* (Loasaceae subfam. Loasoideae) during the uplift of the Central Andes. Org. Divers. Evol. 17, 29–41.

Stull, G.W., Herrera, F., Manchester, S.R., Jaramillo, C., Tiffney, B.H., 2012. Fruits of an "Old World" tribe (Phytocreneae; Icacinaceae) from the Paleogene of North and South America. Syst. Bot. 37, 784–794.

Taylor, D.W., 1991. Paleobiogeographic relationships of Andean angiosperms of Cretaceous to Pliocene age. Palaeogeog. Palaeoclimatol. Palaeoecol. 88, 69–84.

Thiv, M., van der Niet, T., Rutschmann, F., Thulin, M., Brune, T., Linder, H.P., 2011. Old-New World and trans-African disjunctions of *Thamnosma* (Rutaceae): intercontinental long-distance dispersal and local differentiation in the succulent biome. Am. J. Bot., 98, 76–87.

Thulin, M., Razafimandimbison, S.G., Chafe, P., Heidari, N., Kool, A., Shore, J.S., 2012. Phylogeny of the Turneraceae clade (Passifloraceae s.l.): Trans-Atlantic disjunctions and two new genera in Africa. Taxon, 61, 308–323.

Todzia, C.A., 1988. Chloranthaceae: *Hedyosmum*. Flora Neotropica Monograph 48, 1–139.

Weigend, M., 1997. *Nasa* and the conquest of South America: Systematic rearrangements in Loasaceae Juss. PhD Thesis, Ludwig-Maximilians-Universität München, Munich.

Weigend, M., 2004. Loasaceae. In: Kubitzki, K. (Ed.), The families and genera of vascular plants Vol. 6. Berlin, Spinger Verlag, pp. 239–254.

Weigend, M., Gottschling, M., Hoot, S., Ackermann, M., 2004. A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on *trn*L (UAA) sequence data, with consequences for systematics and historical biogeography. Org. Divers. Evol. 4, 73–90.

Wilf, P., Cúneo, N.R., Escapa, I.H., Pol, D., Woodburne, M.O., 2013. Splendid and Seldom Isolated: The Paleobiogeography of Patagonia. Annu. Rev. Earth Planet. Sci. 41, 561–603.

Xiang, Q.-Y.J., Thomas, D.T., Xiang, Q.P., 2011. Resolving and dating the phylogeny of Cornales – effects of taxon sampling, data partitions, and fossil calibrations. Mol. Phylogenet. Evol. 59, 123–138.

Zachos, J.C., Dickens, G.R., Zeebe, R.E., 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. Nat. 451, 279–283.

Zhang, Q., Antonelli, A., Feild, T.S., Kong, H.-Z., 2011) Revisiting taxonomy, morphological evolution, and fossil calibration strategies in Chloranthaceae J. Syst. Evol. 49, 315–329.

CHAPTER 7

Diversification patterns of *Nasa* (Loasaceae subfam. Loasoideae) in the Neotropics

Flower of *Nasa tabularis*. This species is endemic to high Andean forests in central Ecuador. Although rarely collected, it can be locally abundant.

Diversification patterns of *Nasa* (Loasaceae subfam. Loasoideae) in the Neotropics

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ABSTRACT

The Andean uplift is recognized as one of the most important events shaping the Neotropical biota. Previous phylogenetic reconstructions of *Nasa*, a mostly tropical Andean genus and probably influenced by Andean uplift, have been insufficient to address its historical biogeography or ancestral character estimations. The main goal of the present contribution is to attempt to fill those voids. Our results show that *Nasa* is formed by four well supported clades, Clade I is sister to the rest of the genus and formed by niche-conservative species. The remaining three clades have experienced frequent habitat shifts. The crown node of *Nasa* was dated to ca. 30 Ma (Oligocene), prior to most Andean uplift, originating in the Central Andes and the Amotape-Huancabamba Zone at middle elevations, in scrub and scree habitats. Ca. 15 Ma, in tandem with increasing complexity of the topography and climate of the Andes, clades II, III and IV radiated and colonization of different habitats started, with most dispersal events into the Northern Andes taking place after this date. Morphological analyses show that the phylogenetic position of most unsampled *Nasa* species could be predicted, but for few species it remains too uncertain. The geographical structure of the molecular data and discordance

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between morphology and phylogenetic position of some species, suggest reticulate evolution. Testing this will require nuclear marker data to be explored.

ADDITIONAL KEYWORDS: Bayesian Inference – Cluster analysis – Dispersal Extinction Cladogenesis – Habitat – Maximum likehood – Morphology – Phylogenetic signal – Tropical Andes

INTRODUCTION

The neotropical flora is one of the richest in the world (Barthlott *et al.*, 2007; Antonelli & Sanmartín, 2011), product of dispersal between biogeographic realms (Christenhusz & Chase, 2013), extinction (Villar de Seoane *et al.*, 2015) and, often extensive, *in situ* radiation (Perret *et al.*, 2013; Pérez-Escobar *et al.*, 2017). Many of the events that shaped to the current flora, were probably influenced by profound climatologic and geologic changes in the Cenozoic, including the Paleocene-Eocene Thermal Maximum (Zachos, Dickens, & Zeebe, 2008), the opening of the Drake passage (Scher & Martin, 2006), the uplift pulses in the Andes (Graham, 2009), the Neogene climatic deterioration (Andersson, 2009) and the closure of the Central American Seaway (Montes *et al.*, 2012).

The Andean uplift pulses have been considered as some of the most important geologic events shaping the biodiversity patterns in the Neotropics during the Neogene (Gentry, 1982; Luebert, Hilger, & Weigend, 2011; Madriñán, Cortés, & Richardson, 2013). Although huge progress has been made in recent years to understand the major geologic events, considerable debate on their exact timing and significant knowledge gaps remain (Horton, 2018; Richardson *et al.*, 2018) The idea that most of the uplift of the tropical Andes took place during the last 10 Ma has been invoked frequently (Gregory-Wodzicki, 2000; Graham, 2009) and albeit the timing of the diversification of many groups of the region agrees with this time frame, divergences from it have also been revealed (Luebert & Weigend, 2014; Acuña *et al.,* in prep).

In the last two decades, dozens of studies dealing with the biogeography and diversity patterns of Andean groups have been published, ranging from microorganisms (Nottingham *et al.*, 2018) to arthropods (De-Silva *et al.*, 2016; Salgado-Roa *et al.*, 2018), vertebrates (Derryberry *et al.*, 2011; Patterson & Costa, 2012; Mendoza *et al.*, 2015; Musilová *et al.*, 2015; Hazzi *et al.*, 2018); ferns (McHenry & Barrington, 2014; Noben *et al.*, 2017) and flowering plants (Luebert *et al.*, 2011; Nürk, Scheriau, & Madriñán, 2013; Perret *et al.*, 2013; Sanín *et al.*, 2016; Pérez-Escobar *et al.*, 2017). Currently, the tropical

Andes are considered as one of the richest and most critically endangered hotspots of biodiversity (Myers *et al.*, 2000; Mittermeier *et al.*, 2004), with a very high percentage (ca. 50%) of endemic plant species (Mittermeier *et al.*, 2004) and one of the highest proportions of species still awaiting formal description (Joppa *et al.*, 2011). Both the high α-diversity and the high degree of differentiation at the community level found in this area are the result of high geodiversity (Barthlott *et al.*, 2007), with different Andean biomes often having contrasting biotic histories when compared against each other (Särkinen *et al.*, 2012).

Nasa Weigend is a predominantly Andean, medium-sized genus that includes almost one third of the entire extant species diversity of Loasaceae (Weigend, 2004a). Morphologically and ecologically, it is highly diverse, with life histories ranging from ephemeral herbs to ligneous shrubs, including rizomatous biennials and subscandent lianescent species (Weigend 1997, 2004a). *Nasa* can be found in most Andean habitats from near sea level in the Lomas formations of the Peruvian and Chilean deserts, and the coastal mistbelt forests in western Ecuador, to elevations above 4300 m in the puna of the Peruvian Andes. The highest species number is, however, encountered at elevations between 2500 and 3500 m in either montane forests edges or Andean scrub communities. *Nasa* ranges from southeastern Mexico to northern Chile and central Bolivia, but the centre of diversity is clearly found from southern Ecuador to northern Peru, in the Amotape-Huancabamba zone (Mutke *et al.*, 2014), an area that is also a major center of diversity for genera such as *Deprea* Raf. (Solanaceae), *Fuchsia* L. (Onagraceae), *Lysipomia* Kunth (Campanulaceae), *Macrocarpaea* (Griseb.) Gilg, *Ribes* L. (Grossulariaceae) and *Urtica* L. (Urticaceae) amongst others (Ayers, 1999; Weigend, 2002a; Berry *et al.*, 2004; Struwe *et al.*, 2009; Deanna, Barboza, & Carrizo García, 2018).

Nasa can be easily identified by its distinctive inflorescence architecture, with a single bract per flower and its nectar scale structure, with well-developed apical wings and, usually, nectar sacs (Weigend, 1997, 2006). It includes four series, defined first by Urban & Gilg (in Gilg, 1894), and formerly included in a paraphyletic, broadly defined *Loasa* Adans.: *Loasa* sect. *Loasa* ser. *Alatae*, *Loasa* sect. *Loasa* ser. *Caruncluatae*, *Loasa* sect. *Loasa* ser. *Grandiflorae* and *Loasa* sect. *Loasa* ser. *Saccatae*. Weigend (1997), using morphological information, hypothesized that *Nasa* was sister to the group informally called “South Andean Loasas”. Early phylogenetic studies based on molecular data, included only a limited sampling of *Nasa* (Hempel *et al.*, 1995; Moody *et al.*, 2001;

Xiang *et al.*, 2002). Hufford *et al.* (2003, 2005) retrieved *Nasa* as sister to a clade including *Aosa* Weigend, *Presliophytum* (Urb. & Gilg) Weigend and the "South Andean Loasas". Although easily characterized based on morphology, the series are doubtfully monophyletic (Weigend, 1997). Even with a small taxonomic sampling, the results of Hufford *et al.* (2003, 2005) showed that that ser. *Saccatae* was paraphyletic. With 28 species, Weigend *et al.* (2004) had by far the most extensive sampling of the genus in the early molecular era, including at least two species from each of the four series. Their analyses retrieved a monophyletic *Nasa*, but polyphyletic sers. *Saccatae* and *Carunculatae* while ser. *Alatae* was retrieved as doubtfully monophyletic. Only ser. *Grandiflorae* could be shown to be a monophyletic. The enigmatic *Nasa venezuelensis* (Steyerm.) Weigend, originally described as a *Caiophora* C.Presl, was also confirmed as a member of *Nasa*.

Weigend & Gottschling (2006) sampled 49 taxa of *Nasa* for a phylogeny based on both plastid and nuclear markers. They retrieved seven clades with generally moderate to high support on a poorly supported backbone, but could demonstrate that sers. *Saccatae*, *Carunculatae* and *Alatae* are artificial, Most of the series have been shown to be artificial, but some species groups within the series (e.g., *Nasa triphylla* (Juss.) Weigend group: Dostert & Weigend, 1999) are retrieved as monophyletic and could be readily identified based on shared autoapomorphies (Weigend & Gottschling, 2006). No well-resolved, well-sampled phylogeny of the genus *Nasa* is available at present and inferences on the historical biogeography of the genus have accordingly not been attempted. Weigend (1997) stated some hypothesis regarding the origins and dispersal patterns of the main clades, but these remained untested.

*Nas*a, with its wide ecological and geographical range, high species number, including numerous narrow-endemic species, and extreme morphological diversity, represents an interesting subject to analyze the phylogeny and biogeography of a mostly tropical Andean plant group. We aim at providing a well resolved phylogenetic reconstruction for the genus, based on a broad sampling. Based on this result, we reconstruct the historical biogeography of *Nasa* and the ancestral character states of two important traits (elevational distribution and habitat). For those taxa that we could not include our molecular data set, we employ a morphological matrix to infer their placement. We also assess the phylogenetic signal of some traits traditionally used in classification. This study is focused on five research questions: 1- Which are the major, well supported clades in *Nasa*, based on a plastid markers? 2- What are the inferred ancestral distribution areas of these clades and patterns and timing of their dispersal? 3- What major geological events correlate with cladogenesis? 4- What is the ancestral ecology of the major clades of *Nasa* and what clades show niche conservatism? 5- Is there congruence between morphology and phylogeny and could morphology offer plausible insights on the phylogenetic relationship of the species not available for molecular analyses?

MATERIALS AND METHODS

PLANT MATERIAL AND OUTGROUP SELECTION

In total we sampled 202 species or subespecies of Cornales, of which 89 belong to *Nasa* (representing ca. 70% of the species and subspecies of the genus), including two yet undescribed species as well as a naturally occurring hybrid. A subsample of 130 species and subspecies (133 accessions), was employed in Maximum Likelihood (ML, Felsenstein, 1981) and Bayesian Inference (BI, Mau, Newton, & Larget, 1999) phylogenetic reconstructions. For these we included the entire sampling of *Nasa* [including two accessions of each *Nasa carunculata* (Urb. & Gilg) Weigend, *Nasa* '*triphylla'* subsp. *rudis* (Benth.) Weigend and *Nasa triphylla* subsp. *triphylla*] and every genus of Loasoideae including: three species of *Aosa*, three of *Blumenbachia* Schrad., six of *Caiophora* C. Presl., two of *Grausa* Weigend & R.H.Acuña, two of *Huidobria* Gay, *Kissenia capensis* Endl., *Klaprothia fasciculata* (C.Presl) Poston, five of *Loasa* Adans., *Plakothira parviflora* J.Florence two of *Pinnasa* Weigend & R.H.Acuña, three of *Presliophytum*, *Scyphanthus elegans* Sweet and *Xylopodia klaprothioides* Weigend. *Cevallia sinuata* Lag., *Eucnide urens* Parry ex Coville, *Gronovia scandens* L., *Mentzelia albescens* (Gillies ex Arn.) Benth. & Hook.f. ex Griseb., *M. aspera* L. and *Petalonyx linearis* Greene, were also included as representatives of non-Loasoid Loasaceae. *Cornus peruviana* J.F.Macbr., *Fendlera rupicola* Engelm. & A.Gray, *Hydrangea oerstedii* Briq. and *Nyssa talamancana* Hammel & N.Zamora were selected as distantly related outgroups based on Hufford *et al.*, (2003), Xiang, Thomas, & Xiang, (2011) and APGIV (2016). The complete voucher specimen information, including additional Cornales samples for the dating and biogeography analyses, is in Table D.1., Appendix D.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA was extracted using the CTAB method (Doyle & Doyle, 1987). Four plastid regions: *trn*L–*trn*F, *mat*K, the *trn*S–*trn*G intergenic spacers, and the *rps*16 intron were amplified. These regions have proved to be informative to infer the phylogenetic relationships in Loasoideae (Weigend *et al.*, 2004; Hufford *et al.*, 2005; Weigend & Gottschling, 2006; Acuña *et al.*, 2017). Although several *Nasa* sequences were generated newly for this study, the bulk of the sequences were employed in previous studies (Acuña *et al.*, 2017, in prep.; Acuña, Chinchilla, & Weigend, 2018; Henning *et al.*, 2018). The *trn*L–*trn*F sequence of *Aosa plumieri* (Urb.) Weigend and the partial *mat*K sequences of *Cevallia sinuata* and *Grausa lateritia* (Gillies ex. Arn.) Weigend & R.H.Acuña were obtained from GenBank (Hufford *et al.*, 2005). The respective GenBank accession numbers for all sequences are shown in Table D.1. Appendix D. We excluded the partial, short *mat*K sequence of *Nasa formosissima* Weigend generated by Acuña *et al.* (in prep.), while the accessions of *Nasa auca* (Weigend) Weigend, *Nasa humboldtiana* (Urb. & Gilg) Weigend subsp. *roseoalba* (Weigend) Dostert and *Nasa triphylla* subsp. *papaverifolia* (Kunth) Weigend were replaced by new ones from more recently collected field material. The matrix is complete for *trn*L–*trn*F, *trn*S–*trn*G and *rps*16, however it was not possible to obtain *mat*K sequences from *Nasa carnea* (Urb. & Gilg) Weigend, *Nasa lindeniana* (Urb. & Gilg) Weigend, *Nasa pascoensis* Weigend, *Nasa stuebeliana* (Urb. & Gilg) Weigend, *Nasa ranunculifolia* (Kunth) Weigend subsp *macrorrhiza* (Urb. & Gilg) Weigend and *Nasa solaria* (J.F.Macbr.) Weigend. Amplification, sequencing and alignment followed the protocols of Acuña *et al.*, (2017).

PHYLOGENETIC ANALYSES

Phylogenetic reconstructions were carried out for 133 Cornales accessions (see plant material and outgroup selection), employing ML in RAxML v. 8.1.X (Stamatakis, 2014), included in RAxMLGUI v. 1.5b1 (Silvestro & Michalak, 2012), and BI in MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001) in the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010). Based on the Akaike information criterion, FindModel (available from http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html), which implements Posada & Crandall's (2001) Modeltest, selected GTR+Γ as the model that fits best each of the four plastid marker datasets. Following Xiang *et al.* (2011), *Cornus peruviana* was chosen to root the trees. The individual marker ML phylogenic reconstructions were inspected visually in order to find significant topological (>75 Bootstrap support) incongruences. Two nodes were considered as significantly incongruent between the different marker phylogenies, these being the position of *Nasa grandiflora* (Desr.) Weigend as sister to *N. peltiphylla* (Weigend) Weigend or *N. jungiifolia* (Weigend) Weigend in *mat*K and *trn*S–*trn*G respectively and *Caiophora hibiscifolia* (Griseb.) Urb. & Gilg as sister to *C. arechavaletae* (Urb.) Urb. or *C. contorta* (Desr. *ex* Lam.) C.Presl in *mat*K and *trn*L–*trn*F respectively. The individual markers were combined in a single matrix with four partitions. The statistical support for the nodes was assessed by 1000 ML thorough bootstrap replicates in 100 runs. The BI were conducted in four independent runs with one cold and three heated chains; the Markov chain had a length of 10 million generations, sampled every 1000 generations. After convergence was assessed in Tracer 1.5 (Rambaut & Drummond 2007), the first 2.5 million generations were discarded as burn-in. Support was assessed as follows: Low = Bootstrap Support (BS): <75, Posterior Probability (PP): <0.90; Moderate = BS: ≥75–<90, PP: ≥0.90–<0.95; High = BS: ≥90, PP: ≥0.95.

MOLECULAR DATING

The entire molecular dataset (202 accessions of Cornales) was prepared in BEAUti v1.8.4 (Drummond *et al.*, 2012) with a substitution model set to GTR+Γ for all the partitions. This model was selected as the best fit for all markers by FindModel (see Phylogenetic Analyses). For each partition we chose an uncorrelated relaxed clock in order to allow clock rates to vary across the tree. Yule Process was chosen as tree prior, which assumes a constant birth rate. We placed the following seven fossil calibration points for Cornales, using absolute ages of Ogg, Ogg, & Gradstein, (2016), corresponding to the youngest boundary of the geologic age to which the fossils have been assigned:

1. The crown node of *Cornus* was set to a minimum age of 72 Ma (end of the Campanian) based on recently described fossil fruits of *Cornus* cf. *piggae* from the Late Campanian (~73 Ma) of Vancouver Island, British Columbia, which have been confidently assigned to the extant Cornelian-Cherry clade (Atkinson, Stockey, & Rothwell, 2016)

2. The node that marks the divergence between *Davidia* Baill. and *Camptotheca* Decne.+ *Nyssa* L. was set to a minimum age of 56 Ma (end of the Thanetian), based on fruits and leaves of *Davidia antiqua* (Newberry) Manchester, from the Late Paleocene (57-55.5 Ma) of Dakota, Montana and Wyoming, USA (Manchester, 2002), displaying many diagnostic

traits of the extant genus. Although older fossils of *Davidia* (fruits, dating as far back as the late Campanian ca. 72 Ma) have been reported (Serbet et al., 2004; Manchester, Grímsson, & Zetter, 2015), a recent morphological analysis by Atkinson (2017, 2018) revealed that these (along with those assigned to *Nyssa* from the same formation and age) cannot be unequivocally assigned to the crown group of Nyssaceae and so we have decided not to include them in our analyses.

3. The stem node of Nyssaceae (sensu APGIV, 2016) was set to a minimum age of 86 Ma (end of the Coniacian) based on the fossil fruits of *Obamacarpa edenensis* Atkinson, Stockey & Rothwell (Atkinson, Stockey, & Rothwell, 2018) from the Early Coniacian (89 Ma) of Vancouver Island, British Columbia. Although these cannot be assigned with certainty to any extant group Cornales, as they show a mosaic of traits that seem to be basal to Cornales, morphological analyses place this genus as more closely related to Nyssaceae than to any other extant group (Atkinson, 2017, 2018).

4. The crown node of Jamesioideae (Hydrangeaceae) was set to a minimum age of 23 Ma (end of the Chattian) based on fossil leaves of *Jamesia caplani* Axelrod from the Late Oligocene (26.5 ± 0.3 Ma) of Colorado (Axelrod, 1987). Although it is not known if every specimen assigned to this name belonged to the same species, at least some show close resemblance to leaves of extant *Jamesia americana* Torr. & A.Gray, indicating that by this time the extant genera of the subfamily had already diverged.

5. The crown node of *Hydrangea* was set to a minimum age of 41 Ma (end of Lutetian) based on *Hydrangea knowltonii* Manchester from the Late Eocene (43-45 Ma) of Oregon (Manchester, 1994; Manchester *et al.*, 2015). This species has dorsoventrally flattened seeds, a morphological trait shared only with *H. anomala* D.Don from Asia among extant Hydrangeaceae (Hufford, 1995). Although not included in our sampling, *H. anomala* was retrieved as sister to *Hydrangea* Sect. *Cornidia* by De Smet *et al.*, (2015)which is represented in our study by *H. oerstedii* Briq. Potentially older (possibly Paleocene) fossils of *Hydrangea* from Washington have been described by Mustoe (2002), but their actual age remains uncertain.

6. The stem node of *Philadelphus*+*Carpenteria* (the latter was found to be nested in the former by Guo Y *et al.* (2013) was set to a minimum age of 23 Ma (end of the Chattian) based on leaf fossils of *Philadelphus creedensis* Axelrod, from the Late Oligocene (26.5

± 0.3 Ma) of Colorado (Axelrod, 1987). According to Axelrod (1987), leaf morphology resembles that of the extant *Philadelphus microphyllus* A.Gray.

7. The crown node of Klaprothieae was set to an age of 28 Ma (end of Rupelian) based on amber preserved structures of *Klaprothiopsis dyscrita* Poinar, Weigend & T.Henning, from Dominican Republic amber. The Dominican amber fossils have not been dated precisely and could have ages between 45 and 15 Ma (Poinar, Weigend, & Henning, 2015). Although *K. dyscrita* lacks many traits found in extant Loasoideae, it bears a closer resemblance to extant *Plakothira* Florence and *Klaprothia* Kunth than to their closest living relative, *Xylopodia* Weigend.

We did not include *Tylerianthus crossmanensis* Gandolfo, Nixon & Crepet because although it has been cited as representing one of the oldest Cornalean taxa (e.g. (Manchester *et al.*, 2015; Soltis et al., 2018) due to its Turonian-Coniacian age (88.5–90.4 Ma) and putative hydrangeaceous affinities (Gandolfo, Nixon, & Crepet, 1998), its age and phylogenetic assignment remain equivocal (Friis et al., 2011; Atkinson et al., 2018).

A single secondary calibration point was placed at the crown node of Cornales based on the result of Magallón *et al.*, (2015). We therefore set a normal distribution with mean 104.6 and standard deviation of 5.45.

In order to assess the influence that alternative calibrations could have in our analysis, we explored two different calibration schemes (a) including all seven fossils: and (b) including only the fossils *Cornus* cf. *piggae*, *Davidia antiqua* and *Hydrangea knowltonii*. The partitioned datasets under each calibration scheme were run in BEAST v1.8.4 (Drummond *et al.*, 2012) in the CIPRES Science Gateway 3.3 (Miller *et al.*, 2010). The Markov Chain Monte Carlo was set to 250 million generations sampled every 12500th. We discarded 10% of the trees as burn-in. The effective size sample (ESS> 200) and plot likelihoods were examined in Tracer 1.6 (Rambaut & Drummond, 2014). The ESS were >200 for all parameters in the scheme (b) and for most in (a) except for the prior (151), speciation (150) and matk mean rate (199). The age intervals for the different clades, reported in the results and discussion correspond to the 95% Highest posterior density intervals (HPDI) for the respective node unless otherwise stated. TreeAnnotator 1.8.4 (Drummond *et al.*, 2012) was used to generate a consensus tree from the 18000 sampled trees. The chronograms provided in the Figure E.1. Appendix E in were prepared using the package ′phyloch’ (Heibl, 2013).

ANCESTRAL AREA RECONSTRUCTION

For the biogeographic analyses we worked only with *Nasa*. The distributional data came from specimens or images of specimens deposited in herbaria in the Americas and Europe (AAU, B, BM, BONN, BR, CHEP, COL, CR, E, F, G, GH, GOET, GUAY, HA, HUA, HUH, HUSA, HUT, INPA, JAUM, JBB, K, LOJA, LPB, M, MEDEL, MEXU, MO, NY, P, PMA, PSO, QCA, QCNE, R, RB, S, UCH, US, USJ, USM, W, and Z) and from the literature (Weigend, Rodríguez & Dostert, 1998; Dostert & Weigend, 1999; Rodríguez & Weigend, 1999, 2004, 2006; Weigend, 2000a,b, 2001, Weigend, 2002b, Weigend, 2004b, 2011; Weigend & Rodríguez, 2000, 2002, 2003; Rodríguez, Weigend & Dostert, 2002; Weigend, Henning, & Schneider, 2003; Weigend & Gottschling, 2006; Morales, 2007; Rodríguez, 2008, Weigend, Grau & Ackermann, 2008; Henning & Weigend, 2009a,b, 2011; Henning, Cano, & Weigend, 2009; Henning, Rodríguez, & Weigend, 2011; Noguera-Savelli, 2012; Weigend & Ackermann, 2015). The ancestral area reconstruction was performed using the Dispersal Extinction Cladogenesis (DEC) approach described by Ree & Smith (2008) implemented in the R-package'biogeoBEARS' 0.2.1 (Matzke, 2013). We defined six geographic areas: A) Northern Middle America (Tropical areas to the North of the Nicaragua Depression), B) Southern Middle America (Between the Nicaragua Depression and Río Atrato in Colombia), C) Northern Andes (to the north of the Río Jubones in Ecuador, including adjacent ranges like the Sierras de Perijá, Santa Marta, la Macarena, Cordillera Chongón-Colonche and the coastal cordilleras of northern Venezuela amongst others), D) Amotape-Huancabamba Zone (= AHZ, between Río Jubones in Ecuardor and Río Chicama in Peru, including adjacent ranges like Cerros de Amotape and Cordillera del Cóndor), E) Central Andes (between Río Chicama in Peru and the Bolivian Orocline - Arica Bend) and F) Pacific dry Lowlands (the current deserts of Western Peru and Northern Chile). These areas were defined based on the works from Weigend (2002a, 2004c), Weigend *et al.* (2010a,b), Luebert & Weigend (2014), Mutke *et al.* (2014) and Mutke & Weigend (2017) regarding Loasaceae and other Andean plant groups.

Using the chronogram from the calibration scheme (a) obtained from BEAST, we ran an analysis setting a maximum of two areas per node. This is justified because this is the maximum number of areas occupied by any extant terminal taxon of *Nasa*. The adjacency matrix was modified manually to exclude non-contiguous area combinations (e.g. AC or

BE among others). One more time this is justified because no living terminal taxon of *Nasa* occurs in areas that are not contiguous to each other.

ANCESTRAL CHARACTER ESTIMATIONS

We obtained the elevational ranges and habitat preferences of each taxon studied, from the references cited in the ancestral area reconstruction section, field observations and herbarium specimen labels. We considered *Nasa* to inhabit Low (<2000m), medium (2000-3500m) and high (>3500m) elevation belts, with only few species found in all three. Four major habitats are characteristically inhabited by *Nasa* species according to the literature and our own observations: Andean scrub and scree, forest edge and subpáramo, forest understory and subpuna and puna, with most species restricted to only one of these habitats. Each terminal taxon was coded according to its ecological preferences. For terminal taxa with multistate characters, the prior probability of each character state was calculated as one divided between the number of character states found in the taxon (e.g. if a taxon showed four different states, each state would be coded as 0.25). Posterior probability estimations of both ecological characters were carried out independently on the consensus tree under calibration scheme (a), as obtained for the dated phylogenetic reconstruction. To take into account the phylogenetic uncertainty, 1000 post-burnin trees from the BEAST results output, were selected randomly using LogCombiner 1.8.4 (Drummond *et al.*, 2012). The 1000 trees were pruned using the function drop.tip() of the package 'ape' v. 5.1 (Paradis *et al.*, 2018), leaving only *Nasa* taxa. We relied on the function make.simmap() of the R-package 'phytools' 0.6-44 (Revell, 2012) to estimate the ancestral states of the characters of the 1000-tree set. We ran 10 simulations on each tree. The function summary() was then used to summarize the stochastic mapped trees into our consensus dated tree.

MORPHOLOGICAL ANALYSES

The morphological similarity between the 124 currently accepted species and subspecies of *Nasa* (plus one undescribed species), was assessed with 26 characters traditionally used in the systematics and taxonomy of *Nasa*: life history, shoot lignification, shoot shape in transversal section, growth form, type of glandular trichomes, type of T shaped trichomes, proportion of the length of the petiole relative to the lamina of the distal leaves, distal phyllotaxy (basal phyllotaxy is uniformly opposite), lamina outline shape, shape of the lamina base, type of leaf blade division, petal texture, petal color, shape of the petal apex, predominant color of neck/upper back of the nectar scale, predominant color of nectar sacs/lower back of nectar scale, angle of the apical wing relative to back of nectar scale, length of apical wings relative to the back of the nectar scale, shape of the nectar sacs, shape of the mature capsule, type of capsule dehiscense, number of placentae per ovary, and the presence or abscence of rhizomes, stolons, basal leaf rosettes and pseudostipules. The corresponding character states can be easily recorded from herbarium specimens, living plants, label information or from published descriptions of the taxa (Urban & Gilg, 1900; Macbride, 1941; Weigend *et al.*, 1998, 2003, 2006; Dostert & Weigend, 1999; Rodríguez & Weigend, 1999, 2004; Weigend 2000a, 2000b, 2001, 2002b, 2004b, 2011; Weigend & Rodríguez, 2000; 2002, 2003; Rodríguez *et al.*, 2002; Rodríguez, 2008; Henning & Weigend 2009a, 2009b; 2011; Henning *et al.*, 2009, 2011). In the case of polymorphic taxa, in order to run analyses in which multi-state characters can be difficult to implement, we coded the most common condition. All character states for each species are provided on request to the first author.

Cluster analyses were run for two datasets including all traits compiled, one including direct observations and expert-opinion (expert-opinion dataset from now on), and another one with only direct observations (observations-only dataset from now on). Seven variables (shoot lignification, type of glandular trichomes, type of t-shaped trichomes, predominant length proportion petiole to lamina, shape of lamina base, angle and length of the apical wings on the nectar scales) were defined as ordinal with the ordered() function of the base package v. 3.5.1 of R (R Core Team, 2014), with the character states coded as integer numbers starting from 0 (the character states, including missing data, NA, were coded as levels). The remaining variables (both binary and discrete, unordered-nominals) were left undefined [automatically recognized as nominal by daisy()]. The daisy() function, implemented in the 'cluster' package v. 2.0.7-1 (Maechler *et al.*, 2018) of R, was employed to compute the pairwise dissimilarities (distances) between all the taxa which were measured using the Gower distance metric (Gower, 1971). According to Maechler *et al.* (2018) "The contribution of a nominal or binary variable to the total dissimilarity is 0 if both values are equal, 1 otherwise. The contribution of other variables is the absolute difference of both values, divided by the total range of that variable". The pairwise dissimilarities were used for hierarchical clustering analyses employing the function hclust() of the R package 'stats' v. 3.5.1 (R Core Team, 2014) with the UPGMA (Unweighted Pair Group Method with Arithmetic Mean), or average, agglomeration method. In this method the algorithm proceeds iteratively joining the two most similar clusters at each stage until a single cluster encompassing all data points is left (R Core Team 2012). The main clusters obtained by this method and the main clades obtained in the phylogenetic reconstruction were compared visually by plotting in the dendrogram cluster, the major clade to which each species, present in the phylogenetic tree, belongs.

For the species sampled in the molecular trees, the phylogenetic signal of each directly observed trait (the number of placentae per ovary was excluded, as only one sampled species deviated from the rest), was evaluated using Pagel's λ (Pagel, 1999), which measures the fit to which the phylogenetic reconstruction predicts the covariance of traits among the species. The function drop.tip() of the package 'ape' v. 5.1 (Paradis *et al.*, 2018) was used to prune the BI consensus phylogram, leaving only one accession per *Nasa* taxon and removing also the hybrid *Nasa picta* x *chenopodiifolia* and *Nasa* cf. *usquiliensis*. We then used the function na.omit() of the R package 'stats' v. 3.5.1 (R Core Team, 2014) for each trait individually, to eliminate the species with missing data (NA) in the morphological dataset. We employed the lambda transform in the fitDiscrete() function of the 'geiger' Package v. 2.0.6 (Pennell *et al.* 2014), set to 10000 permutations, one trait at a time. The lambda values obtained can vary between 0 (no phylogenetic structure) and 1 (Brownian motion phylogenetic structure, i.e., characters change randomly during a given time interval). Three different transition rate models: "equal rates" (ER: the same parameter for all transitions), "symmetric" (SYM: forward and reverse transitions of a character share the same parameter) and "all rates different" (ARD: there is a unique parameter for each transition) were compared according to their Akaike information criterions (AIC). The transition model with the lowest AIC was compared to the Pagel's λ of a fully unresolved phylogenetic reconstruction (our null hypothesis) under the same transition model. This phylogenetic reconstruction, based on the pruned BI phylogram, was obtained with the function lambdaTree() of the package 'geiger' v.2.0.6 (Pennell *et al.*, 2014) with the value set to 0. Because the two models share the same transition rate model, direct comparison of both the lnL and AIC is possible to evaluate how much they deviate from each other.

For directly observed binary traits (the number of placentae per ovary was excluded), the functions phylo.d() of the package 'caper' v. 1.0.1 (Orme *et al.* 2018) and phylosignal() of the package 'picante' v. 1.7 (Kembel *et al.* 2018) were used to obtain alternative measures of phylogenetic signal. The phylosignal() function also employed for the directly observed ordinal variables. The previously obtained pruned BI phylogram was used in all instances. The function phylo.d was run for 10000 iterations for each binary trait; it implements Fritz & Purvis' D (Fritz & Purvis, 2010): the obtained values of D can vary from < 0, i.e. phylogenetically highly conserved traits to > 1, i.e. overdispersed traits), and provides a significance test in order to assess if the probability of D is significantly different from 1 or 0. The function phylosignal () calculates Blomberg's K (Blomberg *et al.*, 2003) and requires the ordinal and binary traits to be coded as integer numbers so the data table was modified accordingly. In case there were species with missing data for a specific trait, the pruned BI phylogram was pruned further, eliminating those species, in order to avoid conflict between the tree and the dataset. The function was set to 10000 iterations. K values above 1 indicate that the traits or related taxa are more similar to each other than expected from pure Brownian motions, and those under 1 being more different than expected by Brownian motion. The P Value of the observed vs. random phylogenetically independent contrast (PIC) variances indicates whether or not these two values differ significantly from each other.

RESULTS

PHYLOGENETIC ANALYSES

The combined dataset contained 6727 aligned positions (3346 distinct alignment patterns) with a 27.80% gaps and undetermined characters. The final ML and BI trees showed no significant topological incongruences. Our phylogenetic analyses retrieve *Nasa* as a highly supported (BS: 100, PP: 1.00) monophylum (Figs. 1--3), sister to the also highly supported (BS: 99, PP: 1.00) clade of (*Aosa*+(*Presliophytum*+South Andean Loasas *sensu* Acuña *et al.*, 2017)). The clade that includes all previously mentioned taxa is retrieved as a highly supported monophylum (BS: 97, PP: 1.00). Four main, highly supported clades can be recognized in *Nasa*. Clade I (BS: 93, PP: 1.00) is sister to the rest of the genus (Fig. 1) and consists of *Nasa carunculata* (Urb. & Gilg) Weigend, *N. limata* (J.F.Macbr.) Weigend, *N. pascoensis* Weigend and most of the *N. poissoniana* (Urb. & Gilg) Weigend species group (*sensu* Henning & Weigend, 2009a), itself a highly supported clade (BS: 95, PP: 1.00). The rest of the genus constitutes a poorly supported monophylum (BS: <50, PP: 0.89) with two highly supported subclades: Clade II (BS: 100, BS: 1.00) and the combined clades III+IV (BS: 100, BS: 1.00). Clade II (Fig. 2) includes most of the *Nasa*

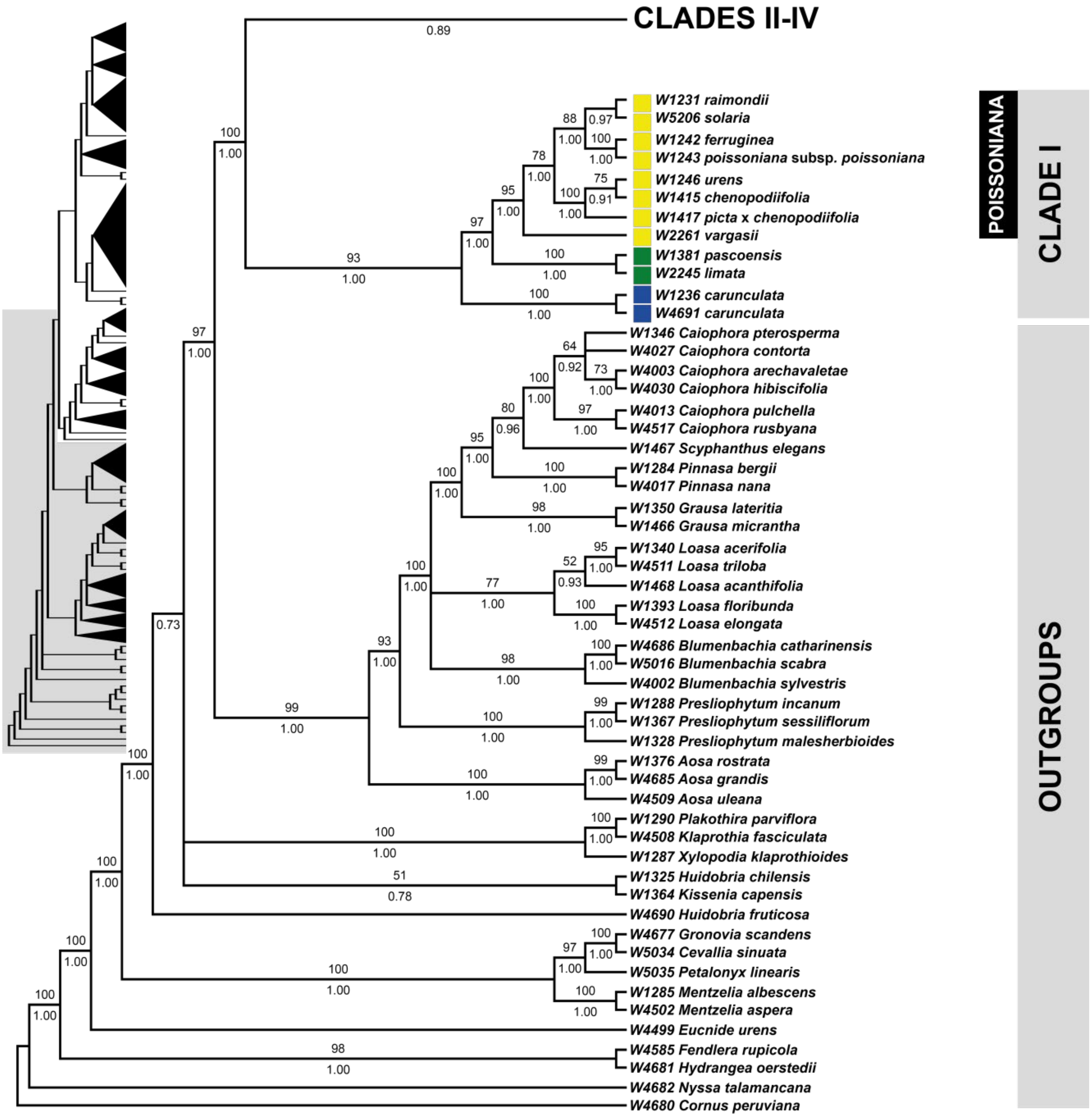

Fig. 1. Bayesian inference consensus tree focusing on *Nasa* Clade I, other Loasaceae and outgroups based on a plastid marker combined dataset (*mat*K, *rps*16, *trn*L-*trn*F, *trn*S-*trn*G). ML bootstrap support values are indicated above branches and Bayesian posterior probabilities below; only values above 50 and 0.5, respectively, are shown. Urban and Gilg's (1894) series of *Nasa* (Weigend, 1997) are indicated by the colors in the column to the left of the respective taxon. Blue = Ser. *Carunculatae*; Green = Ser. *Alatae*; Yellow = Ser. *Saccatae*. Major clades of *Nasa* and monophyletic species groups are indicated to the right of the epithet names. Laboratory accession numbers also included.

series *Alatae* (*sensu* Weigend, 2000a,b, 2001, 2004b) as well as *N. ramirezii* (Weigend) Weigend, *N. herzogii* (Urb. & Gilg) Weigend, *N. sanchezii* T.Henning & Weigend, *N. laxa* (J.F.Macbr.) Weigend and most species of ser. *Carunculatae* (*sensu* Weigend *et al.*, 2003) these last two taxa forming a well-supported (BS: 100, PP: 1.00) monophylum we call *N. laxa* group. Clades III (Fig. 2) and IV (Fig. 3) are both highly supported (BS: 100,

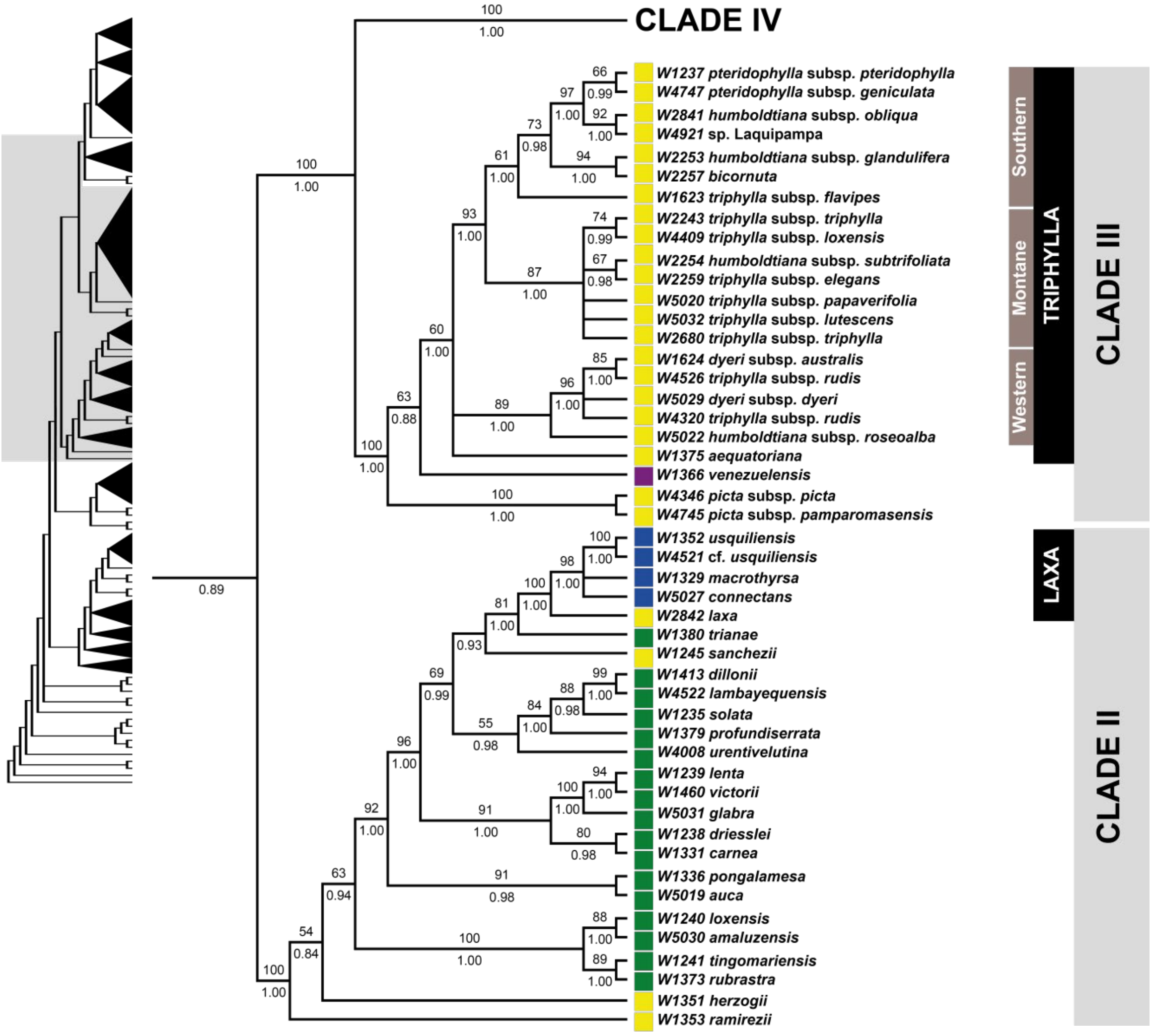

Fig. 2. Bayesian inference consensus tree focusing on *Nasa* Clades II and III based on a plastid marker combined dataset (*mat*K, *rps*16, *trn*L-*trn*F, *trn*S-*trn*G). ML bootstrap support values are indicated above branches and Bayesian posterior probabilities below; only values above 50 and 0.5, respectively, are shown. Urban and Gilg's (1894) series of *Nasa* (Weigend, 1997) are indicated by the colors in the column to the left of the respective taxon. Blue = Ser. *Carunculatae*; Green = Ser. *Alatae*; Purple = *Nasa venezuelensis* group (unknown at the time of Urban & Gilg's publications); Yellow = Ser. *Saccatae*. Major clades and some monophyletic species groups are indicated to the right of the epithet names. Laboratory accession numbers also included.

BS: 1.00). The first is formed by *N. picta* (Hook.) Weigend, *N. venezuelensis* (Steyerm.) Weigend and the *N. triphylla* species group (Dostert & Weigend, 1999). The relationships within the *N. triphylla* species group (BS: 60, PP: 1.00) are moderately well resolved, and neither *N. humboldtiana* nor *N. triphylla* as currently defined are retrieved as monophyletic (Fig. 2). Clade IV has a basal trichotomy (Fig. 3). *N. poissoniana* subsp. *glandulifera* T.Henning & Weigend and *N. weigendii* E.Rodr. are sister to each other

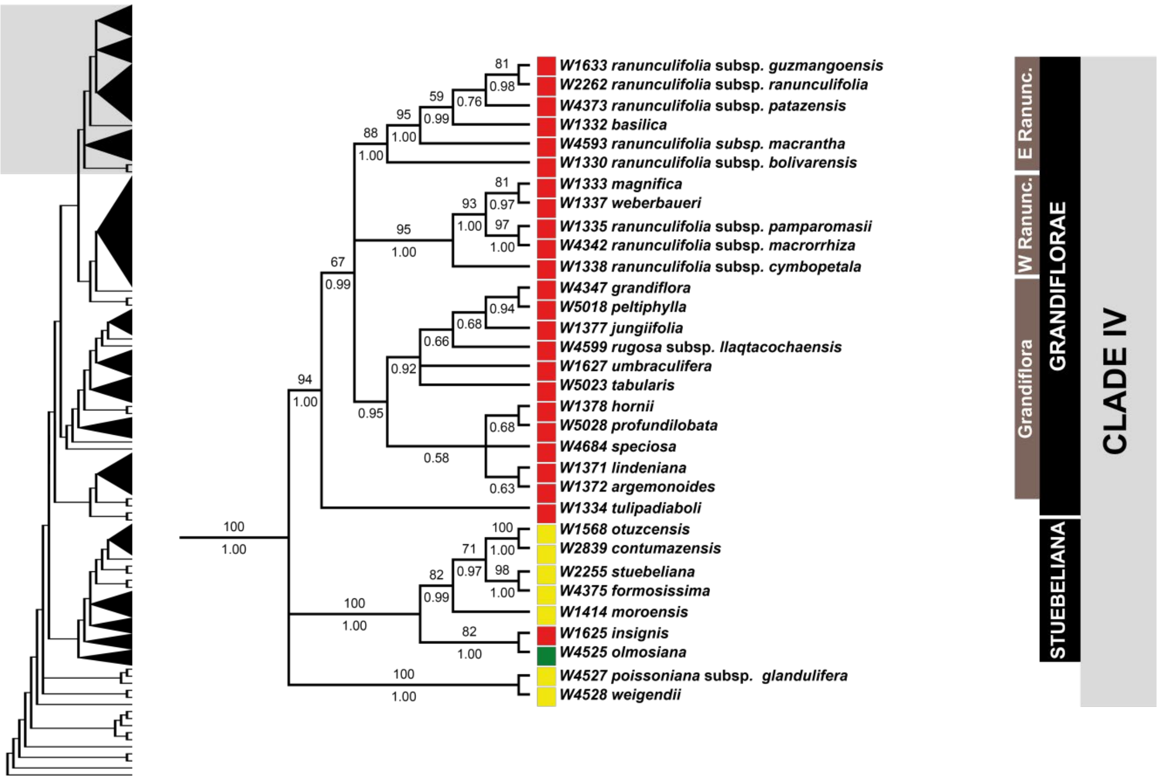

Fig. 3. Bayesian inference consensus tree focusing on *Nasa* Clade IV based on a plastid marker combined dataset (*mat*K, *rps*16, *trn*L-*trn*F, *trn*S-*trn*G). ML bootstrap support values are indicated above branches and Bayesian posterior probabilities below; only values above 50 and 0.5, respectively, are shown. Urban and Gilg's (1894) series of *Nasa* (Weigend, 1997) are indicated by the colors in the column to the left of the respective taxon. Green = Ser. *Alatae*; Red = Ser. *Grandiflorae*; Yellow = Ser. *Saccatae*. Major clades, and some monophyletic species groups are indicated to the right of the epithet names. Laboratory accession numbers also included. Ranunc.= *Nasa ranunculifolia* groups.

forming a well supported clade (BS: 100, PP: 1.00). The *N. stuebeliana* group forms a second well supported clade (BS: 100, PP: 1.00) and includes both *N. olmosiana* (Gilg ex. J.F.Macbr.) Weigend and *N. insignis* Weigend & E.Rodr. Series *Grandiflorae* is retrieved as a highly supported clade (BS: 94, PP: 1.00) with moderately resolved internal relationships. *Nasa tulipadiaboli* T.Henning & Weigend is sister to the rest of the series, which constitutes a moderately supported clade (BS: 67, PP: 0.99). Three subclades that can be recognized in this clade, the first formed mostly by Northern Andean species (*N. grandiflora* group) with moderate support and retrieved only in BI analyses (BS: <50, PP: 0.95), and two additional clades (BS: 95 & 88, PP: 1.00) formed by most of the *N. ranunculifolia* (Kunth) Weigend species group (*sensu* Henning *et al.*, 2011).

MOLECULAR DATING

The divergence times for the major nodes retrieved in the BEAST analysis of both calibration schemes diverge marginally and are shown in Table 1 and Fig. 4. In the ages obtained from calibration scheme (a) are lower than the ages obtained from calibration scheme (b). We will detail only the dates of calibration scheme (a), focusing exclusively in *Nasa*. The divergence estimate between stem *Nasa* and its sister clade was retrieved with a mean age dating to the Eocene (ca. 49 Ma), while the mean age of the crown node of the genus was dated to the Oligocene (ca. 29 Ma). The mean age of the crown node of Clade I is slightly lower (ca. 25 Ma), while the crown node of *N. poissoniana* species group was dated to the Oligocene (25.12) to Miocene (17.63 Ma). Clade II appears to have diverged from clades III and IV in the Oligocene (31.79 Ma) to Miocene (22.92 Ma). The median crown node age of Clade II was retrieved as Miocene (ca. 16.5 Ma). The crown node age of *N. laxa* group was dated Miocene (6.01 Ma) to Pleistocene (2.35 Ma). We estimate that the divergence between Clades III and IV took place in the Oligocene (23.91 Ma) to Miocene (16.12 Ma). The crown node of Clade III was retrieved as having the lowest median age estimate (ca. 11.5 Ma) of any of the crown nodes of four major clades, with crown node of the *N. triphylla* group dated to the Miocene (10. 31-5.95 Ma). The crown node of Clade IV had a median age dated to the Miocene (ca. 13 Ma). The crown nodes of *N. stuebeliana* group and ser. *Grandiflorae* were also retrieved dated to the Miocene (ca. 6.5 and 10.5 Ma respectively). Additional divergence times and the PP estimations can be seen in Figure E.1. Appendix E.

Table 1 Comparison between the divergence time estimates in Ma for some major nodes of *Nasa*, under two different calibration schemes from this study: (a) Employing seven primary calibration points (*Cornus* cf. *piggae*, *Davidia antiqua, Obamacarpa edenensis*, *Jamesia caplani*, *Hydrangea knowltonii*, *Philadelphus creedensis, Klaprothiopsis dyscrita*) and (b) including a subset of three of those calibration points (*Cornus* cf. *piggae*, *Davidia antiqua, Hydrangea knowltonii*), and calibration scheme (a) from Acuña *et al.* (in prep.). The dating of the crown node of Loasoideae is also included. Numbers in parentheses refer to 95% highest posterior density intervals of the ages of the respective node. Refer to the Material and Methods section for the details on each calibration scheme.

	Calibration schemes		Acuña *et al.* (in prep.)
Node	(a)	(b)	
Crown node Loasoideae	53.14 (45.99-60.30)	55.97 (49.42-63.01)	52.08 (44.78-59.73)

Stem node *Nasa*	48.85 (42.41-55.28)	51.27 (45.33-57.50)	47.83 (41.30-54.78)
Crown node *Nasa*	29.21 (24.81-33.80)	30.55 (26.18-35.57)	27.66 (23.12-32.49)
Crown node Clade I	25.23 (21.08-29.44)	26.39 (22.24-30.94)	23.76 (19.46-28.16)
Crown node Clade II	16.47 (13.01-19.91)	17.23 (14.05-20.92)	16.03 (12.84-19.50)
Crown node Clade III	11.49 (8.83-14.52)	12.03 (9.19-15.05)	10.93 (8.00-14.19)
Crown node Clade IV	12.99 (10.35-16.00)	13.62 (10.86-16.59)	11.78 (9.08-14.64)
Crown node *N. stuebeliana* group	6.70 (4.52-9.30)	7.00 (4.71-9.69)	5.88 (3.85-8.28)
Crown node ser. *Grandiflorae*	10.42 (8.37-12.81)	10.90 (8.79-13.35)	9.17 (7.06-11.54)

ANCESTRAL AREA RECONSTRUCTION

The DEC analysis had a lnL value of -175.80 (Fig. 4). The most probable ancestral area for crown *Nasa* was retrieved as the combination of Central Andes + AHZ. For Clade I we obtained the Central Andes as its most probable ancestral area. This clade appears to have been restricted to this area during most of its history with only closely related *N. urens* (Jacq.) Weigend and *N. chenopodiifolia* (Desr.) Weigend dispersing into the adjacent Pacific deserts. The most probable area for the remaining of *Nasa* was either the AHZ or Central Andes + AHZ. The ancestral area for crown Clade II has both the combination of Northern Andes + AHZ or Central Andes + AHZ as the most probable areas in almost equal proportions. Excluding its two basal-most nodes, AHZ is the most probable ancestral area for the majority of the backbone nodes of the extant Clade II (Fig. 4), with a minimum of four independent dispersal events into the Northern Andes and two into the Central Andes. The most probable ancestral area for the rest of the genus was retrieved as Central Andes + AHZ followed closely by the AHZ. The area combinations of Central Andes + AHZ and Northern Andes + AHZ being retrieved in similar proportions as the most probable ancestral areas for Clade III. Excluding *Nasa picta*, sister to the rest of the clade, the most probable area for this clade is Northern Andes+AHZ, A single dispersal event into Central America from the Northern Andes is detected. Clade IV had the Central Andes + AHZ as the most probable ancestral area

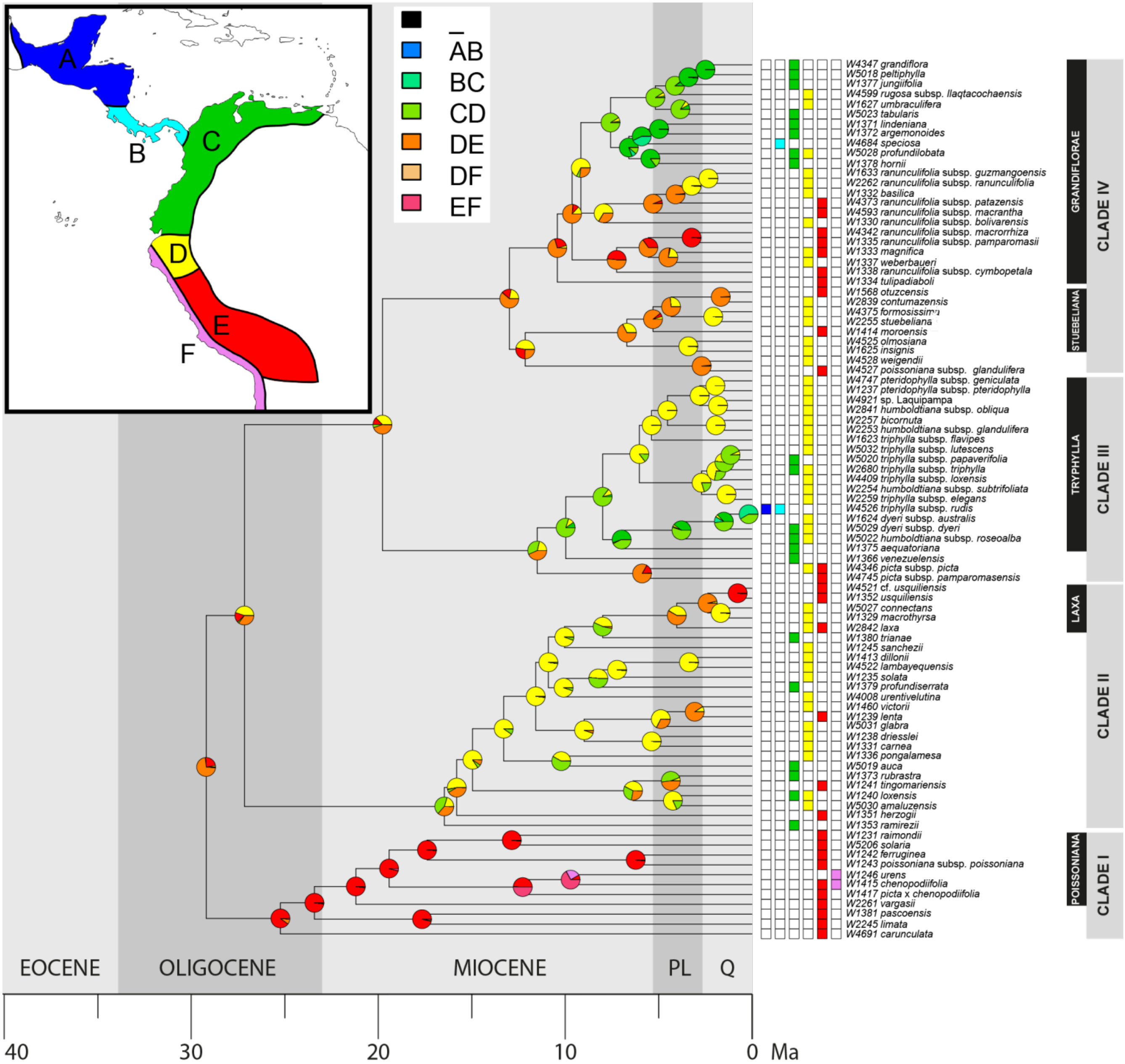

Fig. 4. Divergence time estimates for the phylogenetic reconstruction of *Nasa* using seven primary calibration points with a maximum of two areas per node and dispersal constrained only into adjacent areas. Colour codes correspond to the areas indicated in the legend (see Materials and Methods) and only the allowed area combinations are indicated. Colored grid to the right of the tips indicate the distribution assigned to the terminal taxa according to the same color code. The Pie charts at the nodes depict relative probabilities of areas as estimated from the DEC analysis. Major clades and a monophyletic species group are indicated to the right of the taxa names. Detailed chronograms indicating branch support and 95% highest posterior density intervals for the dating of both calibration schemes of this study, are provided in Appendix E in Supporting Information. Map outline based on Rivas-Martínez *et al.* (2011) and Google Maps.

combination, with the same also applying to both the *N. stuebeliana* group and the ser. *Grandiflorae*. For this clade a single dispersal into the Northern Andes was detected followed by subsequent radiation, and from there into southern Central America.

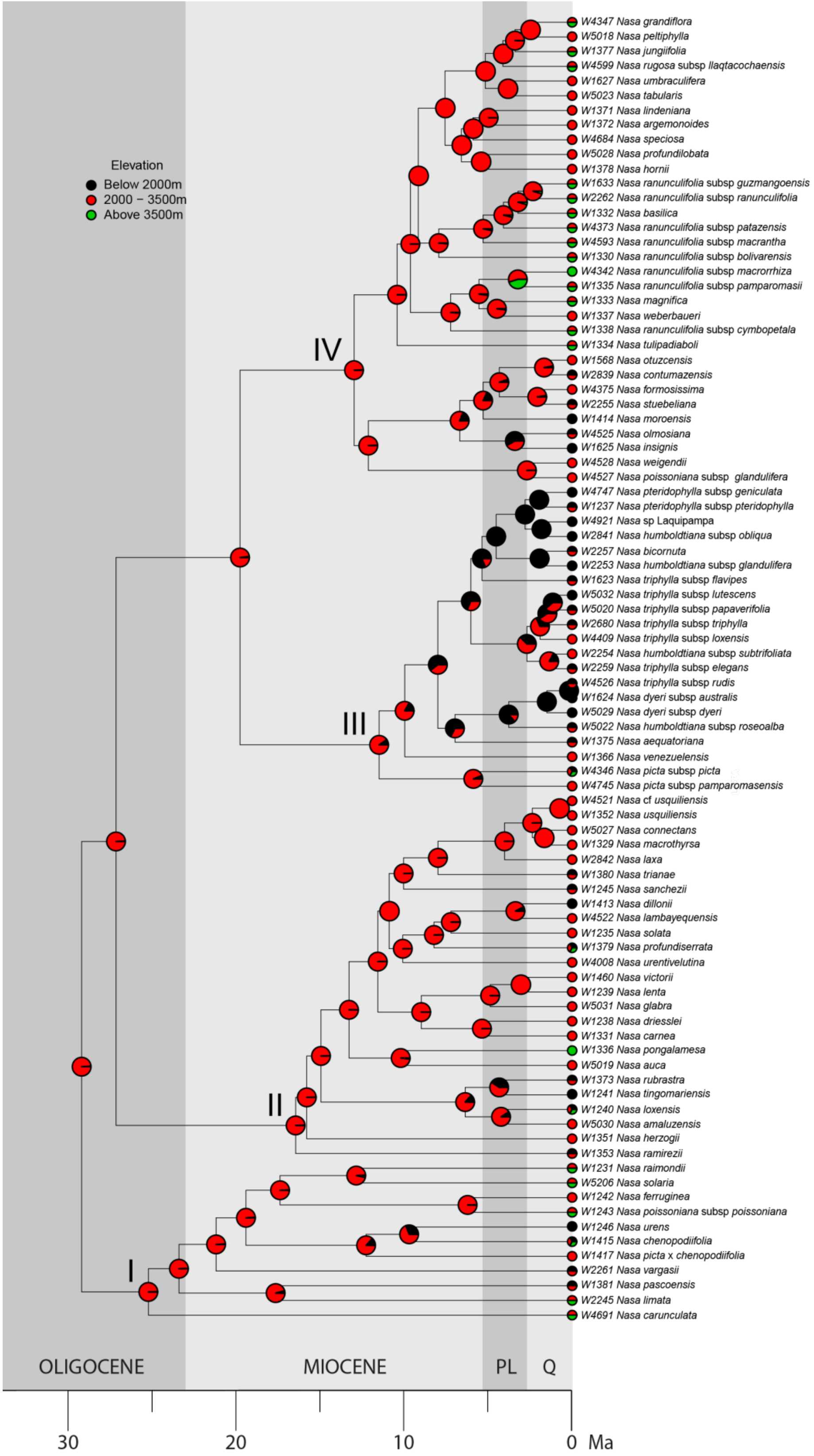

Fig. 5. Ancestral ecological character estimations obtained using simmap for the *Nasa* dated phylogenetic reconstruction under calibration scheme (a). (A) Estimation of the ancestral elevations. Roman numerals indicate the four main clades retrieved in this study

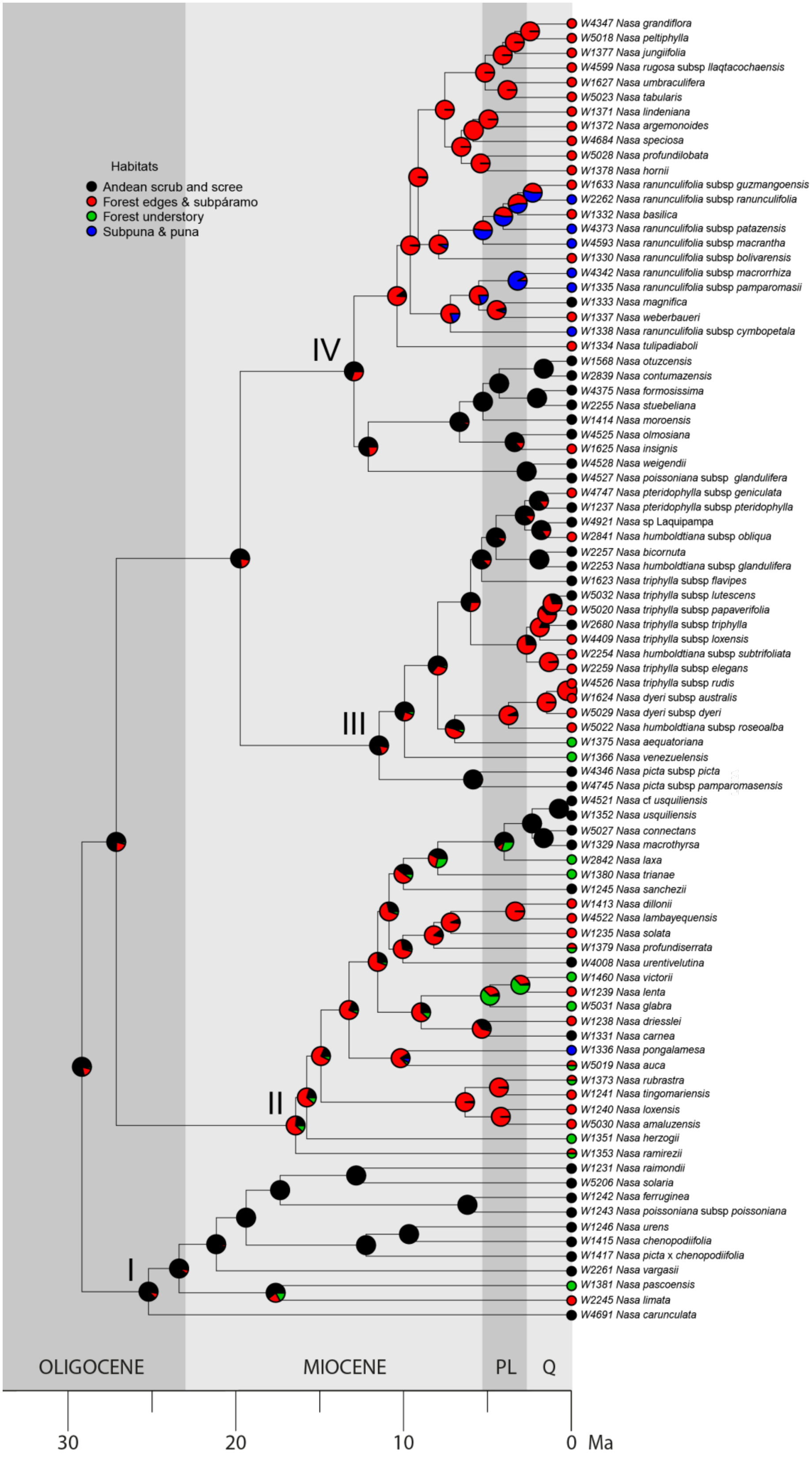

Fig. 5. Ancestral ecological character estimations obtained using simmap for the *Nasa* dated phylogenetic reconstruction under calibration scheme (a). (B) Estimation of the ancestral habitats. Roman numerals indicate the four main clades retrieved in this study

ANCESTRAL CHARACTER ESTIMATIONS

The ancestral elevation estimation for *Nasa* shows that the bulk of the diversity is found at middle elevations and that most of the diversification seems to have taken place at that elevation zone (Fig. 5A). The *triphylla*-group and the *stuebeliana*-group (clades III and IV) show a considerable expansion to lower elevations, especially in the Amotape-Huancabamba Zone. Conversely, the *Grandiflorae* show multiple transitions to high elevations across their range. Ancestral habitat reconstruction indicates that much of the early diversification likely took place in Andean scrub and scree habitats (Fig. 5B), with four diversifications in forest edge and subparamo habitats, namely in Clade II*,* in ser. *Grandiflorae* (Clade IV) and twice in the *Nasa triphylla* group (Clade III). Within ser. *Grandiflorae* both *Nasa ranunculifolia* group clades have diversified into subpuna and puna habitats. Forest understory species are mainly found across Clade II and amongst the early-diverging grade of Clade III.

MORPHOLOGICAL ANALYSES

It is possible to recognize four large clusters in the dendrograms based on the expert-opinion and observations-only datasets (Fig. 6, Fig. E.2. Appendix E). The larger clusters are mostly identical in species composition in both analyses, although the internal topologies of the species in each cluster may differ. The three most important topological differences detected involve: 1- *Nasa panamensis* Weigend, retrieved as part of cluster B in the expert-opinion dataset and as close to clusters C and D in the observations-only dataset; 2- *N. magnifica* (Urb. & Gilg) Weigend, *N. profundilobata* (Werderm.) Weigend and *N. tulipadiaboli* T.Henning & Weigend retrieved as the most dissimilar cluster in the expert-opinion dataset and as forming part of cluster A in the observed-only datset; and 3- *N. glandulosissima* Weigend, *N. insignis* and *N. olmosiana*, are in cluster B in the expert-opinion dataset and in cluster C in the observed-only dataset. Clusters A, B and D are composed and include most of the species of the Clades IV, II and III respectively, while all but two species of Clade I (*N. limata* and *N. pascoensis*) group in Cluster A. Cluster A has species of all the clades of our molecular analyses including *N. ramirezii*, *N. herzogii* and the *N. laxa* species group (Clade II), both subspecies of *N. picta* (Clade III) and *N. poissoniana* subsp. *glandulifera, N. weigendii* and most the *N. stuebeliana* species group (Clade IV). Although Cluster B also has species from the four major clades, a very high proportion of the species belongs to Clade II, with few representatives of the remaining

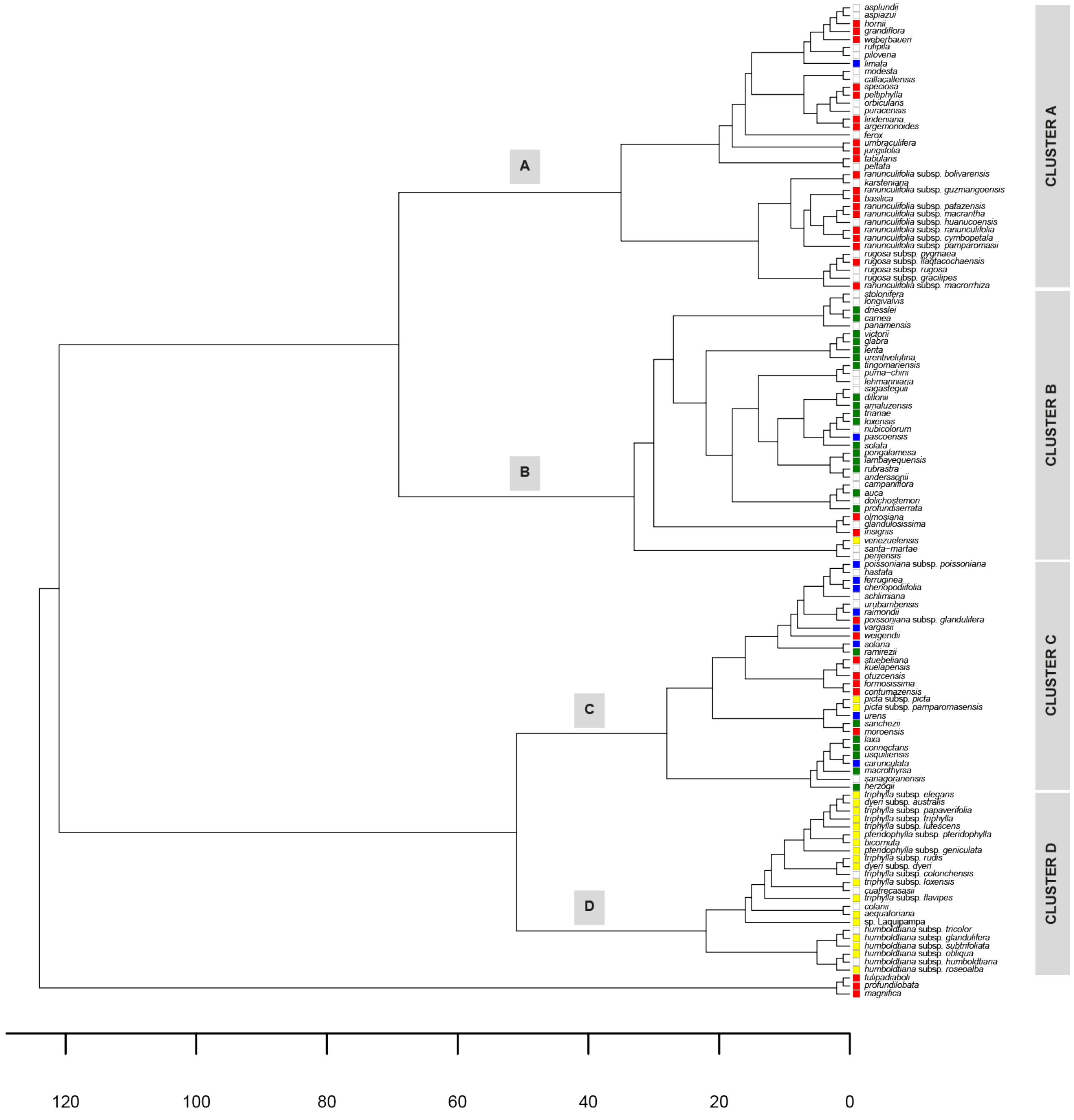

Fig. 6. Dendrogram of the hierarchical cluster analysis of *Nasa* obtained from the expert-opinion dataset. The dissimilarity distances were calculated from data coming from 26 directly observed and expert-opinion inferred morphological character states. The coloured grid to the left of each name represents the clade to which the respective species was retrieved in the phylogenetic analyses. Blue: Clade I, Green: Clade II, Yellow: Clade III, Red: Clade IV.

clades (including *N. pascoensis* from Clade I, and the *N. venezuelensis* species group from Clade III).

Many of the traits analyzed for the species included in the phylogenetic analyses have a phylogenetic signal that is not statistically different from the null hypotheses (no phylogenetic signal) of the respective analyses (NS in Table 2), however most of these traits were tested only for Pagel's λ due to input data restrictions of the other analyses (i.e. unordered nominal traits were not be analyzed using Blomberg's K nor Fritz & Purvis' D). The presence of stolons was the only trait tested using the three methods, where we obtained no significant phylogenetic signal in either. At the other end of the spectrum, distal phyllotaxy shows a strong signal for all three methods (Table 2). Shoot lignification, growth habit, petal texture, angle and length of apical wings on the nectar scales, fruit dehiscence and the presence or abscence of rhizomes, basal leaf rosettes and pseudostipules, showed significant phylogenetic signals, allowing the rejection of the null hypothesis in at least two of the methods employed. Blomberg's K results for both trichome traits studied and shape of lamina base are quite low, but significantly different from the null hypothesis (Table 2). The characters with the strongest signals are plotted onto the BI consensus phylogenetic reconstruction in Fig. E.3. Appendix E. No synapomorphies or diagnostic traits were detected for any of the major clades using our dataset.

DISCUSSION

PHYLOGENETIC RECONSTRUCTIONS

With ca. 70% of the infrageneric taxa sampled, our results confirm that *Nasa* is a well-supported monophyletic group and related to (*Aosa*+(*Presliophytum*+South Andean Loasas) confirming the results of Hufford *et al.* (2003, 2005), Weigend *et al.* (2004), Weigend & Gottschling (2006) and Acuña *et al.* (2017). The four main clades we recognize do not coincide exactly with the series of Urban & Gilg (1900), which turn out to be largely artificial. Species that would be included in ser. *Saccatae*, are retrieved in each of the four major Clades (Figs. 1--3), while ser. *Carunculatae* species are found in Clades I and II and species of ser. *Alatae* in Clades I, II and IV.

The high statistic support obtained for the major clades and many of the internal relationships contrasts with the low support of previous studies (Weigend et al.; 2004;Weigend & Gottschling, 2006). Similar topologies to our own, with Clade I as sister to the rest of *Nasa*, were also obtained by Hufford *et al.* (2003) and Weigend *et al.* (2004).

Table 2 Phylogenetic signal of 25 morphological traits in *Nasa*. For Pagel's λ, the value of λ is indicated for the transition model with the lowest AIC (model chosen: AIC value in parentheses) and compared to the respective value of a null hypothesis under the same transition model AIC value in parenthesis). For Blomberg's K, the K values are indicated (P-value of the observed vs random variance of PICs in parentheses). For Fritz & Purvis' D, the D value is indicated (P-values of it being significantly different from 0/1, respectively, in parentheses). Traits with Pagel's λ < 1.00, P-value of the K statistics > 0.05 (no significant differences from null hypothesis) and Fritz & Purvis' D > 0 are indicated with NS. NA indicates that the trait was not evaluated under the respective test.

Trait	Pagel's λ	Blomberg's K	Fritz & Purvis' D
Life history	NS	NA	NA
Shoot lignification	1.00 (ARD: 139.28)/ 0.00 (184.17)	0.54 (<0.01)	NA
Shoot shape in transversal section	1.00 (ARD: 49.95)/ 0.00 (114.05)	NA	NA
Presence or absence of rhizomes	1.00 (ER: 39.04)/ 0.00 (57.01)	0.72 (<0.01)	-0.87 (<0.01/0.95)
Presence or absence of stolons	NS	NS	NS
Presence or absence of basal leaf rosettes	1.00 (ARD: 43.54)/ 0.00 (71.00)	0.85 (<0.01)	-0.75 (<0.01/0.95)
Growth form	1.00 (ER: 38.16)/ 0.00 (48.11)	0.54 (<0.01)	-0.41 (<0.01/0.77)
Type of glandular trichomes	NS	0.44 (<0.01)	NA

Type of T shaped trichomes	NS	0.39 (0.03)	NA
Presence or absence of pseudostipules	1.00 (ARD: 24.60)/ 0.00 (31.74)	NS	-1.58 (<0.01/0.96)
Predominant longitude of the petiole relative to the lamina	NS	0.47 (<0.01)	NA
Distal phyllotaxy	1.00 (ER: 62.30)/ 0.00 (118.56)	1.02 (<0.01)	-0.52 (<0.01/0.92)
Lamina outline shape	NS	NA	NA
Shape of the lamina base	NS	0.35 (<0.01)	NA
Type of leaf blade division	NS	NA	NA
Petal texture	1.00 (ARD: 57.90)/ 0.00 (85.64)	0.61 (<0.01)	-0.57 (<0.01/0.91)
Petal color	NS	NA	NA
Shape of the petal apex	NS	NA	NA
Predominant color of neck/upper back of the nectar scale	NS	NA	NA
Predominant color of nectar sacs/lower back of nectar scale	NS	NA	NA
Angle of the apical wing relative to back of the nectar scale	1.00 (SYM: 88.10)/ 0.00 (140.28)	0.78 (<0.01)	NA

Length apical wing relative to length of the back of the nectar scale	1.00 (SYM: 122.39)/ 0.00 (150.51)	0.58 (<0.01)	NA
Shape of the nectar sac	NS	NA	NA
Shape of the mature capsule	NS	NA	NA
Capsule dehiscense	1.00 (ARD: 32.82)/ <0.01 (48.77)	0.46 (<0.01)	-1.25 (<0.01/0.97)

Nasa pascoensis and *N. limata*, have striking ecologic and morphologic differences relative to the rest of Clade I and their relationship is not easily explained based on morphology (Weigend 2000b, 2004b). These are the southernmost representatives typically considered ser. *Alatae* (Weigend 2000b, 2004b) and the only ones found south of Dept. Huánuco.

Clade II has *Nasa ramirezii* and *N. herzogii* as part of a 'basal *Saccatae* grade'. This morphology has been considered plesiomorphic in the genus (Weigend 2000a, 2001; Weigend & Gottschling, 2006; Henning & Weigend, 2009a). The affinities of both species were, until this study, unclear. Of the 30 accepted species of ser. *Alatae* (Weigend 2000a,b, 2001, 2002b, 2004; Rodríguez & Weigend 2004) 20 were included in our analyses. Some species groups retrieved within Clade II have moderate to high statistical support, and potential autoapomorphies are currently under study. The *N. laxa* species group on the other hand, is strikingly different from the rest of the clade, having mainly "*Carunculatae* morphology" (Weigend *et al.*, 2003): shrubs with alternate leaves, white, spreading petals and short, erect apical wings. This group is retrieved as nested amongst "*Alatae* morphology" clades and with significantly higher support than in the studies of Weigend et al., (2004) and Weigend & Gottschling (2006).

In clade III, *Nasa picta* is distinctive florally, with the most contrastingly bicolorous petals in the genus and diagnostic basically unicolored nectar scales that have a long narrow neck with four or more transversal calli. Vegetatively, this species does not show the distinctive compound leaves of the rest of the clade, however, *Nasa picta* subsp.

pamparomasensis E.Rodr. & Weigend ex Molinari may, develop trifoliate bracts (Weigend & Rodríguez, 2000). *N. venezuelensis* has leaves that are virtually identical to several taxa of the *N. triphylla* group (Weigend 2001, Weigend & Gottschling, 2006; Noguera-Savelli, 2012) so, even considering the very different floral morphology, a close relationship between these taxa is plausible. Within the *N. triphylla* group three moderately to highly supported subclades can be recognized (Fig. 2). A “western” group found at relatively low (usually <2000 m) elevations in mesic areas from central Ecuador to northeastern Peru, and in Central America; a “southern” group found at relatively low elevations, mainly in seasonally dry habitats in extreme southwestern Ecuador and northwestern Peru. And a montane group in usually mesic conditions at higher elevations (usually >2000 m) from northern Venezuela to northern Peru.

In clade IV, there is no clear morphologic trait that could link *Nasa weigendii* and *N. poissoniana* subsp. *glandulifera* to the rest of the clade, except the irregularly folded testa seen in *N. weigendii* (Rodríguez, 2008; Henning & Weigend, 2009a) that is shared with some species of the *N. stuebeliana* species group. Both taxa are usually considered the northernmost representatives of *N. poissoniana* species group, with *N. weigendii* entering the AHZ (Henning & Weigend, 2009a). The *N. stuebeliana* species group as defined here is a florally heterogeneous clade, but thepresence of amplexicaul bracts and irregularly folded seed testas are common (Weigend & Rodríguez, 2003). *N. olmosiana*, has been considered either an aberrant member of *Nasa* ser. *Alatae* (Weigend 2000a,b), or, along with *N. insignis*, as not belonging to any traditional series (Weigend *et al.*, 1998), Our results, as well as bract and seed morphology (Weigend 2000a,b; Weigend & Rodríguez, 2003), link it to *N. stuebeliana* group. The three moderately to highly supported internal clades of ser. *Grandiflorae* do not fully agree with the *Nasa ranunculifolia* nor *N. grandiflora* species groups as defined by Weigend & Rodríguez (2002) and Henning *et al.* (2011).

Geographic distance may leave an important footprint in the clustering of plastid molecular signal in groups like Espeletiinae (Asteraceae, Diazgranados & Barber, 2017; Pouchon *et al.*, 2018), probably due to occasional hybridization, horizontal gene transfer or organelle capture in sympatric taxa (Rieseberg & Soltis, 1991; Morales-Briones *et al.*, 2018). We hypothesize that these events could account for the geographic-patterned clades we see in the *Nasa triphylla* species group and why polytypic, widely-distributed species like *N. humboldtiana, N. ranunculifolia* and *N. triphylla* are not retrieved as monophyletic, but instead with individual subspecies more closely related to taxa growing in geographical proximity (Fig. 2, Fig. 3). Hybridization is a common phenomenon in angiosperms (Mallet, 2007), albeit relatively infrequent in extant *Nasa* (Weigend, obs. pers.), but maybe this was not the case in the geological past, and a parallel could be drawn with *Caiophora*, a genus that radiated much more recently than *Nasa* (Strelin *et al.*, 2017, Fig. E.1. Appendix E) and in which hybridization takes place frequently (Ackermann, Achatz, & Weigend, 2008; Slanis, Perea, & Grau, 2016).

HISTORICAL BIOGEOGRAPHY

The crown node of *Nasa* is ca. 20 My younger than its stem node. Large gaps like this could be result of high extinction and /or turnover rates in a lineage (Condamine *et al.*, 2015; Leslie *et al.*, 2018). The four major clades we recognize in this study diverged not later than the Middle Miocene (> 16 Ma) and probably even as far back as the late Oligocene (> 23 Ma), preceding the major uplift pulses of the Andes during the Neogene (Gregory-Wodzicki, 2000), although Leier *et al.*, (2013) suggest that ca. 20-15 Ma, some areas of the Andes reached elevations of 2500 m. According to our results, the Central Andes and the AHZ were important areas in the early history of *Nasa*. While AHZ is rich in both clades and species, the Central Andes are rich in clades, but with lower diversity at the species and subspecies level. Many 'early diverging' branches (as could be the case of entire Clade I, *Nasa herzogii*, *Nasa picta*, *Nasa moroensis* and *Nasa tulipadiaboli*), are found in the Central Andes. Clade I represents one of the few major radiations in this area (the others being the *N. ranunculifolia* clades) and it is composed by species that diverged a long time (≥ 10 Ma) from their closest living relatives. Many species in this clade inhabit dry interandean valleys, which, according to Särkinen *et al.* (2012), may have acted as stable and persistent ecological islands for up to tens of millions of years.

For the remaining clades, the AHZ seems to have been one of the most important sources of lineages. It is debatable if the AHZ could have had habitat adequate for *Nasa* before the closure of the West Andean Portal by the Late Middle Miocene (Hoorn *et al.*, 1995) but it has been shown to be an important area for paleo- and neoendemics (Weigend, 2002a), as well as an ancestral area for Andean plant groups (Struwe *et al.*, 2009; Deanna *et al.*, 2018) and a corridor for species of seasonally dry habitats (Quintana *et al.*, 2017). It is worth noting that *Nasa* does not seem to require continuous mountain chains to disperse, judging by its current distribution in the Sierra Nevada de Santa Marta, Cordillera Chongón-Colonche and Central America, although isolated ranges tend to be relatively poor in species. The onset of the subduction of the Nazca Ridge ca. 15 Ma seems to have triggered relief building in the Cordillera Occidental of Peru (Margirier *et al.*, 2015), and may have changed the sedimentary rates (Calvès *et al.*, 2018) and precipitation patterns in the Central Andes (Poulsen, Ehlers & Insel, 2010) due to the increased height of the Cordilleras. These important changes point out at increasing topographic and climatic complexity in the cordillera and are synchronous or precede most divergence events within clades II, III and IV. The mosaic of deeply-dissected, new landscapes may have favored cladogenesis in the genus, as it is hypothesized it happened in the AHZ with diverse plant clades (Weigend 2002a).

At around the same time or slightly after, the Western Andean Portal would have closed (Hoorn *et al.*, 1995; Eakin, Lithgow-Bertelloni, & Dávila, 2014). This process probably facilitated the dispersal of *Nasa* into the Northern Andes: most of the dispersal events into this area took place >13 Ma (the sole possible exception is *N. ramirezii*). In the Northern Andean region the Ecuadorean Andes and Cordilleras Central and Occidental in Colombia are considered to be older than the Cordilleras Oriental in Colombia and the Mérida Andes in Venezuela (Spikings *et al.*, 2001; Villagómez & Spikings, 2013; Bermúdez *et al.*, 2017; Richardson *et al.,* 2018) which could partly explain the higher richness in taxa of the former compared to the latter. The Northern Andes harbored radiations of the *N. venezuelensis* and *N. triphylla* western groups of Clade III and the *N. grandiflora* group of Clade IV (Fig. 4). From this area, *Nasa* dispersed independently twice into Central America, possibly as far back as ca 8.5 Ma, but even if the Central American Seaway was closed then (Montes *et al.*, 2015), most evidence indicates that montane habitats in southern Central America were not available at that time (Gräfe *et al.*, 2002; Driese *et al.*, 2007).

ANCESTRAL ECOLOGY ESTIMATIONS

Weigend (1997) proposed that ancestrally, *Nasa* were mesophytic herbs of intermediate elevation tropical forests. Our results however, show that ancestrally, *Nasa* likely grew in seasonal scrub at middle elevations and this seems to agree with the probable ancestral habitat of Loasoideae, as the 'basal grade' of the subfamily (*Huidobria*, *Kissenia*, *Xylopodia*, most *Aosa*, *Presliophytum*: Appendix E), inhabits seasonally dry or desert environments (Weigend, 2004a). Seasonally dry habitats in the Andes have persisted for a long time (Särkinen *et al.*, 2012) and this would explain why several lineages inhabiting these environments have been so long lived. Colonization of high altitudes appears to have happened repeatedly and seemingly independently, mostly in Clades I and IV. These events affected most prominently Clade IV in the last 10 Ma, probably because these high Andean habitats only became extensive enough in the Late Miocene (Gregory-Wodzicki, 2000; Graham, 2009). The colonization into lower elevation habitats also took place several times independently, prominently in the *N. triphylla* group of clade III. These habitats in tropical latitudes are relatively poor in Loasoideae and thus unsaturated and probably represented an opportunity for *N. triphylla* group to radiate.

Forested (undergrowth and edge) environments represent major habitats for the diversification of all clades except Clade I. Seven reversals back into seasonal Andean scrub and scree habitats were detected with the most notable being the small radiation of the *N. laxa* group in clade II (Weigend & Gottschling, 2006). The recent age of origin of the Puna, (Graham, 2009) is mirrored by the low age of the lineages that colonized it. In terms of habitat, only Clade I can be considered as conservative, while the other clades have had frequent transitions and reversals into different habitats, in particular in the complex mosaic of landscapes found in the AHZ (Weigend 2002a, 2004c).

MORPHOLOGY OF *NASA* IN A PHYLOGENETIC CONTEXT

The species in Cluster A are strongly defined morphologically by their cordate (or peltate) leaf bases, palmate venation and obvious adaptations for ornithophily (campanulate orange corollas, nectar scales with long erect wings and large nectar sacks: Weigend 2000a, 2001; Weigend & Gottsching, 2006, Henning *et al.*, 2011). The topology of the *Nasa magnifica*, *N. profundilobata* and *N. tulipadiaboli* cluster in the expert-opinion dataset (Fig. 6) is probably due to character states in these species that are rare in *Nasa*: palmatisect leaf shape and biennial habit, as well as the presence of branched glandular trichomes in the first two species (Henning et al., 2011). We infer that the unsampled species forming part of cluster A could reasonably belong to the monophyletic ser. *Grandiflorae* of Clade IV.

The vast majority of the species included in Cluster B are well defined morphologically by their opposite distal phyllotaxis, shallow, pinnate-lobed leaves and nectar scales with horizontal to semi-erect apical wings (Weigend 2000a,b. 2004b). Although these traits are universally present in most species of this cluster, six species are clearly divergent. The closest relatives of *N. olmosiana*, *N. insignis* and N. *glandulosissima* have been persistently difficult to infer (Weigend, 1997, 2000a,b; Weigend et al., 1998) and although hybridization between clades II and IV could have taken place, morphological convergence, in particular regarding floral structures (petal morphology and coloration, nectar scale morphology) cannot be discarded. The three species of the *N. venezuelensis* group also show similarities to the rest of the cluster, particularly in petal morphology and coloration as well as nectar scale morphology. However their leaf structure is radically different, with these being compound and triangular in outline, almost identical to the leaves of several species in Cluster D. Excluding these six species, we infer that the species of cluster B would be part of Clade II.

The Cluster C includes species assigned to ser. *Saccatae,* with no evident apomorphies (Henning & Weigend 2009a): annual herbs to perennial shrubs with usually alternate pinnatilobate leaves, white to yellow, membranous petals, nectar scales with tiny erect wings, and often with narrow-cylindrical capsules. This Cluster encompases ser. *Carunculatae*, *N. poissoniana* and *N. stuebeliana* groups, *N. picta, N. laxa, N. ramirezii, N. herzogii. N. sanchezii* and *N. schlimiana*. This cluster is the least homogeneous phylogenetically, and includes many of the "basal grade species" retrieved by our molecular analyses (Fig. 1--3). The phylogenetic position of the unsampled species in this cluster is too uncertain to make plausible inferences.

Cluster D is composed exclusively by the *N. triphylla* group, these have deeply pinnatisect to compound leaves, usually grooved (irregularly or not) stems, filiform petal apices, nectar scales with tiny, erect apical wings and sometimes, unique medifixed trichomes (Dostert & Weigend 1999, Henning & Weigend 2009b). Ecologically they inhabit usually lower elevation habitats than most species of florally similar Cluster C. We infer that the unsampled taxa retrieved in this cluster likely belong to Clade III.

The morphological traits analyzed in this study, although systematically useful, show distinct strengths in their phylogenetic signals. Labile traits that evolved independently several times within a clade, show weak phylogenetic signal. Corolla color is usually seen as a labile trait (Rausher, 2008) and we expect the same could be the case for nectar scale color patterns. In Andean Iochrominae (Solanaceae), Muchhala, Johnsen, & Smith (2014) found out that the phylogenetic signal of flower color was statistically indistinguishable from 0. These authors argued that evolution in sympatry may drive the evolution of new colors among closely related taxa due to competitive interactions for pollinators. Leaf shape could also be labile. Weigend, Kufer, & Müller (2000), already suggested that different species having diverse leaf shapes may be an adaptive advantage when invertebrate herbivores are a major evolutionary force in Loasaceae. Dell'Aglio, Losada, & Jiggins (2016) report that *Heliconius* butterflies use visual cues to find places for oviposition and that these could drive negative-frequency dependent selection in their host plants, that may favor leaf shape diversity in *Passiflora* L.

We hypothesize that nectar sac shape also could represent a labile trait, because although taxa have a predominant morphology, genetically related plants may develop different nectar sac shapes in a single generation as Henning & Weigend (2009a) mention for *Nasa vargasii* and *Nasa poissoniana* subsp. *poissoniana*. This also applies to closely related subspecies of both *N. dyeri* (Urb. & Gilg) Weigend and *N. triphylla* (Dostert & Weigend, 1999).

DISCORDANCE BETWEEN MOLECULAR AND MORPHOLOGICAL INFORMATION

Each major clade retrieved in this study, is composed by a majority of species of morphologically homogeneous groups (*Nasa poissoniana* group in Clade I, *Nasa* ser. *Alatae* in Clade II, *Nasa triphylla* group in Clade III, *Nasa* ser. *Grandiflora* and *Nasa stuebeliana* group in Clade IV). However, we recognize three groups in which the molecular and morphological information seem to be at major discordance with each other. These are *Nasa pascoensis* + *N. limata*, the *N. laxa* group, and *N. weigendii* + *N. poissoniana* subsp. g*landulifera* respective to the clades I, II and IV. If hybridization took place, we hypothesize the parental species belonged different clades of *Nasa.* But if that were the case, why is the overall morphology, in particular the floral structures, so different from the rest of the species of their respective clade?. Campbell (2003) reports that pollinators (hummingbirds, in her case) have driven *Ipomopsis* Michx. (Polemoniaceae) hybrids to approach one of their parental species phenotypes in a clear example of directional selection. Although the first generations of hybrids in *Nasa* could have had phenotypes intermediate between the parental species, these could have been subject to directional or disruptive selection by pollinators and/or the physical environment, analogous to Campbell's (2003) and Hendry *et al's.*, (2009), observations on *Ipomopsis* and Darwin's finches respectively. Under these selective pressures, the respective daughter populations (and eventually species) would, given enough time, attain morphologies very different from each other (disruptive selection) or similar to just one of their parental species (directional selection). Alternatively, convergent evolution even in the absence of reticulate phylogenies, cannot be dismissed. Pollinator shifts, often in tandem with habitat expansions, are responsible of striking examples of morphological convergence, as Weigend & Gottschling (2006) have argued for the multiple origins of funnel flowers in *Nasa*, and Smith & Kriebel, (2018) have tested for Iochrominae corolla shape.

Obtaining informative nuclear markers for *Nasa* should be the next step in order to confirm if the hypothetical hybridization here proposed, could have taken place. The works on Andean Catasetiinae (Orchidaceae) by Pérez-Escobar et al. (2016) and *Lachemilla* Rydb. (Rosaceae) by Morales-Briones et al., (2018), are examples of the use of procrustrean and phylogenetic network approaches to compare between plastid and nuclear data to find evidence of reticulate speciation. Employing similar methods in *Nasa* could prove enlightening.

CONCLUSIONS

We provide the most complete and resolved phylogenetic reconstruction of *Nasa,* retrieving four well-supported clades that are predominantly formed by species of morphologically homogeneous groups. However our research could not identify synapomorphies that could apply to the entirety of any of these major clades. The geographical structure of the reconstructions as well as the apparent discordance between morphological and molecular information in three of the clades may indicate that hybridization or horizontal gene transfer could have taken place, however it will be necessary to have access to nuclear DNA data in order for test these hypotheses. The historical biogeography of the genus indicates that *Nasa* has a long history, preceding Andean uplift as it diverged from its sister group in the early Eocene (>42 Ma). Ancestrally *Nasa* apparently inhabited seasonally dry habitats, one of the most ancient and underappreciated biomes in the Andes. By mid-Miocene (ca. 15 Ma) expansion into new habitats like forest edges and undergrowth may have also started allowing further diversification in the genus. During this time, the topographic and climatic complexity of the Andes probably evolved in response to uplift result of the subduction of the oceanic ridges and the closure of the West Andean portal shortly after. The AHZ could be recognized both as a cradle due to recent cladogenesis, and as a museum of ancient lineages. This contrasts with the Central Andes, which can be considered mostly a museum, with ancient, species-poor lineages represented. Critical reexamination of the historical biogeography patterns in other mid-elevation taxa with high diversity in the AHZ, would reveal the predominant patterns and timing of cladogenesis of the biota, and if they have parallels with those of *Nasa*. Although on a global scale, plant diversity reaches its peak at middle elevations (Fischer, Blaschke, & Bässler, 2011; Guo, Q. *et al.*, 2013), it remains relatively poorly investigated phylogenetically, particularly in the tropics. Due to its ecological and biogeographical idiosyncrasy, it seems reasonable to use *Nasa* as a model to study diversification and biogeography in middle elevations of the tropical Andes. Exploration of phylogenetic diversity metrics (Posadas et al., 2001) on *Nasa* and taxa with spatially-similar diversity patterns like *Calceolaria* L., *Deprea*, *Fuchsia, Macrocarpaea*, *Passiflora* L., *Ribes* or *Urtica* could offer promising data to assess and identify areas where conservation efforts should focus, particularly in endangered mosaic landscapes such as those in the AHZ and the Andes at large.

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this work can be found in Appendix E.

REFERENCES

Ackermann M, Achatz M & Weigend M. **2008**. Hybridization and crossability in *Caiophora* (Loasaceae subfam. Loasoideae): Are interfertile species and inbred populations results of a recent radiation? *American Journal of Botany* **95**: 1109–1121.

Acuña R, Chinchilla IF & Weigend M. **2018**. An unusual disjunction in Loasaceae: Central American *Chichicaste grandis* is nested in Brazilian *Aosa*. *Phytotaxa* **365**: 273–287.

Acuña R, Fließwasser S, Ackermann M, Henning T, Luebert F & Weigend M.2017. Phylogenetic relationships and generic re-arrangements in 'South Andean Loasas' (Loasaceae). *Taxon* **66**: 365–378.

Andersson C. **2009**. Neogene Climates. In: Gornitz V, ed. *Encyclopedia of Paleoclimatology and Ancient Environments*. Dordrecht: Springer Netherlands, 609–612.

Antonelli A & Sanmartín I. **2011**. Why are there so many plant species in the Neotropics? *Taxon* **60**: 403–414.

APGIV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181**: 1–20.

Atkinson BA. 2017. *Unearthing the Cretaceous Diversification of Cornales.* Ph.D. Thesis, Oregon State University, Corvallis, Oregon.

Atkinson BA. 2018. The critical role of fossils in inferring deep-node phylogenetic relationships and macroevolutionary patterns in Cornales. *American Journal of Botany* **105**: 1401–1411.

Atkinson BA, Stockey RA & Rothwell GW. **2016**. Cretaceous origin of dogwoods: an anatomically preserved *Cornus* (Cornaceae) fruit from the Campanian of Vancouver Island. *PeerJ* **4**: e2808.

Atkinson BA, Stockey RA & Rothwell GW. **2018**. Tracking the Initial Diversification of Asterids: Anatomically Preserved Cornalean Fruits from the Early Coniacian (Late Cretaceous) of Western North America. *International Journal of Plant Sciences* **179**: 21–35.

Ayers T. 1999. Biogeography of *Lysipomia* (Campanulaceae), a high elevation endemic: An illustration of species richness at the Huancabamba Depression, Peru. *Arnaldoa* **6**: 13–28.

Barthlott W, Hostert A, Kier G, Küper W, Kreft H, Mutke J, Rafiqpoor MD & Sommer JH. 2007. Geographic patterns of vascular plant diversity at continental to global scales. *Erdkunde* **61**: 305–315.

Bermúdez MA, Hoorn C, Bernet M, Carrillo E, van der Beek PA, Garver JI, Mora JL & Mehrkian K. 2017. The detrital record of late-Miocene to Pliocene surface uplift and exhumation of the Venezuelan Andes in the Maracaibo and Barinas foreland basins. *Basin Research* **29**: 370–395.

Berry PE, Hahn WJ, Sytsma KJ, Hall JC & Mast A. 2004. Phylogenetic relationships and biogeography of *Fuchsia* (Onagraceae) based on noncoding nuclear and chloroplast DNA data. *American Journal of Botany* **91**: 601–614.

Blomberg SP, Garland T & Ives AR. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Calvès G, Calderón Y, Hurtado Enriquez C, Brusset S, Santini W & Baby P. 2018. Mass Balance of Cenozoic Andes-Amazon Source to Sink System—Marañón Basin, Peru. *Geosciences* **8**: 167.

Campbell DR. **2003**. Natural selection in *Ipomopsis* hybrid zones: implications for ecological speciation. *New Phytologist* **161**: 83–90.

Christenhusz MJ & Chase MW. **2013**. Biogeographical patterns of plants in the Neotropics–dispersal rather than plate tectonics is most explanatory. *Botanical Journal of the Linnean Society* **171**: 277–286.

Condamine FL, Nagalingum NS, Marshall CR & Morlon H. 2015. Origin and diversification of living cycads: a cautionary tale on the impact of the branching process prior in Bayesian molecular dating. *BMC Evolutionary Biology* **15**: 65.

De Smet Y, Granados Mendoza C, Wanke S, Goetghebeur P & Samain MS. 2015. Molecular phylogenetics and new (infra)generic classification to alleviate polyphyly in tribe Hydrangeeae (Cornales: Hydrangeaceae). *Taxon* **64**: 741–753.

Deanna R, Barboza GE & Carrizo-García C. **2018**. Phylogenetic relationships of *Deprea*: New insights into the evolutionary history of physaloid groups. *Molecular Phylogenetics and Evolution* **119**: 71–80.

Dell'Aglio DD, Losada ME & Jiggins CD. **2016**. Butterfly Learning and the Diversification of Plant Leaf Shape. *Frontiers in Ecology and Evolution* **4**: 81.

Derryberry EP, Claramunt S, Derryberry G, Chesser RT, Cracraft J, Aleixo A, Pérez-Emán J, Remsen Jr. JV & Brumfield RT. 2011. Lineage Diversification and Morphological Evolution in A Large-Scale Continental Radiation: The Neotropical Ovenbirds and Woodcreepers (Aves: Furnariidae). *Evolution* **65**: 2973–2986.

De-Silva DL, Elias M, Willmott K, Mallet J & Gay JJ. 2016. Diversification of clearwing butterflies with the rise of the Andes. *Journal of Biogeography* **43**: 44–58.

Diazgranados M & Barber JC. **2017**. Geography shapes the phylogeny of frailejones (Espeletiinae Cuatrec., Asteraceae): a remarkable example of recent rapid radiation in sky islands. *PeerJ* **5**: e2968.

Dostert N & Weigend M. **1999**. A synopsis of the *Nasa triphylla* complex (Loasaceae), including some new species and subspecies. *Harvard Papers in Botany* **4**: 439–467.

Doyle JJ & Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.

Driese SG, Orvis KH, Horn SP, Li ZH, Jennings DS. 2007. Paleosol evidence for Quaternary uplift and for climate and ecosystem changes in the Cordillera de Talamanca, Costa Rica. *Palaeogeography, Palaeoclimatology, Palaeoecology* **248**: 1–23.

Drummond AJ, Suchard MA, Xie D & Rambaut A. 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.

Eakin CM, Lithgow-Bertelloni C & Dávila FM. **2014**. Influence of Peruvian flat-subduction dynamics on the evolution of western Amazonia. *Earth and Planetary Science Letters* **404**: 250–260.

Felsenstein J. **1981**. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* **17**: 368–376.

Fischer A, Blaschke M & Bässler C. **2011**. Altitudinal gradients in biodiversity research: the state of the art and future perspectives under climate change aspects. *Waldökologie, Landschaftsforschung und Naturschutz* **11**: 35–47.

Friis EM, Crane PR & Pedersen KR. 2011. *Early Flowers and Angiosperm Evolution***.** Cambridge University Press.

Fritz SA & Purvis A. **2010**. Selectivity in Mammalian Extinction Risk and Threat Types: a New Measure of Phylogenetic Signal Strength in Binary Traits: Selectivity in Extinction Risk. *Conservation Biology* **24**: 1042–1051.

Gandolfo MA, Nixon KC & Crepet WL. **1998**. *Tylerianthus crossmanensis* gen. et sp. nov.(aff. Hydrangeaceae) from the Upper Cretaceous of New Jersey. *American Journal of Botany* **85**: 376–386.

Gentry AH. 1982. Neotropical floristic diversity: phytogeographical connections between Central and SouthAmerica, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* **69**: 557–593.

Gilg E. 1894. Loasaceae. In: Engler A & Prantl K, eds. *Die natürlichen Pflanzenfamilien III: Teil. 6. Abteilung a.* Leipzig, Wilhelm Engelmann, 100–121.

Gräfe K, Frisch W, Villa IM & Meschede M. 2002. Geodynamic evolution of southern Costa Rica related to low-angle subduction of the Cocos Ridge: constraints from thermochronology. *Tectonophysics* **348**: 187–204.

Graham A. **2009**. The Andes: a Geological Overview from a Biological Perspective. *Annals of the Missouri Botanical Garden* **96**: 371–385.

Gregory-Wodzicki KM. **2000**. Uplift history of the Central and Northern Andes: a review. *Geological Society of America Bulletin* **112**: 1091–1105.

Gower JC. 1971. A general coefficient of similarity and some of its properties. *Biometrics* **27**: 857–874.

Guo Q, Kelt DA, Sun Z, Liu H, Hu L, Ren H & Wen J. 2013. Global variation in elevational diversity patterns. *Scientific Reports* **3**: 3007.

Guo YL, Pais A, Weakley AS & Xiang QY. 2013. Molecular phylogenetic analysis suggests paraphyly and early diversification of *Philadelphus* (Hydrangeaceae) in western North America: New insights into affinity with *Carpenteria*. *Journal of Systematics and Evolution* **51**: 545–563.

Hazzi NA, Moreno JS, Ortiz-Movliav C & Palacio RD. 2018. Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. *Proceedings of the National Academy of Sciences* **115**: 7985–7990.

Heibl, C. 2013. 'phyloch': interfaces and graphic tools for phylogenetic data in R. Available: http://www.christophheibl.de/Rpackages.html.

Hempel AL, Reeves PA, Olmstead RG & Jansen RK. 1995. Implications of *rbc*L sequence data for higher order relationships of the Loasaceae and the anomalous aquatic plant *Hydrostachys* (Hydrostachyaceae). *Plant Systematics and Evolution* **194**: 25–37.

Hendry AP, Huber SK, De Leon LF, Herrel A & Podos J. 2009. Disruptive selection in a bimodal population of Darwin's finches. *Proceedings of the Royal Society B: Biological Sciences* **276**: 753–759.

Henning T, Cano A & Weigend M. **2009**. A new shrubby species of *Nasa* Weigend ser. *Caruncul*atae (Urb. & Gilg) Weigend (Loasaceae) from the Amotape-Huancabamba Zone. *Revista Peruana de Biología* **16:** 151–156.

Henning T, Mittelbach M, Ismail SA, Acuña R & Weigend M. 2018. A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation. *Scientific Reports* **8**: 14018.

Henning T, Rodriguez E & Weigend M. **2011**. A revision of the *Nasa ranunculifolia* group (*Nasa* ser. *Grandiflorae* pro parte, Loasaceae). *Botanical Journal of the Linnean Society* **167**: 47–93.

Henning T & Weigend M. **2009a**. Systematics of the *Nasa poissoniana* group (Loasaceae) from Andean South America. *Botanical journal of the Linnean Society* **161**: 278–301.

Henning T & Weigend M. **2009b**. Two novel and critically endangered subspecies of *Nasa humboldtiana* (Loasaceae) from Peru. *Botanische Jahrbücher* **127**: 473–488.

Henning T & Weigend M. **2011**. Two new species of *Nasa* (Loasaceae) from Andean South America. *Phytotaxa* **26**: 1–8.

Hoorn C, Guerrero J, Sarmiento GA, & Lorente MA. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology* **23**: 237–240.

Horton BK. **2018**. Sedimentary record of Andean mountain building. *Earth-Science Reviews* **178**: 279–309.

Huelsenbeck JP & Ronquist F. **2001**. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Hufford L. **1995**. Seed morphology of Hydrangeaceae and its phylogenetic implications. *International Journal of Plant Sciences* **156**: 555–580.

Hufford L, McMahon MM, O'Quinn R & Poston ME. **2005**. A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. *International Journal of Plant Sciences* **166**: 289–300.

Hufford L, McMahon MM, Sherwood AM, Reeves G & Chase MW. **2003**. The major clades of Loasaceae: phylogenetic analysis using the plastid *matK* and *trnL-trnF* regions. *American Journal of Botany* **90**: 1215–1228.

Joppa LN, Roberts DL, Myers N & Pimm SL. 2011. Biodiversity hotspots house most undiscovered plant species. *Proceedings of the National Academy of Sciences* **108**: 13171–13176.

Kembel SW, Ackerly DD, Blomberg SP, Cornwell WK, Cowan PD, Helmus MR, Morlon H & Webb CO. 2018. 'picante' Integrating Phylogenies and Ecology. Available: https://cran.r-project.org/web/packages/picante/

Leier A, McQuarrie N, Garzione C & Eiler J. 2013. Stable isotope evidence for multiple pulses of rapid surface uplift in the Central Andes, Bolivia. *Earth and Planetary Science Letters* **371–372**: 49–58.

Leslie AB, Beaulieu J, Holman G, Campbell CS, Mei W, Raubeson LR & Mathews S. 2018. An overview of extant conifer evolution from the perspective of the fossil record. *American Journal of Botany* **105**: 1–14

Luebert F, Hilger HH & Weigend M. **2011**. Diversification in the Andes: Age and origins of South American *Heliotropium* lineages (Heliotropiaceae, Boraginales). *Molecular Phylogenetics and Evolution* **61**: 90–102.

Luebert F & Weigend M. **2014**. Phylogenetic insights into Andean plant diversification. *Frontiers in Ecology and Evolution* **2**: 27.

Macbride F. 1941. Loasaceae. In: Macbride F, ed. *Flora of Peru*. *Fieldiana, Botany*: **13 Part 4(1)**: 143–181.

Madriñán S, Cortés AJ & Richardson JE. **2013**. Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics* **4**: 192.

Maechler M, Rousseeuw P, Stryuf A, Hubert M, Hornik K, Studer M, Roudier P, González J & Kozlowski B. 2018. 'cluster' Finding Groups in Data: Cluster Analysis Extended. Available: https://cran.r-project.org/web/packages/cluster/

Magallón S, Gómez-Acevedo S, Sánchez-Reyes LL & Hernández-Hernández T. 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist* **207**: 437–453.

Mallet J. **2007**. Hybrid speciation. *Nature* **446**: 279–283.

Manchester SR. **1994**. Fruits and seeds of the middle Eocene Nut Beds Flora, Clarno Formation, Oregon. *Palaeontographia Americana* **58**: 1–205.

Manchester SR. 2002. Leaves and fruits of *Davidia* (Cornales) from the Paleocene of North America. *Systematic Botany* **27**: 368–382.

Manchester SR, Grímsson F & Zetter R. **2015**. Assessing the Fossil Record of Asterids in the Context of Our Current Phylogenetic Framework. *Annals of the Missouri Botanical Garden* **100**: 329–363.

Margirier A, Robert X, Audin L, Gautheron C, Bernet M, Hall S & Simon-Labric T. 2015. Slab flattening, magmatism, and surface uplift in the Cordillera Occidental (northern Peru). *Geology* **43**: 1031–1034.

Matzke NJ. **2013**. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* **5**: 4.

Mau B, Newton MA & Larget B. **1999**. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* **55**: 1–12.

McHenry MA & Barrington DS. **2014**. Phylogeny and biogeography of exindusiate Andean *Polystichum* (Dryopteridaceae). *American Journal of Botany* **101**: 365–375.

Mendoza ÁM, Ospina OE, Cárdenas-Henao H & García JC. 2015. A likelihood inference of historical biogeography in the world's most diverse terrestrial vertebrate genus: Diversification of direct-developing frogs (Craugastoridae: *Pristimantis*) across the Neotropics. *Molecular Phylogenetics and Evolution* **85**: 50–58.

Miller MA, Pfeiffer W & Schwartz T. **2010**. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *2010 Gateway Computing Environments Workshop (GCE)*. New Orleans, LA, USA: IEEE, 1–8.

Mittermeier RA, Robles-Gil P, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J & da Fonseca GAB. 2004. *Hotspots revisited – Earth's biologically richest and most endangered terrestrial ecoregions*. Mexico City, CEMEX.

Montes C, Cardona A, Jaramillo C, Pardo A, Silva JC, Valencia V, Ayala C, Pérez-Ángel LC, Rodríguez-Parra LA, Ramírez V & Niño H. 2015. Middle Miocene closure of the Central American seaway. *Science* **348**: 226–229.

Montes C, Bayona G, Cardona A, Buchs DM, Silva CA, Morón S, Hoyos N, Ramírez DA, Jaramillo CA & Valencia V. 2012. Arc-continent collision and orocline formation: Closing of the Central American seaway. *Journal of Geophysical Research* **117**: B04105.

Moody ML, Hufford L, Soltis DE & Soltis PS. 2001. Phylogenetic relationships of Loasaceae subfamily Gronovioideae inferred from matK and ITS sequence data. *American Journal of Botany* **88**: 326–336.

Morales J. 2007. Loasaceae. In Hammel B, Grayum M, Herrera C & Zamora N, eds, *Manual de Plantas de Costa Rica. Vol. VI. Monographs in Systematic Botany from the Missouri Botanical. Garden Vol.111*: 202–206.

Morales-Briones DF, Romoleroux K, Kolář F & Tank DC. 2018. Phylogeny and Evolution of the Neotropical Radiation of *Lachemilla* (Rosaceae): Uncovering a History of Reticulate Evolution and Implications for Infrageneric Classification. *Systematic Botany* **43**: 17–34.

Muchhala N, Johnsen S & Smith SD. **2014**. Competition for Hummingbird Pollination Shapes Flower Color Variation in Andean Solanaceae. *Evolution* **68**: 2275–2286

Musilová Z, Říčan O, Říčanová Š, Janšta P, Gahura O & Novák J. 2015. Phylogeny and historical biogeography of trans-Andean cichlid fishes (Teleostei: Cichlidae). *Vertebrate Zoology* **65**: 333–350.

Mustoe GE. Hydrangea Fossils from the Early Tertiary Chuckanut Formation. *Washington Geology* **30**: 17–20.

Mutke J, Jacobs R, Meyers K, Henning T & Weigend M. 2014. Diversity patterns of selected Andean plant groups correspond to topography and habitat dynamics, not orogeny. *Frontiers in Genetics* **5**: 351.

Mutke J & Weigend M. Mesoscale patterns of plant diversity in Andean South America based on combined checklist and GBIF data. *Berichten der Reinhold-Tüxen-Gesellschaft* 29: 83–97.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB & Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.

Noben S, Kessler M, Quandt D, Wiegand A, Wicke S, Krug M & Lehnert M. 2017. Biogeography of the Gondwanan tree fern family Dicksoniaceae-A tale of vicariance, dispersal and extinction. *Journal of Biogeography* **44**: 2648–2659.

Noguera-Savelli E. 2012. A taxonomic revision of Loasaceae from Venezuela. *Caldasia* **34**: 43–67.

Nottingham AT, Fierer N, Turner BL, Whitaker J, Ostle NJ, McNamara NP, Bardgett RD, Leff JW, Salinas N, Silman M, Kruuk L & Meir P. 2018. Microbes follow Humboldt: temperature drives plant and soil microbial diversity patterns from the Amazon to the Andes. *Ecology* doi: 10.1002/ecy.2482

Nürk NM, Scheriau C & Madriñán S. 2013. Explosive radiation in high Andean *Hypericum*—rates of diversification among New World lineages. *Frontiers in Genetics* **4**: 175.

Ogg JG, Ogg G & Gradstein FM. 2016. *A concise geologic time scale 2016*. Amsterdam, Elsevier.

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N & Pearse W. 2018. 'caper' Comparative Analyses of Phylogenetics and Evolution in R. Available: https://cran.r-project.org/web/packages/caper/

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.

Paradis E, Blomberg S, Bolker B, Brown J, Claude J, Cuong HS & Desper R. 2018. 'ape' Analyses of Phylogenetics and Evolution. Available: http://ape-package.ird.fr/

Patterson BD & Costa LP. 2012. *Bones, Clones, and Biomes: The History and Geography of Recent Neotropical Mammals*. University of Chicago Press.

Pennell MW, Eastman JM, Slater GJ, Brown JW, Uyeda JC, FitzJohn RJ, Alfaro ME & Harmon LJ. 2014. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* **15**: 2216–2218.

Pérez-Escobar OA, Balbuena JA, Gottschling M. 2016. Rumbling orchids: how to assess divergent evolution between chloroplast endosymbionts and the nuclear host. *Systematic Biology* **65**: 51–65.

Pérez-Escobar OA, Chomicki G, Condamine FL, Karremans AP, Bogarín D, Matzke N, Silvestro D & Antonelli A. 2017. Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. *New Phytologist* **215**: 891–905.

Perret M, Chautems A, De Araujo AO & Salamin N. 2013. Temporal and spatial origin of Gesneriaceae in the New World inferred from plastid DNA sequences. *Botanical Journal of the Linnean Society* **171**: 61–79.

Poinar Jr GO, Weigend M & Henning T. **2015**. *Klaprothiopsis dyscrita* gen. et sp. nov., Loasaceae) in mid-tertiary Dominican amber. *Journal of the Botanical Research Institute of Texas* **9**: 369–379.

Posada D & Crandall KA. **2001**. Selecting the best-fit model of nucleotide substitution. *Systematic Biology* **50**: 580–601.

Posadas P, Miranda-Esquivel DR & Crisci JV. 2001. Using Phylogenetic Diversity Measures to Set Priorities in Conservation: an Example from Southern South America. *Conservation Biology* **15**: 1325–1334.

Pouchon C, Fernández A, Nassar JM, Boyer F, Aubert S, Levergne S & Mavárez J. 2018. Phylogenomic Analysis of the Explosive Adaptive Radiation of the *Espeletia* Complex (Asteraceae) in the Tropical Andes. *Systematic Biology* **67**: 1041–1060.

Poulsen CJ, Ehlers TA & Insel N. **2010**. Onset of convective rainfall during gradual late Miocene rise of the central Andes. *Science* **328**: 490–493.

Quintana C, Pennington RT, Ulloa CU & Balslev H. 2017. Biogeographic Barriers in the Andes: Is the Amotape—Huancabamba Zone a Dispersal Barrier for Dry Forest Plants? *Annals of the Missouri Botanical Garden* **102**: 542–550.

R Core Team. 2014. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna.

Rambaut A & Drummond AJ. 2014. Tracer v.1.6. Available at: http://tree.bio.ed.ac.uk/software/tracer/.

Rausher MD. **2008**. Evolutionary Transitions in Floral Color. *International Journal of Plant Sciences* **169**: 7–21.

Ree RH & Smith SA. **2008**. Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. *Systematic Biology* **57**: 4–14.

Revell LJ. **2012**. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223.

Richardson JE, Madriñán S, Gómez-Gutiérrez MC, Valderrama E, Luna J, Banda K, Serrano J, Torres MF, Jara OA, Aldana AM, Cortés R, Sánchez D & Montes C. 2018. Using dated molecular phylogenies to help reconstruct geological, climatic, and biological history: Examples from Colombia. *Geological Journal* **2018**: 1–9.

Rieseberg LH & Soltis DE. **1991**. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* **5**: 65–84.

Rivas-Martínez S, Navarro G, Penas A, & Costa M. 2011. Biogeographic map of South America. A preliminary survey. *International Journal of Geobotanical Research* **1:** 21–40 + Map.

Rodríguez E. 2008. *Nasa weigendii* (Loasaceae: Loasoideae) una nueva especie del Perú. *Arnaldoa* **15**: 21–29.

Rodríguez E & Weigend M. 1999. *Nasa umbracullifera* (Loasaceae: Loasoideae), una nueva especie con hojas peltadas del Perú. *Arnaldoa* **6**: 49–56.

Rodríguez E & Weigend M. 2004. *Nasa longivalvis*, una nueva especie del Departamento de La Libertad, Perú. *Arnaldoa* **11**: 67–78.

Rodríguez E & Weigend M. 2006. Loasaceae endémicas del Peru. *Revista Peruana de Biología*. **Número especial 13**: 391s–402s.

Rodríguez E, Weigend M & Dostert N. 2002. Sobre la validez de *Nasa dyeri* subsp. *dyeri* (Loasaceae) como un nuevo reporte para la flora peruana. *Arnaldoa* **9**: 21–25.

Salgado-Roa FC, Pardo-Diaz C, Lasso E, Arias Cf, Solferini VN & Salazar C. 2018. Gene flow and Andean uplift shape the diversification of *Gasteracantha cancriformis* (Araneae: Araneidae) in northern South America. *Ecology and Evolution* **2018**: 7131–7142.

Sanín MJ, Kissling WD, Bacon CD, Borschenius F, Galeano G, Svenning JC, Olivera J, Ramírez R, Trénel P & Pintaud JC. 2016. The Neogene rise of the tropical Andes facilitated diversification of wax palms (*Ceroxylon*: Arecaceae) through geographical colonization and climatic niche separation. *Botanical Journal of the Linnean Society* **182**: 303–317.

Särkinen T, Pennington RT, Lavin M, Simon MF & Hughes CE. 2012. Evolutionary islands in the Andes: persistence and isolation explain high endemism in Andean dry tropical forests: Evolutionary islands in the Andes. *Journal of Biogeography* **39**: 884–900.

Scher HD & Martin EE. **2006**. Timing and climatic consequences of the opening of Drake Passage. *Science* **312**: 428–430.

Serbet R, Manchester SR, Aulenbach K & Braman D. 2004. Nyssaceae among the dinosaurs: Anatomically preserved fruits from the Upper Cretaceous Horseshoe Canyon Formation, Drumheller, Alberta, Canada. Abstract. Botany 2004 Conference. Available at: http://2004.botanyconference.org/engine/search/index.php?func=detail&aid=485

Silvestro D & Michalak I. **2012**. raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* **12**: 335–337.

Slanis AC, Perea MC & Grau A. **2016**. Revisión taxonómica del género *Caiophora* (Loasaceae) para Argentina: *C. sleumerii* una nueva especie. *Darwiniana, nueva serie* **4**: 138–191.

Smith SD & Kriebel R. **2018**. Convergent evolution of floral shape tied to pollinator shifts in Iochrominae (Solanaceae). *Evolution* **72**: 688–697.

Soltis D, Soltis P, Endress P, Chase M, Manchester S, Judd W, Majure L & Mavrodiev E. 2018. *Phylogeny and Evolution of the Angiosperms: Revised and Updated Edition.* University of Chicago Press.

Spikings R., Winkler W, Seward D & Handler R. 2001. Along-strike variations in the thermal and tectonic response of the continental Ecuadorian Andes to the collision with heterogeneous oceanic crust. *Earth and Planetary Science Letters* **186**: 57–73.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Strelin MM, Arroyo JI, Fließwasser S & Ackermann M. 2017. Diversification of *Caiophora* (Loasaceae subfam. Loasoideae) during the uplift of the Central Andes. *Organisms Diversity & Evolution* **17**: 29–41.

Struwe L, Haag S, Heiberg E & Grant JR. 2009. Andean speciation and vicariance in neotropical *Macrocarpaea* (Gentianaceae–Helieae). *Annals of the Missouri Botanical Garden* **96**: 450–469.

Urban I & Gilg E. 1900. Monographia Loasacearum. *Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum* **76**: 1–368.

Villagómez D & Spikings R. 2013. Thermochronology and tectonics of the Central and Western Cordilleras of Colombia: Early Cretaceous–Tertiary evolution of the Northern Andes. *Lithos* **160–161**: 228–249.

Villar de Seoane L, Cúneo NR, Escapa I, Wilf P & Gandolfo MA. 2015. *Ginkgoites patagonica* (Berry) comb. nov. from the Eocene of Patagonia, Last Ginkgoalean Record in South America. *International Journal of Plant Sciences* **176**: 346–363.

Weigend M. 1997. Nasa *and the conquest of South America: Systematic rearrangements in Loasaceae Juss.*. Ph.D. Thesis. Ludwig-Maximilians Universität München. Germany.

Weigend M. 2000a. Loasaceae No. 132. In: Andersson L, Harling G, eds. *Flora of Ecuador* **64**: 1–92.

Weigend M. **2000b**. A revision of the Peruvian species of *Nasa* ser. *Alatae* (Loasaceae). *Nordic Journal of Botany* **20**: 15–31.

Weigend M. 2001. Loasaceae. In: Bernal R, Forero E, eds. *Flora de Colombia* **22***:* 1–100.

Weigend M. **2002a**. Observations on the biogeography of the Amotape-Huancabamba zone in northern Peru. *The Botanical Review* **68**: 38–54.

Weigend M. **2002b**. *Nasa panamensis*, a new species of *Nasa* (Loasaceae) from Central America. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **124**: 211–216.

Weigend M. 2004a. Loasaceae. In: Kubitzki K, ed. *The families and genera of vascular plants Vol. 6.* Berlin, Spinger, 239–254.

Weigend M. 2004b. Four new species of *Nasa* ser *Alatae* (Loasaceae) in the Amotape-Huancabamba zone of Peru. *Novon* **14**: 134–146.

Weigend M. 2004c. Additional observations on the biogeography of the Amotape-Huancabamba zone in Northern Peru: Defining the South-Eastern limits. *Revista Peruana de Biología* **11**: 127–134.

Weigend M. 2006. Validating subfamily, genus and species names in Loasaceae (Cornales). *Taxon* **55**: 463–468.

Weigend M. 2011. Loasaceae 173A. In: Devidse G, Sousa M, Knapp S, Chiang F, eds. *Flora Mesoamericana 3(2) Erythroxylaceae a Icacinaceae*. Universidad Autónoma de México. Accessed from:http://www.tropicos.org/docs/meso/loasaceae.pdf

Weigend M & Ackermann M. 2015. Loasaceae. In Jørgensen PM, Nee M & Beck S, eds. *Catálogo de las Plantas Vasculares de Bolivia. Monographs in Systematic Botany from the Missouri Botanical Garden, Vol. 127*. Missouri: Missouri Botanical Garden Press, 763–765.

Weigend M, Cano A, Rodríguez E & Breitkopf H. 2010a. Four New Species of *Ribes* (Grossulariaceae), Primarily from the Amotape–Huancabamba Zone in Northern Peru. *Novon* **20**: 228–238.

Weigend M, Grau J & Ackermann M. 2008. Loasaceae. In Zuloaga FO, Morrone O & Belgrano MJ, eds. *Catálogo de las Plantas Vasculares del Cono Sur (Argentina, Sur de Brasil, Chile, Paraguay y Uruguay). Monographs in Systematic Botany from the Missouri Botanical Garden, Vol. 107*. Missouri: Missouri Botanical Garden Press, 2413–2424.

Weigend M & Gottschling M. **2006**. Evolution of Funnel-Revolver Flowers and Ornithophily in *Nasa* (Loasaceae). *Plant Biology* **8**: 120–142.

Weigend M, Gottschling M, Hilger HH & Nürk N. 2010b. Five new species of *Lithospermum* L.(Boraginaceae tribe Lithospermeae) in Andean South America: another radiation in the Amotape-Huancabamba zone. *Taxon* **59**: 1161–1179.

Weigend M, Gottschling M, Hoot S & Ackermann M. 2004. A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on *trn*L (UAA) sequence data, with consequences for systematics and historical biogeography. *Organisms Diversity & Evolution* **4**: 73–90.

Weigend M, Henning T & Schneider C. **2003**. A revision of *Nasa* ser. *Carunculatae* (Loasaceae subfam. Loasoideae). *Systematic Botany* **28**: 765–781.

Weigend M, Kufer J & Müller AA. **2000**. Phytochemistry and the systematics and ecology of Loasaceae and Gronoviaceae (Loasales). *American journal of botany* **87**: 1202–1210.

Weigend M & Rodríguez E. 2000. *Nasa picta* subsp. *pamparomasii* (Loasaceae: Loasoideae) una nueva subespecie de Ancash, Perú. *Arnaldoa* **7**: 19–26.

Weigend M & Rodríguez E. 2002. Las especies arbustivas de *Nasa* ser. *Grandiflorae* en el Norte de Peru, con la descripción de una especie nueva de la Abra del Barro Negro (Callacalla), Dpto. Amazonas. *Arnaldoa* **9**: 7–20

Weigend M & Rodriguez E. 2003. A revision of the *Nasa stuebeliana* group [*Nasa* ser. *Saccatae* (Urb. & Gilg) Weigend, Loasaceae] with notes on morphology, ecology and distribution. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **124**: 345–382.

Weigend M, Rodríguez E & Dostert N. 1998. *Nasa insignis* y *Nasa glangulosissima* (Loasaceae: Loasoideae), dos nuevas especies con hojas peltadas del norte de Perú. *Arnaldoa* **5**: 151-157.

Xiang QY, Moody ML, Soltis DE, *et al.* 2002. Relationships within Cornales and circumscription of Cornaceae—*mat*K and *rbc*L sequence data and effects of outgroups and long branches. *Molecular Phylogenetics and Evolution* **24**: 35–57.

Xiang QY, Thomas DT & Xiang QP. **2011**. Resolving and dating the phylogeny of Cornales – Effects of taxon sampling, data partitions, and fossil calibrations. *Molecular Phylogenetics and Evolution* **59**: 123–138.

Zachos JC, Dickens GR & Zeebe RE. **2008**. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* **451**: 279–283.

CHAPTER 8

A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation[i]

Nasa macrothyrsa a species endemic to the Amotape Huancabamba zone. This species is hardy and can survive on roadsides. It has been subject to extensive research regarding male function fitness and stamen thigmonasty. Photograph courtesy of Tilo Henning.

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A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation

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Obvious movements of plant organs have fascinated scientists for a long time. They have been studied extensively, but few behavioural studies to date have dealt with them, and hardly anything is known about their evolution. Here, we present a large experimental dataset on the stamen movement patterns found in the Loasaceae subfam. Loasoideae (Cornales). An evolutionary transition from autonomous-only to a combination of autonomous and thigmonastic stamen movement with increased complexity was experimentally demonstrated. We compare the stamen movement patterns with extensive pollinator observations and discuss it in the context of male mating behavior. Thigmonastic pollen presentation via stamen movements appears to be a crucial component of floral adaptation to pollinator behaviour, evolving in concert with complex adjustments of flower signal, reward and morphology. We hypothesize that rapid adjustments of pollen presentation timing may play a significant role in the diversification of this plant group, representing a striking example for the evolutionary significance of plant behaviour.

Plant behaviour. Plant behavioural studies are gradually being accepted as a branch of plant science[1–4]. Numerous aspects of plant intelligence, including neurobiology and behavioural responses dealing with biotic and abiotic stimuli, have been invoked to describe and explain complex reactions of plants to stimuli. Only recently have studies documented plant learning[5] and even discussed visual cognition[6,7]. Without trying to summarize the numerous aspects of plant behaviour that have been described in recent years[1,4,8], it is clear that plants have long been perceived as passive organisms.

Most scientific evidence on plant behaviour circumscribes individual phenomena or compares distantly related taxa, missing a possible linkage between behaviour and evolutionary processes[9]. Behaviour is fundamental for understanding the fitness of an individual organism, as has been amply documented in the animal kingdom, but it also conveys competitive advantages at the population and meta-population level and is thus instrumental in driving natural selection. Behavioural diversification has long been known to be a driver of diversification in the animal kingdom (e.g. birds[10]; poison frogs – *Oophaga granulifera*[11]). Behavioural isolation, often concerning mating behaviour, has been instrumental in circumscribing animal species, (crabs – *Uca* sp[12–16]). The potential evolutionary implications of plant behaviour, however, have not yet been studied – since there are few known examples and previous investigations have focused on individual species, and such investigations precluded any comparative or phylogenetic analyses.

Stamen Movement. The active movement of plant organs, in particular those that are fast and therefore obvious, have fascinated scientists ever since their first discovery[17,18]. Rapid movements of specialized organs have been studied quite extensively, e.g., the trap mechanisms of *Dionaea muscipula* or *Aldrovanda vesiculosa*[19]

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or the leaf movements of *Mimosa pudica*[20] or *Albizzia julibrissin*[21]. These movements serve to protect the plant body from physical damage or to catch animal prey for plant nutrition. Conversely, a wide spectrum of less obvious movements of floral organs can be observed in the context of pollination ecology. Among these, stamen movements are the most common type and have been reported from a range of plant families (see[19]). Stamen movements have been known for a long time (*Berberis* – Berberidaceae[22]; *Parietaria* – Urticaceae[23]). The functional interrelation between these movements and flower visitors[23] and the process of pollination (*Nigella* – Ranunculaceae[24]) was reported as early as the 19th century. Several more or less spectacular cases of stamen movements have been reported from a variety of plant families. These movements are either singular movements driven by unrepeatable releases of stored energy (e.g. *Ricinus* – Euphorbiaceae[25]; *Trophis* – Moraceae[26,27]; *Catasetum* – Orchidaceae[28]; *Cornus canadensis* – Cornaceae[29]), or are slower, cascade-like movements that lead to the consecutive movement of stamens within the flower (*Tropaeolum* – Tropaeolaceae and *Parnassia* – Celastraceae[30]) or the movement can be repeatedly triggered by flower visitors (e.g. *Berberis*[31]). For *Ruta graveolens* (Rutaceae), Ren and Tang[32] revealed a combination of an autonomous, successive movement complemented by an accelerated stamen uplift triggered by an increased number of pollinator visits on the flower. Such thigmonastic stamen movements (thigmonasty = nastic response to touch or vibration – in stamens = triggered by the contact with flower visitors) have been reported for several plant families: Aizoaceae, Berberidaceae, Cactaceae, Cistaceae, Malvaceae, Portulacaceae, and Tiliaceae[33–38] but are often restricted to a single taxon. The majority of these movements follow uniform patterns: a single stimulus leads to the simultaneous, unrepeatable movement of all stamens in a fixed direction in order to achieve maximum pollen deposition on a pollinator. In almost all cases, this movement is triggered by stimulating the stamen (usually at the filament). Only few examples of more complex responses have been reported. In *Stylidium* (Stylidiaceae) the stamens and style form a columnar complex that can perform repeated rapid movements from one side of the flower to the other[39]. In *Berberis*, the intensity of the stimulus determines the number of stamens that move in response[22] and in *Opuntia lindheimeri*, the direction of the movement is determined by the exact location of the stimulus[40]. In many other species of *Opuntia* the stamens, upon stimulation, repeatably perform a bidirectional movement from the petals towards the style and back, regardless of the specific site of contact[41,42]. Finally, cascade movement mechanisms (whether thigmonastic or not) often occur in combination with subsequent autonomous movements to avoid anther-anther interference during pollination (e.g. *Parnassia*[43]; *Ruta graveolens*[32]). Moreover, in the latter case all stamens repeat their movement towards the style simultaneously at the end of anthesis to ensure pollination through selfing as a backup mechanism[32].

Members of the Loasaceae subfam. Loasoideae have an even more complex stamen presentation. Sequentially maturing stamens individually move into the centre of the flower, ancestrally this movement appears to be exclusively autonomous, but in the derived condition appears to be triggered and thigmonastic[44]. Unlike in most other plants with thigmonastic stamens, the stimulation does not lead to the indiscriminate movement of all, or multiple stamens, but only a small and relatively fixed number of stamens reacts to each stimulus[44–47]. Individual stamens can be triggered throughout the staminate phase for as long as fresh stamens are available. Finally, the mechanical stimulus is not received by the stamen itself, but by the so-called nectar scales (see below). Flower visitors manipulate these scales in order to access the nectar and this stimulus is transmitted to the stamen fascicles, linking actual nectar harvest to pollen dispensation[47]. The stimulus thus has to be transmitted through the receptacle from the nectar scale to the stamen. This remarkably complex mechanism has been widely documented for representatives of subfam. Loasoideae, but nowhere else in the plant kingdom[46]. In Loasaceae, this reaction is one aspect of the considerable diversification of floral morphology and function. It has been argued that thigmonastic stamen presentation is a highly specialized case of pollen partitioning and a mechanism to increase male fitness, and data have been presented indicating that the specific timing of pollen presentation is likely to increase pollen export[45].

The stamen movement observed in Loasaceae subfam. Loasoideae is in line with the predictions made in the context of the pollen-presentation theory[48,49]: Plants can increase male fitness by adjusting pollen presentation to pollinator quality and quantity. If the mechanism of pollen presentation adjusts to a certain pollinator's traplining behaviour and makes use of pollinator revisits, then outbreeding success would likely increase. LeBuhn and Holsinger (p. 119[50]) concluded that: "A plant should allocate pollen such that all pollinators that visit remove pollen". Such a system of pollen packaging and dispensing would require either a very constant frequency of revisits or a mode of pollen presentation that can adjust to the pollinator activity. LeBuhn and Holsinger (p. 119–120[50]) called this the "unlikely case in which the number of visits to be received is highly predictable and the individual plant possess the ability to adjust pollen-dispensing schedules accordingly" by which"plant fitness may increase substantially"[51]. Flower visitation has been shown to be remarkably regular in several species of Loasaceae subfam. Loasoideae for which detailed observations are available[44,45,52]. The floral behaviour reported for this plant group thus complies with the theoretical ideal proposed by LeBuhn and Holsinger[50] and hence constitutes a prime example to study the evolution of such an elaborate pollen dispensation system.

Floral function in Loasaceae subfam. Loasoideae. Loasaceae are a small, predominantly neotropical plant family with a center of diversity in Andean South America (Colombia to Chile). The family comprises ca. 350 species in 21 genera. Molecular studies have largely confirmed earlier systematic re-arrangements based on morphology (e.g.[53]), and the phylogeny of the group can be considered as well-resolved[54–56]. In spite of its relatively moderate number of species, the family is morphologically highly diversified (Fig. 1). Numerous studies have revealed a high level of diversity for growth- and life-forms[57], leaf morphology and wood anatomy[58–61], pollen- and seed morphology[62,63], indumentum[64–66] and especially floral morphology[67–70]. Most of the floral diversification is found in subfam. Loasoideae comprising ca. two thirds of all species (200 spp.) in 14 genera. Loasoideae are clearly distinguished from the other subfamilies by their deeply boat-shaped petals, into which the immature stamens are initially reflexed, and the highly modified staminodial complex, consisting of an

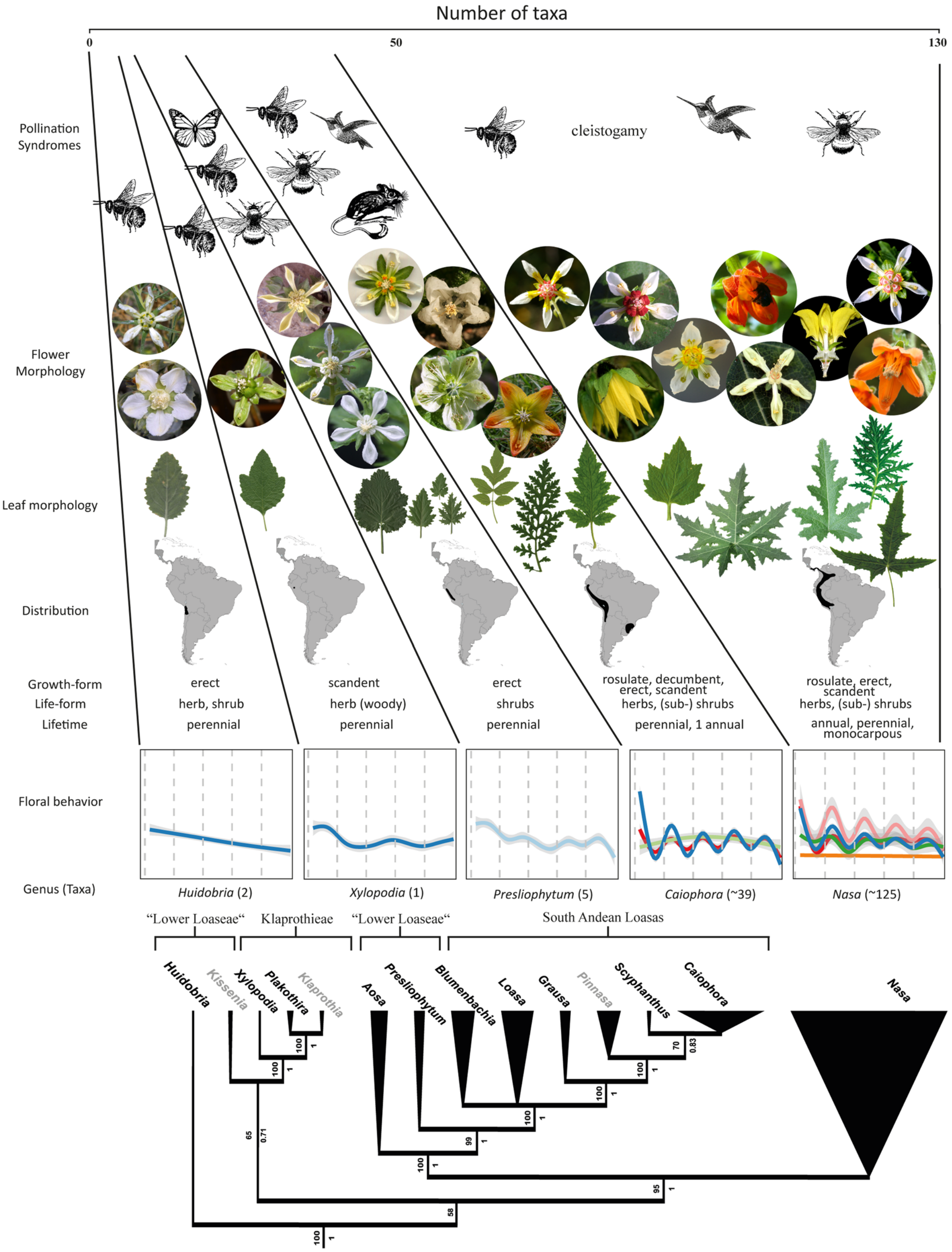


Figure 1. Graphical summary of the diversity found in selected genera of the Loasaceae subfam. Loasoideae. Five genera have been chosen exemplarily to illustrate the general evolutionary trends determined. The tree at the bottom shows the phylogenetic relationship of the whole subfamily with the width of the branches indicating the number of taxa currently accepted (bootstrap values above, posterior probabilities indicated below branches).

outer, fused floral scale and inner, free staminodia (Fig. 2)[71]. Overall flower morphology is relatively conserved throughout the subfamily, but two tribes are recognized based i.a. on the number of floral organs: tetramerous Klaprothieae (3 genera) and mostly pentamerous Loaseae (11 genera Fig. 1).

The flowers of Loasoideae are polyandrous (many stamens) and show both dichogamy (male and female organs mature at different times) and protandry (stamens/pollen is presented before the stigma becomes receptive), two very common strategies to promote outcrossing in angiosperms[72]. The 10 to 250 stamens are arranged into antepetalous fascicles. They are initially reflexed into the spreading, boat-shaped petals and typically oriented at an

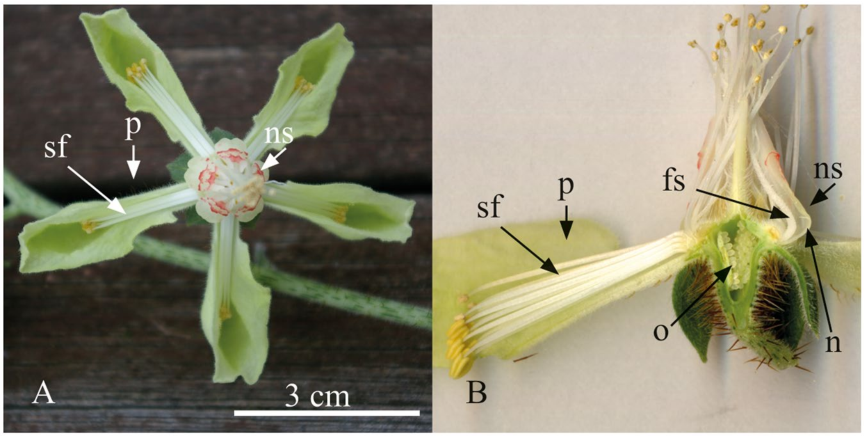


Figure 2. Typical flower of Loasaceae subfam. Loasoideae (*Nasa macrothyrsa*). (**A**) Frontal view, note the stamen fascicles (sf) hidden in the boat shaped petals (p) and the nectar scales (ns) providing a visual cue, structures to hold on and guide the pollinator to the nectar (n). (**B**) Longitudinal section through the flower. The nectar is secreted from the margins of the receptacle (bearing the ovules (o)) into the nectar scales (ns) and accumulates at their base. Two free inner staminodes (fs) direct the pollinator to the nectar.

angle of 40–140° to the style (Fig. 2). During the staminate phase, stamens mature sequentially and gradually present their pollen[47,67–69]. As the anthers mature, the filaments successively and individually curve at their bases, thus bending the anthers into the center of the flower, where pollen is presented to the flower visitors. The details of the mechanistic principles in the Loasoideae remain unexplained to date but there is evidence that the stamen movement was preceeded by an increased synorganization of the vasculature system in the receptacle, which is known to play a key role in the transduction of electric signals[47]. It can be assumed that the underlying molecular mechanisms to perceive (sense and transmit the stimulus) and respond (stamen movement) to the stimulation of the nectar scale in Loasoideae are the same that are generally recognised for the mechanoperception in plants[37,73,74].

The movement is fast enough to be observed with the naked eye and typically takes only 1–3 minutes. Throughout the staminate phase autonomous movement takes place during the daylight hours of 2–3 consecutive days, ensuring that fresh, viable pollen for potential pollinators is continuously available in the centre of the flower and pollen offerings in the flower remain more or less constant throughout the staminate phase[46,47]. Additionally, thigmonastic stamen presentation occurs in most genera. Here, the presentation of fresh anthers in the flower centre is triggered by pollinator visits (Supplementary Video). The peculiar staminodial complexes alternate with the filament fascicles and typically consist of five staminodes; the outer three of which are fused into a scale-shaped structure (floral scale) and two of those close this scale towards the center of the flower (Fig. 2). These staminodial complexes have a range of different roles in plant-pollinator interaction (for details see[69,70]). Nectar is secreted from the margins of the receptacle into the floral scales, with the nectar continuously replenished[45]. To access the nectar, pollinators insert their proboscis or beak into the floral scale forcing it to bend outwards. This manipulation of the floral scale triggers the thigmonastic movement of filaments[44,75]. Unlike the autonomous movement, this thigmonastic motion is a direct reaction to a floral visit and thus plant behaviour that is active and responsive[45–47]. Unlike autonomous stamen presentation, thigmonastic stamen presentation replenishes the pollen offerings of the flower immediately after a pollinator visit. Therefore, the time period where the flower is not able to dispense pollen to a flower visitor is reduced. This complex floral behaviour has been demonstrated for a range of species from different genera (*Blumenbachia*, *Caiophora*, *Loasa*, *Nasa*, *Presliophytum*) in Loasaceae subfam. Loasoideae[45–47,52,76,77], and has not yet been reported from representatives outside this subfamily that lack both reflexed stamens and floral scales. Comparative data have not been provided on floral responses across different taxa, nor has an evolutionary assessment been attempted. To the best of our knowledge the present study is the first attempt to explain plant behaviour – in our case a highly specific, thigmonastic response to flower visits – in a phylogenetic context across many (in our case 44) species representing circa ¾ of all genera (11 out of 14) of the subfamily.

Aims. Based on what is known, the floral function of Loasaceae represents a unique system for an evolutionary study on plant behaviour, in this case the specific reaction of stamens to pollinator visits. The present paper presents a large experimental data set on the behavioural diversity of thigmonastic stamen presentation and places it in the context of a phylogenetic framework of a molecular phylogeny and data on the pollination syndromes of neotropical Loasaceae subfam. Loasoideae. Based on these data we aim at:

1. Investigating the presence and characteristics of the stamen presentation across the subfamily.
2. Documenting the extent of diversification of thigmonastic stamen presentation as plant behaviour.
3. Contextualizing the patterns of thigmonasty with the phylogeny of the group and the pollination syndromes that have been documented.
4. Assessing and discussing thigmonasty as a behavioural expression and investigating its possible significance for the evolutionary history and diversification of this plant group in the overall context of flower function.

Material and Methods

Plant material. A total of 44 taxa from 11 genera of Loasaceae subfam. Loasoideae were investigated (*Aosa* (2 species), *Blumenbachia*[3], *Caiophora*[12], *Grausa*[1], *Huidobria*[1], *Loasa*[5], *Nasa* (13 species and subspecies), *Plakothira*[1], *Presliophytum*[2], *Scyphanthus*[2] and *Xylopodia*[1], for a complete list incl. taxonomic information see Supplementary Table 1). The data for *Huidobria fruticosa* were obtained from plants in their natural habitat. All other datasets were obtained from plants in cultivation. All species were raised from seed collected in the wild, with the only exception of *Blumenbachia insignis* and *B. hieronymi*, which were obtained from cultivated material of unknown provenance from botanical gardens (see Supplementary Table 1 for detailed voucher information). Plants were cultivated in the greenhouses at the Institut für Biologie, Freie Universität Berlin (2001 to 2008) and the Nees Institut für Biodiversität der Pflanzen, Universität Bonn (2012). For detailed information on cultivation see[41].

Pollinator Data. Pollinator data for the taxa studied were either extracted from the literature or are based on our own field observations. For some taxa the pollination syndrome were extrapolated from the overall flower morphology and data available on closely related taxa. Pollination syndromes are generalized to the principal types observed in the Loasoideae[70,75–78]. Six different groups of pollinators have been previously reported for Loasoideae: short-tongued bees, long tongued bees, flies, butterflies, hummingbirds and mammals (Supplementary Table 2). Based on field observations, observations in cultivation and literature data the taxa examined were assigned to eight different pollination syndromes for the present study: short-tongued bees, long tongued bees, long tongued bees and hummingbirds, flies, various insects (i.a. butterflies), hummingbirds, mammals and cleistogamy.

Thigmonastic stamen movement. Depending on the quantity of flowers available, experiments were either conducted with isolated inflorescence branches placed into glass vials in the laboratory or were carried out directly on living plants in the greenhouse. Flowers were individually marked and mature stamens that already had moved into the center of the flower were cut off one hour prior to the first stimulation experiment. Depending on flower availability, 10–35 flowers were used for individual sets of experimental observations with control groups of 5–22 flowers. Stamen movement was triggered by imitating a pollinator visit by slightly bending all five nectar scales outwards with a needle. Anthers of the newly moved stamens were carefully cut off to preclude double counting. Five consecutive stimuli with 30 minute intervals between the individual stimuli were carried out. This stimulus interval was chosen based on field observations indicating an average interval between two visits to individual flowers of ca. 25 minutes for one of the species[45]. This follows the rationale that the timing of experimental visits to flowers should reflect the natural visitation rate[51] and at the same time serves the purpose to standardize the resulting dataset. For purposes of recording, the overall interval of 30 minutes was subdivided into fractions of 5 minutes each and the anthers moved in each of these 5 minute sub-intervals were pooled to ease data capture and analyses, resulting in a rate of stamens moved per 5 minute intervals.

Statistical analysis. In order to test for the presence of thigmonastic stamen movement in the species investigated, we applied multiple Generalized Additive Models (GAMs), as implemented in the gam() function of the mgcv package[79] in the R framework[80]. We used one smoother per treatment (control vs. stimulation) and the treatment as categorical variable to predict the average number of moved stamens per 5 minute interval after the manual impulse. To account for false discovery rate due to multiple comparisons, we adjusted p-values using the Benjamini-Hochberg procedure[81]. Details of data exploration procedure and modeling terms can be found in Supplementary Material 3.

For the comparison of thigmonastic patterns between pollination syndromes across phylogenetic placements, we applied a global Generalized Additive Mixed Model (GAMM), as implemented in the function gamm() in the mgcv package, to the whole dataset from which control flowers were removed. Details on model term selection and model validation can be found in Supplementary Material 3. In brief, we predicted the number of moved stamen per 5 minute interval by the respective impulse and the pollination syndrome. To account for phylogenetic relatedness of sampled species, we included the distance to the root as calculated with the function distRoot() in the adephylo package[82] for the phylogenetic tree as described below. Since we performed multiple stimuli at single flowers, which are not independent of each other, we included the impulse period into the modeling term and included the taxon ID as random factor.

To test if phylogenetic radiation impacts the thigmonastic stamen presentation in flowers pollinated by short-tongued bees, we removed control treatments and other pollination syndromes from the dataset and calculated a separate GAMM. We predicted the number of moved stamens per impulse period, with the impulse, the absolute experimental time, and the genus ID ordered according to phylogenetic placement. We added a correlation structure for the impulse period, and the species ID as random factor. Model selection and validation can be found in detail in Supplementary Material 3.

Molecular methods. The taxon sampling for the molecular data conforms exactly to that of the pollination data and thigmonastic stamen movement. Whenever it was possible, we attempted to use the same voucher specimens for the experimental as well as the molecular data. Some taxa were represented by more than one accession (*Nasa moroensis*, *N. olmosiana* and *N. triphylla* subsp. *triphylla*) if the taxa were morphologically variable. Additionally *Gronovia scandens*, *Mentzelia albescens*, *Eucnide urens* and *Deutzia discolor* were included in the analyses as outgroups. Outgroups were selected based on the phylogenetic studies of Weigend *et al.*[54] and Hufford *et al.*[55]. All sampled plant material with its geographic origin, herbarium voucher, and GenBank accession numbers is listed in Supplementary Table 1.

DNA was extracted from 0.5–1 cm^2 samples of silica gel dried leaves or herbarium leaf material with a modified CTAB method[83]. We sequenced the plastid regions *trn*L-*trn*F, *mat*K, the *trn*S-*trn*G intergenic spacers, and the *rps*16 intron (taxon sampling was complete for all markers). The PCR amplification and sequencing protocols follow Acuña *et al.*[56]. Sequences were assembled in Geneious v. 8.0.1[84] using the default *De Novo* assemble settings.

Assembled sequences were aligned in Mafft v. 7[85], followed by manual adjustments using PhyDE v. 0.9971[86]. Alignment files are available from the corresponding authors on request. FindModel (available from http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html), which implements Posada & Crandall's[87] Modeltest, selected GTR+Gamma as the model that best fits all four plastid markers. Phylogenetic reconstructions for Maximum Likelihood (ML)[88], were conducted in RAxML v. 8[89] included in RAxMLGUI v. 1.5 Beta[90]. Bayesian Inference (BI)[91] was conducted in MrBayes 3.2.2[92], in the CIPRES Science Gateway computing facility[93]. Each marker was at first analyzed separately. In the absence of topological conflict (defined as incongruence in the topologies of nodes with bootstrap support >80%) the markers were combined. ML analyses were implemented using the GTRCAT approximation, because it works in an analogous way to GTR+Gamma and yields similar results but with less intensive computational costs[89]. The statistical support for the nodes was assessed by 1000 ML thorough bootstrap replicates with 100 runs under the same analysis conditions. The BI was conducted, with four independent runs with one cold and three heated chains, the Markov chain had a length of 10 million generations, sampled every 1000 generations. After convergence was assessed in Tracer 1.5[94], the first 2.5 million generations were discarded as burn-in.

Phylogenetic effects. Traits of any kind are usually more similar between closely related species than between more distantly related species and therefore, they cannot be regarded as independent samples[95]. Therefore, it is necessary to account for phylogenetic distance in any analysis of attributes across related species[96]. Comparative phylogenetic methods have been used to investigate whether traits of species are influenced by their ancestral state[95,97,98]. Testing for phylogenetic signal thus permits an evaluation of whether phenotypic differentiation of a given species trait is equal to, higher than or less than what would be expected under a Brownian motion (BM) model of evolution[97,98]. A given trait can be treated as independent of phylogenetic history if there is no significant phylogenetic signal[96].

To investigate whether variation of thigmonastic stamen movement between species is influenced by phylogenetic history, we calculated Blomberg's K[98] and Pagel's λ[97] and tested these values for significance. As continuous variables of thigmonastic stamen movement, we used the average number of stamens moved within the first 5 minutes and the average number of stamens moved after 30 minutes (note that the average number of stamens moved after 30 minutes corresponds the below mentioned **s**tamen movement per stimulus per flower in the 30 Min-interval following an individual **s**timulus (sps30 hereinafter)). As a measure of stamen movement speed we calculated the percentage of stamens which moved within the first 5 minutes relative to the stamens moved after 30 minutes. These variables were tested for phylogenetic signal for the stimulation treatment as well as for the control treatment. This results in six variables which were tested for a significant phylogenetic signal: four variables of stamen movement and two of stamen movement speed.

The underlying branch lengths were based on the rooted maximum likelihood phylogenetic reconstruction computed with RAxML v. 8[89] as described in the previous section. Branch lengths of the trees are proportional to the substitution rates per site and so the distance to the root will differ for the different tips. Smith & Donoghue[99] and Lanfear *et al.*[100] have shown that rates of molecular evolution in plants could change according to life history and growth form. Accordingly, we assume that molecular markers can have variable evolutionary rates.

Prior to testing for phylogenetic signal, the outgroups used for constructing the phylogeny were trimmed from the tree with the drop.tip() function in the R package ape[101]. Blomberg's K and Pagel's λ were calculated with the phylosig() function implemented in the R package phytools[102]. For testing if the observed K value is significant we applied a randomization test implemented in the phylosig() function based on 10000 randomizations of the trait datasets to generate a null distribution. For testing the significance of λ a likelihood ratio test implemented in the phylosig() function was applied. This test indicates whether the reported λ significantly differs from a λ equal to zero (i.e., a "star phylogeny") where relatedness does not explain the trait similarity between species.

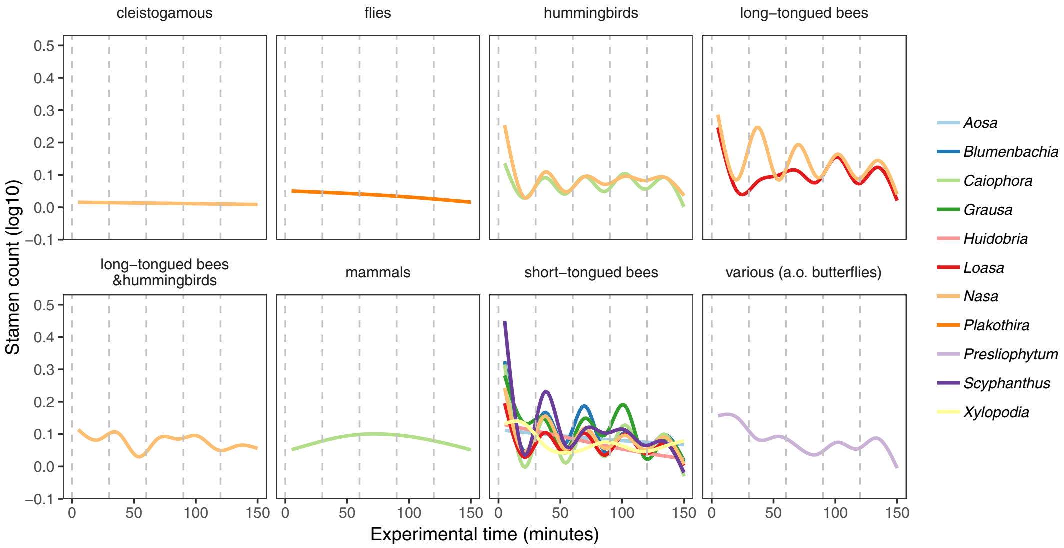
Results

Thigmonasty. 38 of the 44 taxa examined show a significant thigmonastic response upon a stimulation of the nectar scales in terms of significant differences in the rate of stamen movements compared to control flowers (Supplementary Material 3: Fig. 8). The remaining six taxa show autonomous stamen movement only, a thigmonastic response is absent. Of these taxa, *Huidobria fruticosa* and *Xylopodia klaprothioides* belong to the early -branching grade of subfam. Loasoideae. *Presliophytum incanum* and *Aosa rupestris* are part of the speciose Higher Loaseae-clade of the Loasoideae. For *A. rupestris*, stamen presentation has already been reported to only be autonomous[103]. Interestingly, stamen presentation in respective sister taxa of these non-thigmonastic species is thigmonastic (*P. heucheraefolium* and *A. parviflora*). The other non-thigmonastic species include mammal-pollinated *Caiophora coronata*[77] and cleistogamous *Nasa chenopodiifolia* (pers. observation). All other taxa examined show a significant reaction upon scale manipulation mimicking pollinator-behaviour and are known to be pollinated by insects and/or hummingbirds (Supplemental Material 3: Fig. 8).

The control groups show random, aperiodic stamen presentation. Theoretically, the autonomous stamen movement should approach a straight line if sample size was large enough and observation period time long enough. Due to the very low overall autonomous stamen presentation rate, single movements have a strong influence on the shape of the curve in our analyses. The resulting shapes (Supplementary Material 3: Fig. S8) thus mostly represent random patterns rather than straight lines.

On average, a total of 0.24–4.24 stamens per flower move in the 30 Min-interval following an individual stimulus (sps30). The thigmonastic reaction is weakest in *Nasa chenopodiifolia* (sps30 = 0.24) and highest in *Scyphanthus stenocarpus* (sps30 = 4.24). A comparison of different taxa reveals considerable differences between

(a) Loess–smoothed averages of stimulated flowers within pollination syndromes and genera



(b) Loess–smoothed averages of stimulated flowers within genera and pollination syndromes

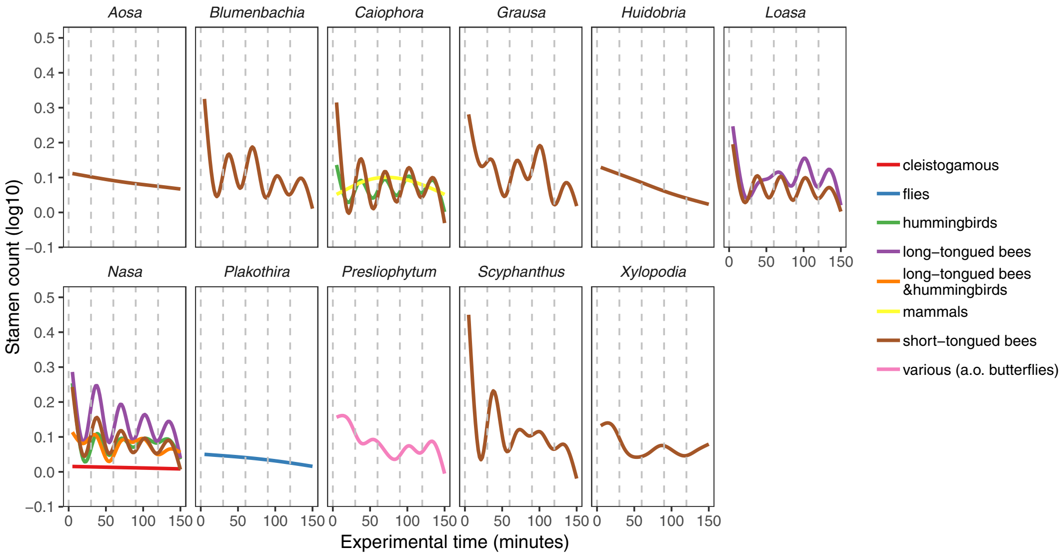


Figure 3. Differences in staminal movements during experimental time in reaction to manual stimulations of the floral organs in flowers. Dashed vertical lines mark stimulations. First evaluation of staminal reaction occured 5 minutes after stimulus. Solid lines are averaged Loess-smooths colored for each pollination syndrome (**a**) or genus (**b**). Shaded ribbons show 95% confidence intervals of smooths.

the genera. Whilst all the species of *Caiophora* show a rather uniform presentation rate of 1.22 to 2.94 sps30, *Nasa* displays a more variable response of 0.24 to 3.39 sps30. Even small genera such as *Presliophytum* and *Scyphanthus* exhibit striking differences between individual taxa with 0.8–2.67 and 2.19–4.24 sps30, respectively. There is no obvious, quantifiable trend in the movement rates either across the whole subfamily or within genera (Fig. 3a). Comparing patterns within pollination syndromes rather than taxa, the movement rate also varies strongly. In hummingbird pollinated taxa, for example, 0.76 to 3.28 sps30 are recorded, in taxa pollinated by short-tongued bees stamen presentation rates vary from 0.66 to 4.24 sps30. The other insect and the rodent pollinated taxa also fall into this range; the only exception is the cleistogamous *N. chenopodiifolia*, displaying the lowest movement of all taxa examined (0.24 sps30).

Figure 3 shows the average stamen movements over time, pooled for the different genera sorted by pollination syndromes (Fig. 3a), respectively pooled for the pollination syndromes and sorted by genera (Fig. 3b). Taxa pollinated by bees and hummingbirds show an overall rhythmic stamen presentation, synchronised by the repeated stimuli. The other syndromes are characterised by an asynchronous presentation pattern (mammals, other insects and mixed pollination), or show no dynamics in the movement at all (cleistogamy). Within the common syndromes in Loasoideae, i.e. bee or hummingbird pollination, a strong variation can be found across the taxa examined. Variation is highest in bee pollination and lowest in hummingbird flowers. Figure 4 summarizes

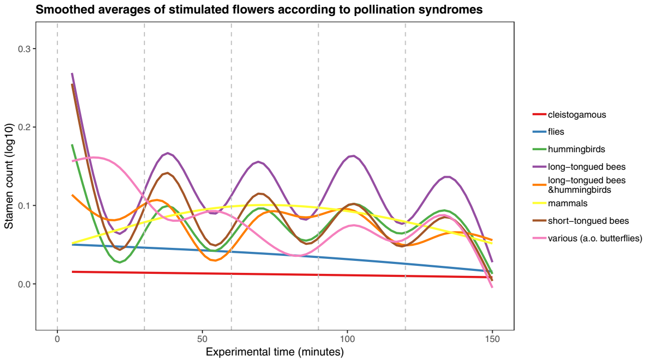


Figure 4. Differences in staminal movements between pollination syndromes during experimental time in reaction to manual stimulations of the floral organs. Dashed vertical lines mark stimulations. First evaluation of staminal reaction occured 5 minutes after stimulus. Solid lines are estimated Loess-smooths colored for each group of pollination syndrome, including species across all genera. Shaded ribbons show 95% confidence intervals of smooths.

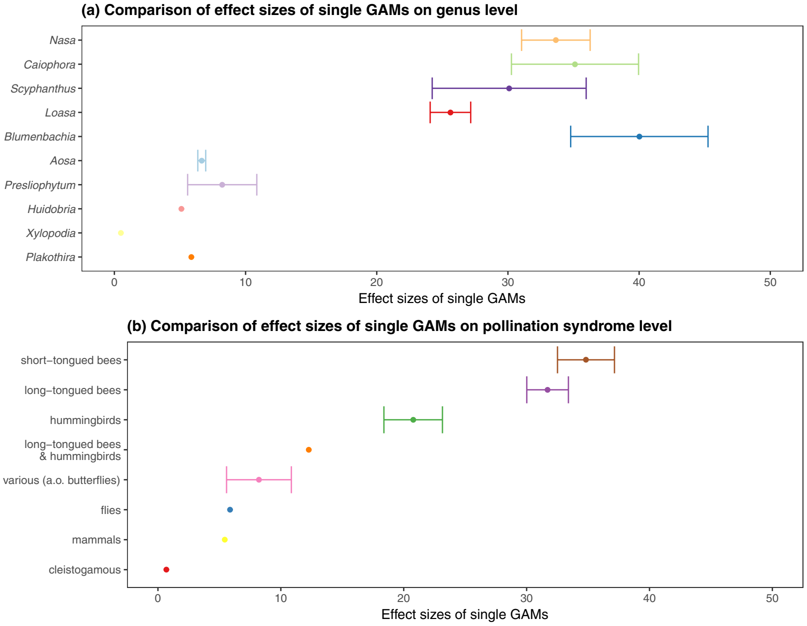


Figure 5. Comparison of GAM effect sizes averaged for different genera (**a**) or pollination synsdromes (**b**) comparing staminal response after experimental stimuli to non-stimulated control flowers within each species. Points show mean effect sizes per group. Bars refer to standard errors. Genera (**a**) are ordered ascendingly to increasing distance to the node in phylogenetic tree. Pollination syndromes are ordered to increasing average effect size.

the overall patterns observed for the different pollination syndromes. It reveals rhythmic patterns that are more or less synchronous to the stimuli for all taxa

Stamen presentation patterns are mainly influenced by pollination syndrome and to a lesser extent by the phylogenetic distance between the taxa (Fig. 5). Comparing the effect size of single GAMs on the pollination syndrome and the genus level, the standard error is smaller (and remarkably uniform) throughout the different pollination syndromes examined. In order to understand the adaptation of individual taxa to a specific pollination syndrome during the evolutionary history of the group, we analyzed a reduced dataset of all taxa pollinated by short-tongued bees only. It has been argued that this pollination mode constitutes the plesiomorphic condition in Loasoideae[53,70,78] and it is universally found in eight of the eleven genera examined, including species-poor *Xylopodia* and *Huidobria* and species-rich *Nasa* and *Caiophora*.)

Representatives from the basal nodes (*Huidobria* and *Xylopodia*) show decreasing, *Aosa* rather random reactions upon repeated stimulation. Figure 3b illustrates that there is an increase in the regularity of the reactions in the other clades (e.g., *Nasa*, *Caiophora* and *Blumenbachia*), in line with the analyses of the effect sizes across all datasets. Furthermore, these taxa maintain a virtually standardized response over repeated flower visits, presenting uniform stamen numbers with largely uniform timing.

		Blomberg's K	Pagel's λ
Movement	Stamen movement 1st 5 min (stimulation)	0.087	0.383
	Stamen movement 1st 5 min (control)	0.100	0.154
	Stamen movement in 30 min (stimulation)	0.096	0.000
	Stamen movement in 30 min (control)	0.165	0.122
Speed	% of stamens moved in 1st 5 min (stimulation)	0.091	0.636*
	% of stamens moved in 1st 5 min (control)	0.089	0.077

Table 1. Phylogenetic signal and statistical tests for variables of stamen movement in the Loasoideae. The asterisk indicates significance at the 95% confidence level based on a randomization test for Blomberg's K and on a likelihood ratio test for Pagel's λ.

Phylogenetic signal in stamen presentation. The phylogenetic placement of individual taxa, included as distance of branch tips to the root (of the phylogenetic tree) in the final GAMM, has only a marginal effect ($F = 0.197$, $p = 0.657$) on thigmonastic patterns (Supplemental Material 3). However, the effect size of single pairwise GAMs – that is the difference in shape between thigmonastic and autonomous movement – increases with increasing number of branches between the respective clade and the common node (Fig. 5a). Testing the phylogenetic signal with Blomberg's K for the average stamen movement after 5 minutes and after 30 minutes as well as for the speed of the stamen movement revealed no significant K value (Table 1). As a more robust approach Pagel's λ only revealed a λ of 0.636 (i.e., significantly different from zero $p = 0.0007$) for the speed of stamen movement in the stimulation treatment. The consistent non-significance of the very low K and of the λ values for stimulated stamen movement (i. e., thigmonastic movement) indicates that if there is an effect of shared ancestry, it is very weak (Table 1).

Discussion

Thigmonastic patterns. *Huidobria*, *Plakothira* and *Xylopodia* only show an autonomous movement (Fig. 1). Some taxa of the basal grade lack floral scales (*Klaprothia mentzeliodes*) and/or are obligate selfers (*K. fasciculata*). Overall, it can be assumed that these early diverging lineages of Loasoideae indeed show autonomous pollen presentation only and that this represents the ancestral condition, although experimental evidence on the two other basally branching taxa (*H. chilensis*, *Kissenia*) would clearly be desirable to corroborate this conclusion. The vast majority of taxa investigated display thigmonastic stamen presentation. A thigmonastic response can be triggered – often with highly predictable timing – by mimicking a pollinator visit by manipulating the floral scale. The analyses further indicate that – in very general terms – the thigmonastic stamen movement increases with increasing distance from the phylogenetic root in effect size, speed, and regularity. This appears to reflect an increasing ability to control and adjust pollen presentation to a given flower visitation scenario. Basally branching taxa show simple, if any thigmonastic stamen presentation and do not fall into a rhythmical pattern of pollen presentation in reaction to periodic re-visits. In representatives of the terminal clades of the Loasoideae, movement patterns are highly predictable and are synchronized with repeated flower visits (Figs 1, 3b and 6). Minor adjustments of the thigmonastic pollen presentation indicate either an adaptation to whole pollination syndromes, or possibly to the idiosyncratic visitation behaviour of individual pollinator species (Fig. 3a). Flowers pollinated by short-tongued bees - the largest subset of the taxa here studied - show a remarkably homogeneous thigmonastic pattern across the genera (Fig. 6). Minor deviations from this relatively uniform floral reaction may be the result of random effects of factors such as flower size and morphology or may go back to fine-tuning in response to different behavioural patterns in this diversely pollinated group. Long-tongued bees and hummingbirds have a larger body surface and are capable of carrying larger pollen loads compared to short-tongued bees. The presentation of a high number of stamens presented may consequently be advantageous for plants pollinated by these larger animals (Fig. 3a). However, additional datasets indicate that pollen load might be adjusted at least partly by increasing anther size and pollen grain number (Henning & Weigend, in prep.) rather than by shifts in the thigmonastic response, i.e. the number of stamens presented. Increasing the rate of anther presentation would automatically diminish the scope for pollen partitioning, since the anther stock would be depleted much faster. It is also obvious that hummingbird-pollinated taxa possess a reduced thigmonastic response to the second stimulus (Fig. 3a), which likely corresponds to specific pollinator behaviour. Hummingbirds are known to be erratic trapliners, foraging over long distances and returning after long and irregular time intervals[104,105], rendering iterative pollen replenishment in short intervals ineffective. The behavioural differences between plant taxa that are visited by different pollinator groups therefore appear to reflect the differential interaction with different pollinators and/or pollinator guilds. Conversely, a secondary loss of thigmonasty can be inferred for *Caiophora coronata*, *Nasa chenopodiifolia*, and possibly *Presliophytum incanum* and *Aosa rupestris*. *Caiophora coronata* is reportedly pollinated by opportunistic rodents whose visitation rate may be highly randomised and possibly with one off visits to individual flowers[77]. *N. chenopodiifolia* is largely autogamous or even cleistogamous – any form of pollen partitioning and timing of pollen presentation would therefore be superfluous. In the case of *Presliophytum* and *Aosa*, thigmonasty is significantly different from experimental controls in only one of two closely related taxa studied (Fig. 8 in Supplementary Material 3). Additional studies on other species of *Aosa* would clearly be of interest, but observations of cultivated individuals indicate that species of *Aosa* cultivated so far are highly autogamous, possibly relaxing the need for fine-tuning pollen presentation to pollinators. *Presliophytum incanum* could be shown to have a very broad range of flower visitors from different insect groups, with

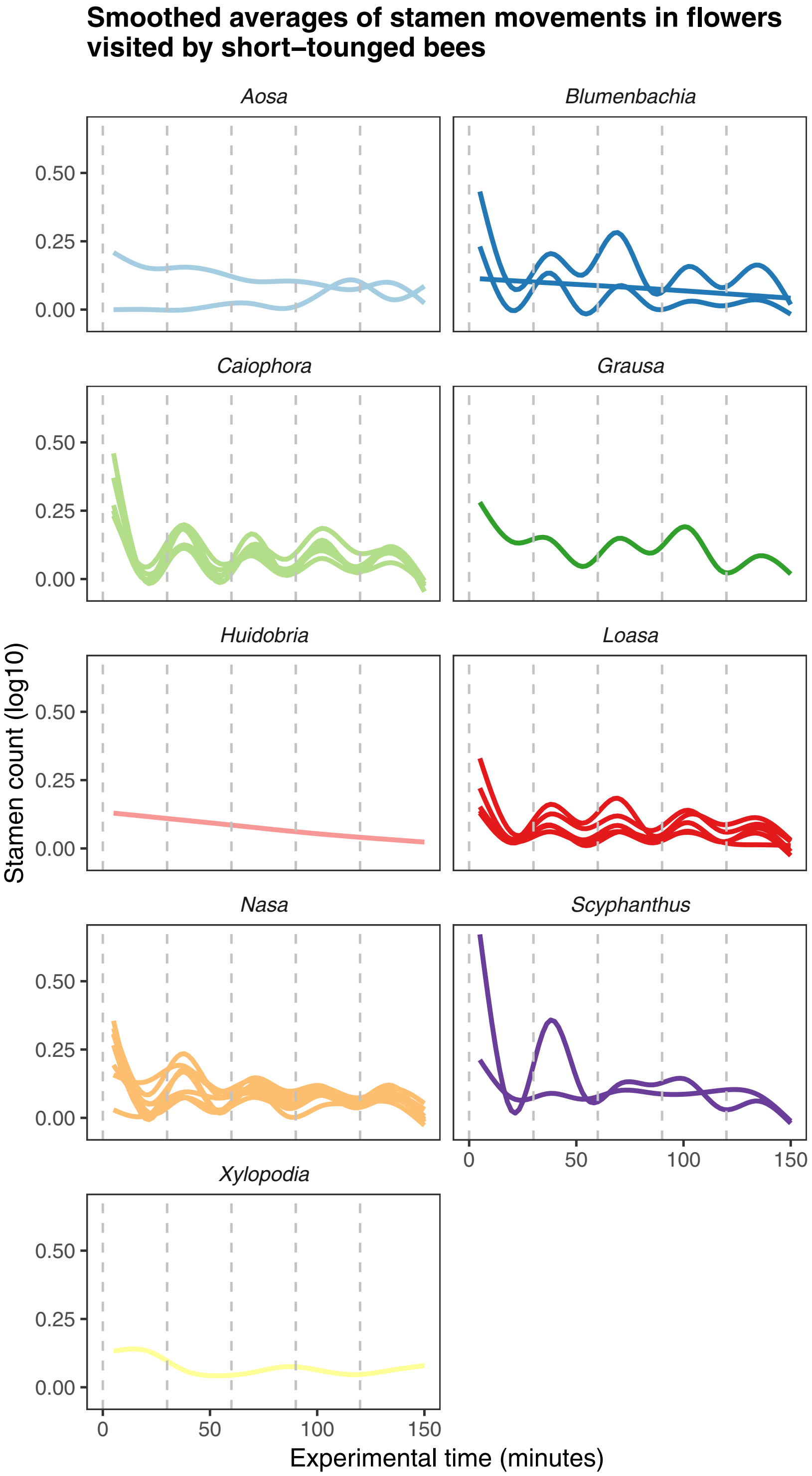


Figure 6. Differences in staminal movements between and within genera during experimental time in reaction to manual stimulations of the floral organs in flowers pollinated by short-tounged bees. Dashed horizontal lines mark stimulations. First evaluation of staminal reaction occured 5 minutes after stimulus. Solid lines are estimated Loess-smooths, colored for each genus.

butterflies representing a considerable proportion of the observed pollinators, and our data show that it does not show a thigmonastic response. Conversely, for *P. heucheraefolium* only a narrow range of visitors has been reported, essentially long-tongued-bees, and it does show a thigmonastic response (*Presliophytum* sp., Fig. 8 in Supplementary Material 3). This would underscore that a thigmonastic response only makes adaptive sense when the range of pollinators is narrow and predictable in its behaviour.

Phylogenetic signal. Patterns of thigmonastic stamen presentation in the plants investigated in the present study indicate an adaptation to pollinator groups rather than a correspondence to phylogenetic placement. As indicators for a more controlled and accurate reaction, we present both the effect size of single GAMs (Fig. 5a) and the speed of stamen movement (Table 1), both of which increase in more speciose clades such as *Blumenbachia*, *Nasa* and *Caiophora* and the latter being the only behavioural trait for which we detected a significant phylogenetic signal, based on Pagel's λ (Table 1). In other words, stamen presentation patterns in distantly related taxa with the same pollination syndromes are more similar than those of closely related taxa with different pollination syndromes.

Furthermore, effect size is positively correlated with the phylogenetic "derived-ness" (Fig. 5a), i.e., the complexity and the intensity of the reaction upon a stimulus increases with the increasing distance from the root of the phylogenetic tree. An increasing precision of the thigmonastic response can also be detected when looking more closely at the average responses of short-tongued-bee pollinated taxa upon individual stimuli. Within the derived genera, such as *Nasa* and *Caiophora*, precision of the response increases towards the crown group, specifically the speed of the thigmonastic response shows a continuous increase. The thigmonastic patterns in flowers visited by short-tongued bees are relatively stable within individual genera, whereas the regularity of these patterns (smooths) seems to increase in the more derived genera.

Floral adaptations to functional pollinator groups have been shown to be closely associated with speciation events[106,107], and our data indicate that this might be also the case in the Loasoideae. Adaptations of floral traits are at the heart of reproductive isolation and have been shown to be subject to significant phylogenetic signal (e.g.[108,109]). The lack of phylogenetic signal for stimulated stamen presentation suggests that the evolutionary adjustment of thigmonastic stamen presentation in Loasoideae is relatively rapid and possibly a *de novo* invention. An absence of a phylogenetic effect has been suggested to either arise through rapid evolution and multiple homoplastic transitions[110] or could be explained by a high degree of adaptability in behavioural responses. Previously, it has been argued that Loasoideae species show a fast evolutionary adjustment of nectar amount and composition with shifts in pollination syndrome[78]. It is possible that the rapid adjustment of pollen presentation schedules is a complementary mechanism to the evolution of nectar characteristics in response to pollinator shifts.

Floral behaviour and speciation. It has been argued that "…much plant taxonomy relies on flower structure in which plasticity is minimized" Trewavas (p. 15[111]) It is undoubtedly true in general terms that the basic architecture of Loasoideae-flowers is remarkably conserved[112]. This argument could be contrasted with the notable behavioural diversity documented here for the first time, but this would underestimate the extreme diversification in the details of flower morphology (Fig. 1), in regards to aspects of function and signalling[60,62,63,70]. Similarly, the primary floral reward in Loasoideae is highly diverse and the broad range of nectar amounts and concentrations has been shown to correlate with pollination syndromes[78]. Consequently, the adjustment of flower behaviour, i.e. the amount, timing and periodicity of pollen presentation in reaction to flower visits, appears to be part of a complex evolution of floral function in tandem with aspects of signal, reward, and morphology. This functional complexity permits multidimensional adaptations to specific individual pollinators or pollinator groups. The high level of diversity and the elevated rate of micro-endemism characteristic of this plant group has been attributed to temporal habitat heterogeneity (e.g., landslides) and repeated re-colonization of Andean habitats, in particular by the annual species (e.g., *Nasa*[113]) In order to ensure the rapid establishment of stable populations after a successful initial colonization of a new habitat, reliable pollen vectors are vital. It has been argued that an increasing adaptation of a plant taxon to a specialized pollinator following its initial recruitment is often followed by a stepwise consolidation of a mutualistic relationship[114], in turn giving rise to pre-mating barriers to the parental population. In Loasoideae, this includes a specific floral signal, morphology and reward (amount and concentration of nectar) and a – possibly rapid – adjustment of the pollen presentation timing to specific pollinators and their idiosyncratic visitation rates. We hypothesize that thigmonastic stamen presentation is a mechanism to increase male fitness[45] and has been one important component in the diversification of Loasoideae in Andean habitats, further strengthening the divergence of populations by adding an additional dimension to potential pre-mating barriers between diverging plant populations. The variation in chromosome number seems an important driver of the diversification of *Mentzelia* (Loasaceae subfam. Mentzelioideae[115,116]) where aneuploidy and polyploidy act as reproductive isolators. There is no evidence for this in Loasoideae, with usually highly conserved karyotypes[53,117,118]. Thigmonastic pollen presentation with characteristic – and apparently evolutionarily labile – timing should be considered in concert with complex adjustments of floral signal, nectar quality and quantity, flower orientation and functional morphology (nectar scales), providing numerous opportunities for adaptation and specialization along multiple functional axes.

We conclude that active floral behaviour may be an underestimated component of flower function. A critical review of other traits such as floral scents and stigmatic reactions or even systemic responses to changing pollination scenarios or flower symmetry on the inflorescence- or individual flower-level would likely provide crucial insights into hitherto overlooked mechanisms of plant adaptation and diversification. Clearly, Loasoideae provides an extreme example due to the complexity, speed and precision of floral responses to pollinator induced stimuli. However, the current pattern of plant behaviour and related floral phenomena suggest that flowers could adjust to pollinator preferences and that this ability might convey competitive evolutionary advantages. It is conceivable that many other evolutionary similarly labile behavioural traits related to plant mating exist but have not been recognized due to their low speed or due to the absence of movement. Dynamic nectar replenishment might be a similar, but subtler behavioural response to preferences and visitation rates of individual pollinators[119,120]. An exemplary survey indicates that such a response is likely common throughout flowering plants[121] and a recent study discusses the characteristics of nectar secretion dynamics in the context of mixed pollination syndromes[122]. Irrespective of future insights, here we demonstrate that Loasoideae flowers show a rather sophisticated behaviour and we are able to provide a general outline of the evolutionary pathways of complex thigmonastic responses. This is the first time such an evolutionary scenario is proposed for plant behaviour. It invites a plethora of further studies, e.g. on the details of plant-pollinator relationships, but more importantly on the physiological details of mechanoreception in plants, the anatomy and physiology of the transmission of the stimulus and the basis of the mechanical response specifically in Loasoideae flowers and for plants in general. Finally, it is evidently time to investigate the genetic basis for plant behaviour – since we demonstrate here that it is a trait, that evolves and diversifies like any other morphological or chemical trait.

Data Availability

The datasets generated during and/or analysed in the current study are available in the [Open Science Framework] repository, [https://osf.io/sd4q9/?view_only=5e9563caee50457d851d16efd7b1440d].

References

1. Silvertown, J. & Gordon, D. M. A Framework for plant behavior. *Annu. Rev. Ecol. Syst.* **20**, 349–366 (1989).
2. Karban, R. Plant behaviour and communication. *Ecol.Lett.* **11**, 727–739 (2008).
3. Metlen, K. L., Aschehoug, E. T. & Callaway, R. M. Plant behavioural ecology: dynamic plasticity in secondary metabolites. *Plant Cell Environ.* **32**, 641–653 (2009).
4. Trewawas, A. Plant Intelligence: An overview. *BioScience* **66**, 542–551 (2016).
5. Gagliano, M., Vyazovskiy, V. V., Borbély, A. A., Grimonprez, M. & Depczynski, M. Learning by Association in Plants. *Sci. Rep.* **6**, 38427 (2016).
6. Baluška, F. & Mancuso, S. Vision in plants via plant-specific ocelli? *Trends in. Plant Sci.* **21**, 727–730 (2016).
7. Mancuso, S. & Baluška, F. Plant ocelli for visually guided plant behavior. *Trends in Plant Sci.* **22**, 5–6 (2016).
8. Calvo Garzón, P. & Keijzer, F. Plants: Adaptive behavior, root brains, and minimal cognition. *Adapt. Behav.* **19**, 155–171 (2011).
9. Zink, A. G. & He, Z. Botanical brilliance. Are plants decision-makers or elaborate fakers? *Science* **347**, 724–725 (2015).
10. del Hoyo, J. & Collar, N. *Illustrated checklist of the birds of the World. Vol 1 Non-Passerines*. (Lynx Editions, 2014).
11. Willink, B., Brenes-Mora, E., Bolaños, F. & Pröhl, H. Not everything is black and white. Color and behavioral variation reveal a continuum between cryptic and aposematic strategies in a polymorphic poison frog. *Evolution* **67**, 2783–2794 (2013).
12. Salmon, M. & Atsaides, S. P. Behavioral, morphological and ecological evidence for two new species of fiddler crabs (Genus *Uca*) from the Gulf Coast of the United States. *Proc. Biol. Soc. Wash.* **81**, 275–289 (1968).
13. Salmon, M., Ferris, S. D., Johnston, D., Hyatt, G. & Whitt, C. S. Behavioral and biochemical evidence of species distinctiveness in the fiddler crabs, *Uca speciosa* and *U. spinicarpa*. *Evolution* **33**, 182–191 (1979).
14. Salmon, M. & Kettler, M. The importance of behavioral and biochemical differences between fiddler crab taxa, with special reference to *Uca rapax* (Smith) and *U. virens* (Salmon and Atsaides). *Contrib. Mar. Sci.* **30**, 63–76 (1987).
15. Knowlton, N. & Keller, B. D. A new, sibling species of snapping shrimp associated with the Caribbean sea anemone *Bartholomea annulata*. *Bull. Mar. Sci.* **33**, 353–362 (1983).
16. Knowlton, N. & Keller, B. D. Two more sibling species of alpheid shrimps associated with the Caribbean sea anemones *Bartholomea annulata* and *Heteractis lucida*. *Bull. Mar. Sci.* **37**, 893–904 (1985).
17. Darwin, C. *The movements and habits of climbing plants*. (John Murray, 1875).
18. Braam, J. In touch: plant responses to mecanical stimuli. *New Phytol.* **165**, 373–389 (2005).
19. Sibaoka, T. Physiology of the rapid movements of higher plants. *Annu. Rev. Plant Physiol.* **20**, 165–184 (1969).
20. Weintraub, M. Leaf movements in *Mimosa pudica* L. *New Phytol.* **50**, 357–382 (1952).
21. Satter, R. L., Applewhite, P. B., Kreis, D. J. & Galston, A. W. Rhythmic leaflet movement in *Albizzia julibrissin*. *Plant Physiol.* **52**, 202–207 (1973).
22. Kölreuter, J. G. *Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen* (Gleditsch, 1761).
23. Smith, J. E. Some observations on the irretability of vegetables. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **78**, 158–165 (1788).
24. Sprengel, C. K. *Das entdeckte Geheimnis der Natur im Bau und in der Befruchtung der Blumen* (Vieweg, 1793).
25. Bianchini, M. & Pacini, E. Explosive anther dehiscense in *Ricinus communis* L. involves cell wall modifications amd relative humidity. *Int. J. Plant Sci.* **157**, 737–745 (1996).
26. Bawa, K. S. & Crisp, J. E. Wind-pollination in the understorey of a rainforest in Costa Rica. *J. Ecol.* **68**, 871–876 (1980).
27. Simons, P. *The Action Plant. Movements and Nervous Behaviour in Plants* (Blackwell, 1992).
28. Romero, G. A. & Nelson, C. E. Sexual dimorphism in *Catasetum* orchids: forcible pollen emplacement and male flower competition. *Science* **232**, 1538–1540 (1986).
29. Edwards, J., Whitaker, D., Klionsky, S. & Laskowski, M. J. A record breaking catapult. *Nature* **435**, 164 (2005).
30. Ren, M.-X. Stamen movements in hermaphroditic flowers: diversity and adaptive significance. *Chin. J. Plant Ecol.* **34**, 867–875 (2010).
31. LeBuhn, G. & Anderson, G. J. Anther tripping and pollen dispensing in *Berberis thunbergii*. *Am. Midl. Nat.* **131**, 257–265 (1994).
32. Ren, M. X. & Tang, J. Y. Up and down: stamen movements in *Ruta graveolens* (Rutaceae) enhance both outcrossing and delayed selfing. *Ann. Bot.* **110**, 1017–1025 (2012).
33. Kabsch, W. Anatomische und physiologische Beobachtungen über die Reizbarkeit der Geschlechtsorgane. *Bot. Zeitung* **19**, 25–40 (1861).
34. Unger, F. Einige Bemerkungen über die Bewegungserscheinungen an den Staubfäden der Centaurien. *Bot. Zeitung* **21**, 349–353 (1863).
35. Juel, H. O. Einige Beobachtungen an reizbaren Staubfäden. In: Botanisker Studier (ed. Kjellmann, F. R.) (Almqvist & Wiksell, 1906).
36. Bünning, E. Die seismonastischen Reaktionen. In: *Handbuch der Pflanzenphysiologie XVII* (ed. Ruhland, W.): 184–238 (Springer, 1959).
37. Guttenberg, H. *Bewegungsgewebe und Perzeptionsorgane. Handbuch der Pflanzenanatomie, Bd. 5, Teil 5* (Bornträger, 1971).
38. Jaffe, M. J., Gibson, C. & Biro, R. Physiological studies of the mechanically stimulated motor responses of flower parts. I: Characterization of the thigmotropic stamens of *Portulaca grandiflora* Hook. *Bot. Gaz.* **138**, 438–447 (1977).
39. Findley, G. P. & Findlay, N. Anatomy and Movement of the column in *Stylidium*. *Aust. J. Plant Physiol.* **2**, 597–621 (1975).
40. Grant, V., Grant, K. A. & Hurd, P. D. Pollination of *Opuntia lindheimeri* and related species. *Plant Syst. Evol.* **132**, 313–320 (1979).
41. Schlindwein, C. & Wittmann, D. Stamen movements in flowers of *Opuntia* (Cactaceae) favour oligolectic pollinators. *Plant Syst. Evol.* **204**, 179–193 (1997).
42. Cota-Sánchez, J. H., Almeida, O. J. G., Falconer, D. J., Choi, H. J. & Bevan, L. Intriguing thigmonastic (sensitive) stamens in the Plains Prickly Pear *Opuntia polyacantha* (Cactaceae). *Flora* **208**, 381–389 (2013).
43. Ren, M. X. & Bu, Z. J. Is there 'anther-anther interference' within a flower? Evidences from one-by-one stamen movement in an insect-pollinated plant. *PLoS One* **9**(1), e86581 (2014).
44. Schlindwein, C. & Wittmann, D. Micro-Foraging routes of *Bicolletes pampeana* (Colletidae) and bee-induced pollen presentation in *Caiophora arechavaletae*. *Bot. Acta* **110**, 177–183 (1997).

45. Weigend, M., Ackermann, M. & Henning, T. Reloading the revolver–male fitness as a simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Loasaceae, Cornales). *Biol. J. Linn. Soc.* **100**, 124–131 (2010).
46. Henning, T. & Weigend, M. Total Control — Pollen Presentation and Floral Longevity in Loasaceae (Blazing Star Family) are Modulated by Light, Temperature and Pollinator Visitation Rates. *PloS One* **7**, e41121 (2012).
47. Henning, T. & Weigend, M. Beautiful, complicated–and intelligent? Novel aspects of the thigmonastic stamend movement in Loasaceae. *Plant Signal.Behav.* **8**, e24605 (2013).
48. Pecival, M. S. The presentation of pollen in certain angiosperms and its collection by *Apis mellifera*. *New Phytol.* **54**, 353–368 (1955).
49. Thomson, J. D., Wilson, P., Valenzuela, M. & Malzone, M. Pollen presentation and pollination syndromes, with special reference to *Penstemon*. *Plant Species Biol.* **43**, 657–661 (2000).
50. LeBuhn, G. & Holsinger, K. A sensitive analyses of pollen dispensing schedules. *Evol. Ecol.* **12**, 111–121 (1998).
51. Harder, L. D. & Wilson, W. G. Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plants. *Evol. Ecol.* **8**, 542–559 (1994).
52. Wittmann, D. & Schlindwein, C. Mellitophilous plants, their pollen and flower visiting bees in southern Brazil. *1. Loasaceae. Biociéncias* **3**, 19–34 (1995).
53. Weigend, M. *Nasa* and the conquest of South America. PhD. *Dissertation, Ludwig-Maximilians-University*, (Munich, 1997).
54. Weigend, M., Gottschling, M., Hoot, S. & Ackermann, M. A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on trnL (UAA) sequence data, with consequences for systematics and historical biogeography. *Org. Divers. Evol.* **4**, 73–90 (2004).
55. Hufford, L., McMahon, M., O'Quinn, R. & Poston, M. A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. *Int. J. Plant Sci.* **166**, 289–300 (2005).
56. Acuña, R. *et al.* Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae). *Taxon* **66**, 365–378 (2017).
57. Henning, T., Rodriguez, E. & Weigend, M. A revision of the Nasa ranunculifolia group (Nasa ser. Grandiflorae pro parte, Loasaceae). *Bot. J. Linn. Soc.* **167**, 47–93 (2011).
58. Weigend, M. Four new species of *Nasa* ser. *Alatae* (Loasaceae) in the Amotape-Huancabamba Zone of Peru. *Novon* **14**, 134–146 (2004).
59. Weigend, M. & Rodríguez, E. Las especies arbustivas de *Nasa* ser. *Grandiflorae* en el Norte de Perú, con la descripción de una especie nueva de la Abra de Barro Negro (Callacalla), Dpto. Amazonas. *Arnaldoa* **9**, 7–20 (2002).
60. Weigend, M. & Rodríguez, E. A revision of the *Nasa stuebeliana* group [*Nasa* ser. *Saccatae* (Urb. & Gilg) Weigend, Loasaceae] with notes on morphology, ecology, and distribution. *Bot. Jahrb. Syst.* **124**, 345–382 (2003).
61. Carlquist, S. Wood anatomy of Loasaceae with relation to systematics, habit, and ecology. *Aliso* **10**, 583–602 (1984).
62. Weigend, M., Aizetmüller, K. & Bruehl, L. The seeds of Loasaceae subfam. Loasoideae (Cornales) I: seed release, seed number and fatty acid composition. *Flora* **199**, 424–436 (2004).
63. Weigend, M., Gröger, A. & Ackermann, M. The seeds of Loasaceae subfam. *Loasoideae (Cornales) II: Seed morphology of "South Andean Loasas" (Loasa, Caiophora, Scyphanthus and Blumenbachia) Flora* **200**, 569–591 (2005).
64. Weigend, M., Henning, T. & Schneider, C. A revision of *Nasa* ser. *Carunculatae* (Loasaceae subfam. *Loasoideae). Syst. Bot.* **28**, 765–781 (2003).
65. Henning, T. & Weigend, M. Two novel and critically endangered subspecies of *Nasa humboldtiana* (Loasaceae) from Peru. *Bot. Jahrb. Syst.* **127**, 473–488 (2009).
66. Henning, T. & Weigend, M. Systematics of the *Nasa poissoniana* group (Loasaceae) from Andean South America. *Bot. J. Linn. Soc.* **161**, 278–301 (2009).
67. Urban, I. Die Bestäubungseinrichtungen der Loasaceen. *Jahrb. Königl. Bot. Gart. Berlin* **4**, 364–388 (1886).
68. Urban, I. Blüten- und Fruchtbau der Loasaceen. *Ber. Deutsch. Bot. Ges.* **10**, 259–265 (1892).
69. Brown, D. K. & Kaul, R. B. Floral structure and mechanisms in Loasaceae. *Am.J. Bot.* **68**, 361–372 (1981).
70. Weigend, M. & Gottschling, M. Evolution of funnel-revolver flowers and ornithophily in *Nasa* (Loasaceae). *Plant Biol.* **8**, 120–142 (2006).
71. Weigend, M. Loasaceae in *The Families and Genera of Vascular Plants 6* (ed. Kubitzki, K.) 239–254 (Springer, 2003).
72. Lloyd, D. G. & Webb, C. J. The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. *New Zeal. J. Bot.* **24**, 135–162 (1986).
73. Monshausen, G. B. & Haswell, E. S. A force of nature: molecular mechanisms of mechanoperception in plants. *J. Exp. Bot.* **64**, 4663–4680 (2013).
74. Telewski, F. W. A unified hypothesis of mechanoperception in plants. *Am. J. Bot.* **93**, 1466–1476 (2006).
75. Schlindwein, C. & Wittmann, D. Specialized pollinators of *Cajophora arechavaletae* (Loasaceae) induced stamen movements and time next visit to delayed pollen presentation *in Congreso Latino-americano de Zoologia 12, e Congresso Brasileiro de Zoologia, 1992, Belém. Resumos*, 73–74. Sociedade Brasileira de Zoologia (1992).
76. Harter, B., Schlindwein, C. & Wittmann, D. Bienen und Kolibris als Bestäuber von Blüten der Gattung *Cajophora* (Loasaceae). *Apidologie* **26**, 356–357 (1995).
77. Cocucci, A. A. & Sérsic, A. N. Evidence of rodent pollination in *Cajophora coronata* (Loasaceae). *Plant Syst. Evol.* **211**, 113–128 (1998).
78. Ackermann, M. & Weigend, M. Nectar, Floral morphology and pollination syndrome in Loasaceae subfam. Loasoideae (Cornales). *Ann. Bot.* **98**, 503–514 (2006).
79. Wood, S. N. Stable and efficient multiple smoothing parameter estimation for generalized additive models. *J. Am. Stat. Assoc.* **99**, 673–686 (2004).
80. R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. : https://www.R-project.org/ (2017).
81. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Stat. Methodol.* **57**, 289–300 (1995).
82. Jombart, T. & Dray, S. Adephylo: exploratory analyses for the phylogenetic comparative method. *Bioinformatics* **26**, 1907–1909 (2016).
83. Doyle, J. J. & Doyle, J. L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull. Bot. Soc. Am.* **19**, 11–15 (1987).
84. Kearse, M. *et al.* Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).
85. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
86. Müller, J., Müller, K., Nienhuis, C. & Quandt, D. PhyDe: Phylogenetic Data Editor. Available from: http://www.phyde.de/ (2010).
87. Posada, D. & Crandall, K. Selecting the best-fit model of nucleotide substitution. *Syst. Biol.* **50**, 580–601 (2001).
88. Felsenstein, J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376 (1981).
89. Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313 (2014).

90. Silvestro, D. & Michalak, I. RAxMLGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* **12**, 335–337 (2012).
91. Mau, B., Newton, M. & Larget, B. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* **55**, 1–12 (1999).
92. Huelsenbeck, P. & Ronquist, F. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**, 754–755 (2001).
93. Miller, M., Pfeiffer, W. & Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees in *Proceedings of the Gateway Computing Environments Workshop (GCE)* 1–8 (New Orleans, 2010).
94. Rambaut, A. & Drummond, A. Tracer v1.4. Available at: http://beast.bio.ed.ac.uk/Tracer (2007).
95. Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15 (1985).
96. Swenson, N. G. Comparative Methods and Phylogenetic Signal in *Functional and Phylogenetic Ecology in R* (ed. Swenson, N. G.) 147–171 (Springer, 2014).
97. Pagel, M. Inferring evolutionary processes from phylogenies. *Zool. Scri.* **26**, 331–348 (1997).
98. Blomberg, S. P., Garland, T. & Ives, A. R. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717–745 (2003).
99. Smith, S. A. & Donoghue, M. J. Rates of molecular evolution are linked to life history in flowering plants. *Science* **322**, 86–89 (2008).
100. Lanfear *et al*. Taller plants have lower rates of molecular evolution. *Nat. Commun.* **4**, 1879 (2013).
101. Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).
102. Revell, L. J. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).
103. Leite, A. V., Nadia, T. & Machado, I. C. Pollination of *Aosa rupestris* (Hook.) Weigend (Loasaceae): are stamen movements induced by pollinators? *Braz. J.Bot.* **39**, 559–567 (2016).
104. Castellanos, M. C., Wilson, P., Keller, S. J., Wolfe, A. D. & Thomson, J. D. Anther evolution: pollen presentation strategies when pollinators differ. *Am. Nat.* **167**, 288–296 (2006).
105. Janzen, D. H. Reproductive Behavior in the Passifloraceae and some of its pollinators in Central America. *Behavior* **32**, 33–48 (1968).
106. Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. Pollination syndromes and floral specialization. *Ann. Rev. Ecol. Evol. Syst.* **35**, 375–403 (2004).
107. Strelin, M., Arroyo, J., Fließwasser, S. & Ackermann, M. Diversification of *Caiophora* (Loasaceae subfam. Loasoideae) during the uplift of the Central Andes. *Org. Divers. Evol.* **17**, 29–41 (2016).
108. Whittall, J. B. & Hodges, S. A. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. *Nature* **447**, 706–709 (2007).
109. Alcantara, S. & Lohmann, L. G. Contrasting phylogenetic signals and evolutionary rates in floral traits of Neotropical lianas. *Biol. J. Linn. Soc.* **102**, 378–390 (2011).
110. Rheindt, F. E., Grafe, T. U. & Abouheif, E. Rapidly evolving traits and the comparative method: how important is testing for phylogenetic signal? *Evol. Ecol. Res.* **6**, 377–396 (2004).
111. Trewawas, A. Aspects of Plant Intelligence. *Ann. Bot.* **92**, 1–20 (2003).
112. Urban, I. & Gilg, W. Monographia Loasacearum. *Nova Acta Acad. Caes. Leop. Car. Nat. Cur.* **76**, 1–368 (1900).
113. Mutke, J., Jacobs, R., Meyers, K., Henning, T. & Weigend, M. Diversity patterns of selected Andean plant groups correspond to topography and habitat dynamics, not orogeny. *Front. Genet.* **5**(10), 3389 (2014).
114. Gervasi, D. L. & Schiestl, F. P. Real-time divergent evolution in plants driven by pollinators. *Nature Commun.* **8**, 14691 (2017).
115. Thompson, H. J. & Lewis, H. Chromosome numbers in *Mentzelia* (Loasaceae). *Madroño* **13**, 102–107 (1955).
116. Thompson, H. J. Cytotaxonomic observations on *Mentzelia*, Sect. *Bartonia (Loasaceae). Madroño* **17**, 16–22 (1963).
117. Poston, M. E. & Thompson, H. J. Cytotaxonomic observations in Loasaceae subfamily Loasoideae. *Syst. Bot.* **2**, 28–35 (1977).
118. Grau, J. Chromosomenzahlen chilenischer Loasaceae. *Mitt. Bot. Staatssamml. München* **27**, 7–14 (1988).
119. Castellanos, M. C., Wilson, P. & Thomson, J. D. Dynamic nectar replenishment in flowers of *Penstemon* (Scrophulariaceae). *Am. J. Bot.* **89**, 111–118 (2002).
120. Ordano, M. & Ornelas, J. F. Generous-like flowers: nectar production in two epiphytic bromeliads and a meta-analysis of removal effects. *Oecologia* **140**, 495–505.
121. Luo, E. Y., Olgivie, J. E. & Thomson, J. D. (2014) Stimulation of flower nectar replenishment by removal: a survey of eleven animal-pollinated plant species. *J. Pollinat. Ecol.* **12**, 52–62 (2004).
122. Salas-Arcos, L., Lara, C. & Ornelas, J. F. Reproductive biology and nectar secretion dynamics of *Penstemon gentianoides* (Plantaginaceae): a perennial herb with a mixed pollination system? *PeerJ* **5**, e3636 (2017).

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Author Contributions

T.H. and M.W. designed the study, collected plant material, established the cultivation and co-wrote the main body of the manuscript. T.H. designed and conducted/supervised the greenhouse/field experiments, generated the data, organized the data transfer and led the writing process. M.M. planned and executed the statistical analysis using GAMMs and prepared the respective graphs and supplements. S.A.I. prepared and conducted the statistical analysis (phylogenetic effects), developed the respective R-code and prepared the tables. R.A.C. generated and provided the molecular data used in all statistical approaches, prepared a tree and complemented the voucher data. All authors participated in completing the manuscript with a special emphasis on those parts regarding their respective focal area.

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CHAPTER 9

General Conclusions

9.1.The systematics of Loasaceae subfam. Loasoideae

9.1.1.Relationships between the currently accepted genera of Loasaceae subfam. Loasoideae

The results presented in this thesis (Chapters 2, 5 and 6) solved, with high statistical, support most of the remaining questions regarding the delimitations and relationships of the genera of Loasaceae subfam. Loasoideae. There is agreement between the nuclear and plastid marker evidence at the genus, and in many cases also at the infrageneric, level (Chapters 2 and 5). Likewise, morphological traits could be used to clearly define most of the larger clades. The most important conclusions derived from research questions 1 and 2 (see section 1.5.1 in Introduction) can be summarized as follows.

The results of Chapters 2, 5 and 6 show that Loasaceae subfam. Loasoideae contains 14 genera, 11 of which are retrieved consistently as monophyletic with high statistical support (*Xylopodia* is monotypic, and only one species of *Plakothira* was sampled. *Huidobria* could remain as the only potentially paraphyletic genus). The best supported trees retrieve *Huidobria fruticosa* as sister to the rest of the subfamily. It differs from all others members of the subfamily in its scabrid trichomes with long, thin, upturned barbs (Ensikat *et al.*, 2017), in the construction of its nectar scales and its striate, fulvous seeds (Urban & Gilg, 1900; Grau, 1997).

The rest of Loasoideae is then divided in two clades. The first clade is moderately supported and widespread (Tropical America, Africa, Oceania), but species poor (nine species) and does not have obvious autoapomorphies. Within this clade, *Huidobria chilensis* is sister to *Kissenia* and Klaprothieae (which are sister to each other). *Huidobria chilensis* is the only loasoid that has linear leaves; its narrowly-acuminate, long (> 1x as long as the petals) calyx lobes are also distinctive. *Kissenia* has very elongated calyx lobes (about 2x as long as the petals), ligulate nectar scales and an indehiscent fruit with only 1-3 seeds unique in Loasoideae (Chapter 4). Within Klaprothieae, *Xylopodia* is retrieved as sister to *Klaprothia* and *Plakothira*. Klaprothieae is well delimited morphologically, especially in regards to its tetramerous flowers (Weigend 2004b, Weigend *et al.*, 2004a), however the current evidence seems to indicate that, if Klaprothieae (and Kissenieae) is considered as a valid tribe, Loaseae is rendered paraphyletic.

The second clade has very high support (Chapters 2, 5, 6 and 7) and is considerably more species rich (ca. 200 species), including all the remaining genera. It was informally called "Core Loaseae" in Chapter 6. This clade appears to be characterized by the presence of stinging trichomes (absent in the rest of the subfamily, but present elsewhere in Loasaceae), nectar scales formed by three completely fused staminodes (putative convergence with *Kissenia*) without a ligule, and thigmonastic stamens (Chapter 8, although apparently lost independently several times). *Nasa* is retrieved as sister to the rest of "Core Loaseae", the monobracteate flowers and nectar scales with well-developed apical wings and nectar sacks are characteristic of this genus (Weigend, 1997a, 2006).

The remaining clade is formed by *Aosa*, *Presliophytum* and the South Andean Loasas (SAL: *Blumenbachia*, *Caiophora*, *Loasa* and *Scyphanthus*). The results in Chapters 2 and 5 notably clarify the evolution and systematics of this group. This group probably had the least satisfactory, and often conflicting, inferred relationships in Loasoideae (Weigend *et al.*, 2004a; Hufford *et al.*, 2005), with most genera still requiring their limits and alliances to be evaluated in depth. This clade seems to lack obvious autoapomorphies, however each genus can be easily characterized. The best trees retrieve *Aosa* as sister to *Presliophytum* + SAL. *Aosa* has mostly ebracteate flowers (not in every species: Chapter 5), and inhabits seasonally dry to mesic low elevation habitats. Weigend (1997a, 2006) had originally defined *Aosa* as including 3 series, corresponding to *Loasa* ser. *Corymbosae*, *Loasa* ser. *Parviflorae* and *Loasa* ser. *Pusillae,* of Urban and Gilg (1900). This has been confirmed with high statistical support for the first time (Chapters 2 and 5). *Chichicaste grandis* was shown to be nested with high support in *Aosa* ser. *Parviflorae* (Chapter 5), however this species does not seem to share most autoapomorphies with its closest extant relatives.

Presliophytum has extensive con- and recaulescence of the bracts (Chapter 3) and is restricted to desert areas. *Loasa sessiliflora* Phil. and *Loasa malesherbioides* Phil., restricted to northern Chile and western Argentina (Chapters 2 and 3), were found to be part of the same clade, as successive sister groups of the Peruvian species, previously included in the genus. These two species share, only with each other, transversally-grooved seeds, while still having conspicuous con-and recaulescent bracts like their congeners.

The major clades retrived in SAL are well supported by the results shown in chapter 2. The systematic placement of *Blumenbachia* sects. *Angulatae* and *Gripidea* had been contentious since Urban & Gilg (in Gilg, 1894) transferred them to *Caiophora,* a decision followed by most taxonomic treatments that dealt with these groups since (Urban & Gilg, 1900; Reiche, 1901; Sleumer, 1955; Santos & Fromm-Trinta, 1985; Crespo & Pérez-Moreau, 1988; Pacheco, 2002; Bovini & Giordano, 2005), however the molecular evidence presented in this thesis is clear in showing, for the first time unequivocally, that these two sections are more closely related to *Blumenbachia* than to *Caiophora* or any other clade (Chapter 2), agreeing strongly with morphological evidence (particularly petal, nectar scale and fruit characters: Weigend, 1997a; Weigend *et al*., 2004a).

According to the results obtained, in order to render *Loasa* as a monophyletic entity (while maintaining long standing *Blumenbachia*, *Caiophora* and *Scyphanthus*), *Loasa* sers. *Acaules* + *Volubiles* and *Loasa* ser. *Pinnatae* (all of which were found to be closer to *Caiophora* than to *Loasa*) were segregated. *Loasa* should then include only four of the 15 series defined by Urban & Gilg (1900): *Loasa* sers. *Deserticolae*, *Floribundae*, *Loasa* and *Macrospermae* (Chapter 2). This genus remains quite variable morphologically and has few obvious autoapomorphies, the most evident being the presence a subterminal hilum or hilar scar in the seed (Weigend, Gröger, & Ackermann, 2005). *Loasa* sers. *Acaules* was found nested in *Loasa* ser. *Volubiles* with this clade as sister to *Loasa* ser. *Pinnatae, Scyphanthus* and *Caiophora*, while *Loasa* ser. *Pinnatae* is sister to the latter two genera. The unusual *Caiophora pulchella* was retrieved as belonging to *Caiophora* (Appendix B), not to *Loasa* as it was suggested by some authors (Pérez-Moreau & Crespo, 1992; Slanis, Perea, & Grau, 2016).

9.1.2.Taxonomic changes in Loasaceae subfam Loasoideae

Molecular studies (Moody *et al.*, 2001; Weigend *et al.*, 2004a; Chapters 2, 5 and 6) have confirmed for the most part, the morphology-based genus level taxonomy established by Weigend (1997a, 2004b, 2006). However, some entities (notably *Loasa*) remained as artificial and correspondingly, taxonomy required adjustments to reflect more accurately their relationships. At the same time the small genera *Presliophytum* and *Kissenia* (Chapters 3 and 4) were identified as requiring modern taxonomic revisions. These are thus, the main conclusions from research questions 3 and 4.

Monotypic *Chichicaste* has been formally synonymized under *Aosa* (Chapter 5). The new combination *Aosa grandis* (Standl.) R.H.Acuña & Weigend was formally published.

The new combinations *Presliophytum malesherbioides* (Phil.) R.H.Acuña & Weigend and *Presliophytum sessiliflorum* (Phil.) R.H.Acuña & Weigend were established. These changes have been included in the latest Catalogue of Vascular plants of Chile (Rodríguez *et al.*, 2018). The first is the only herbaceous species of the genus, it has subentire leaves, linear sepals, contrastingly colored nectar scales and 10-100 seeds per capsule. The second is a subshrub with regularly lobate leaves, ovate sepals, nectar scales of the same color as the corolla and only four seeds per capsule. The remaining three taxa are all shrubs with hundreds of tiny seeds per capsule. *Presliophytum heucheraefolium* has reniform leaves and sepals shorter than the petals; *Presliophytum arequipense* has ovate leaves and small flowers with sepals about as long as the petals; *Presliophytum incanum* has ovate leaves with sepals shorter than the petals (Chapters 2 and 3).

The new genera *Grausa* (five species) and *Pinnasa* (four species) were described, including the species of *Loasa* sers. *Acaules* + *Volubiles* and *Loasa* ser. *Pinnatae* respectively (Chapter 2). Several new combinations were provided. The first is a small genus with usually ternate leaf laminas (Urban & Gilg, 1900; Chapter 2) and seeds often with a conspicuous hilar cone (Weigend *et al.*, 2005). The second genus is characterized by its deeply pinnatifid to pinnate leaves and laciniate petals (Urban & Gilg, 1900). These taxonomic changes have been as well included in the latest Catalogue of Vascular plants of Chile (Rodríguez *et al.*, 2018).

After more than 170 years, the taxonomic standings of *Kissenia* R.Br. *ex* Endl. and *Cnidone* E.Mey. *ex* Fenzl have been clarified (Chapter 4) with a formal proposal for the conservation of the former name against the latter (Appendix C, to be submitted to Taxon). Likewise the correct name for the northeast African –southwest Arabian species should be *Kissenia spathulata* R.Br. ex. Dandy instead of *Kissenia arabica* Arn. *ex* Chiov. Corresponding species names were lectotypified (Chapter 4). Both *Kissenia* species are very similar morphologically with *Kissenia spathulata* differing from *Kissenia capensis* in its usually wider leaf laminas, and irregularly shaped, thread-like, often deeply divided, nectar scale ligula (as opposed to the lamelliform, entire, rarely shallowly cleft ligula of the latter).

9.1.3.Systematic relationships and morphology in *Nasa*

Unlike the highly supported subclades of *Loasa* and *Blumenbachia* that correspond very precisely to series and sections of Urban & Gilg's classification system (Gilg, 1894; Urban & Gilg, 1900; Chapter 2), the series of *Nasa*, except ser. *Grandiflorae* in the strict sense, have been repeatedly retrieved as artificial (Chapter 7), this is in agreement with previous research (Hufford *et al.*, 2003; Weigend *et al.*, 2004a; Weigend & Gottschling, 2006). The following are the main conclusions to research questions 6 and 7.

The relationships between the major clades of *Nasa* had low support in previous works (Weigend *et al.*, 2004a; Weigend & Gottschling 2006). In the present contribution, most of the phylogenetic relationships were obtained with moderate to high support (Chapter 7) especially regarding the four major clades. Clade I is retrieved as sister to the rest of the genus. It is restricted to the Central Andes and is the only major clade absent from the Amotape-Huancabamba Zone (AHZ). Most species in this clade correspond to the *Nasa poissoniana* species group (Henning & Weigend, 2009) and are annual herbs, with alternate leaves (except *Nasa solaria*), fully spread to reflexed, white to yellow petals and nectar scales with short, erect apical wings, found in seasonally dry Andean scrub and scree (as well as in the Lomas). It is parsimonious to infer these traits as plesiomorphic in *Nasa* (Weigend & Gottschling, 2006; Henning & Weigend, 2009) considering that the species that share most of these, are retrieved as early branching taxa in each of the four major clades. There are however two deviations from this ‘Bauplan’ in Clade I: 1- *Nasa carunculatae*, retrieved as sister to the rest of the clade, is a coarse shrub and 2- *Nasa limata* and *Nasa pascoensis* (sister to the *Nasa poissoniana* group) have campanulate corollas, concave, red petals, and nectar scales with semierect to horizontal apical wings. These two species also inhabit forest edges or undergrowth.

Clade II is sister to Clades III and IV, and is distributed in the Northern and Central Andes as well as in AHZ, where it reaches its highest diversity. Most members in this clade correspond to ser. *Alatae* (Weigend, 2000a,b, 2001, 2004d) and have opposite leaves, campanulate corollas, concave, orange to red (more rarely yellow or pink) petals, nectar scales with semierect to horizontal apical wings and inhabit forest understory or edges. The basal grade of this group, *Nasa ramirezii* and *Nasa herzogii*, differs mostly in the spread, white petals and nectar scales with short, erect apical wings. These two species inhabit forest habitats and have opposite leaves like most other species of Clade II. The well supported *Nasa laxa* group appears to be nested amongst *Alatae* morphology clades, and has floral traits similar to the basal grade, but unlike them, these species have alternate leaves and inhabit mainly seasonal Andean scrub and scree (Weigend, Henning, & Schneider, 2003; Henning, Cano, & Weigend, 2009).

Clade III is sister to clade IV and is mainly found in AHZ and the Northern Andes. Most of this clade corresponds to the morphologically well-defined and monophyletic *Nasa triphylla* group, having *Nasa venezuelensis* and *Nasa picta* as successive sister groups. Although sharing most traits with *Nasa poissoniana* group (see above), they differ in the trifoliate to pinnate leaves, shortly clavate to ovoid capsules and, frequently, presence of t-shaped trichomes (symmetrical or not) and filiform petal apices (Dostert & Weigend, 1999). The leaf morphology of the florally very different *Nasa venezuelensis*, suggests a close relationship to the *Nasa triphylla* group (Weigend, 2001).

Most of the species of Clade IV belong to the monophyletic ser. *Grandiflorae,* a clade of biennial to perennial plants, from forest edge, subparamo and puna habitats with usually dense-glandular indumentum, palmatilobate to palmatisect leaves, campanulate corollas, red or orange (more rarely yellow) petals and nectar scales with long, erect apical wings (Weigend 2000a, 2001; Henning, Rodríguez, & Weigend, 2011). Related to this series is the *Nasa stuebeliana* group, which do not have many obvious autoapomorphies. In most traits they are similar to *Nasa poissoniana* group, however the presence of amplexicaul bracts and/or pseudostipules and irregularly undulate seed testas is mostly restricted to this clade. *Nasa weigendii* and *Nasa poissoniana* subsp. *glandulifera*, are also retrieved as part of Clade IV but their closest relatives within the clade are uncertain. These two taxa are morphologically almost indistinguishable from the *Nasa poissoniana* group.

It is evident that the bulk of each major clade is composed by morphologically homogeneous groups (*Nasa poissoniana* group in clade I, *Nasa* ser. *Alatae* in clade II, *Nasa triphylla* group in clade III, *Nasa* ser. *Grandiflora* and *Nasa stuebeliana* group in clade IV). Three groups can however be considered as morphologically discordant within the clade they were retrieved: 1- *Nasa pascoensis* and *Nasa limata* in Clade I (from the Central Andes), 2- the *Nasa laxa* group in Clade II (AHZ) and 3- *Nasa weigendii* and *Nasa poissoniana* subsp. *glandulifera* in clade IV (just entering the southeastern most apart of the AHZ). Their retrieved position in the trees could be result of hybridization between sympatric taxa of different clades. Alternatively, (or additionally) unexpected events of horizontal gene transfer or organelle capture (Rieseberg & Soltis, 1991; Mahelka *et al.*, 2017) cannot be discarded as another potential reason beneath these surprising placements. Hybridization and/or horizontal gene transfer between sympatric species could explain at least in part, why there appears to be strong geographic signal in *Nasa* as is the case for other Andean groups, particularly regarding plastid marker phylogenies (Diazgranados & Barber, 2017; Morales-Briones *et al.*, 2018; Pouchon *et al.*, 2018). It is noteworthy that although hybridization apparently takes place infrequently amongst living taxa in *Nasa* (M. Weigend, pers comm) this does not mean this has always been the case. During early cladogenesis events in *Nasa,* something analogous to the situation of *Caiophora* nowadays, in which hybridization is frequent (Sleumer, 1955; Ackermann & Weigend, 2007; Ackermann, Achatz, & Weigend, 2008; Slanis *et al.*, 2016), could have occurred.

9.2. Historical Biogeography of Loasaceae subfam. Loasoideae with emphasis on *Nasa* and the Tropical Andes

Although the study of the Biogeography of Loasoideae is not new (Dandy, 1926; Weigend, 1997a), it is just now that the phylogenetic results, alpha taxonomy and distribution data of the taxa have reached a resolution that allows the use of complex quantitative biogeography tools on the subfamily. Loasoideae is widely distributed in the Neotropics and temperate South America, it has a high proportion of narrow endemics and thus is an adequate model to study speciation on a continental scale, but particularly in the hyperdiverse Andean region. The main conclusions to research questions 5 and 7 are the following.

It is likely that the most recent common ancestor of Loasoideae was already established in South America by the Early Eocene, ca. 50 Ma, probably deriving from North American-Mexican ancestors (Weigend 1997a; Schenk *et al.*, 2017). Most of the genus level lineages of the subfamily diverged between ca. 53 and 37 Ma (Chapter 6) during the Eocene, long time before the main Andean uplift pulses (Gregory-Wodzicki, 2000; Graham, 2009). However, the divergence of the deserticolous clades (*Huidobria*, *Presliophytum*) in this time frame suggests the persistence of arid environments in western South America for tens of millions of years, like Hartley *et al.* (2005) propose. Other elements of the desert flora such as *Heliotropium* L. sect *Cochranea* or *Nolana* L.f. diversified much more recently (Dillon *et al.*, 2009; Luebert, Hilger, & Weigend, 2011), closer in time to the inferred establishment of hyperarid conditions in the Atacama Desert.

Nasa and its sister clade appear to have been allopatric for most of their history, with *Nasa* restricted to tropical Andes and its sister clade mainly to Eastern and Southern South America. The fossil record of southern South America is better known than that of other parts of the continent (Wilf *et al.*, 2013). Patagonia was covered by dense forests until the end of the Eocene, when these were replaced by more open habitats (Dunn *et al.*, 2015). These changes could have allowed the SAL to establish in this area. The divergence events in the major genus-level clades of SAL preceded major orogenic events of the Andes (22-8 Ma Giambiagi *et al.*, 2016; Gianni *et al.*, 2016). On the other hand, the retrieved age of many of the crown nodes of mostly Andean clades, like *Blumenbachia* sect. *Angulatae*, *Caiophora* and *Pinnasa* (ca. 16-6 Ma), overlap considerably with Andean uplift. These pulses also caused the establishment of a latitudinal high elevation corridor in the last 10 Ma, facilitating the northward expansion of clades of probable austral origin, including *Caiophora* (Chapter 6), *Azorella* Lam. (Nicolas & Plunkett, 2014) and *Juania* Drude - *Ceroxylon* Bonpl. (Sanín *et al.*, 2016).

In the case of *Nasa*, initial diversification of the crown group may have taken place as far back as ca. 30 Ma (Oligocene) in seasonally dry habitats, at moderate elevations (Chapter 7). In these initial phases and until about 15 Ma (Middle Miocene) the diversification of the genus was apparently gradual, and mostly happening in Clade I. Särkinen *et al.* (2012) compared seasonally dry habitats in the tropical Andes to ecologically stable islands with isolated and evolutionarily persistent biotas, as suggested by Fabaceae molecular dating. This agrees with Clade I, as it has a long story, with ancient divergence events. By 15 Ma the subduction of the Nazca Ridge may have triggered significant changes in the relief of the Central Andes (Margirier *et al.*, 2015; Calvès *et al.*, 2018). This may have in turn increased the topographic (Antonelli *et al.*, 2009) and climatic (Poulsen, Ehlers, & Insel, 2010) complexity of the Andes. By that time, the remaining three major Clades of *Nasa* had already diverged, with their divergence intervals overlapping with those of species-rich, mid-elevation, mostly-Andean groups like *Fuchsia* L. sect. *Fuchsia*, Gesneriaceae tribe Episcieae and *Vasconcellea* A.St.-Hil. (Berry *et al.*, 2004; Carvalho & Renner, 2012; Perret *et al.*, 2013).

By ca. 13 Ma (Late Miocene) the west Andean Portal closed and probably facilitated dispersal of *Nasa* northwards, as Rubiaceae tribe Cinchoneae (Antonelli *et al.*, 2009) and *Vasconcellea* (Carvalho & Renner, 2012) did southwards. The northern Andes can be considered a "sink" of lineages, because although important radiations took place in clades III and IV, the dispersal of these lineages outside the Northern Andes, appears to have been limited. By 10-8 Ma *Nasa* may have started to colonize lower elevations and by ca. 7-5 Ma, it dispersed into Central America and colonized High Andean habitats (which were probably unavailable previously). High Andean groups such as *Diplostephium* Kunth (Vargas, Ortiz, & Simpson, 2017), *Hypericum* L. (Nürk, Scheriau, & Madriñán, 2013) and *Lachemilla* (Focke) Rydb (Morales-Briones *et al.*, 2018) also radiated considerably since 5 Ma. At the same time, although the definitive closure of the Central American Seaway is still debated (Montes *et al.*, 2015), biological evidence suggests that movement of the South American Biota northwards may have started as far back as 23 Ma (Bacon *et al.*, 2015).

The heterogeneity of the landscape and frequent events of isolation and connection of the different habitats in South America during the late Neogene were proposed by Weigend (1997a) as important drivers of secondary speciation in Loasaceae. This appears to be the case in the AHZ were different species substitute their relatives in the same habitats over short distances (*Nasa stuebeliana* and *Nasa triphylla* groups). The biogeographic results presented in Chapter 7 show that the AHZ acts as a cradle of recently diverged lineages for *Nasa* but also as a museum of ancient lineages for all major clades, except Clade I. Although it is possible that the same would apply to other groups of angiosperms like *Macrocarpaea* (Griseb.) Gilg (Struwe *et al.*, 2009), *Deprea* Raf. (Deanna, Barboza, & Carrizo-García, 2018) and *Fuchsia* (Berry *et al.*, 2004), previous biogeographical analyses lacked either dated reconstructions or quantitative biogeography analyses.

9.3.Mating behavior in Loasaceae subfam. Loasoideae in a phylogenetic context

Plant behavior is an emerging scientific discipline, and although processes related to it have been studied for a long time (Darwin & Darwin, 1898), it is recently that it is starting to receive considerable attention (Silvertown & Gordon, 1989). Due to its importance in the survival of populations and species in time, plant reproductive behavior must have important evolutionary implications yet to be determined (Chapter 8). The following are the main conclusions of research question 8.

Thigmonasty seems to be widely distributed in Loasoideae, at least in the “Core Loaseae”. It has been argued that this mechanism increases the male fitness of a plant (Weigend, Ackermann, & Henning, 2010) as it allows a more efficient partition of the pollen delivery across time. The results in Chapter 8 show that important differences in the thigmonasty patterns exist, even among closely related species (e.g. *Aosa parviflora* and *Presliophytum heucheraefolium* have thigmonastic stamens, even though *Aosa rupestris* and *Presliophytum* *incanum* do not) and that the effect of shared ancestry on the flower response is quite weak in Loasoideae.

Species sharing similar pollinators on the other hand, were detected as more similar to each other than expected based on phylogenetic relatedness. In the case of species pollinated by short tongued bees the response of the stamens is cyclical, probably reflecting the foraging strategy of this pollinator-guild. Many bees tend to have systematic foraging patterns in both the timing and the routes followed while visiting a flower patch (Janzen, 1971; Siriani-Oliveira, Oliveira, & Schlindwein, 2018) and in some cases may even prefer previously visited flowers as long as they proved rewarding or, alternatively, to deny freshly produced resources to competitors (Corbet *et al.*, 1984). Species pollinated by guilds with less flower fidelity or frequence of visits (large bees and hummingbirds) do not behave the same way and often show a decrease in the stamen movement response after repeated stimulation.

From an evolutionary perspective due the high proportion of microendemic species in the family (Weigend 2000a,b, 2001, 2004d; Henning & Weigend 2011), population survival would presumably rely on very effective pollination mechanisms in order to produce enough descendants. The capacity of the thigmonastic strategy to be adjusted rapidly to different pollinator-guild behaviors may even allow the effective pollinators to get "sorted" by thigmonastic strategies and in time, act as an additional reproductive barrier between populations.

9.4.Topics for future systematic research in Loasaceae subfam. Loasoideae

The research conducted in the last four years on the phylogenetics and systematics of Loasaceae subfam. Loasoideae has not only lead to uncover very interesting patterns in the phylogeny, biogeography, evolution and ecology of the subfamily but also to the identification of areas that may merit the attention for further research.

To fully clarify the generic relationships in the subfamily, two areas remain contentious: First is the polytomy involving the deepest two nodes of the SAL and second is the poor resolution of the deepest nodes of Loasaceae subfam. Loasoideae. According to the dating results shown in Chapter 6, both the deep nodes of crown Loasoideae and crown SAL seem to have experienced quick divergence events. Deciphering the topologies of nodes arising due to fast diversification events remains a major challenge in evolutionary biology, with consequences in the interpretation of relationships as well as character evolution and biogeography (Whitfield & Lockhart, 2007). Considering the satisfactory (although still not complete) taxon sampling density in both cases, testing an increased molecular sequence sampling may be an appropriate solution to tackle this persistent issue.

At the infrageneric level, the two largest genera of Loasoideae, *Caiophora* and *Nasa* may need to be revisited with new phylogenetic tools. The support of the published phylogenies of *Caiophora*, especially at the backbone level, is not satisfactory, (Chapter 2). Frequent hybridization is reported in the field (Ackermann, et al., 2008) and it is plausible that phylogenetic reconstructions may better be represented in a reticulate pattern.

Due to their biparental inheritance, nuclear markers can offer insights whether introgression (or other kind of hybridization) has played a role in the evolution of a group (Rothfels, Pryer, & Li, 2017). Identifying novel, single-copy nuclear markers in *Nasa* would be an important step to avoid the complications of the phylogenetic interpretation of multi-copy genes, but this process is often time consuming due to the 'trial and error' process in the early phases (cf. Appendix A). PacBio sequencing (Rhoads & Au, 2015) could offer an alternative and probably more time-efficient solution, as even multi-copy genes (as it is suspected could be the case for ITS in *Nasa*) are sequenced one molecule at a time, with high accuracy reads, which then can be sorted out using bioinformatics to find the homologue copies (Rothfels *et al.*, 2017). For both *Caiophora* and *Nasa,* sampling from several populations of the same taxon would be recommended, in order to detect variability in the genetic signal, identify possible non-monophyletic taxa and cryptic speciation. The comparisons between plastid and nuclear data to find evidence of hybridization, horizontal gene transfer or plastid capture, could be done by applying procrustean approaches to cophylogeny estimation, coalescent based estimations and phylogenetic networks as it was done for Andean Catasetiinae (Orchidaceae) and *Lachemilla* by Pérez-Escobar, Balbuena, & Gottschling (2016) and Morales-Briones *et al.* (2018), groups in which reticulate speciation has been shown to be common.

Morphological studies on *Nasa* in a phylogenetic context may reveal ecological or phylogenetic patterns that have not been identified before. Lower stem/underground structures (basal leaf rosettes, stolons, rhizomes) and branching patterns remain understudied (except maybe in the *Nasa poissoniana* and *Nasa ranunculifolia* and species groups: Henning & Weigend 2009; Henning et al., 2011) and impossible to study in most herbarium specimens. The morphological diversity in the seeds of *Nasa* is much more modest than in SAL (Weigend, 1997a; Weigend *et al.*, 2005), and although species representing most major clades have been studied it in detail (M. Weigend unpl. data), data on relatively few species has been published (Weigend, 1997a; Weigend *et al.*, 2003; Weigend & Rodriguez, 2003). These morphological patterns and the phylogenetic interpretations remain challenging.

Something that became apparent when examining herbarium material for the biogeographic studies, is that most genera of the SAL may be in need of modern taxonomic revisions: e.g. the last time this was done for *Blumenbachia* sect. *Angulatae*, *Grausa*, *Loasa*, *Pinnasa* and *Scyphanthus* was by Urban & Gilg (1900), and for Peruvian *Caiophora* by Macbride (1941), and these sources are currently insufficient to deal with the wealth of material now available to researchers. The Loasaceae catalogues of the Southern Cone (Weigend et al., 2008) and Bolivia (Weigend & Ackermann, 2015), regional revisions of Loasaceae for Argentina (Crespo & Pérez Moreau, 1988; Pérez-Moreau & Crespo, 2003), and relevant works on *Caiophora* (Weigend, 1997b; Weigend & Ackermann, 2003; Ackermann & Weigend, 2007, 2013) offer valuable insights and solve many questions, but the taxonomic status of many taxa still remains obscure. Field studies and thorough examination of type specimens from Argentinean and Chilean herbaria (in particular those of species described by R.A. Philippi in SGO) are needed to understand the species limits in these clades.

Finally, the results of the phylogenetic and systematic studies presented in this thesis, would not only concern taxonomists or biogeographers but also ecologists and authorities involved in conservation policies. As Posadas, Esquivel, & Crisci (2001) state "Conservation of biodiversity requires knowledge of its history". Although species richness and complementarity of sites (i.e. sites with very different yet rich biotas) are some of the most used metrics to consider the establishment protected areas (Rodrigues & Gaston, 2002), the incorporation of phylogenetic diversity metrics has also proved to be valuable (Posadas *et al.*, 2001; Pio *et al.*, 2011). Due to limited investment of public resources in protected areas in Latin America (World Bank, 2012), the establishment of new areas and protection of the existing ones should be carefully planned. The extensive wild fires in the AHZ in the last years (Mutke *et al.*, 2017) represent a dramatic example of the threats that protected areas in Latin America face due to scarcity of resources. The wild fires have caused dramatic losses in the cloud forest belt and affected 11 protected areas (NASA Earth Observatory, 2017). The AHZ not only harbors a high diversity and endemism of Loasaceae, the subject of this thesis, but also of Asteraceae, Calceolariaceae, Campanulaceae, Onagraceae, Orchidaceae, Passifloraceae and many other groups (Weigend, 2002a, 2004a; Schulenberg *et al.,* 2007). Phylogenetic diversity metrics in Loasaceae subfam. Loasoideae and other Andean groups could be used as proxies and offer valuable information for the identification of priority areas still in need of formal protection, or that require it to be strengthened. This applies both in the Andes and elsewhere in Latin America.

SUMMARY

Acuña-Castillo, Rafael. 2018. Phylogeny, Biogeography and Systematics of Loasaceae subfam. Loasoideae. Doctoral thesis, Mathematisch-Naturwissenschaftliche Fakultät, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany.

Loasaceae subfam. Loasoideae is a mostly South American group of angiosperms that reaches its highest diversity in the Andean mountain ranges but also extends into Central America, Africa and Oceania. Most species of this clade are herbaceous but other growth forms, like shrubs or vines, are also common. Most neotropical habitats from sea level to ca. 5000 m a.s.l. are inhabited by species of Loasoideae, however these are mostly absent from low elevation tropical forests. Although some molecular studies dealing with the relationships of this clade have been published in the last 20 years, none has provided satisfactorily resolved phylogenetic reconstructions for the whole subfamily, with several clades requiring their limits to be unequivocally established. At the same time no quantitative historical biogeography studies have been attempted for the entirety of the subfamily.

The major aims of this thesis are to clarify the phylogenetic relationships of the genera of Loasaceae subfam. Loasoideae, including extensive taxonomic sampling covering every major clade in the subfamily, with special emphasis on *Nasa* and the South Andean Loasas clade. Based on the phylogenetic results, to address the historical biogeography of *Nasa* and Loasaceae subfam. Loasoideae, and to identify and solve the major discrepancies between the currently accepted taxonomy and the molecular phylogenetic results.

The South Andean Loasas are a clade comprised by the genera *Blumenbachia*, *Caiophora*, *Loasa* and *Scyphanthus* and mostly restricted to southern South America. The results presented in Chapter 2, based on plastid marker (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) and ITS sequences, confirm: 1- that *Loasa* ser. *Malesherbioideae* could be included in *Presliophytum*, 2- that sects. *Angulatae* and *Gripidea* are more closely related to *Blumenbachia* than to *Caiophora*, 3- that *Loasa* has to be redefined to include only sers. *Deserticolae*, *Floribundae*, *Loasa* and *Macrospermae,* and 4- that *Loasa* sers. *Volubiles* + *Acaules* and *L.* ser. *Pinnatae* are phylogentically closer to *Caiophora* and *Scyphanthus* than to *Loasa*. To eliminate paraphyletic genera concepts, the most conservative solution taxonomically, is to describe two new genera (*Grausa* and *Pinnasa*). These newly required names and some of the new combinations are formalized here.

Presliophytum is a small genus of five species endemic to arid western South America. Until recently, the genus comprised only three well-defined species, but subsequent molecular studies confirmed a close relationship to two Chilean species, traditionally placed in *Loasa*. A thourough revision and description of *Presliophytum* had not been published before and the

present study aims at filling this gap. Data on morphology, distribution and ecology of the five species are provided, as well as a key for the now newly defined *Presliophytum* (Chapter 3).

Kissenia is the sole African genus of the mostly American family Loasaceae. The nomenclature of this clade has had an unexpectedly convolute history that still needs to be resolved. In this work a full account on the status of every name applied to *Kissenia* and its constituent species is provided, including the appropriate lectotypifications and correct usage of the names. Our results show that the correct names for the species of the genus should be *Kissenia capensis* and *K. spathulata.* Detailed descriptions of the species as well as data on morphology, distribution and ecology of both species are provided (Chapter 4).

Relationships of the highly distinctive genus *Chichicaste* have been doubtful in the absence of critical morphological analyses (particularly of living plants) and convincing molecular data. The present study resolves the phylogenetic relationships of *C. grandis* using plastid marker (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) and ITS sequences, as well as morphological data. *Chichicaste* is clearly placed in *Aosa* ser. *Parviflorae*. A critical morphological re-examination indicates that considering *C. grandis* as part of *Aosa* is plausible. Based on these results the genus *Chichicaste* is synonymized with *Aosa* and an amended key for an expanded *Aosa,* is also presented (Chapter 5).

Loasaceae subfam. Loasoideae with its wide distribution and numerous narrow endemics is an interesting subject for quantitative historical biogeography analyses. Plastid markers (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) sequences from ca. 70% of the species of Loasaceae subfam. Loasoideae, were used to generate chronograms and conduct dispersal extinction cladogenesis analyses, to reconstruct the historical biogeography of the subfamily as shown in Chapter 6. Loasoideae diverged from its sister group in the Late Cretaceous-early Paleocene (83–62 Ma). Most extant genera-level clades, diverged from their sister groups by the Eocene, preceding the Andean orogenic events. Divergence within extant lineages appears to have happened more or less parallel to Andean uplift pulses. For crown Loasoideae, we retrieved different area combinations as the most probable ancestral areas, but the tropical Andes and Pacific deserts were obtained most frequently. Although particularly rich in taxa, southern South America appears to have been colonized relatively late by members of the Loasoideae, while eastern South America could have played a more important role in the early evolutionary history of the group than expected based on its low extant diversity. Long distance dispersal appears to have been infrequent in Loasoideae and involved in the distribution of the small clades *Kissenia, Plakothira* and *Aosa.*

The genus *Nasa* is the largest genus in Loasaceae, however, previous phylogenetic studies were unsatisfactory in resolving the internal relationships in the group. Plastid marker (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) sequences from ca. 70% of the species and subspecies of

Nasa, and morphological matrices including 26 traits for all the species of the genus, were used to produce phylogenetic reconstructions, conduct historical biogeography analyses and ancestral character estimations. The results in Chapter 7 show four well supported clades retrieved in the molecular phylogenetic reconstructions. The majority of the species of each clade share similar morphologies, hence, inferences on the phylogenetic position of most species based on morphology is possible. However, hybridization or horizontal gene transfer and directional selection may be responsible for the lack of agreement between morphology and phylogenetic placement in some species like *Nasa limata*, the *Nasa laxa* group and *Nasa weigendii*. *Nasa* diverged from its sister group ca. 49 Ma but its crown node was dated to 29 Ma, the most probable ancestral areas for the genus appear to have been the Amotape Huancabamba Zone and the Central Andes, probably at mid-elevations and in seasonally dry Andean scrub and scree. Divergence of the four main clades took place between ca. 29-9 Ma. For Clades II, III and IV, the Amotape Huancabamba Zone has been a crucial ancestral area for many of the internal nodes. Dispersal into the Northern Andes took place mostly (or entirely) in the last 15 Ma. By 10-8 Ma *Nasa* may have started to colonize lower elevations and by ca. 7-5 Ma, it dispersed into Central America and colonized High Andean habitats.

The study of plant behavior in an evolutionary framework has rarely been attempted. Using a large experimental dataset on the patterns of stamen movement in Loasaceae subfam. Loasoideae, as well as data on pollinators (from both, own field observations and literature) and phylogenetic reconstructions based on plastid marker (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) sequences, the work presented in Chapter 8 shows that the phylogenetic effect on the thigmonastic patterns is minor compared to the effect of the main pollinator-guilds to which the taxa are adapted. This appears to indicate that thigmonastic pollen presentation in Loasoideae could be a crucial component and another dimension in the complex plant-pollinator interactions, along with other seemingly phylogenetically-labile traits like color, scent, reward quantity and quality. Adaptive variability in the thigmonastic movement patterns potentially acts as a pre-mating barrier between individual populations.

ZUSAMMENFASSUNG

Acuña-Castillo, Rafael. 2018. Phylogenie, Biogeographie und Systematik von Loasaceae subfam. Loasoideae. Doktorarbeit, Mathematisch-Naturwissenschaftliche Fakultät, Rheinische Friedrich-Wilhelms-Universität Bonn, Deutschland.

Die Unterfamilie Loasoideae aus der Familie der Loasaceae ist eine Gruppe innerhalb der Angiospermen, die neben einigen wenigen Vertretern in Mittelamerika, Afrika und Ozeanien ihre größte Vielfalt in den südamerikanischen Anden erreicht. Die meisten Vertreter dieser Unterfamilie sind krautig Pflanzen, es finden sind aber auch Arten deren Wuchsform als strauchig oder kletternd zu charakterisieren ist. Die Arten der Loasoideae haben die meisten neotropischen Lebensräume auf Meereshöhe bis auf Höhen von ca. 5000 m ü. NN erobert, in tropischen Wälder des Tieflandes fehlen sie jedoch meist. Obwohl in den letzten 20 Jahren einige molekulargenetische Studien zu den Verwandtschaftsbeziehungen dieser Gruppe publiziert wurden, war keine dieser Arbeiten in der Lage die Phylogenie der gesamten Unterfamilie zufriedenstellend zu rekonstruieren, obschon einige Gruppen eindeutig umgrenzt werden konnten. Auch quantitative Studien zur historischen Biogeographie für die gesamte Unterfamilie wurden bisher nicht durchgeführt.

Die Hauptziele dieser Arbeit sind, mit Hilfe eines extensiven taxonomischen Samplings unter Einbeziehung aller relevanten Gruppen der Unterfamilie (mit besonderem Schwerpunkt auf der artenreichen Gattung *Nasa* und der südandinen *Loasa*-Gruppe), die phylogenetischen Beziehungen der Gattungen der Loasaceae subfam. Loasoideae zu klären. Basierend auf den resultierenden phylogenetischen Ergebnissen, soll die historische Biogeographie von *Nasa* und Loasaceae subfam. Loasoideae analysiert werden. Anschließend soll die weitreichende Diskrepanzen zwischen den molekulargenetischen Informationen und der aktuellen Taxonomie identifiziert und abschließend bearbeitet werden.

Der hauptsächlich auf das südliche Südamerika beschränkte Verwandtschaftsbereich der südandinen Loasas bildet eine Gruppe, welche die Gattungen *Blumenbachia*, *Caiophora*, *Loasa* und *Scyphanthus* umfasst. Die Ergebnisse in Kapitel 2, basierend auf Plastidenmarker- (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) sowie Kerngenomsequenzen (ITS), bestätigen dass: 1. *Loasa* ser. *Malesherbioideae* zu *Presliophytum* gehört, 2. die Sektionen *Angulatae* und *Gripidea* enger mit *Blumenbachia* verwandt sind als mit *Caiophora*, 3. die Gattung *Loasa* neu definiert werden muss und nur Sers. *Deserticolae*, *Floribundae*, *Loasa* und *Macrospermae* umfasst, und 4. *Loasa* sers. *Volubiles* + *Acaules* sowie *L.* ser. *Pinnatae* phylogenetisch *Caiophora* und *Scyphanthus* näher stehen als *Loasa*. Zur Beseitigung paraphyletischer Gattungsbegriffe ist die taxonomisch sparsamste Lösung die Beschreibung zwei neuer Gattungen (*Grausa* und *Pinnasa*). Diese neuen Namen und einige der neuen Kombinationen sind bereits formalisiert.

Presliophytum ist eine kleine Gattung von fünf endemischen Arten im trockenen westlichen Südamerika die bis zum Jahr 2000 drei Arten umfasste. Molekularphylogenetische Studien bestätigten jedoch eine enge Beziehung zu zwei chilenischen Arten, die traditionell *Loasa* zugerechnet wurden. Eine detaillierte Überarbeitung und Beschreibung von *Presliophytum* war bisher nicht veröffentlicht worden und die vorliegende Studie zielt darauf ab, diese Lücke zu schließen. Daten zur Morphologie, Verbreitung und Ökologie der fünf Arten sowie ein Schlüssel für alle Arten werden präsentiert (Kapitel 3).

Die einzige afrikanische Gattung der ansonsten überwiegend amerikanischen Loasaceae ist *Kissenia*. Die Nomenklatur dieser Gruppe hat eine unerwartet verworrene Geschichte vorzuweisen, die noch gelöst werden muss. In dieser Arbeit wird ein vollständiger Bericht über den Status aller sich auf *Kissenia* beziehenden taxonomischen Namen gegeben, einschließlich neuer Lectotypifizierungen sowie der korrekten Verwendung dieser Namen. Unsere Ergebnisse zeigen, dass die korrekten Namen für die Arten in dieser Gattung *Kissenia capensis* und *K. spathulata* sein sollten. Dieser Teil der Arbeit umfasst detaillierte Beschreibungen aller Arten, sowie Daten zur Morphologie, Verbreitung und Ökologie (Kapitel 4).

In Ermangelung kritischer morphologischer Analysen (insbesondere lebender Pflanzen) und überzeugender molekularer Daten waren die verwandtschaftlichen Beziehungen der hochdifferenzierten Gattung *Chichicaste* bislang zweifelhaft. Die vorliegende Studie klärt die phylogenetischen Beziehungen von *C. grandis* mit Hilfe von Plastidenmarker- (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) und Kerngenomsequenzen (ITS) sowie morphologischen Daten. *Chichicaste* ist in *Aosa* ser. *Parviflorae* eingebettet. Eine kritische morphologische Überprüfung zeigt, dass *C. grandis* als Teil von *Aosa* plausibel ist. Basierend auf diesen Ergebnissen wird die Gattung *Chichicaste* mit *Aosa* synonymisiert. Ein geänderter Schlüssel für eine erweiterte Gattung *Aosa* wird ebenfalls vorgestellt (Kapitel 5).

Die Loasaceae subfam. Loasoideae mit ihrer weiten Verbreitung und zahlreichen eng endemischen Arten ist ein interessantes Thema für quantitative historisch-biogeographische Analysen. Plastidenmarkersequenzen (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) von ca. 70% der Arten der Loasaceae subfam. Loasoideae wurden für die Datierung der Gruppe verwendet darüber hinaus wurde eine dispersal-extinction-cladogenesis Analysen durchzuführen, um die historische Biogeographie der Unterfamilie, wie in Kapitel 6 gezeigt, zu rekonstruieren. Die Loasoideae divergierten von ihrer Schwestergruppe in der späten Kreidezeit bis zum frühen Paläozän (83-62 Ma). Die meisten existierenden Gruppen auf Gattungsniveau zweigten von den Schwestergruppen bereits im Eozän und damit vor der

Andenorogenese ab. Die Abweichung innerhalb der rezenten Linien scheint mehr oder weniger parallel zu den Schüben der Andenauffaltung stattgefunden zu haben. Für die Gruppe Loasoideae, haben die Analysen verschiedene Gebietskombinationen als wahrscheinlichste Ahnengebiete ergeben, wobei die tropischen Anden und pazifischen Wüsten die höchste Unterstützung erhalten. Obwohl das südliche Südamerika besonders artenreich ist, scheint es relativ spät von Vertretern der Loasoideae besiedelt worden zu sein. Hingegen scheint das östliche Südamerika eine wichtigere Rolle in der frühen Geschichte der Gruppe gespielt zu haben, was aufgrund der geringen Artenvielfalt nicht zu erwartet gewesen wäre. Fernausbreitung scheint in den Loasoideae nur selten stattgefunden zu haben und ist lediglich für die Verbreitung der kleinen Gattungen *Kissenia*, *Plakothira* und *Aosa* anzunehmen.

Die Gattung *Nasa* ist die größte Gattung in den Loasaceae. Frühere phylogenetische Studien haben jedoch nur unbefriedigend Einblicke in die verwandtschaftlichen Beziehungen zwischen den Arten der Gruppe gewährt. Plastidenmarkersequenzen (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16) von ca. 70% der Arten und Unterarten von *Nasa* sowie eine morphologische Matrix, basierend auf 26 Merkmalen für alle Arten der Gattung, wurden verwendet, um eine phylogenetische Rekonstruktionen zu erstellen, eine historische Biogeographieanalyse sowie eine Abschätzung der ursprünglichen Merkmalszustände durchzuführen. Die Ergebnisse in Kapitel 7 zeigen vier gut gestützte Kladen. Die Mehrheit der Arten innerhalb jeder dieser Gruppen weisen morphologische Ähnlichkeiten auf, so dass Rückschlüsse auf die phylogenetische Position der meisten Arten basierend auf der Morphologie möglich sind. Hybridisierung oder horizontaler Gentransfer sowie direktionale Selektion könnten für die fehlende Übereinstimmung zwischen Morphologie und phylogenetischer Plazierung bei einigen Taxa wie *Nasa limata*, *Nasa laxa* und *Nasa weigendii* verantwortlich sein. *Nasa* spaltete sich von seiner Schwestergruppe vor ca. 49 Ma ab, aber sein Kronenknoten wurde auf 29 Ma datiert. Die wahrscheinlichsten Ursprungsgebiete der Gattung scheinen die Amotape-Huancabamba-Zone und die Zentralanden gewesen zu sein, wahrscheinlich in mittleren Höhenlagen, in saisonal trockenem Anden-Gebüsch und Geröllhalden. Die Auftrennung der vier Hauptgruppen fand zwischen ca. 29-9 Ma statt. Insbesondere für die Abstammungslinien der Kladen II, III und IV war die Amotape-Huancabamba-Zone ein entscheidendes Ursprungsgebiet. Die Ausbreitung in die nördlichen Anden fand größtenteils (oder vollständig) in den letzten 15 Ma statt. Vor 10-8 Ma begann *Nasa* niedrigere Lagen zu kolonisieren, vor etwa 7-5 Ma, breitete sich die Gattung in Mittelamerika aus und eroberte hochandiene Lebensräume.

Das Studium von Pflanzenverhalten in einem evolutionären Kontext wurde nur selten untersucht. Mit einem großen experimentellen Datensatz zu den Charakteristika der Staubblattbewegung zahlreicher Arten der Loasaceae subfam. Loasoideae, die mit Bestäuberdaten (sowohl aus Feld als auch Literatur) und phylogenetischen Rekonstruktionen, basierend auf Plastidenmarkersequenzen (*trn*L-*trn*F, *mat*K, *trn*S-*trn*G and *rps*16), in Beziehung gesetzt wurden, zeigt die in Kapitel 8 vorgestellte Arbeit die Evolutionsmuster der Thigmonastie innerhalb der Unterfamilie. Dabei zeigte sich, dass die individuellen Muster der Thigmonastie weniger unter dem Einfluss der Phylogenie variieren als bspw. durch das jeweilige Bestäubungssyndrom und die häufig enge Beziehung zu einzelnen Bestäubergruppen. Dies scheint darauf hinzuweisen, dass die thigmonastische Pollenpräsentation in den Loasoideae eine entscheidende Komponente darstellt, die zusammen mit anderen, phylogenetisch ähnlichen, wenig aussagekräftigen Eigenschaften wie Blütenfarbe und -duft, sowie Nektarmenge und -qualität höchst komplexe Pflanzen-Bestäuber-Interaktionen orchestriert. Die Thigmonastie als potentiell sehr schnell veränderbare Komponente könnte für Artbildungseffekte durch eine Bestäubungsbarriere zwischen Populationen von Bedeutung sein.

LITERATURE

Ackermann M, Achatz M & Weigend M. **2008**. Hybridization and crossability in *Caiophora* (Loasaceae subfam. Loasoideae): Are interfertile species and inbred populations results of a recent radiation? *American Journal of Botany* **95**: 1109–1121.

Ackermann M & Weigend M. **2007**. Notes on the genus *Caiophora* (Loasoideae, Loasaceae) in Chile and neighbouring countries. *Darwiniana* **45**: 45–67.

Ackermann M & Weigend M. **2013**. A revision of loasoid *Caiophora* (*Caiophora pterosperma*-group, Loasoideae, Loasaceae) from Peru. *Phytotaxa* **110**: 17.

Acuña R, Chinchilla IF & Weigend M. **2018**. An unusual disjunction in Loasaceae: Central American *Chichicaste grandis* is nested in Brazilian *Aosa*. *Phytotaxa* **365**: 273–287.

Acuña R, Fließwasser S, Ackermann M, Henning T, Luebert F & Weigend M.2017. Phylogenetic relationships and generic re-arrangements in 'South Andean Loasas' (Loasaceae). *Taxon* **66**: 365–378.

Acuña R & Weigend M. 2017. A revision of the western South American genus *Presliophytum* (Loasaceae). *Phytotaxa* **329**: 51–68.

Adanson M. 1763. *Familles des plantes I. Partie.* Paris: Chez Vincent.

Albert JS & Reis RE. 2011. *Historical Biogeography of Neotropical Freshwater Fishes*. Berkeley: University of California Press.

Antonelli A, Nylander JA, Persson C & Sanmartín I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences* **106**: 9749–9754.

APG. 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* **85**: 531–553.

APGIV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181**: 1–20.

Armijo R, Lacassin R, Coudurier-Curveur A & Carrizo D. 2015. Coupled tectonic evolution of Andean orogeny and global climate. *Earth-Science Reviews* **143**: 1–35.

Auboin JA, Borrello AV, Cecione G, Charrier R, Chotin P, Frutos J, Thiele R & Vicente JC. 1973. Esquisse paleogeographique et structurale des Andes meridionales. *Revue de géographie physique et de géologie dynamique* **15**: 11–71.

Bacon CD, Silvestro D, Jaramillo C, Smith BT, Chakrabarty P & Antonelli A. 2015. Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences* **112**: 6110–6115.

Bacon CD, Velásquez-Puentes F, Hinojosa LF, Schwartz T, Oxelman B, Pfeil B, Kalin-Arroyo MT, Wanntorp, L & Antonelli A. 2018. Evolutionary persistence in *Gunnera* and the contribution of southern plant groups to the tropical Andes biodiversity hotspot. *PeerJ* **6**: e4388.

Barnes JB & Ehlers TA. **2009**. End member models for Andean Plateau uplift. *Earth-Science Reviews* **97**: 105–132.

Bar-On YM, Phillips R & Milo R. **2018**. The biomass distribution on Earth. *Proceedings of the National Academy of Sciences* **115**: 6506–6511.

Barthlott W. **1981**. Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nordic Journal of Botany* **1**: 345–355.

Barthlott W, Hostert A, Kier G, Küper W, Kreft H, Mutke J, Rafiqpoor MD & Sommer JH. 2007. Geographic patterns of vascular plant diversity at continental to global scales. *Erdkunde* **61**: 305–315.

Barthlott W, Mail M, Bhushan B & Koch K. 2017. Plant Surfaces: Structures and Functions for Biomimetic Innovations. *Nano-Micro Letters* **9**: 23.

Barthlott W, Rafiqpoor D, Kier G & Kreft H. 2005. Global Centers of Vascular Plant Diversity. *Nova Acta Leopoldina NF92* **342**: 61–83.

Behnke HD & Barthlott W. **1983**. New evidence from the ultrastructural and micromorphological fields in angiosperm classification. *Nordic Journal of Botany* **3**: 43–66.

Bermúdez MA, Hoorn C, Bernet M, Carrillo E, van der Beek PA, Garver JI, Mora JL & Mehrkian K. 2017. The detrital record of late-Miocene to Pliocene surface uplift and exhumation of the Venezuelan Andes in the Maracaibo and Barinas foreland basins. *Basin Research* **29**: 370–395.

Bermúdez MA, Kohn BP, van der Beek PA, Bernet M, O'Sullivan PB & Shagam R. 2010. Spatial and temporal patterns of exhumation across the Venezuelan Andes: Implications for Cenozoic Caribbean geodynamics. *Tectonics* **29**: TC5009.

Bernal R, Gradstein SR & Celis M. 2015. *Catálogo de plantas y líquenes de Colombia.* Bogotá: Instituto de Ciencias Naturales, Universidad Nacional de Colombia. Available from: http://catalogoplantasdecolombia.unal.edu.co

Berry PE. **1982**. The Systematics and Evolution of *Fuchsia* Sect. *Fuchsia* (Onagraceae). *Annals of the Missouri Botanical Garden* **69**: 1–198.

Berry PE, Hahn WJ, Sytsma KJ, Hall JC & Mast A. 2004. Phylogenetic relationships and biogeography of *Fuchsia* (Onagraceae) based on noncoding nuclear and chloroplast DNA data. *American Journal of Botany* **91**: 601–614.

Bonpland A, Humboldt A & Kunth CS. 1823. *Nova genera et species plantarum*. Paris: Gide Filium.

Borsdorf A & Stadel C. **2015**. *The Andes*. New York, Berlin, Heidelberg: Springer.

Bovini MG & Giordano LC da S. **2005**. Loasaceae Lindl. in the State of Rio de Janeiro, Brazil. *Acta Botanica Brasilica* **19**: 265–271.

Brako L & Zarucchi JL. 1993. *Catalogue of the Flowering Plants and Gymnosperms of Peru. Monographs in Systematic Botany from the Missouri Botanical Garden Vol. 45*. Missouri: Missouri Botanical Garden Press.

Brokaw JM & Hufford L. 2010a. Phylogeny, introgression, and character evolution of diploid species in *Mentzelia* section *Trachyphytum* (Loasaceae). *Systematic botany* **35**: 601–617.

Brokaw JM & Hufford L. **2010b**. Origins and introgression of polyploid species in *Mentzelia* section *Trachyphytum* (Loasaceae). *American Journal of Botany* **97**: 1457–1473.

Brooks TM, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Rylands AB, Konstant WR, Flick P, Pilgrim J, Oldfield S, Magin G & Hilton-Taylor C. 2002. Habitat Loss and Extinction in the Hotspots of Biodiversity. *Conservation Biology* **16**: 909–923.

Brown DK & Kaul RB. **1981**. Floral Structure and Mechanism in Loasaceae. *American Journal of Botany* **68**: 361–372.

Burman J. 1756. *Plantarum americanarum Fasciculus secundus.* Amsterdam and Leiden: Viduam & Filium S. Schouten and Gerard Potvliet & Theodor Haak.

Calderón E, Galeano G & García N. 2002. *Libro rojo de las plantas fanerógamas de Colombia, Volumen 1: Chrysobalanaceae, Dichapetalaceae y Lecythidaceae*. Bogotá: Instituto Humboldt y Universidad Nacional de Colombia.

Calderón E, Galeano G & García N. 2005. *Libro rojo de plantas de Colombia, Volumen 2: Palmas, Frailejones y Zamias.* Bogotá: Instituto Humboldt y Universidad Nacional de Colombia.

Calvès G, Calderón Y, Hurtado Enriquez C, Brusset S, Santini W & Baby P. 2018. Mass Balance of Cenozoic Andes-Amazon Source to Sink System—Marañón Basin, Peru. *Geosciences* **8**: 167.

Carlquist S. **1984**. Wood anatomy of Loasaceae with relation to systematics, habit, and ecology. *Aliso: A Journal of Systematic and Evolutionary Botany* **10**: 583–602.

Carlquist S. **1987**. Wood anatomy of *Plakothira* (Loasaceae). *Aliso: A Journal of Systematic and Evolutionary Botany* **11**: 563–569.

Carlquist S. **1992**. Wood anatomy of sympetalous dicotyledon families: a summary, with comments on systematic relationships and evolution of the woody habit. *Annals of the Missouri Botanical Garden* **79**: 303–332.

Carvalho FA & Renner SS. **2012**. A dated phylogeny of the papaya family (Caricaceae) reveals the crop's closest relatives and the family's biogeographic history. *Molecular Phylogenetics and Evolution* **65**: 46–53.

Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM & Palmer TM. 2015. Accelerated modern human-induced species losses: Entering the sixth mass extinction. *Science Advances* **1**: e1400253.

Chacón J, de Assis MC, Meerow AW & Renner SS. 2012. From East Gondwana to Central America: historical biogeography of the Alstroemeriaceae. *Journal of Biogeography* **39**: 1806–1818.

Chen LY, Zhao SY, Mao KS, Les DH, Wang QF & Moody MF. 2014. Historical biogeography of Haloragaceae: An out-of-Australia hypothesis with multiple intercontinental dispersals. *Molecular Phylogenetics and Evolution* **78**: 87–95.

Corbet SA, Kerslake CJC, Brown D & Morland NE. 1984. Can Bees Select Nectar-Rich Flowers in a Patch? *Journal of Apicultural Research* **23**: 234–242.

Crespo S & Pérez-Moreau RL. 1988. Loasaceae. In: Correa MN, ed. *Flora Patagónica. Vol. 8 (5)*. Buenos Aires: Colección Científica del Instituto Nacional de Tecnología Agropecuaria, 199–217.

Cronquist A. 1981. *An integrated system of classification of flowering plants.* New York: Columbia University Press.

Cronquist A. 1988. *The evolution and classification of flowering plants 2nd ed*. New York: New York Botanical Garden.

Dandy JE. **1926**. Notes on *Kissenia* and the Geographical Distribution of the Loasaceae. *Bulletin of Miscellaneous Information (Royal Gardens, Kew)* **1926**: 174–180.

Dahlgren R. 1975a. A system of classification of the angiosperms to be used to demonstrate the distribution of characters. *Botaniska Notiser* **128**: 119–147.

Dahlgren R. 1975b. The distribution of characters within an angiosperm system. I. Some embryological characters. *Botaniska Notiser* **128**: 181–197.

Darwin C & Darwin F. 1898. *The power of movement in plants*. New York: D. Appleton & Company.

Davis WS & Thompson HJ. 1967. A revision of *Petalonyx* (Loasaceae) with a consideration of affinities in subfamily Gronovioideae. *Madroño* **19**: 1–32.

de Candole, AP. 1828. *Prodromus systematis naturalis regni vegetabilis*. Paris: Treutel & Würtz.

De Smet Y, De Clerck O, Uemachi T, Granados-Mendoza C, Wanke S, Goetghebeur & Samain MS. 2017. Multilocus coalescent species delimitation to evaluate traditionally defined morphotypes in *Hydrangea* sect. *Asperae* (Hydrangeaceae). *Molecular Phylogenetics and Evolution* **114**: 415–425.

Deanna R, Barboza GE & Carrizo-García C. **2018**. Phylogenetic relationships of *Deprea*: New insights into the evolutionary history of physaloid groups. *Molecular Phylogenetics and Evolution* **119**: 71–80.

Denham S, Zavala-Gallo L, Johnson L & Pozner RE. 2016. Insights into the phylogeny and evolutionary history of Calyceraceae. *Taxon* **65**: 1328–1344.

Desrousseaux LAJ, Poiret JLM & Savigny JC. 1797. *Encyclopédie méthodique. Botanique. Tome 4eme. pt. I.* Paris: Chez H. Agasse.

Diazgranados M & Barber JC. **2017**. Geography shapes the phylogeny of frailejones (Espeletiinae Cuatrec., Asteraceae): a remarkable example of recent rapid radiation in sky islands. *PeerJ* **5**: e2968.

Dillon MO, Tu T, Xie L, Quipuscoa-Silvestre V& Wen J. 2009. Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution* **47**: 457–476.

Dostert N & Weigend M. **1999**. A synopsis of the *Nasa triphylla* complex (Loasaceae), including some new species and subspecies. *Harvard Papers in Botany* **4**: 439–467.

Drummond AJ, Suchard MA, Xie D & Rambaut A. 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969–1973.

Dunn RE, Strömberg CAE, Madden RH, Kohn MJ & Carlini AA. 2015. Linked canopy, climate, and faunal change in the Cenozoic of Patagonia. *Science* **347**: 258–261.

Eisner T, Eisner M & Hoebeke ER. **1998**. When defense backfires: detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. *Proceedings of the National Academy of Sciences* **95**: 4410–4414.

Endlicher S. 1836-40. *Genera Plantarum secundum Ordines Naturales disposita*. Vienna: Fr. Beck.

Ensikat HJ, Geisler T & Weigend M. **2016**. A first report of hydroxylated apatite as structural biomineral in Loasaceae – plants' teeth against herbivores. *Scientific Reports* **6**: 26073.

Ensikat HJ, Mustafa A & Weigend M. **2017**. Complex patterns of multiple biomineralization in single-celled plant trichomes of the Loasaceae. *American Journal of Botany* **104**: 195–206.

Eude A, Roddaz M, Brichau S, Brusset S, Calderon Y, Baby P & Soula JC. 2015. Controls on timing of exhumation and deformation in the northern Peruvian eastern Andean wedge as inferred from low-temperature thermochronology and balanced cross section: E. *Tectonics* **34**: 715–730.

Evert RF. 2006. E*sau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development. 3rd ed.* New Jersey: Wiley Interscience.

Felsenstein J. **1981**. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* **17**: 368–376.

Fernández de Oviedo G. 1851. *Historia general y natural de Las Indias* (José Amador de los Ríos Ed.). Madrid: Imprenta de la Real Academia de la Historia.

Feuillée L. 1714. *Journal des observations physiques, mathematiques et botaniques. Tome Second*. Paris: Chez Pierre Giffart.

Folguera A, Naipauer M, Sagripanti L, Ghiglione MC, Orts DL & Giambiagi L. **2016**. *Growth of the Southern Andes*. Cham: Springer International Publishing.

Folguera A & Ramos VA. **2011**. Repeated eastward shifts of arc magmatism in the Southern Andes: A revision to the long-term pattern of Andean uplift and magmatism. *Journal of South American Earth Sciences* **32**: 531–546.

Fritsch PW, Manchester SR, Stone RD, Cruz BC & Almeda F. 2015. Northern Hemisphere origins of the amphi-Pacific tropical plant family Symplocaceae. *Journal of Biogeography* **42**: 891–901.

Garcia V. 1962. Embryological Studies in the Loasaceae: development of endosperm in *Blumenbachia hieronymi* Urb. *Phytomorphology* **12**: 307–312.

García de Albano ME & Slanis AC. **2006**. Estudio palinologico de las especies argentinas de *Mentzelia* (Loasaceae). *Revista del Museo Argentino de Ciencias Naturales* **8**: 165–170.

Garzione CN, McQuarrie N, Perez ND, Ehlers TE, Beck SL, Kar N, Eichelberger N, Chapman AD, Ward KM, Ducea MN, Lease RO, Poulsen CJ, Wagner LS, Saylor JE, Zandt G & Horton BK. 2017. Tectonic Evolution of the Central Andean Plateau and Implications for the Growth of Plateaus. *Annual Review of Earth and Planetary Sciences* **45**: 529–559.

Gentry AH. 1982. Neotropical floristic diversity: phytogeographical connections between Central and SouthAmerica, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* **69**: 557–593.

Ghiglione MC. **2016**. *Geodynamic Evolution of the Southernmost Andes*. Cham: Springer International Publishing.

Ghosh P. **2006**. Rapid Uplift of the Altiplano Revealed Through 13C-18O Bonds in Paleosol Carbonates. *Science* **311**: 511–515.

Giambiagi L, Mescua J, Bechis F, Hoke G, Suriano J, Spagnotto S, & Pagano DS. 2016. Cenozoic Orogenic Evolution of the Southern Central Andes (32–36°S). In Folguera A, Naipauer M, Sagripanti L, Ghiglione MC, Orts DL & Giambiagi L, eds. *Growth of the Southern Andes*. Cham: Springer International Publishing, 63–98.

Gianni, G., Folguera, A., Navarrete, C., Encinas, A. & Echaurren, A. 2016. The North Patagonian Orogen: Meso-Cenozoic Evolution from the Andes to the Foreland Area. In Folguera A, Naipauer M, Sagripanti L, Ghiglione MC, Orts DL & Giambiagi L, eds. *Growth of the Southern Andes*. Cham: Springer International Publishing, 173–200.

Gilg E. 1894. Loasaceae. In: Engler A & Prantl K, eds. *Die natürlichen Pflanzenfamilien III: Teil. 6. Abteilung a.* Leipzig, Wilhelm Engelmann, 100–121.

Gilg E. 1925. Loasaceae. In: Engler A & Prantl K, eds. *Die natürlichen Pflanzenfamilien 2nd ed, Teil. 21: Parietales*. Leipzig, Wilhelm Engelmann, 522–543.

Graham A. **2009**. The Andes: a Geological Overview from a Biological Perspective. *Annals of the Missouri Botanical Garden* **96**: 371–385.

Grau J. 1997. *Huidobria*, eine isolierte Gattung der Loasaceae aus Chile. *Sendtnera* **4**: 77–93.

Gregory M. 1998. Hydrangeaceae. In: Cutler DF & Gregory M, eds. *Anatomy of the dicotyledons, 2nd ed. , vol. 4. Saxifragales.* Oxford: Oxford University Press, 87–108.

Gregory-Wodzicki KM. **2000**. Uplift history of the Central and Northern Andes: a review. *Geological Society of America Bulletin* **112**: 1091–1105.

Grissom JL. 2014. *Phylogenetics and Biogeography of* Mentzelia *section* Mentzelia *(Loasaceae).* M.Sc. Thesis, Washington State University, Pullman, Washington.

Haddad NM, Brudvig LA, Clobert J, Davies KF, Gonzalez A, Holt RD, Lovejoy TE, Sexton JO, Austin MP, Collins CD, Cook WM, Damschen EI, Ewers RM, Foster BL, Jenkins CN, King AJ, Laurance WF, Levey DJ, Margules CR, Melbourne BA, Nicholls AO, Orrock JL, Song DX, & Townshend JR. 2015. Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances* **1**: e1500052.

Hallier H. 1905. Provisional scheme of the natural (phylogenetic) system of flowering plants. *New Phytologist* **4**: 151–162.

Harling G & Sparre B (founding eds.). 1973-ongoing. *Flora of Ecuador, Vol 1+*. Göteborg & Stockholm: University of Göteborg & Swedish Museum of Natural History.

Harter B, Schlindwein C & Wittmann D. 1995. Bienen und Kolibris als Bestäuber von Blüten der Gattung *Cajophora* (Loasaceae). *Apidologie* **26**: 356–357.

Hartley AJ, Chong G, Houston J & Mather AE. 2005. 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile. *Journal of the Geological Society* **162**: 421–424.

Harvey WH. 1859. *Thesaurus capensis: or illustrations of the South African flora. Vol. I.* Dublin: Hodges, Smith & Co.

Hazzi NA, Moreno JS, Ortiz-Movliav C & Palacio RD. 2018. Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. *Proceedings of the National Academy of Sciences* **115**: 7985–7990.

Hempel AL, Reeves PA, Olmstead RG & Jansen RK. 1995. Implications of *rbc*L sequence data for higher order relationships of the Loasaceae and the anomalous aquatic plant *Hydrostachys* (Hydrostachyaceae). *Plant Systematics and Evolution* **194**: 25–37.

Henning T, Cano A & Weigend M. 2009. A new shrubby species of *Nasa* Weigend ser. *Carunculatae* (Urb. & Gilg) Weigend (Loasaceae) from the Amotape-Huancabamba Zone. *Revista Peruana de Biología* **16:** 151–156.

Henning T, Mittelbach M, Ismail SA, Acuña R & Weigend M. 2018. A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation. *Scientific Reports* **8**: 14018.

Henning T, Rodríguez E & Weigend M. 2011. A revision of the *Nasa ranunculifolia* group (*Nasa* ser. *Grandiflorae* pro parte, Loasaceae). *Botanical Journal of the Linnean Society* **167**: 47–93.

Henning T & Weigend M. 2009. Systematics of the *Nasa poissoniana* group (Loasaceae) from Andean South America. *Botanical journal of the Linnean Society* **161**: 278–301.

Henning T & Weigend M. 2012. Total Control – Pollen Presentation and Floral Longevity in Loasaceae (Blazing Star Family) Are Modulated by Light, Temperature and Pollinator Visitation Rates. *PLoS ONE* **7**: e41121.

Henning T & Weigend M. 2013. Beautiful, complicated—and intelligent? Novel aspects of the thigmonastic stamen movement in Loasaceae. *Plant Signaling & Behavior* **8**: e24605.

Herrera S, Pinto L, Deckart K, Cortés J & Valenzuela JI. 2017. Cenozoic tectonostratigraphic evolution and architecture of the Central Andes in northern Chile based on the Aquine region, Western Cordillera (19°-19°30’ S). *Andean Geology* **44**: 87–122.

Hill RJ. **1976**. Taxonomic and Phylogenetic Significance of Seed Coat Microsculpturing in *Mentzelia* (Loasaceae) in Wyoming and Adjacent Western States. *Brittonia* **28**: 86–112.

Hokche O, Berry P & Huber O. 2008. *Nuevo Catálogo de la flora vascular de Venezuela*. Caracas: Fundación Instituto Botánico de Venezuela Dr. Tobias Lasser.

Hoorn C, Wesselingh FP, Ter Steege H, Bermúdez MA, Mora A, Sevink J, Sanmartín I, Sánchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T, Antonelli A. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* **330**: 927–931.

Hufford L. **1988**. Seed Morphology of *Eucnide* and Other Loasaceae. *Systematic Botany* **13**: 154–167.

Hufford L. **1989**. The structure and potential loasaceous affinities of *Schismocarpus*. *Nordic journal of botany* **9**: 217–227.

Hufford L. **2003**. Homology and Developmental Transformation: Models for the Origins of the Staminodes of Loasaceae Subfamily Loasoideae. *International Journal of Plant Sciences* **164**: S409–S439.

Hufford L. 2016. Loasaceae. In: Flora of North America Editorial Committee, eds. *Flora of North America North of Mexico Vol. 12*. New York and London: Oxford University Press, 491–545.

Hufford L, McMahon MM, O’Quinn R & Poston ME. **2005**. A phylogenetic analysis of Loasaceae subfamily Loasoideae based on plastid DNA sequences. *International Journal of Plant Sciences* **166**: 289–300.

Hufford L, McMahon MM, Sherwood AM, Reeves G & Chase MW. **2003**. The major clades of Loasaceae: phylogenetic analysis using the plastid *matK* and *trnL-trnF* regions. *American Journal of Botany* **90**: 1215–1228.

Hufford L, Schenk JJ & Brokaw JM. 2016. *Mentzelia*. In: Flora of North America Editorial Committee, eds. *Flora of North America North of Mexico Vol. 12*. New York and London: Oxford University Press, 496–543.

Hughes CE. **2016**. The tropical Andean plant diversity powerhouse. *New Phytologist* **210**: 1152–1154.

Hughes CE, Nyffeler R & Linder HP. **2015**. Evolutionary plant radiations: where, when, why and how? *New Phytologist* **207**: 249–253.

Hughes CE, Pennington RT & Antonelli A. 2013. Neotropical Plant Evolution: Assembling the Big Picture:. *Botanical Journal of the Linnean Society* **171**: 1–18.

Humboldt A & Bonpland A. 1805. *Essai sur la géographie des plantes*. Paris: Chez Levrault, Schoell et compagnie, libraires.

Janzen DH. **1971**. Euglossine Bees as Long-Distance Pollinators of Tropical Plants. *Science* **171**: 203–205.

Jabaily RS & Sytsma KJ. **2013**. Historical biogeography and life-history evolution of Andean *Puya* (Bromeliaceae). *Botanical Journal of the Linnean Society* **171**: 201–224.

Jennings WB & Berry KH. **2015**. Desert Tortoises (*Gopherus agassizii*) Are Selective Herbivores that Track the Flowering Phenology of Their Preferred Food Plants. *PLOS ONE* **10**: e0116716.

Jensen SR, Nielsen BJ & Dahlgren R. 1975. Iridoid compounds, their occurrence and systematic importance in the angiosperms. *Botaniska Notiser* **128**: 148–180.

Joppa LN, Roberts DL, Myers N & Pimm SL. 2011. Biodiversity hotspots house most undiscovered plant species. *Proceedings of the National Academy of Sciences* **108**: 13171–13176.

Jørgensen PM & León-Yánez S. 1999. *Catalogue of the Vascular Plants of Ecuador. Monographs in Systematic Botany from the Missouri Botanical Garden Vol. 75*. Missouri: Missouri Botanical Garden Press.

Jørgensen PM, Nee MH & Beck SG. 2015. *Catálogo de las plantas vasculares de Bolivia, Monographs in Systematic Botany from the Missouri Botanical Garden, Vol. 127*. Missouri: Missouri Botanical Garden Press

Josse C, Cuesta F, Navarro G, Barrena V, Becerra MT, Cabrera E, Chacón-Moreno E, Ferreira W, Peralvo M, Saito J, Tovar A & Naranjo LG. Physical Geography and Ecosystems in the Tropical Andes. In: Herzog SK, Martínez R, Jørgensen PM & Tiessen H, eds. *Climate Change and Biodiversity in the Tropical Andes*, MacArthur Foundation, IAI, SCOPE, 152–169.

Jussieu AL. 1789. *Genera Plantarum secundum Ordines Naturales disposita*. Paris: Viduam Herissant and Theophilum Barrois.

Jussieu AL. 1804. Mémoire sur le *Loasa*, genre de plantes qui devra constituer avec *Mentzelia*, une nouvelle famille. *Annales du Muséum d'histoire naturelle* **5**: 18–27.

Kessler, M. 2010. Biogeography of Ferns. In: Mehltreter K, Walker LR & Sharpe JM, eds. *Fern Ecology*. Cambridge: Cambridge University Press.

Kooiman P. **1974**. Iridoid glycosides in the Loasaceae and the taxonomic position of the family. *Acta Botanica Neerlandica* **23**: 677–679.

Lagomarsino LP, Antonelli A, Muchhala N, Timmermann A, Mathews S & Davis CC. 2014. Phylogeny, classification, and fruit evolution of the species-rich Neotropical bellflowers (Campanulaceae: Lobelioideae). *American Journal of Botany* **101**: 2097–2112.

Lagomarsino LP, Forrestel EJ, Muchhala N & Davis CC. 2017. Repeated evolution of vertebrate pollination syndromes in a recently diverged Andean plant clade. *Evolution* **71**: 1970–1985.

Lamarck JB. 1789. *Encyclopédie méthodique. Botanique. Tome 3eme. pt. I.* Paris and Liége: Chez Panckoucke and Chez Plomteux.

Lamarck JB. 1792. *Encyclopédie méthodique. Botanique. Tome 3eme. pt. II.* Paris and Liége: Chez Panckoucke and Chez Plomteux.

Lamb S. **2016**. Cenozoic uplift of the Central Andes in northern Chile and Bolivia—reconciling paleoaltimetry with the geological evolution. *Canadian Journal of Earth Sciences* **53**: 1227–1245.

Lasser T (founding ed.). 1964-ongoing. *Flora de Venezuela, Vol. 1+*. Caracas: Fondo Editorial Acta Científica Venezolana.

Leier A, McQuarrie N, Garzione C & Eiler J. **2013**. Stable isotope evidence for multiple pulses of rapid surface uplift in the Central Andes, Bolivia. *Earth and Planetary Science Letters* **371–372**: 49–58.

Leite AV, Nadia T & Machado IC. **2016**. Pollination of *Aosa rupestris* (Hook.) Weigend (Loasaceae): are stamen movements induced by pollinators? *Brazilian Journal of Botany* **39**: 559–567.

León B, Pitman N & Roque J. 2006. El libro rojo de las plantas endémicas del Perú. *Revista Peruana de Biología* **13(2)**.

León-Yánez S, Valencia-Reyes R, Pitman NCA, Endara L, Ulloa-Ulloa C & Navarrete H. 2011. *Libro Rojo de las Plantas Endémicas del Ecuador, 2nd ed.* Quito: Herbario QCA, Pontificia Universidad Católica del Ecuador.

Linnaeus C. 1753. *Species Plantarum Tomus I.* Stockholm: Impensis Laurentii Salvii.

Liogier AH. 1981. *Antillean studies I. Flora of Hispaniola: Part I. Celastrales, Rhamnales, Malvales Thymeleales, Violales. Phytologia Memoirs 3.* New Jersey: HN Moldenke & AL Moldenke.

Llamozas S, Duno de Stefano R, Meier W, Riina R, Stauffer F, Aymard G, Huber O & Ortiz R. 2003. *Libro Rojo de la Flora Venezolana.* Caracas: PROVITA/ Fundación Empresas Polar/ Fundación Instituto Botánico de Venezuela "Dr. Tobias Lasser"/ Conservación Internacional.

Luebert F, Hilger HH & Weigend M. **2011**. Diversification in the Andes: Age and origins of South American *Heliotropium* lineages (Heliotropiaceae, Boraginales). *Molecular Phylogenetics and Evolution* **61**: 90–102.

Luebert F & Weigend M. **2014**. Phylogenetic insights into Andean plant diversification. *Frontiers in Ecology and Evolution* **2**: 27.

Macbride F. 1941. Loasaceae. In: Macbride F, ed. *Flora of Peru*. *Fieldiana, Botany*: **13 Part 4(1)**: 143–181.

Machado AFP, Rønsted N, Bruun-Lund S, Pereira RAS & de Queiroz LP. 2018. Atlantic forests to the all Americas: Biogeographical history and divergence times of Neotropical *Ficus* (Moraceae). *Molecular Phylogenetics and Evolution* **122**: 46–58.

Madriñán S, Cortés AJ & Richardson JE. **2013**. Páramo is the world's fastest evolving and coolest biodiversity hotspot. *Frontiers in Genetics* **4**: 192.

Mahelka V, Krak K, Kopecký D, Fehrer J, Šafař J, Bartoš J, Hobza R, Blavet N & Blattner FR. 2017. Multiple horizontal transfers of nuclear ribosomal genes between phylogenetically distinct grass lineages. *Proceedings of the National Academy of Sciences* **114**: 1726–1731.

Marchese C. **2015**. Biodiversity hotspots: A shortcut for a more complicated concept. *Global Ecology and Conservation* **3**: 297–309.

Margirier A, Robert X, Audin L, Gautheron C, Bernet M, Hall S & Simon-Labric T. 2015. Slab flattening, magmatism, and surface uplift in the Cordillera Occidental (northern Peru). *Geology* **43**: 1031–1034.

Maroon EA, Frierson DMW & Battisti DS. **2015**. The Tropical Precipitation Response to Andes Topography and Ocean Heat Fluxes in an Aquaplanet Model. *Journal of Climate* **28**: 381–398.

Martin-Gombojav N & Winkler W. **2008**. Recycling of Proterozoic crust in the Andean Amazon foreland of Ecuador: implications for orogenic development of the Northern Andes: Multiple recycling of Proterozoic crust in the Northern Andes. *Terra Nova* **20**: 22–31.

Matzke NJ. **2013**. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* **5**: 4.

Mau B, Newton MA & Larget B. **1999**. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* **55**: 1–12.

McQuarrie N, Horton BK, Zandt G, Beck S & DeCelles PG. 2005. Lithospheric evolution of the Andean fold–thrust belt, Bolivia, and the origin of the central Andean plateau. *Tectonophysics* **399**: 15–37.

Michalak MJ, Hall SR, Farber DL, Audin L & Hourigan JK. 2016. (U-Th)/He thermochronology records late Miocene accelerated cooling in the north-central Peruvian Andes. *Lithosphere* **8**: 103–115.

Mittermeier RA, Robles-Gil P, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J & da Fonseca GAB. 2004. *Hotspots revisited – Earth's biologically richest and most endangered terrestrial ecoregions*. Mexico City, CEMEX.

Montes C, Cardona A, Jaramillo C, Pardo A, Silva JC, Valencia V, Ayala C, Pérez-Ángel LC, Rodríguez-Parra LA, Ramírez V & Niño H. 2015. Middle Miocene closure of the Central American seaway. *Science* **348**: 226–229.

Moody ML & Hufford L. 2000. Floral ontogeny and morphology of *Cevallia, Fuertesia* , and *Gronovia* (Loasaceae Subfamily Gronovioideae). *International Journal of Plant Sciences* **161**: 869–883.

Moody ML, Hufford L, Soltis DE & Soltis PS. 2001. Phylogenetic relationships of Loasaceae subfamily Gronovioideae inferred from *mat*K and ITS sequence data. *American Journal of Botany* **88**: 326–336.

Moonlight PW, Richardson JE, Tebbitt MC, Thomas DC, Hollands R, Peng CI & Hughes M. 2015. Continental-scale diversification patterns in a megadiverse genus: the biogeography of Neotropical *Begonia*. *Journal of Biogeography* **42**: 1137–1149.

Morales-Briones DF, Romoleroux K, Kolář F & Tank DC. 2018. Phylogeny and Evolution of the Neotropical Radiation of *Lachemilla* (Rosaceae): Uncovering a History of Reticulate Evolution and Implications for Infrageneric Classification. *Systematic Botany* **43**: 17–34.

Morrone JJ. 2017. *Neotropical biogeography, regionalization and evolution*. Boca Raton: CRC Press.

Murillo JC, Stuessy TF & Ruiz E. 2016. Explaining disjunct distributions in the flora of southern South America: evolutionary history and biogeography of *Myrceugenia* (Myrtaceae). *Journal of Biogeography* **43**: 979–990.

Mustafa A. 2018. *Morphology, Ontogeny, and Biomineralization of Trichomes of Selected Higher Plants*. Ph.D. Thesis, University of Bonn, Bonn, Germany.

Mustafa A, Ensikat HJ & Weigend M. 2017. Ontogeny and the process of biomineralization in the trichomes of Loasaceae. *American Journal of Botany* **104**: 367–378.

Mutke J, Böhnert T & Weigend M. 2017. Save last cloud forests in western Andes. *Nature* **541**: 157.

Mutke J, Jacobs R, Meyers K, Henning T & Weigend M. 2014. Diversity patterns of selected Andean plant groups correspond to topography and habitat dynamics, not orogeny. *Frontiers in Genetics* **5**: 351.

Mutke J & Weigend M. Mesoscale patterns of plant diversity in Andean South America based on combined checklist and GBIF data. *Berichten der Reinhold-Tüxen-Gesellschaft* 29: 83–97.

Naeem S, Chazdon R, Duffy JE, Prager C & Worm B. 2016. Biodiversity and human well-being: an essential link for sustainable development. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20162091.

Naiman Z, Goodman PJ, Krasting JP, Malyshev SL, Russell JL, Stouffer RJ & Wittenberg AT. 2017. Impact of Mountains on Tropical Circulation in Two Earth System Models. *Journal of Climate* **30**: 4149–4163.

NASA Earth Observatory. 2017. Finding fires in Peru. Available from: https://earthobservatory.nasa.gov/images/89620/finding-fires-in-peru

Neill DA & Ulloa-Ulloa C. 2011. *Adiciones a la Flora del Ecuador: Segundo Suplemento, 2005-2010*. Quito: Funtación Jatun Sacha.

Nicolas AN & Plunkett GM. 2014. Diversification Times and Biogeographic Patterns in Apiales. *The Botanical Review* **80**: 30–58.

Noguera-Savelli E, Ruiz T & Jáuregui D. 2009. Morfología del polen de las especies de Loasaceae Juss. presentes en Venezuela. *Ernstia* **19**: 67–79.

Nürk NM, Scheriau C & Madriñán S. 2013. Explosive radiation in high Andean *Hypericum*—rates of diversification among New World lineages. *Frontiers in Genetics* **4**: 175.

Pacheco AMF. 2002. Loasaceae**.** In: Wanderley MGL, Shepherd GJ, Giulietti AM, Melhem TS, Bittrich V & Kameyama C, eds. *Flora Fanerogâmica do Estado de São Paulo*, *vol. 2*. São Paulo: Instituto de Botânica, 159–162.

Payer, JB. 1857. *Traité d'organogénie comparée de la fleur.* Paris: Libraire de Victor Masson.

Pérez-Escobar OA, Balbuena JA & Gottschling M. 2016. Rumbling Orchids: How To Assess Divergent Evolution Between Chloroplast Endosymbionts and the Nuclear Host. *Systematic Biology* **65**: 51–65.

Pérez-Escobar OA, Chomicki G, Condamine FL, Karremans AP, Bogarín D, Matzke N, Silvestro D & Antonelli A. 2017. Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. *New Phytologist* **215**: 891–905.

Pérez-Moreau RL & Crespo S. 1992. Notas sobre Loasaceae IV. *Loasa pulchella* nueva combinación. *Hickenia* **2(14)**: 67–68.

Pérez-Moreau RL & Crespo S. 2003. Loasaceae. In Kiesling R, ed, *Flora de San Juan Volumen 2*. Buenos Aires: Estudios Sigma, 152–160

Perret M, Chautems A, De Araujo AO & Salamin N. 2013. Temporal and spatial origin of Gesneriaceae in the New World inferred from plastid DNA sequences. *Botanical Journal of the Linnean Society* **171**: 61–79.

Pimm SL & Joppa LN. **2015**. How Many Plant Species are There, Where are They, and at What Rate are They Going Extinct? *Annals of the Missouri Botanical Garden* **100**: 170–176.

Pimm SL & Raven PH. **2017**. The Fate of the World's Plants. *Trends in Ecology & Evolution* **32**: 317–320.

Pinto P (founding ed.). 1983-ongoing. *Flora de Colombia, Vol. 1+*. Bogotá: Universidad Nacional de Colombia.

Pio DV, Broennimann O, Barraclough TG, Reeves G, Rebelo AG, Thuiller W, Guisan A & Salamin N. 2011. Spatial Predictions of Phylogenetic Diversity in Conservation Decision Making: Patterns of Phylogenetic Diversity. *Conservation Biology* **25**: 1229–1239.

Pirie MD, Maas PJM, Wilschut RA, Melchers-Sharrot, H & Chatrou LW. 2018. Parallel diversifications of *Cremastosperma* and *Mosannona* (Annonaceae), tropical rainforest trees tracking Neogene upheaval of South America. *Royal Society Open Science* **5**: 171561.

Plumier C. 1703. *Nova plantarum americanarum genera*. Paris: Joannem Boudot.

Posadas P, Miranda-Esquivel DR & Crisci JV. 2001. Using Phylogenetic Diversity Measures to Set Priorities in Conservation: an Example from Southern South America. *Conservation Biology* **15**: 1325–1334.

Poston ME & Nowicke JW. **1993**. Pollen Morphology, Trichome Types, and Relationships of the Gronovioideae (Loasaceae). *American Journal of Botany* **80**: 689–784.

Pouchon C, Fernández A, Nassar JM, Boyer F, Aubert S, Levergne S & Mavárez J. 2018. Phylogenomic Analysis of the Explosive Adaptive Radiation of the *Espeletia* Complex (Asteraceae) in the Tropical Andes. *Systematic Biology* **67**: 1041–1060.

Poulsen CJ, Ehlers TA & Insel N. **2010**. Onset of convective rainfall during gradual late Miocene rise of the central Andes. *Science* **328**: 490–493.

Quade J, Dettinger MP, Carrapa B, DeCelles P, Murray KE, Huntington KW, Cartwright A, Canavan RR, Gehrels G & Clementz M. 2015. The growth of the central Andes, 22°S–26°S. In DeCelles P, Ducea MN, Carrapa B & Kapp PA, eds. *Geodynamics of a Cordilleran Orogenic System: The Central Andes of Argentina and Northern Chile*. *Geological Society of America Memoirs*, **212**: 277–308.

Raimúndez-Urrutia E & Varela C. **2005**. Predispersal reproductive ecology of *Gronovia scandens* L.(Loasaceae), a plant from disturbed habitats1. *The Journal of the Torrey Botanical Society* **132**: 581–589.

Ree RH & Smith SA. **2008**. Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. *Systematic Biology* **57**: 4–14.

Reiche K. 1901. Estudios críticos sobre la flora de Chile. *Anales de la Universidad de Chile* **108**: 707–751.

Reichenbach, L. 1837. *Handbuch des natürlichen Pflanzensystems*. Dreden and Leipzig: Arnoldischen Buchhandlung.

Restrepo-Moreno S, Foster D, Stockli D & Parra-Sánchez LN. 2009. Long-term erosion and exhumation of the "Altiplano Antioqueño", Northern Andes (Colombia) from apatite (U–Th)/He thermochronology. *Earth and Planetary Science Letters* **278**: 1–12.

Rhoads A & Au KF. **2015**. PacBio Sequencing and its Applications. *Genomics, Proteomics & Bioinformatics* **13**: 278–289.

Richardson JE, Madriñán S, Gómez-Gutiérrez MC, Valderrama E, Luna J, Banda K, Serrano J, Torres MF, Jara OA, Aldana AM, Cortés R, Sánchez D & Montes C. 2018. Using dated molecular phylogenies to help reconstruct geological, climatic, and biological history: Examples from Colombia. *Geological Journal* **2018**: 1–9.

Rieseberg LH & Soltis DE. **1991**. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* **5**: 65–84.

Rivas-Martínez S, Navarro G, Penas A, & Costa M. 2011. Biogeographic map of South America. A preliminary survey. *International Journal of Geobotanical Research* **1:** 21–40 + Map.

Rodrigues AS. & Gaston KJ. **2002**. Maximising phylogenetic diversity in the selection of networks of conservation areas. *Biological Conservation* **105**: 103–111.

Rodríguez R, Marticorena C, Alarcón D, Baeza C, Cavieres L, Finot VL, Fuentes N, Kiessling A, Mihoc M, Pauchard A, Ruiz E, Sánchez P & Marticorena A. 2018. Catálogo de las plantas vasculares de Chile. *Gayana Botánica* **75**: 1–430.

Roncal J, Kahn F, Millan B, Couvreur TLP & Pintaud JC. **2012**. Cenozoic colonization and diversification patterns of tropical American palms: evidence from *Astrocaryum* (Arecaceae). *Botanical Journal of the Linnean Society* **171**: 120–139.

Ronquist F. **1997**. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46**: 195–203.

Rothfels CJ, Pryer KM & Li FW. **2017**. Next-generation polyploid phylogenetics: rapid resolution of hybrid polyploid complexes using PacBio single-molecule sequencing. *New Phytologist* **213**: 413–429.

Salariato DL, Zuloaga FO, Franzke A, Mummenhoff K & Al-Shehbaz I. 2016. Diversification patterns in the CES clade (Brassicaceae tribes Cremolobeae, Eudemeae, Schizopetaleae) in Andean South America. *Botanical Journal of the Linnean Society* **181**: 543–566.

Sanín MJ, Kissling WD, Bacon CD, Borschenius F, Galeano G, Svenning JC, Olivera J, Ramírez R, Trénel P & Pintaud JC. 2016. The Neogene rise of the tropical Andes facilitated diversification of wax palms (*Ceroxylon*: Arecaceae) through geographical colonization and climatic niche separation. *Botanical Journal of the Linnean Society* **182**: 303–317.

Santos E & Fromm-Trinta E. 1985. *Loasáceas*. In: Reitz PR, ed. *Flora Ilustrada Catarinense*. Santa Catarina: Herbário Barbosa Rodrigues, 1–20.

Särkinen T, Pennington RT, Lavin M, Simon MF & Hughes CE. 2012. Evolutionary islands in the Andes: persistence and isolation explain high endemism in Andean dry tropical forests: Evolutionary islands in the Andes. *Journal of Biogeography* **39**: 884–900.

Schenk JJ. 2013. Biogeographical diversification of *Mentzelia* section *Bartonia* in western North America. J*ournal of Biogeography* **40**: 455–465.

Schenk JJ & Hufford L. 2011. Phylogeny and Taxonomy of *Mentzelia* Section *Bartonia* (Loasaceae). *Systematic Botany* **36**: 711–720.

Schenk J, Jacobs S & Hufford L. 2017. Comparative Diversification Analyses of Hydrangeaceae and Loasaceae: The Role of Continental Dispersal in Generating Species Diversity. Abstract. Botany2017 Conference. Available at: http://2017.botanyconference.org/engine/search/index.php?func=detail&aid=82

Schulenberg, TS, Stotz, DF, Lane DF, O'Neill JP & Parker TA. 2007. *Birds of Peru*. Princeton: Princeton University Press.

Schlindwein C & Wittmann D. 1997. Micro-Foraging Routes of *Bicolletes pampeana* (Colletidae) and Bee-Induced Pollen Presentation in *Cajophora arechavaletae* (Loasaceae). *Botanica Acta* **110**: 177–183.

Serrano J, Richardson JE, Pennington TD, Cortés R., Cárdenas D, Elliot A & Jiménez I. 2018. Biotic homogeneity of putative biogeographic units in the Neotropics: A test with Sapotaceae. *Diversity and Distributions* **24**: 1121–1135.

Silvertown J & Gordon DM. 1989. A framework for plant behavior. *Annual Review of Ecology and Systematics* **20**: 349–366.

Siriani-Oliveira S, Oliveira R & Schlindwein C. 2018. Pollination of *Blumenbachia amana* (Loasaceae): flower morphology and partitioned pollen presentation guarantee a private reward to a specialist pollinator. *Biological Journal of the Linnean Society* **124**: 479–491.

Sklenář P, Dušková E & Balslev H. 2011. Tropical and Temperate: Evolutionary History of Páramo Flora. *The Botanical Review* **77**: 71–108.

Sklenář P, Hedberg I & Cleef AM. **2014**. Island biogeography of tropical alpine floras. *Journal of Biogeography* **41**: 287–297.

Slanis AC, Perea MC & Grau A. **2016**. Revisión taxonómica del género *Caiophora* (Loasaceae) para Argentina: *C. sleumerii* una nueva especie. *Darwiniana, nueva serie* **4**: 138–191.

Sleumer H. 1955. Die Loasaceen Argentiniens. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **76**: 411–462.

Spikings R., Winkler W, Seward D & Handler R. 2001. Along-strike variations in the thermal and tectonic response of the continental Ecuadorian Andes to the collision with heterogeneous oceanic crust. *Earth and Planetary Science Letters* **186**: 57–73.

Strelin MM, Arroyo JI, Fließwasser S & Ackermann M. 2017. Diversification of *Caiophora* (Loasaceae subfam. Loasoideae) during the uplift of the Central Andes. *Organisms Diversity & Evolution* **17**: 29–41.

Strelin MM, Benítez-Vieyra S, Ackermann M & Cocucci A. 2016a. Flower reshaping in the transition to hummingbird pollination in Loasaceae subfam. Loasoideae despite absence of corolla tubes or spurs. *Evolutionary Ecology* **30**: 401–417.

Strelin MM, Benítez-Vieyra S, Fornoni J, Klingenberg CP & Cocucci A. 2016b. Exploring the ontogenetic scaling hypothesis during the diversification of pollination syndromes in *Caiophora* (Loasaceae, subfam. Loasoideae). *Annals of Botany* **117**: 937–947.

Struwe L, Haag S, Heiberg E & Grant JR. 2009. Andean speciation and vicariance in neotropical *Macrocarpaea* (Gentianaceae–Helieae). *Annals of the Missouri Botanical Garden* **96**: 450–469.

Takhtajan, A. 1973. *Evolution und Ausbreitung der Blütenpflanzen*. Stuttgart: Gustav Fischer Verlag.

Takhtajan, A. 1997. *Diversity and classification of flowering plants*. New York: Columbia University Press.

Tassara A & Yáñez G. 2003. Relación entre el espesor elástico de la litósfera y la segmentación tectónica del margen andino (15–47°S). *Revista Geológica de Chile* **30**: 159–186.

Thompson HJ & Ernst WR. 1967. Floral Biology and Systematics of *Eucnide* (Loasaceae). *Journal of the Arnold Arboretum* **48**: 56–88.

Trénel P, Gustafsson MHG, Baker WJ, Asmussen-Lange CB, Dransfield, J & Borchsenius F. 2007. Mid-Tertiary dispersal, not Gondwanan vicariance explains distribution patterns in the wax palm subfamily (Ceroxyloideae: Arecaceae). *Molecular Phylogenetics and Evolution* **45**: 272–288.

Urban I. 1886. Die Bestäubungseinrichtungen bei den Loasaceen. J*ahrbuch des Königlichen botanischen Gartens und des botanischen Museums zu Berlin* **4**: 364–388.

Urban I. 1892a. Die Blüthenstände der Loasaceen. *Berichte der Deutschen Botanischen Gesellschaft* **10**: 259–265.

Urban I. 1892b. Blüthen- und Fruchtbau der Loasaceen. *Berichte der Deutschen Botanischen Gesellschaft* **10**: 259–265.

Urban I. 1910. Zwei neue Loasaceen von Sto. Domingo. *Berichte der Deutschen Botanischen Gesellschaft* **28**: 515–523.

Urban I & Gilg E. 1900. Monographia Loasacearum. *Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum* **76**: 1–368.

Vargas OM, Ortiz EM & Simpson BB. **2017**. Conflicting phylogenomic signals reveal a pattern of reticulate evolution in a recent high-Andean diversification (Asteraceae: Astereae: *Diplostephium*). *New Phytologist* **214**: 1736–1750.

Vijayaraghavan MR & Prabhakar K. 1984. The endosperm. In: Johri BM, ed. *Embryology of angiosperms*. Berlin, Heidelberg and New York: Springer, 319–376.

Villagómez D & Spikings R. **2013**. Thermochronology and tectonics of the Central and Western Cordilleras of Colombia: Early Cretaceous–Tertiary evolution of the Northern Andes. *Lithos* **160–161**: 228–249.

Villagómez D, Spikings R, Mora A, Guzmán G, Ojeda G, Cortés E & van der Lelij R. **2013**. Vertical tectonics at a continental crust-oceanic plateau plate boundary zone: Fission track thermochronology of the Sierra Nevada de Santa Marta, Colombia. *Tectonics* **30**: TC4004.

Weigend M. 1997a. Nasa *and the conquest of South America: Systematic rearrangements in Loasaceae Juss.*. Ph.D. Thesis. Ludwig-Maximilians Universität München. Germany.

Weigend M. 1997b. Names and types in *Caiophora* K.Presl.s.str. (Loasaceae). *Sendtnera* **4**: 221–242.

Weigend M. 2000a. Loasaceae No. 132. In: Andersson L, Harling G, eds. *Flora of Ecuador* **64**: 1–92.

Weigend M. **2000b**. A revision of the Peruvian species of *Nasa* ser. *Alatae* (Loasaceae). *Nordic Journal of Botany* **20**: 15–31.

Weigend M. 2001. Loasaceae. In: Bernal R, Forero E, eds. *Flora de Colombia* **22***:* 1–100.

Weigend M. **2002a**. Observations on the biogeography of the Amotape-Huancabamba zone in northern Peru. *The Botanical Review* **68**: 38–54.

Weigend M. **2002b**. *Nasa panamensis*, a new species of *Nasa* (Loasaceae) from Central America. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **124**: 211–216.

Weigend M. **2004a**. Additional observations on the biogeography of the Amotape-Huancabamba zone in Northern Peru: Defining the South-Eastern limits. *Revista Peruana de Biología* **11**: 127–134.

Weigend M. 2004b. Loasaceae. In: Kubitzki K, ed. *The families and genera of vascular plants Vol. 6.* Berlin, Spinger, 239–254.

Weigend M. 2004c. Loasaceae. In: Smith N, Mori SA, Henderson A, Stevenson DW & Heald SV, eds. *Flowering Plants of the Neotropics*. Princeton: Princeton University Press, 217-219.

Weigend M. 2004d. Four new species of *Nasa* ser *Alatae* (Loasaceae) in the Amotape-Huancabamba zone of Peru. *Novon* **14**: 134–146.

Weigend M. 2006. Validating subfamily, genus and species names in Loasaceae (Cornales). *Taxon* **55**: 463–468.

Weigend M. 2009. Neotropical Loasaceae. In: Milliken W, Klitgård B & Baracat A, eds. *Neotropikey-Interactive key and information resources for flowering plants of the Neotropics.* Available from:http://www.kew.org/science/tropamerica/neotropikey/families/Loasaceae.htm.

Weigend M & Ackermann M. 2003. Los nombres antiguos en el género *Caiophora* (Loasáceas subfam. Loasoídeas) y una clasificación infragenérica preliminar. *Arnaldoa* **10**: 75–94.

Weigend M & Ackermann M. 2015. Loasaceae. In Jørgensen PM, Nee M & Beck S, eds. *Catálogo de las Plantas Vasculares de Bolivia. Monographs in Systematic Botany from the Missouri Botanical Garden, Vol. 127*. Missouri: Missouri Botanical Garden Press, 763–765.

Weigend M, Ackermann M & Henning T. **2010**. Reloading the revolver–male fitness as a simple explanation for complex reward partitioning in *Nasa macrothyrsa* (Loasaceae, Cornales). *Biological Journal of the Linnean Society* **100**: 124–131.

Weigend M, Aitzetmüller K & Bruehl L. **2004b**. The seeds of Loasaceae subfam. Loasoideae (Cornales) I: Seed release, seed numbers and fatty acid composition. *Flora-Morphology, Distribution, Functional Ecology of Plants* **199**: 424–436.

Weigend M & Gottschling M. **2006**. Evolution of Funnel-Revolver Flowers and Ornithophily in *Nasa* (Loasaceae). *Plant Biology* **8**: 120–142.

Weigend M, Gottschling M, Hoot S & Ackermann M. 2004a. A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on *trn*L (UAA) sequence data, with consequences for systematics and historical biogeography. *Organisms Diversity & Evolution* **4**: 73–90.

Weigend M, Grau J & Ackermann M. 2008. Loasaceae. In Zuloaga FO, Morrone O & Belgrano MJ, eds. *Catálogo de las Plantas Vasculares del Cono Sur (Argentina, Sur de Brasil, Chile, Paraguay y Uruguay). Monographs in Systematic Botany from the Missouri Botanical Garden, Vol. 107*. Missouri: Missouri Botanical Garden Press, 2413–2424.

Weigend M, Gröger A & Ackermann M. **2005**. The seeds of Loasaceae subfam. Loasoideae (Cornales) II: Seed morphology of "South Andean Loasas" (*Loasa*, *Caiophora*, *Scyphanthus* and *Blumenbachia*). *Flora - Morphology, Distribution, Functional Ecology of Plants* **200**: 569–591.

Weigend M, Henning T & Schneider C. **2003**. A revision of *Nasa* ser. *Carunculatae* (Loasaceae subfam. Loasoideae). *Systematic Botany* **28**: 765–781.

Weigend M, Kufer J & Müller AA. **2000**. Phytochemistry and the systematics and ecology of Loasaceae and Gronoviaceae (Loasales). *American Journal of Botany* **87**: 1202–1210.

Weigend M & Rodriguez E. 2003. A revision of the *Nasa stuebeliana* group [*Nasa* ser. *Saccatae* (Urb. & Gilg) Weigend, Loasaceae] with notes on morphology, ecology and distribution. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **124**: 345–382.

Wen J & Ickert-Bond SM. **2009**. Evolution of the Madrean-Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *Journal of Systematics and Evolution* **47**: 331–348.

Whitfield JB & Lockhart PJ. **2007**. Deciphering ancient rapid radiations. *Trends in Ecology & Evolution* **22**: 258–265.

Wilf P, Cúneo NR, Escapa IH, Pol D & Woodburne MO. 2013. Splendid and Seldom Isolated: The Paleobiogeography of Patagonia. *Annual Review of Earth and Planetary Sciences* **41**: 561–603.

Winterton C, Richardson JE, Hollingsworth M, Clark A, Zamora N & Pennington RT. 2014. Historical biogeography of the neotropical legume genus *Dussia*: the Andes, the Panama Isthmus and the Chocó. *Paleobotany and Biogeography: A Festschrift for Alan Graham in his 80th Year*, Missouri: Missouri Botanical Garden Press, 389–404.

World Bank. 2012. *Expanding Financing for Biodiversity Conservation: experiences from Latin America and the Caribbean*. Washington: International Bank for Reconstruction and Development / The World Bank.

Wunderlich R. **1959**. Zur Frage der Phylogenie der Endospermtypen bei den Angiospermen. *Österreichische Botanische Zeitschrift* **106**: 203–293.

Xiang QY, Moody ML, Soltis DE, Fan CZ & Soltis PS. 2002. Relationships within Cornales and circumscription of Cornaceae—*mat*K and *rbc*L sequence data and effects of outgroups and long branches. *Molecular Phylogenetics and Evolution* **24**: 35–57.

Xiang QY, Soltis DE & Soltis PS. **1998**. Phylogenetic relationships of Cornaceae and close relatives inferred from *mat*K and *rbc*L Sequences. *American Journal of Botany* **85**: 285–297.

Xiang QY, Thomas DT & Xiang QP. **2011**. Resolving and dating the phylogeny of Cornales – Effects of taxon sampling, data partitions, and fossil calibrations. *Molecular Phylogenetics and Evolution* **59**: 123–138.

Xu H, Wang Y & Xie SP. **2004**. Effects of the Andes on Eastern Pacific Climate: A Regional Atmospheric Model Study. *Journal of Climate* **17**: 589–602.

Yang T, Lu LM, Wang W, Li JH, Manchester SR, Wen J & Chen ZD. 2018. Boreotropical range expansion and long-distance dispersal explain two amphi-Pacific tropical disjunctions in Sabiaceae. *Molecular Phylogenetics and Evolution* **124**: 181–191.

APPENDIX A (Chapter 2: Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae))

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Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae)

Rafael Acuña, Stella Fließwasser, Markus Ackermann, Tilo Henning,
Federico Luebert & Maximilian Weigend

Taxon 66: 365–378

Table S1. List of the nuclear regions for which DNA amplification was attempted for SAL.

Nuclear region	Forward primers	Reverse primers	References
DAL1	ATG63900-1F, ATG63900-2F	ATG63900-1R, ATG63900-2R	Granados-Mendoza & al. (2015)
ETS	ETS-1F, AST-1, ETS-MentF	18S-ETS	Baldwin & Markos (1998), Linder & al. (2000), Markos & Baldwin (2001), Schenk & Hufford (2011)
G3pdh	GPDX7F	GPDX9R	Strand & al. (1997)
LFY	LFYF2	LFYR1	Howarth & Baum (2005)
PHYC	PHYC upstream	PHYC downstream	Mathews & Donoghue (1999)
TIF3H1	ATG10840-2F, ATG10840-3F	ATG10840-2R, ATG10840-3R	Granados-Mendoza & al. (2015)
TOPO6	Top6_6F_1175, Topo6P_8_700F	Top6_9R_1958, Top6_11R_2338	Jakob & Blattner (2010), Blaner & al. (2014), Blattner (2016)
waxy	waxy1F	waxy9R	Evans & al. (2000)

The references describe the primers and their respective amplification protocols.

Literature cited

Baldwin, B.G. & Markos, S. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molec. Phylogen. Evol.* 10: 449–463. https://doi.org/10.1006/mpev.1998.0545

Blaner, A., Schneider, J. & Röser, M. 2014. Phylogenetic relationships in the grass family (Poaceae) based on the nuclear single copy locus topoisomerase 6 compared with chloroplast DNA. *Syst. Biodivers.* 12: 111–124. https://doi.org/10.1080/14772000.2014.890137

Blattner, F.R. 2016. *TOPO6*: A nuclear single-copy gene for plant phylogenetic inference. *Pl. Syst. Evol.* 302: 239–244. https://doi.org/10.1007/s00606-015-1259-1

Evans, R.C., Alice, L.A., Campbell, C.S., Kellogg, E.A. & Dickinson, T.A. 2000. The granule-bound starch synthase (GBSSI) gene in the Rosaceae: Multiple loci and phylogenetic utility. *Molec. Phylogen. Evol.* 17: 388–400. https://doi.org/10.1006/mpev.2000.0828

Granados-Mendoza, C., Naumann, J., Samain, M.-S., Goetghebeur, P., De Smet, Y. & Wanke, S. 2015. A genome-scale mining strategy for recovering novel rapidly-evolving nuclear single-copy genes for addressing shallow-scale phylogenetics in *Hydrangea*. *B. M. C. Evol. Biol.* 15: 132. https://doi.org/10.1186/s12862-015-0416-z

Howarth, D.G. & Baum, D.A. 2005. Genealogical evidence of homoploid hybrid speciation in an adaptive radiation of *Scaevola* (Goodeniaceae) in the Hawaiian Islands. *Evolution* 59: 948–961. https://doi.org/10.1111/j.0014-3820.2005.tb01034.x

Jakob, S.S. & Blattner, F.R. 2010. Two extinct diploid progenitors were involved in allopolyploid formation in the *Hordeum murinum* (Poaceae: Triticeae) taxon complex. *Molec. Phylogen. Evol.* 55: 650–659. https://doi.org/10.1016/j.ympev.2009.10.021

Linder, C.R., Goertzen, J.R., Heuvel, B.V, Francisco-Ortega, J. & Jansen, R. 2000. The complete external transcribed spacer of 18S-26S rDNA: Amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely allied families. *Molec. Phylogen. Evol.* 14: 285–303. https://doi.org/10.1006/mpev.1999.0706

Markos, S. & Baldwin, B.G. 2001. Higher-level relationships and major lineages of *Lessingia* (Compositae, Astereae) based on nuclear rDNA internal and external transcribed spacer (ITS and ETS) sequences. *Syst. Bot.* 26: 168–183.

Mathews, S. & Donoghue, M. 1999. The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286: 947–950. https://doi.org/10.1126/science.286.5441.947

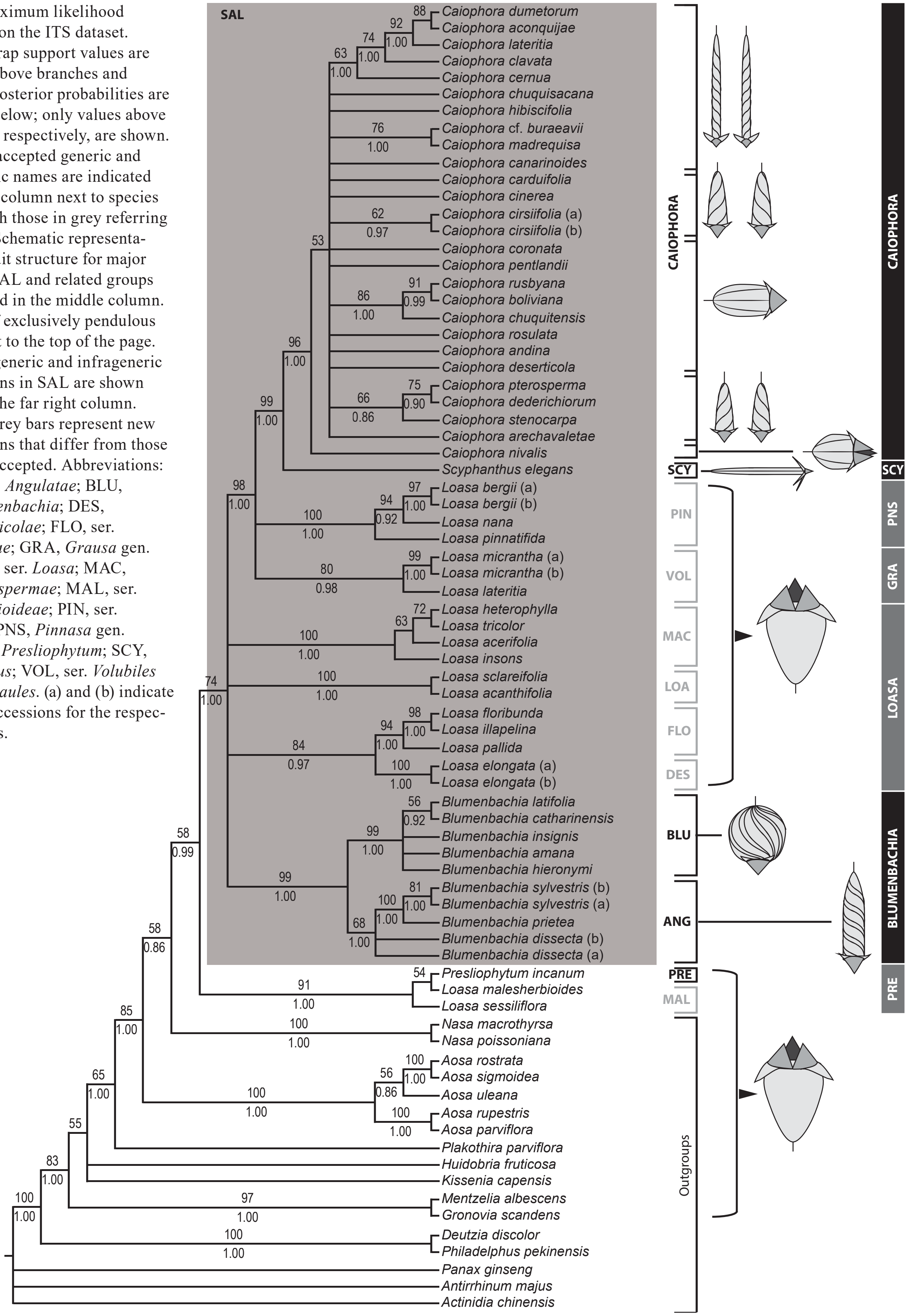
Schenk, J.J. & Hufford, L. 2011. Phylogeny and taxonomy of *Mentzelia* section *Bartonia* (Loasaceae). *Syst. Bot.* 36: 711–720. https://doi.org/10.1600/036364411X583673

Strand, A.E., Leebens-Mack, J. & Milligan, G. 1997. Nuclear DNA based markers for plant evolutionary biology. *Molec. Ecol.* 6: 113–118. https://doi.org/10.1046/j.1365-294X.1997.00153.x

Table S2. Comparison of previous classifications of South Andean Loasas sensu Weigend (1997) (*Blumenbachia* Schrad., *Caiophora* C.Presl, *Loasa* Adans., *Scyphanthus* Sweet) with the one proposed in this study.

Urban & Gilg (1900)	Type species of basionym	Weigend (1997)	This study
Blumenbachia	*Blumenbachia insignis* Schrad.	***Blumenbachia*** sect. *Blumenbachia*	***Blumenbachia*** sect. *Blumenbachia*
Caiophora sect. *Angulatae*	*Blumenbachia sylvestris* Poepp.	***Blumenbachia*** sect. *Angulatae*	***Blumenbachia*** sect. *Angulatae*
Caiophora sect. *Bialatae*	*Gripidea scabra* Miers	***Blumenbachia*** sect. *Gripidea*	***Blumenbachia*** sect. *Gripidea*
Caiophora sect. *Bicallosae*	*Blumenbachia arechavaletae* Urb.	***Caiophora*** sect. *Bicallosae*	***Caiophora***
Caiophora sect. *Dolichocarpae*	*Loasa contorta* Desr. ex Lam.	***Caiophora*** sect. *Caiophora*	***Caiophora***
Caiophora sect. *Orthocarpae* ser. *Pentamerae*	*Loasa coronata* Gillies ex Arn.	***Caiophora*** sect. *Orthocarpae*	***Caiophora***
Caiophora sect. *Orthocarpae* ser. *Pleiomerae*	*Loasa chuquitensis* Meyen	***Caiophora*** sect. *Orthocarpae*	***Caiophora***
Caiophora sect. *Platypetalae*	*Illairea canarinoides* Lenné & K. Koch	***Caiophora*** sect. *Caiophora*	***Caiophora***
Loasa sect. *Euloasa* ser. *Acanthifoliae*	*Loasa acanthifolia* Lam.	***Loasa*** ser. *Loasa*	***Loasa*** ser. *Loasa*
Loasa sect. *Euloasa* ser. *Acaules*	*Loasa lateritia* Gillies ex Arn.	***Loasa*** ser. *Acaules*	***Grausa*** gen. nov. (p.p.)
Loasa sect. *Euloasa* ser. *Deserticolae*	*Loasa elongata* Hook. & Arn.	***Loasa*** ser. *Deserticolae*	***Loasa*** ser. *Deserticolae*
Loasa sect. *Euloasa* ser. *Floribundae*	*Loasa floribunda* Hook & Arn.	***Loasa*** ser. *Floribundae*	***Loasa*** ser. *Floribundae*
Loasa sect. *Euloasa* ser. *Macrospermae*	*Loasa nitida* Lam.	***Loasa*** ser. *Macrospermae*	***Loasa*** ser. *Macrospermae*
Loasa sect. *Euloasa* ser. *Malesherbioideae*	*Loasa malesherbioide*s Phil.	***Loasa*** ser. *Malesherbioideae*	***Presliophytum*** (p.p.)
Loasa sect. *Euloasa* ser. *Pinnatae*	*Loasa volubilis* Juss.	***Loasa*** ser. *Pinnatae*	***Pinnasa*** gen. nov.
Loasa sect. *Euloasa* ser. *Volubiles*	*Loasa micrantha* Poepp.	***Loasa*** ser. *Volubiles*	***Grausa*** gen. nov. (p.p.)
Scyphanthus	*Scyphanthus elegans* Sweet	***Scyphanthus***	***Scyphanthus***

Fig. S1. Maximum likelihood tree based on the ITS dataset. ML bootstrap support values are indicated above branches and Bayesian posterior probabilities are indicated below; only values above 50 and 0.5, respectively, are shown. Currently accepted generic and infrageneric names are indicated in the first column next to species names, with those in grey referring to *Loasa*. Schematic representations of fruit structure for major clades of SAL and related groups are depicted in the middle column. Pedicels of exclusively pendulous fruits point to the top of the page. Proposed generic and infrageneric delimitations in SAL are shown as bars in the far right column. The dark grey bars represent new delimitations that differ from those currently accepted. Abbreviations: ANG, sect. *Angulatae*; BLU, sect. *Blumenbachia*; DES, ser. *Deserticolae*; FLO, ser. *Floribundae*; GRA, *Grausa* gen. nov.; LOA, ser. *Loasa*; MAC, ser. *Macrospermae*; MAL, ser. *Malesherbioideae*; PIN, ser. *Pinnatae*; PNS, *Pinnasa* gen. nov.; PRE, *Presliophytum*; SCY, *Scyphanthus*; VOL, ser. *Volubiles* and ser. *Acaules*. (a) and (b) indicate different accessions for the respective species.



APPENDIX B (The generic affinity of *Caiophora pulchella* (Loasaceae, Loasoideae))

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THE GENERIC AFFINITY OF *CAIOPHORA PULCHELLA* (LOASACEAE, LOASOIDEAE)

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Abstract. Acuña, R. & M. Weigend. 2018. The generic affinity of *Caiophora pulchella* (Loasaceae, Loasoideae). *Darwiniana*, nueva serie 6(1): 94-98.

The history of the nomenclatural changes and past hypotheses on the relationships of *Caiophora pulchella* are presented. The identity of voucher specimens, identified as *C. nivalis* in previous phylogenetic studies, is reassessed based on fruit morphology. The validity of *C. pulchella* is reconsidered and can be confirmed as a member of the genus *Caiophora* based on both morphological and molecular data. Alternative generic placements are rejected.

Keywords. Argentina; *Caiophora*; *Loasa*; Mendoza; morphology; phylogeny; San Juan.

Resumen. Acuña, R. & M. Weigend. 2018. La afinidad genérica de *Caiophora pulchella* (Loasaceae, Loasoideae). *Darwiniana*, nueva serie 6(1): 94-98.

Se presentan la historia nomenclatural e hipótesis previas acerca de las relaciones de *Caiophora pulchella* (Loasaceae, Loasoideae). La identidad de especímenes "voucher", identificados como *C. nivalis* en estudios filogenéticos previos, es reevaluada de acuerdo a la morfología de los frutos. La validez de *C. pulchella* es reconsiderada y se confirma como una especie de *Caiophora* de acuerdo a evidencias morfológicas y moleculares. Su vinculación alternativa en otros géneros es rechazada.

Palabras clave. Argentina; *Caiophora*; filogenia; *Loasa*; Mendoza; morfología; San Juan.

INTRODUCTION

Caiophora pulchella Urb. & Gilg is a very distinctive species of *Caiophora* C.Presl., endemic to the Provinces of San Juan and Mendoza in west-central Argentina (Pérez-Moreau & Crespo, 2003). Although considered part of *Caiophora* even before its valid description (Urban & Gilg in Kurtz, 1893; Urban & Gilg, 1900; Sleumer, 1955), its placement in this genus was called into doubt by Pérez-Moreau & Crespo (1992), the first botanists to describe and study this species' unusual fruits. The new combination *Loasa pulchella* (Urb. & Gilg) R.L.Pérez-Mor. & Crespo was created and the species reconsidered as probably related to *Loasa* Adans. ser. Loasa (≡ Acanthifoliae Urb. & Gilg). The same authors used this new name in posterior works (Pérez-Moreau & Crespo, 2003). In their excellent recent revision of the Argentinean species of *Caiophora*, Slanis et al. (2016) excluded *C. pulchella*, following the same recommendation, considering it as part of *Loasa*. Conversely, Weigend (1997), Weigend & Ackermann (2003), and Weigend et al. (2008) retain this species in *Caiophora*, considering it as a close ally of *C. nivalis* Lillo.

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Recent molecular analyses of the "South Andean Loasas" (defined as the group that includes the genera *Blumenbachia* Schrad. *Caiophora*, *Loasa* and *Scyphanthus* Sweet, as well as recently segregated *Grausa* Weigend & R.H.Acuña and *Pinnasa* Weigend & R.H.Acuña) identified the major clades within this speciose group, mostly restricted to temperate South America and the High Andes (Strelin et al., 2017; Acuña et al., 2017). However, the affinities of *Caiophora pulchella* were not discussed in these works. The goal of this study is to evaluate and clarify the affinities of *C. pulchella* based on both morphological and molecular evidence.

MATERIALS AND METHODS

Detailed examination of preserved specimens of *Caiophora pulchella* and *C. nivalis* (including extant type material) was carried out on specimens or photographs of specimens deposited at the herbaria BM, CORD, F, L, LIL, P and SI (Thiers, 2017).

RESULTS

A re-examination of herbarium specimens of *Caiophora* from the Provinces of San Juan and Mendoza revealed that the vouchers *Cocucci et al. 2219* (CORD) and *Cocucci & Sérsic 4840* (CORD), were incorrectly determined as *C. nivalis* in previous molecular studies (Weigend et al., 2004, as *Cocucci s.n.* = *Cocucci et al. 2219*; Strelin et al., 2017; Acuña et al., 2017). The capsules in these specimens (Fig. 1), even if still mostly immature, are clearly diagnostic and typical of *Caiophora pulchella* (Pérez-Moreau & Crespo 1992, Weigend 1997). In *C. pulchella*, the capsules are mostly superior and dehiscent above the sepals (Fig. 1), something unique in *Caiophora*, while in the morphologically similar *C. nivalis*, as in the rest of the genus, these are mostly inferior and dehiscent below the sepals (Slanis et al., 2016: Figs. 24E, 25F). The collection locality of these specimens is likewise in agreement with the known geographic range of *C. pulchella*, not with *C. nivalis* (known only from the Provinces of Jujuy, Salta, Catamarca and Tucumán), which has not been recorded from the more southerly Province of Mendoza. (Weigend et al., 2008; Slanis et al., 2016).

Weigend et al. (2004), Strelin et al. (2017) and Acuña et al. (2017) all reach similar conclusions in their analyses: *Cocucci et al. 2219* and *Cocucci & Sérsic 4840*, and by extension *Caiophora pulchella*, are retrieved unequivocally as part of a monophyletic and highly supported *Caiophora*, both based on plastid and nuclear markers. Although the relationships within the genus are not fully resolved, trees from both Strelin et al. (2017) and Acuña et al. (2017) indicate that *C. pulchella* may represent an early diverging branch in the genus, agreeing with the interpretation of the morphological evidence by Weigend et al. (2005) mostly regarding the characteristic seeds of the species.

There are a range of similarities shared between *Caiophora pulchella* and *C. nivalis*, such as habitat (from areas above the tree line usually at altitudes well above 2000 m a.s.), growth habit (rosulate herbs < 10 cm in height, with well-developed rhizomes), leaf morphology (leaves < 10 cm long, with pinnatifid to pinnate blades) and floral morphology (flowers axillary, erect and solitary; sepals reduced and inconspicuous; corollas white, half to full spreading; nectar scales contrastingly coloured yellow to orange, with three dorsoventrally flattened dorsal threads, attached to the distal-most third of the scale's back). On the other hand, other putative, close relatives to *C. pulchella* such as *Loasa* series Loasa (Pérez-Moreau & Crespo, 1992) are not just only distantly related to *C. pulchella* based on molecular evidence (Strelin et al., 2017; Acuña et al., 2017), but also differ in ecology and morphology, as both *L. acanthifolia* Lam. and *L. sclareifolia* Juss. inhabit forested (or formerly forested) habitats at lower elevations (usually below 2000 m), and are very robust biennial or perennial herbs often well over 1 m tall, with pinnately lobed, large (up to ca. 30 cm) simple leaves, deflexed flowers with conspicuous and well developed sepals, yellow to red corollas, mostly red nectar scales with flag shaped (laterally expanded and obovoid in shape) dorsal threads and large, globose seeds with shallowly reticulate testas (instead of deeply pitted as in *Caiophora*, Weigend et al., 2004, 2005; Acuña et al. 2017). Another group formerly included in *Loasa* that bears a superficial resemblance to *C. pulchella* (and often sharing similar habitat and habit), is the genus *Pinnasa* (≡ *Loasa* ser. Pinnatae Urb. & Gilg). However, no author has suggested a close relationship between both taxa and the phylogenetic data retrieve them as only distantly related (Weigend et al., 2004; Strelin et al., 2017; Acuña et al., 2017): *C. pulchella* is more closely related to other *Caiophora* than to *Pinnasa*. Morphologically, *Pinnasa* differs from *C. pulchella* in the virtual abscence of stinging trichomes, deeply serrate to laciniate petal margins (entire in *C. pulchella* and *C. nivalis*) and mostly inferior capsules opening solely with apical valves (a combination not found in any *Caiophora*, Urban & Gilg, 1900; Weigend et al., 2004; Acuña et al., 2017). Though not suggested in the literature, the only other plant that could be reasonably confused with *C. pulchella* due to habitat and morphology is *Grausa lateritia* (Gillies ex Arn.) Weigend & R.H.Acuña, in particular the white flowered plants called *Loasa acaulis* (Phil.) Urb. & Gilg. *Grausa lateritia,* however, also lacks stinging trichomes, its sepals are conspicuous, ca. half the length of the petals, its floral scales are the same colour as the petals with dorsal threads that are apically expanded (instead of being linear), its apically dehiscent capsules are mostly inferior and its seeds often have a conspicuous hilar cone that is found only in *Grausa* (Weigend et al., 2004, 2005; Acuña et al., 2017).

Based on the evidence presented here, both molecular and morphological data clearly underscore that *Caiophora pulchella* is indeed part of *Caiophora*. Its closest relative is likely *C. nivalis*, but future molecular studies should investigate these relationships in more detail. Clades included in the traditional concept of *Loasa* are only distantly related to this species.

Representative specimens examined

Caiophora pulchella Urb. & Gilg, Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 76: 272-273. 1900. TYPE: Argentina, Mendoza, Depto. Malargüe, Cumbre entre el Valle Hermoso y el del Río Salado. Oben am Schnee, 18-I-1888, *F. Kurtz 5865* (Lectotype, here designated: CORD 00003381!; B† [photo F Neg. No.10163!]).

ARGENTINA. **San Juan.** Depto. Calingasta, de La Invernada a Las Minitas, 2950-3100 m s.m., 21-II-1988, *Kiesling et al. 6981* (SI); Reserva Estricta El Leoncito: Quebrada de las Vaquitas Muertas (Arroyo y zona de influencia), 25-I-1995, *Apochian et al. 220* (SI); Sierra del Tontal, 3500 m s.m., 22-I-1987, *Kiesling & Meglioli 6538* (SI); Sierra del Tontal al N de Barreal camino a la antena, 3550-3750 m s.m., 5-II-1989, *Kiesling et al. 7350* (SI);del Observatorio El Leoncito al Portezuelo del Tontal, 3300 m s.m., 1-III-1984, *Kiesling 4677* (SI); El Leoncito, Ciénaga de las Cabeceras, 3300 m s.m., 24-II-1999, *Kiesling et al. 9289* (SI); Reserva natural estricta El Leoncito, Quebrada del arroyo Portezuelo, 3730 m s.m., 9-IV-1999, *Haene 1994* (SI). **Mendoza**. Depto. San Carlos, Tres Esquinas, 2200 m s.m., I-1921, *Carette 278* (SI); Depto. Las Heras, Cordillera del Tigre, 3000 m s.m., 15-XII-1927, *King 328* (BM). Depto. Luján de Cuyo, Vallecitos cerca del refugio Club de Regatas Mendoza, 3020 m s.m., 11-I-2003, *Cocucci et al. 2219* (CORD); dentro del Centro de Esquí Vallecitos, 3074 m s.m., 20-I-1998, *Herrera & Jiménez 477* (SI). Depto. Las Heras, Vallecitos, 18-I-2012, *Cocucci & Sérsic 4840* (CORD). Depto. San Carlos, Quebrada del Paso de La Cruz de Piedra, 15-I-1949, *Ruiz Leal 11692* (P); camino a Laguna del Diamante, 3000 m s.m., 27-I-1950, *Araque 1390* (L); camino a la Laguna Diamante, afluente del Arroyo Papagallos, 3-II-1950, *Soriano 4125* (SI); Los Paramillos, camino a Lag. Diamante, 23-I-1989, *Gómez-Sosa 343* (SI).

Caiophora nivalis Lillo, Prim. Reun. Nac. Soc. Argent. Cien. Nat. [Tucuman, 1916] Secc. 3, Bot.: 229. 1919. TYPE: Argentina, Tucumán, Depto. Tafí del Valle, Cumbres Calchaquíes, lagunas, campo en la cima, 4700 m s. m., 4-II-1903, *M. Lillo 3090* (Holotype LIL-78055 [000993]!).

ARGENTINA. **Jujuy.** Depto. Tilcara, Omgeving van Tilcara, 2500 m s.m., 1953, *Sleumer s.n.* (L). **Salta.** Depto. Cafayate (?), La Laguna, Cerro del Cajón, 2900 m s.m., 25-I-1914, *Rodríguez 1314* (SI). **Tucumán.** Depto. Tafí del Valle, El Pelado, habita faldas y peñas, 4000 m s.m., 19-III-1912, *Rodríguez 579* (SI); Quebrada Honda, 3800 m s.m., 28-I-1952, *Sparre et al. 9395* (L); Cumbres Calchaquíes, Huaca Huasi, alrededores laguna Nostra, suelo arenoso, 4300 m s.m., 13-III-1984, *Gómez-Sosa & Múlgura 179* (SI).

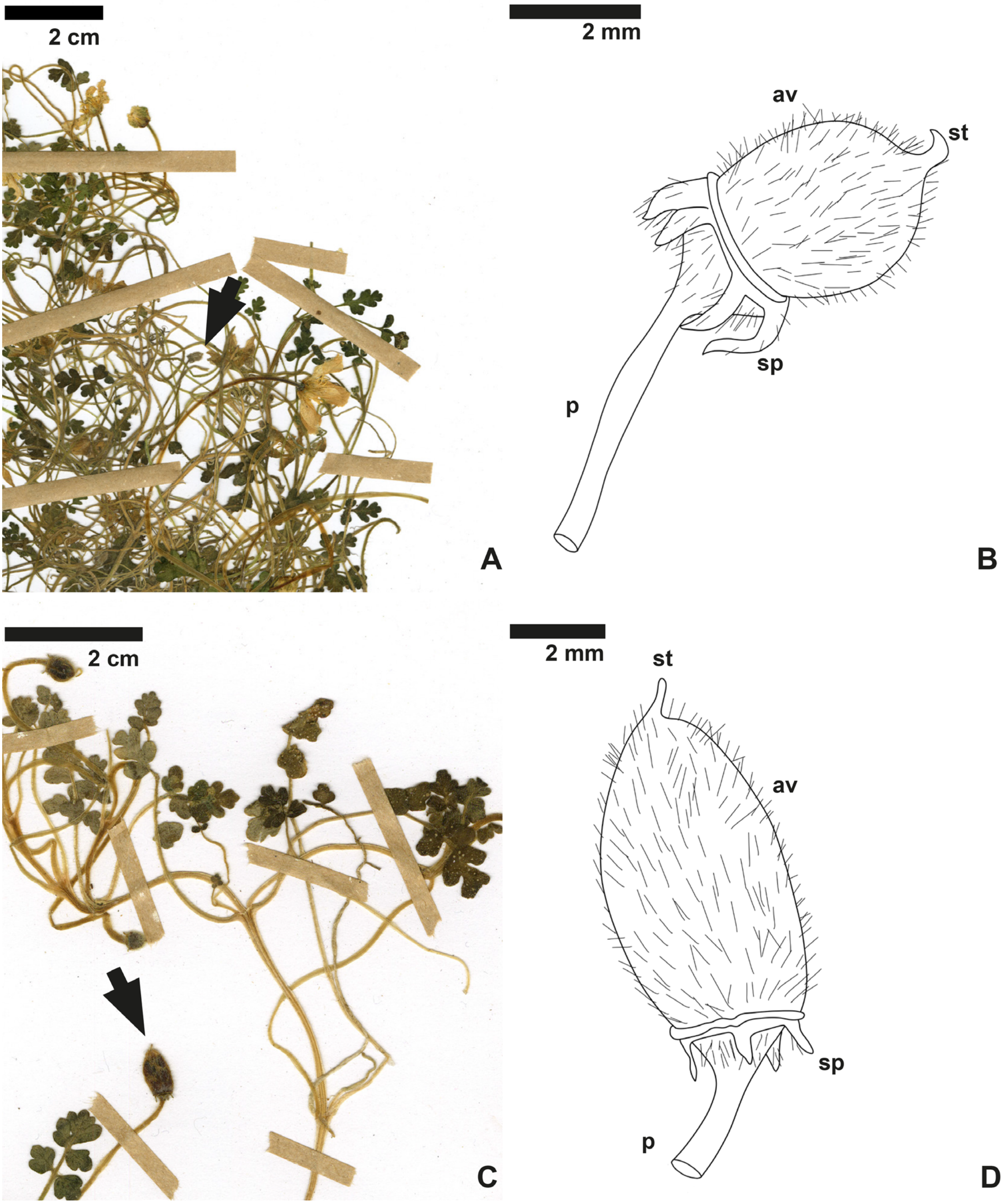

Fig. 1. Voucher specimens of *Caiophora pulchella* used in the molecular studies of Weigend et al. (2004), Strelin et al. (2017) and Acuña et al. (2017). **A,** *Cocucci & Sérsic 4840* (CORD), overview. **B,** detail of an immature capsule. **C,** *Cocucci et al. 2219* (CORD), overview. **D,** detail of an immature capsule. Abbreviations: **av**, apical valve region; **p**, pedicel; **sp**, sepal; **st**, persistent style. Color version at http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/780/755

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BIBLIOGRAPHY

Acuña, R.; S. Fließwasser; M. Ackermann; T. Henning; F. Luebert & Weigend, M. 2017. Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae).*Taxon* 66: 365-378. DOI: 10.12705/662.5

Kurtz, F. 1893. Dos viajes botánicos al Río Salado superior (Cordillera de Mendoza). *Boletín de la Academia Nacional de Ciencias en Córdoba (República Argentina)* 13: 171-210

Pérez-Moreau, R. L. & S. Crespo. 1992. Notas sobre Loasaceae IV. *Loasa pulchella* nueva combinación. *Hickenia* 2(14): 67-68.

Pérez-Moreau, R. L. & S. Crespo. 2003. Loasaceae, in R. Kiesling (ed.), *Flora de San Juan Volumen 2,* pp. 152–160. Buenos Aires: Estudios Sigma.

Slanis, A. C.; M. C. Perea & A. Grau. 2016. Revisión taxonómica del género *Caiophora* (Loasaceae) para Argentina: *C. sleumerii* una nueva especie. *Darwiniana*, nueva serie 4(2): 138-191. DOI: 10.14522/darwiniana.2016.42.685

Sleumer, H. 1955. Die Loasaceen Argentiniens. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 76: 411-462.

Strelin, M.; J. Arroyo; S. Fließwasser & M. Ackermann. 2017. Diversification of *Caiophora* (Loasaceae subfam. Loasoideae) during the uplift of the Central Andes. *Organisms Diversity and Evolution* 17: 29-41. DOI: 10.1007/s13127-016-0312-4

Thiers, B. [continuously updated, accessed 2017]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium; available from: http://sweetgum.nybg.org/ih/ (accessed Nov 2017).

Urban, I & E. Gilg. 1900. Monographia Loasacearum. *Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curosiorum* 76: 1-368.

Weigend, M. 1997. Names and types in *Caiophora* K.Presl.s.str. (Loasaceae). *Sendtnera* 4: 221-242.

Weigend, M. & M. Ackermann. 2003. Los nombres antiguos en el género *Caiophora* (Loasáceas subfam. Loasoídeas) y una clasificación infragenérica preliminar. *Arnaldoa* 10: 75-94.

Weigend, M.; M. Gottschling; S. Hoot & M. Ackermann. 2004. A preliminary phylogeny of Loasaceae subfam. Loasoideae (Angiospermae: Cornales) based on trnL (UAA) sequence data, with consequences for systematics and historical biogeography. *Organisms Diversity and Evolution* 4: 73-90. DOI: 10.1016/j.ode.2003.12.001

Weigend, M.; J. Grau & M. Ackermann. 2008. Loasaceae, in: F. O. Zuloaga, O. Morrone & M. J. Belgrano (eds.), Catálogo de las Plantas Vasculares del Cono Sur (Argentina, Sur de Brasil, Chile, Paraguay y Uruguay) *Monographs in Systematic Botany from the Missouri Botanical Garden* 107(3): 2413–2424.

Weigend, M.; A. Gröger; & M. Ackermann. 2005. The seeds of Loasaceae subfam. Loasoideae (Cornales) II: Seed morphology of 'South Andean Loasas' (*Loasa*, *Caiophora*, *Scyphanthus* and *Blumenbachia*). *Flora* 200: 569-591. DOI: 10.1016/j.flora.2005.06.009

APPENDIX C

Proposal to conserve the name *Kissenia*, with a correction of the original spelling *Fissenia*, against its homotypic earlier synonym *Cnidone*

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***Kissenia* (as "*Fissenia"*)** R.Br. ex Endl. in Gen. Pl. [Endlicher] Suppl. 2: 76. 1842 [Angiosp.: *Loas*.] nom. cons. prop.
("Herbae capenses et arabicae […] Fissenia R. Brown msc. Cnidone E.Meyer msc. Fenzl Not. msc. (character e sola F. capensi. Cnidone mentzelioide E. Mey.")
Typus (hic designatus): *Kissenia capensis* Endl. ex Harv. (in Thes. Cap. 1: 61, Pl. XCVIII. 1859)
(=) ***Cnidone*** E.Mey. ex Fenzl in Denkschr. Königl.-Baier. Bot. Ges. Regensburg 3: 199. 1841. nom. rej. prop.

There are two species currently accepted as belonging to *Kissenia* R.Br. ex Endl. (Dandy in Kew Bull. 20: 451–453. 1966; Codd in Fl. S. Africa [ed. L.E.W. Codd et al.] 22: 134–136. 1976; Gilbert in Fl. Ethiopia & Eritrea 2(1): 73. 2000). They are morphologically similar but widely disjunct. One species occurs in the arid south-western Africa and the other species in north-eastern Africa and on the southern Arabian peninsula (Dandy in Bull. Misc. Inf. Kew 1926(4): 174–180. 1926). The taxonomic distinctness of the genus was recognized independently by Ernst Heinrich Friedrich Meyer (1791–1858) on herbarium slips and by Robert Brown (1773–1858) in an unpublished manuscript (B.65 24 399) in the 1830s. Both names, Meyer's *Cnidone* and Brown's *Kissenia* were effectively published later. However, *Kissenia* was published with an aberrant spelling, *Fissenia*, by Endlicher (Gen. Pl. [Endlicher] Suppl. 2: 76. 1842). In 1860 (J. Proc. Linn. Soc., Bot. 5 (Suppl. 1): 43), Anderson pointed out that the spelling *Fissenia* was erroneous, as he thought it was based on the name "Kissen", allegedly a collector in Arabia. Indeed, the name *Kissenia* is derived from "Kissen", although it is not a collector but the westernised spelling of a town called Qishn (Al Mahra, Yemen), as Dandy and Exell pointed out after examining Brown's notes (J. Bot. 70: 198–199. 1932).

Closer inspection of the nomenclatural history resulted in the discovery that *Fissenia* is not the oldest available name for the genus. Eduard Fenzl (Denkschr. Königl.-Baier. Bot. Ges. Regensburg 3: 199. 1841) published a description of the genus *Cnidone* based on *Cnidone mentzelioides* by Meyer, who worked on the vast South African collections by Johann Franz (Jean François) Drège (1794–1881). In the same paper, Fenzl announced an upcoming and more detailed description of this genus by Endlicher. Endlicher did describe a new genus based on Drège's material. He was also aware of a manuscript by Fenzl, who was his successor as curator of the *Hofnaturalien-Cabinette* (today: Naturhistorisches Museum Wien) after 1839 (Stearn in J. Arn. Arb. 28(4): 424–429. 1947). However, Endlicher apparently learned about another, older name for the same plant through Arnott (J. Bot. (Hooker) 3: 259. 1841). Arnott pointed out that, according to George Bentham (1800–1884), the two unpublished names "*Cnidone mentzelioides* E.Mey." and "*Fissenia arabica* R.Br." describe plants that are so similar that it would be best to consider them as belonging to the same genus. Apparently, Endlicher (1842) adopted Arnott's note and changed *Cnidone* to Arnott's erroneously spelt *Fissenia*, probably unaware of Fenzl's publication (1841) in the meantime. Fenzl's *Cnidone* – with priority over *Kissenia* – was subsequently forgotten. None of the three species names published under *Kissenia* was ever combined with *Cnidone.* Therefore, application of the rule of priority would result in the need to create new combinations for both currently accepted species names: *Kissenia capensis* Endl. ex Harv. (in Thes. Cap. 1: 61, Pl. XCVIII. 1859) and *Kissenia arabica* Arn. ex Chiov. (Fl. Somala 1: 174–175. 1929). Additionally, Arnott's publication of the mere names (*nom. nud.*) proves that the aberrant spelling was not Endlicher's mistake but either Arnott's or Bentham's. Endlicher's original name *Fissenia* was therefore intentional and not a typographical error on his side. In order to retain stability, however, we also propose to conserve Anderson's corrected spelling *Kissenia.* It is 1) the one Brown intended and 2) the one mainly in use in the literature since then, despite Anderson's incorrect etymological derivation.

APPENDIX D (Chapters 6 & 7: Major lineages of Loasaceae subfam. Loasoideae diversified along with the Andean uplift & Diversification patterns of *Nasa* (Loasaceae subfam. Loasoideae) in the Neotropics)

Table D.1. List of taxa sampled for the molecular analyses with their respective voucher specimen (herbarium acronyms in parentheses), geographic origins and GenBank accession numbers. Dashes (–) indicate missing data.

Taxon	Voucher	County of Origin	GenBank Codes			
			trnL-trnF	*matK*	*trnS-trnG*	*rps16*
Alangium platanifolium var. *trilobum* (Siebold & Zucc.) Harms	*Acuña 1208* (BONN)	Germany (cultivated)				
Aosa grandis (Standl.) R.H.Acuña & Weigend	*Acuña et al. 1264* (USJ)	Costa Rica	MF972120	MF972100	MF972129	MF972110
Aosa parviflora (Schrad. ex DC.) Weigend	*Grant 4650* (BSB)	Brazil	KY286967	KY286698	KY286877	KY286787
Aosa plumieri (Urb.) Weigend	*Acevedo-Rodríguez et al. 13065* (US)	Dominican Republic	-	MF972102	MF972131	MF972112
Aosa plumieri (Urb.) Weigend	*Moody et al. 47* (JBSD)	Dominican Republic	AY254225	-	-	-
Aosa rostrata (Urb.) Weigend	*Salino 3042* (M)	Brazil	KY286948	KY286679	KY286858	KY286768
Aosa rupestris (Gardner) Weigend	*Weigend 7138* (BSB)	Brazil	KY286925	KY286657	KY286835	KY286745
Aosa sigmoidea Weigend	*Guedes & al. 10360* (ALCB)	Brazil	KY286995	KY28675	KY286905	KY286815
Aosa uleana Weigend	*Joßberger 342* (BONN)	Brazil	KY286998	KY286728	KY286908	KY286818
Blumenbachia amana T.Henning & Weigend	*Acuña 1204* (BONN)	Brazil	KY287001	KY286731	KY286911	KY286821
Blumenbachia catharinensis Urb. & Gilg	*Trevisan 1723* (BONN)	Brazil	KY287006	KY286736	KY286916	KY286826
Blumenbachia dissecta (Hook. & Arn.) Weigend & Grau	*Weigend & al. 6816* (B)	Argentina	KY286961	KY286692	KY286871	KY286781
Blumenbachia hieronymi Urb.	*Ackermann 601* (BSB)	Germany (cultivated)	KY286966	KY286697	KY286876	KY286786
Blumenbachia insignis Schrad.	*Weigend 7475* (B)	Germany (cultivated)	KY286964	KY286695	KY286874	KY286784
Blumenbachia latifolia Cambess.	*Schwabe s.n.* (B)	Brazil	KY286949	KY286680	KY286859	KY286769
Blumenbachia prietea Gay	*Weigend & al. 6823* (B)	Argentina	KY286963	KY286694	KY286873	KY286783,
Blumenbachia	*Lombardi &*	Brazil	MF972121	MF972101	MF972130	MF972111

scabra (Miers) Urb.	*Saka 8631* (UPCB)					
Blumenbachia sylvestris Poepp.	*Cocucci & Sérsic 4780* (CORD)	Argentina	KY286969	KY286700	KY286879	KY286789
Caiophora aconquijae Sleumer	*Strelin AAC-5482* (CORD)	Argentina	KY286974	KY286705	KY286884	KY286794
Caiophora andina Urb. & Gilg	*Moreira & Luebert 2379* (SGO)	Chile	KY287005	KY286735	KY286915	KY286825
Caiophora arechavaletae (Urb.) Urb.	*Weigend 9330* (BSB)	Brazil	KY286970	KY286701	KY286880	KY286790
Caiophora boliviana Urb. & Gilg	*Badcock 619* (K)	Bolivia	KY286955	KY286686	KY286865	KY286775
Caiophora canarinoides (Lenné & K.Koch) Urb. & Gilg	*Ackermann 375* (BSB)	Peru	KY286975	KY286706	KY286885	KY286795
Caiophora carduifolia C.Presl	*Ackermann & Kollehn 288* (BSB)	Peru	KY286939	KY286671	KY286849	KY286759
Caiophora cernua (Griseb.) Urb. & Gilg ex Kurtz	*Ackermann 1100* (BONN)	Argentina	KY286972	KY286703	KY286882	KY286792
Caiophora cf. *buraeavii*	*Grant & al. 10-4619* (BSB)	Peru	KY286981	KY286712	KY286891	KY286801
Caiophora chuquisacana Urb. & Gilg	*Ritter & Wood 1498* (LPB)	Bolivia	KY286982	KY286713	KY286892	KY286802
Caiophora chuquitensis (Meyen) Urb. & Gilg	*Ackermann 1101* (BONN)	Argentina	KY286983	KY286714	KY286893	KY286803
Caiophora cinerea Urb. & Gilg	*Weigend & al. 5715* (BSB)	Peru	KY286953	KY286684	KY286863	KY286773
Caiophora cirsiifolia C.Presl	*Ackermann 610* (BSB)	Peru	KY286984	KY286715	KY286894	KY286804
Caiophora clavata Urb. & Gilg	*Ackermann 1102* (BONN)	Argentina	KY287002	KY286732	KY286912	KY286822
Caiophora contorta (Desr. ex Lam.) C.Presl	*Weigend & Brokamp 9110* (BSB)	Ecuador	KY286985	KY286716	KY286895	KY286805
Caiophora coronata (Gillies ex Arn.) Hook. & Arn.	*Cocucci & Sersic 4845* (CORD)	Argentina	KY286973	KY286704	KY286883	KY286793
Caiophora dederichiorum Mark.Ackermann & Weigend	*Henning & Schulz 19* (BSB)	Peru	KY286977	KY286708	KY286887	KY286797
Caiophora deserticola Weigend & Mark.Ackermann	*Muñoz-Schick 4296* (BSB)	Chile	KY286952	KY286683	KY286862	KY286772
Caiophora dumetorum Urb. & Gilg	*Strelin AAC-5481* (CORD)	Argentina	KY286986	KY286717	KY286896	KY286806
Caiophora grandiflora (G.Don)	*Henning & Brokamp 3* (BSB)	Peru	KY286987	KY286718	KY286897	KY286807

Weigend & Mark.Ackermann						
Caiophora hibiscifolia (Griseb.) Urb. & Gilg	*Ackermann 1103* (BONN)	Argentina	KY286988	KY286719	KY286898	KY286808
Caiophora lateritia Klotzsch	*Ackermann 1104* (BONN)	Argentina	KY286989	KY286720	KY286899	KY286809
Caiophora madrequisa Killip	*Weigend & Weigend 2000/440* (HUSA)	Peru	KY286954	KY286685	KY286864	KY286774
Caiophora pentlandii (Paxton ex Graham) G.Don ex Loudon	*Ackermann 360* (BSB)	Peru	KY286938	KY286670	KY286848	KY286758
Caiophora pterosperma (Ruiz & Pav. ex G.Don) Urb. & Gilg	*Weigend & al. 5484* (BSB)	Peru	KY286940	KY286672	KY286850	KY286760
Caiophora pulchella Urb. & Gilg	*Cocucci & Sérsic 4840* (CORD)	Argentina	KY286976	KY286707	KY286886	KY286796
Caiophora rosulata subsp. *taraxacoides* (Killip) Weigend & Mark.Ackermann	*Schlumpberger & Brokamp 675* (BSB)	Bolivia	KY287004	KY286734	KY286914	KY286824
Caiophora rusbyana Urb. & Gilg ex Rusby	*Schlumpberger & Brokamp 627* (BSB)	Bolivia	KY287003	KY286733	KY286913	KY286823
Caiophora stenocarpa Urb. & Gilg	*Ackermann & al. 758* (BSB)	Peru	KY286978	KY286709	KY286888	KY286798
Camptotheca acuminata Decne.	*Acuña1212* (BONN)	Germany (cultivated)				
Carpenteria californica Torr.	*Acuña 1210* (BONN)	Germany (cultivated)				
Cevallia simuata Lag.	*Spencer 418* (RSA)	Mexico		-		
Cevallia simuata Lag.	*Waterbrook 175* (WS)		-	AF503301	-	-
Cornus alternifolia L.f.	*Acuña 1213* (BONN)	Germany (cultivated)				
Cornus canadensis L.	*Acuña 1214* (BONN)	Germany (cultivated)				
Cornus cf. *walteri*	*Acuña 1222* (BONN)	Germany (cultivated)				
Cornus disciflora Moc. & Sessé ex DC.	*Acuña et al. 1239* (USJ)	Costa Rica				
Cornus kousa var. *chinensis* (Osborn) Q.Y.Xiang	*Acuña 1216* (BONN)	Germany (cultivated)				
Cornus mas L.	*Acuña 1207* (BONN)	Germany (cultivated)				
Cornus officinalis Siebold & Zucc.	*Acuña 1217* (BONN)	Germany (cultivated)				
Cornus	*Acuña et al.*	Costa Rica				

peruviana J.F.Macbr.	*1230* (USJ)					
Curtisia dentata (Burm.f.) C.A.Sm.	*Acuña 1218* (BONN)	Germany (cultivated)				
Davidia involucrata var. *vilmoriniana* (Dode) Holub	*Acuña 1202* (BONN)	Germany (cultivated)				
Deutzia discolor Hemsl.	*Weigend 5615* (BSB)	Germany (cultivated)	KY286929	KY286661	KY286839	KY286749
Deutzia rubens Rehder	*Weigend 5613* (BSB)	Germany (cultivated)	KY286928	KY286660	KY286838	KY286748
Diplopanax stachyanthus Hand.-Mazz.	*Acuña 1203* (BONN)	Germany (cultivated)				
Eucnide urens Parry ex Coville	*Weigend 9153* (BSB)	United States	KY286996	KY286726	KY286906	KY286816
Fendlera rupicola Engelm. & A.Gray	*Acuña 1220* (BONN)	Germany (cultivated)				
Fendlerella utahensis (S.Watson) A.Heller	*Acuña 1221* (BONN)	Germany (cultivated)				
Grausa gayana (Urb. & Gilg) Weigend & R.H.Acuña	*Weigend et al. 7057* (MSB)	Chile	KY286962	KY286693	KY286872	KY286782
Grausa lateritia (Gillies ex Arn.) Weigend & R.H.Acuña	*Werdermann 1342* (M)	Chile	KY286941	-	KY286851	KY286761
Grausa lateritia (Gillies ex Arn.) Weigend & R.H.Acuña	*Marticorena Mattei* 930(F)	Chile	-	AY781453	-	-
Grausa micrantha (Poepp.) Weigend & R.H.Acuña	*Grau s.n.* (M)	Chile	KY286957	KY286688	KY286867	KY286777
Gronovia scandens L.	*Jiménez & Majure 2895* (USJ)	Costa Rica				
Huidobria chilensis Gay	*Ackermann 490* (BSB)	Chile	KY286931	KY286663	KY286841	KY286751
Huidobria fruticosa Phil.	*Luebert & Moreira 2991* (SGO)	Chile				
Hydrangea ampla (Chun) Y.De Smet & Granados	*Acuña 1201* (BONN)	Germany (cultivated)				
Hydrangea arborescens L.	*Acuña 1205* (BONN)	Germany (cultivated)				
Hydrangea caerulea (Stapf) Y.De Smet & Granados	*Acuña 1219* (BONN)	Germany (cultivated)				
Hydrangea oerstedii Briq.	*Acuña et al. 1231* (USJ)	Costa Rica				
Jamesia americana Torr. & A.Gray	*Weigend 5610 (*BSB)	Germany (cultivated)				
Kirengeshoma palmata Yatabe	*Acuña 1206* (BONN)	Germany (cultivated)				
Kissenia arabica R.Br.	*Kilian & Oberprieler YP*	Yemen				

ex Chiov.	*2008* (B)					
Kissenia capensis Endl.	*Greuter 21627* (B)	South Africa	KY286944	KY286675	KY286854	KY286764
Klaprothia fasciculata (C.Presl) Poston	*Weigend et al. 7553* (B)	Peru				
Klaprothia mentzelioides Kunth	*Acuña et al. 1227* (USJ)	Costa Rica				
Loasa acanthifolia Desr.	*Weigend et al. 6924* (M)	Argentina	KY286959	KY286690	KY286869	KY286779
Loasa acerifolia Dombey ex Juss.	*Weigend et al. 6848* (M)	Argentina	KY286937	KY286669	KY286847	KY286757
Loasa elongata Hook. & Arn.	*Weigend 9333* (BSB)	Chile	KY287000	KY286730	KY286910	KY286820
Loasa floribunda Hook. & Arn.	*Weigend et al. 5937* (BSB)	Chile	KY286951	KY286682	KY286861	KY286771
Loasa heterophylla Hook. & Arn.	*Weigend et al. 5920* (BSB)	Chile	KY286930	KY286662	KY286840	KY286750
Loasa illapelina Phil.	*Ackermann 519* (BSB)	Chile	KY286950	KY286681	KY286860	KY286770
Loasa insons Poepp.	*Ackermann 536* (BSB)	Chile	KY286943	KY286674	KY286853	KY286763
Loasa nitida Lam.	*Weigend & al. 7346* (BSB)	Peru	KY286936	KY286668	KY286846	KY286756
Loasa pallida Gillies ex Arn.	*Ackermann 1319* (BONN)	Chile	KY286991	KY286722	KY286901	Y286811
Loasa sclareifolia Juss.	*Weigend* 8183 (B)	Chile	KY286994	KY286724	KY286904	KY286814
Loasa tricolor Ker Gawl.	*Luebert 3021* (B)	Chile	KY286979	KY286710	KY286889	KY286799
Loasa triloba Dombey ex Juss.	*Luebert & Bidart 3014b* (B)		KY286999	KY286729	KY286909	KY286819
Mentzelia albescens (Gillies ex Arn.) Benth. & Hook.f. ex Griseb.	*Weigend et al. 6865* (BSB)	Argentina	KY286921	KY286653	KY286831	KY286741
Mentzelia aspera L.	*Weigend et al. 8421* (B)	Peru	MF972116	MF972096	MF972125	MF972106
Mentzelia decapetala (Pursh) Urb.	*T.Joßberger s.n.* (BONN)	Germany (cultivated)				
Mentzelia parvifolia Urb. & Gilg ex Kurtz	*Weigend et al. 8361* (B)	Peru				
Mentzelia scabra subsp. *chilensis* (Gay) Weigend	*Weigend & al. 98/470 (F)*	Peru	KY286922	KY286654	KY286832	KY286742
Nasa aequatoriana (Urb. & Gilg) Weigend	*Weigend & Jaramillo 3937* (F)	Ecuador	KY286947	KY286678	KY286857	KY286767
Nasa argemonoides (Juss.) Weigend	*Weigend s.n.* (M)	Colombia				
Nasa auca (Weigend) Weigend	*Boysen-Larsen & Eriksen 45376* (AAU)	Ecuador				
Nasa basilica T.Henning & Weigend	*Weigend & al. 97/370* (F)	Peru	KY286935	KY286667	KY286845	KY286755

Nasa bicornuta (Weigend) Weigend	*Weigend et al. 8600* (MO)	Peru				
Nasa carnea (Urb. & Gilg) Weigend	*Weigend & Dostert 98/117* (M)	Peru				
Nasa carunculata (Urb. & Gilg) Weigend	*Weigend et al. 5091* (BSB)	Peru				
Nasa chenopodiifolia (Desr.) Weigend	*Weigend et al. 7685* (M)	Peru	MF972162	MF972134	MF972176	MF972148
Nasa contumazensis Weigend & E.Rodr.	*Henning & Schulz 35* (USM)	Peru				
Nasa dillonii Weigend	*Weigend 7556* (B)	Peru	MF972160	MF972132	MF972174	MF972146
Nasa driesslei Weigend	*Henning & Schneider 243* (BSB)	Peru	KY286917	KY286649	KY286827	KY286737
Nasa dyeri subsp. *australis* Dostert & Weigend	*Dostert 98/80* (MSB)	Peru	MF972165	MF972137	MF972179	MF972151
Nasa ferruginea (Urb. & Gilg) Weigend	*Weigend &. Weigend 2000/199* (HUSA)	Peru				
Nasa formosissima Weigend	*Henning & Brokamp 13* (B)	Peru				
Nasa grandiflora (Desr.) Weigend	*Weigend s.n.* (BONN)	Ecuador				
Nasa herzogii (Urb. & Gilg) Weigend	*Müller & Heinrichs 6596* (LPB)	Bolivia				
Nasa hornii (Weigend) Weigend	*Weigend & Horn 3815* (M)	Ecuador				
Nasa humboldtiana subsp. *glandulifera* Weigend & T.Henning	*Weigend et al. 8543* (B)	Peru				
Nasa humboldtiana subsp. *obliqua* Dostert & Weigend	*Henning & Schulz 40* (BSB)	Peru				
Nasa humboldtiana subsp. *roseoalba* (Weigend) Dostert	*Weigend & Horn 3812* (M)	Ecuador				
Nasa humboldtiana subsp. *subtrifoliata* Weigend & T.Henning	*Weigend et al. 8622* (USM)	Peru				
Nasa insignis Weigend & E.Rodr.	*Weigend et al. 7563* (M)	Peru				
Nasa jungiifolia (Weigend)	*Weigend & Horn 3838* (M)	Ecuador				

Weigend						
Nasa lambayequensis Weigend	*Weigend et al. 9669* (BONN)	Peru				
Nasa laxa (J.F.Macbr.) Weigend	*Henning & Schulz 29* (B)	Peru				
Nasa lenta (J.F.Macbr.) Weigend	*Weigend et al. 2001/446* (BSB)	Peru				
Nasa limata (J.F.Macbr.) Weigend	*Weigend & Weigend 2000/382* (HUSA)	Peru				
Nasa loxensis (Kunth) Weigend	*Grant & Struwe 01-4063* (BSB)	Ecuador				
Nasa macrothyrsa (Urb. & Gilg) Weigend	*Weigend et al. 97/s.n.* (M)	Peru	KY286934	KY286666	KY286844	KY286754
Nasa magnifica (Urb. & Gilg) Weigend	*Weigend et al. 97/468* (F)	Peru				
Nasa moroensis Weigend	*Weigend 7694* (B)	Peru	MF972161	MF972133	MF972175	MF972147
Nasa olmosiana (Gilg ex J.F.Macbr.) Weigend	*Weigend et al. 8541* (B)	Peru	MF972171	MF972143	MF972185	MF972157
Nasa otuzcensis Weigend & E.Rodr.	*Rodríguez et al. 2374* (HUT)	Peru				
Nasa pascoensis Weigend	*Foster & Smith 9073* (M)	Peru				
Nasa picta subsp. *pamparomasensis* E.Rodr. & Weigend ex Molinari	*Weigend et al. 7683* (B)					
Nasa picta subsp. *picta* (Hook.) Molinari	*Henning & Schulz 33* (BSB)	Peru				
Nasa poissoniana subsp. *glandulifera* T.Henning & Weigend	*Weigend & Schwarzer 8007* (B)	Peru	MF972172	MF972144	MF972186	MF972158
Nasa poissoniana subsp. *poissoniana* (Urb. & Gilg) Weigend	*Weigend & Weigend 00/208* (NY)	Peru	KY286918	KY286650	KY286828	KY286738
Nasa	*Weigend et al.*	Peru	MF972114	MF972094	MF972123	MF972104

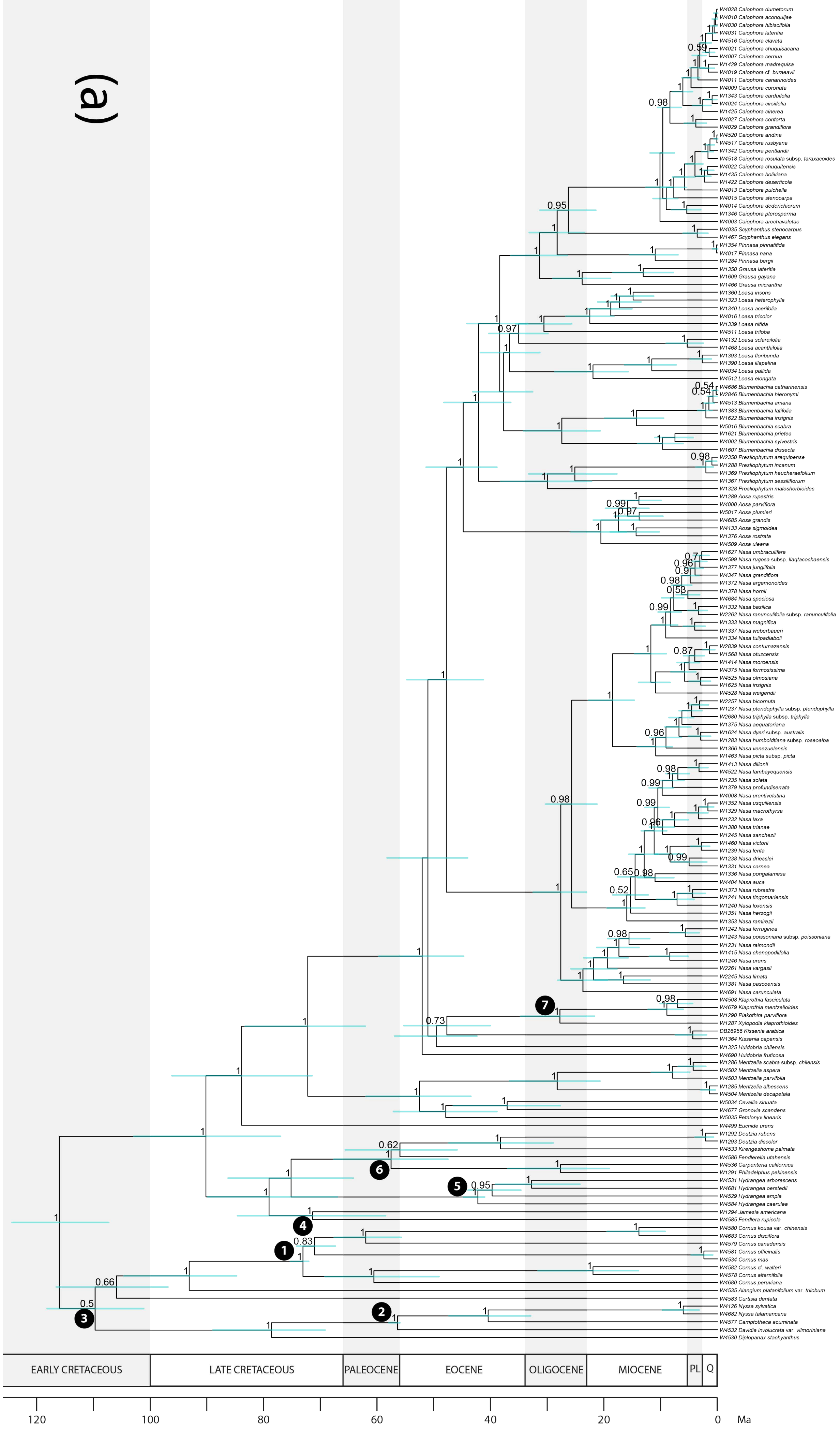
pongalamesa Weigend	*2000/752* (BSB)					
Nasa profundiserrata Weigend	*Weigend et al. 3626* (M)	Colombia				
Nasa pteridophylla subsp. *geniculata* Weigend & Dostert Weigend & Dostert	*Weigend et al. 7552* (B)	Peru				
Nasa pteridophylla subsp. *pteridophylla* Weigend & Dostert	*Weigend et al. 97/307C* (M)	Peru				
Nasa raimondii (Standl. & F.A.Barkley) Weigend	*Weigend & Weigend 2000/289* (HUSA)	Peru	MF972113	MF972093	MF972122	MF972103
Nasa ramirezii (Weigend) Weigend	*Weigend & Ramírez 3523* (M)	Colombia				
Nasa ranunculifolia subsp. *bolivarensis* T.Henning, E.Rodr. & Weigend	*Weigend et al. 2000/816* (USM)	Peru				
Nasa ranunculifolia subsp. *cymbopetala* (Urb. & Gilg) Weigend	*Weigend et al. 97/466* (F)	Peru				
Nasa ranunculifolia subsp. *guzmangoensis* T.Henning, E.Rodr. & Weigend	*Weigend et al. 7639* (F)	Peru				
Nasa ranunculifolia subsp. *macrantha* (Urb. & Gilg) Weigend	*M. Weigend et al. 5660* (B)	Peru				
Nasa ranunculifolia subsp. *macrorrhiza* (Urb. & Gilg) Weigend	*T. Henning & J. Schulz 26* (BSB)	Peru				
Nasa ranunculifolia subsp. *pamparomasii* T.Henning, E.Rodr. & Weigend	*Weigend et al. 7458* (BSB)	Peru				
Nasa ranunculifolia subsp. *patazensis* T.Henning,	*Weigend & Schwarzer 7941* (B)	Peru				

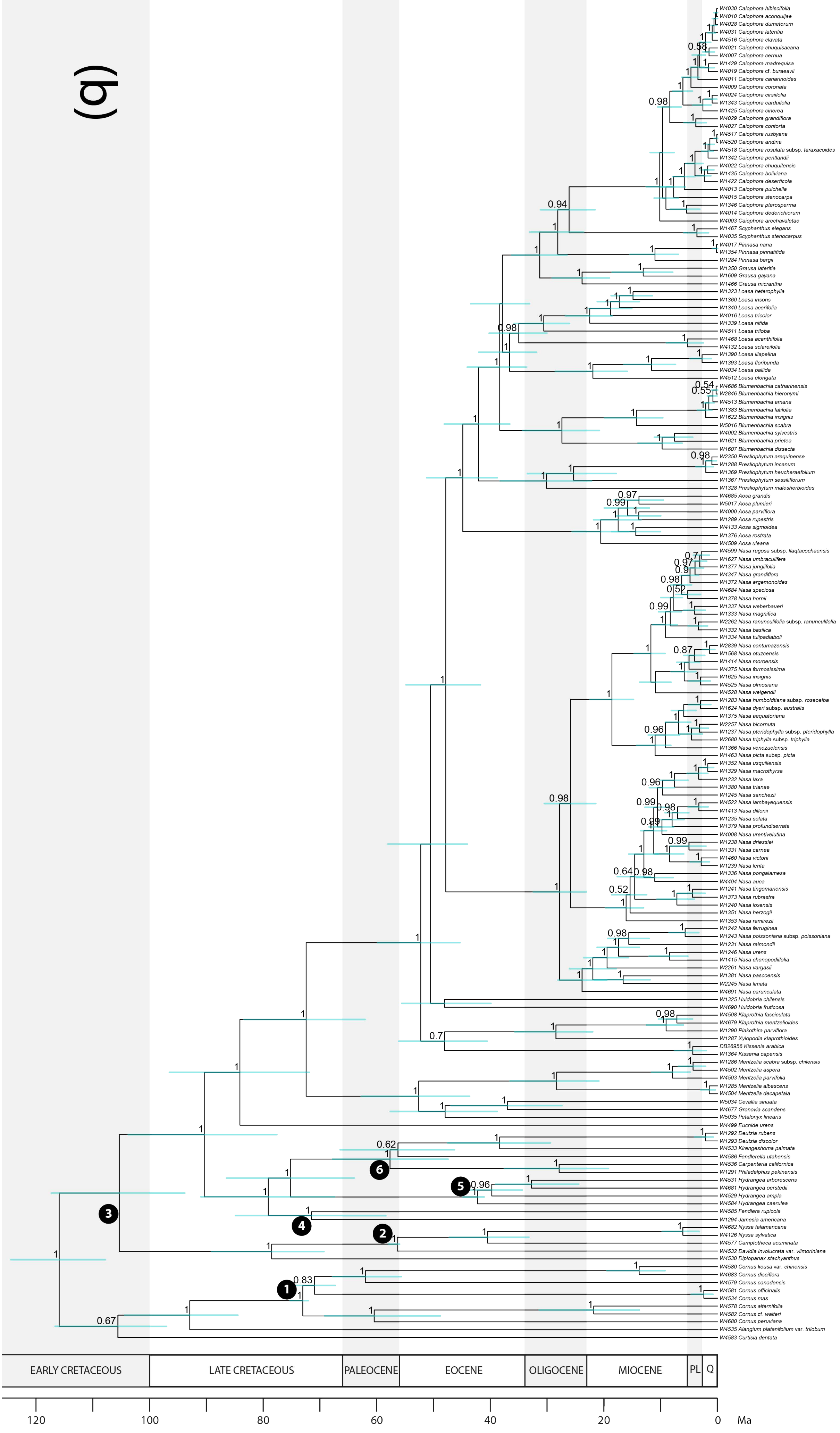
E.Rodr. & Weigend						
Nasa ranunculifolia subsp. *ranunculifolia* (Kunth) Weigend	*Henning 06/05* (B)	Peru	MF972169	MF972141	MF972183	MF972155
Nasa rubrastra (Weigend) Weigend	*Schwerdtfeger 22207* (GOET)	Ecuador				
Nasa rugosa subsp. *llaqtacochaensis* T.Henning, E.Rodr. & Weigend	*Henning & Schneider 303* (B)	Peru				
Nasa sanchezii T.Henning & Weigend	*Henning & Schneider 242* (B)	Peru				
Nasa solata (J.F.Macbr.) Weigend	*Weigend & Dostert 98/259* (F)	Peru				
Nasa sp.	*García 333* (HUT)	Peru				
Nasa speciosa (Donn.Sm.) Weigend	*Acuña et al. 1261* (USJ)	Costa Rica	MF972119	MF972099	MF972128	MF972109
Nasa tingomariensis (J.F.Macbr.) Weigend	*Weigend et al. 5302* (BSB)	Peru				
Nasa trianae (Urb. & Gilg) Weigend	*Weigend et al. 3610* (M)	Colombia				
Nasa triphylla subsp. *elegans* Dostert & Weigend	*Dostert 98/103* (B)	Peru				
Nasa triphylla subsp. *flavipes* Weigend & Dostert	*Weigend & Dostert 98/203* (M)	Peru	MF972164	MF972136	MF972178	MF972150
Nasa triphylla subsp. *loxensis* Dostert & Weigend	*Freire-Fierro et al. 2478* (QCA)	Ecuador				
Nasa triphylla subsp. *papaverifolia* (Kunth) Weigend Dostert & Weigend	*McCook 1116* (QCA)	Ecuador				
Nasa triphylla subsp. *rudis* (Benth.) Weigend	*Acuña et al. 1211* (BONN)	Costa Rica	MF972117	MF972097	MF972126	MF972107
Nasa triphylla subsp. *triphylla* (Juss.) Weigend	*Weigend & Brokamp 9098* (B)	Ecuador	MF972170	MF972142	MF972184	MF972156
Nasa tulipadiaboli T.Henning & Weigend	*Weigend et al. 01/443* (B)	Peru				
Nasa umbraculifera E.Rodr. & Weigend	*Henning & Schneider 291* (BSB)	Peru				
Nasa urens	*Weigend &*	Peru				

(Jacq.) Weigend	*Förther 97/542* (USM)					
Nasa urentivelutina Weigend	*Henning & Brokamp 9* (B)	Peru				
Nasa usquiliensis Weigend, T.Henning & C.Schneid.	*González et al. 2708* (USM)	Peru				
Nasa vargasii (J.F.Macbr.) Weigend	*Weigend et al. 5463* (B)	Peru	MF972168	MF972140	MF972182	MF972154
Nasa venezuelensis (Steyerm.) Weigend	*Weigend 3604* (M)	Venezuela				
Nasa victorii Weigend	*Weigend et al. 2000/929* (BSB)	Peru				
Nasa weberbaueri (Urb. & Gilg) Weigend	*Weigend & Dostert 98/261* (F)	Peru				
Nasa weigendii E.Rodr.	*Weigend & Schwarzer 7913C* (BONN)	Peru	MF972173	MF972145	MF972187	MF972159
Nyssa sylvatica Marshall	*Römer s.n.* (BONN)	Germany (cultivated)				
Nyssa talamancana Hammel & N.Zamora	*Acuña et al. 1237* (USJ)	Costa Rica				
Petalonyx linearis Greene	*Bell 6232* (RSA)	United States				
Philadelphus pekinensis Rupr.	*Weigend 5614* (BSB)	Germany (cultivated)	KY286927	KY286659	KY286837	KY286747
Pinnasa bergii (Hieron.) Weigend & R.H.Acuña	*Weigend 6800* (BSB)	Argentina	KY286920	KY286652	KY286830	KY286740
Pinnasa nana Phil.	*Weigend et al. 7080* (BSB)	Argentina	KY286980	KY286711	KY286890	KY286800
Pinnasa pinnatifida (Gillies ex Arn.) Weigend & R.H.Acuña	*Weigend et al. 6880* (BSB)	Argentina	KY286942	KY286673	KY286852	KY286762
Plakothira parviflora J.Florence	*Weigend s.n.* (BSB)	France (Marquesas Islands)	KY286926	KY286658	KY286836	KY286746
Presliophytum arequipense Weigend	*Ortiz 121* (BONN)	Peru	KY286965	KY286696	KY286875	KY286785
Presliophytum heucheraefolium (Killip) Weigend	*Weigend et al. 7368* (BSB)	Peru	KY286946	KY286677	KY286856	KY286766
Presliophytum incanum (Graham) Weigend	*Weigend & Förther 97/848* (F)	Peru	KY286924	KY286656	KY286834	KY286744
Presliophytum malesherbioides (Phil.) R.H.Acuña & Weigend	*Wagenknecht 18509* (M)	Chile	KY286933	KY286665	KY286843	KY286753
Presliophytum	Ehrhardt	Chile	KY286945	KY286676	KY286855	KY286765

sessiliflorum (Phil.) R.H.Acuña & Weigend	s.n.(M)					
Scyphanthus elegans Sweet	*Grau & Ehrhardt 2-093* (M)	Chile	KY286958	KY286689	KY286868	KY286778
Scyphanthus stenocarpus (Poepp.)	*Gardner & Knees 8351 C* (BSB)	Chile	KY286992	KY286723	KY286902	KY286812
Xylopodia klaprothioides Weigend	*Weigend & al. 97/450* (M)	Peru	KY286923	KY286655	KY286833	KY286743

Fig. D.1. Chronograms of Cornales under different calibration schemes and .tre files of the maximum clade credibility trees. Posterior probability support values (above each branch, only values ≥ 0.5 included) and 95% highest posterior density intervals for the age of the respective node (grey bars) are shown. Black circles numbered 1–7 indicate the fossil calibration points as described in Materials and Methods: 1. *Cornus* cf. *piggae*, 2. *Davidia antiqua,* 3. *Obamacarpa edenensis*, 4. *Jamesia caplani*, 5. *Hydrangea knowltonii*, 6. *Philadelphus creedensis,* 7. *Klaprothiopsis dyscrita*. **(a)** Calibration scheme including all seven calibration points. **(b)** Calibration scheme including all calibration points except 7. **(c)** Calibration scheme including only points 1, 2 and 5. (d) .tre files of the maximum clade credibility trees under the respective calibration schemes **(a)**, **(b)** and **(c)**. Scale bars in millions of years before present (Ma). PL= Pliocene, Q = Quaternary.


(a)
W4028 Caiophora dumetorum
W4010 Caiophora aconquijae
W4030 Caiophora hibiscifolia
W4031 Caiophora lateritia
W4516 Caiophora clavata
W4021 Caiophora chuquisacana
W4007 Caiophora cernua
W1429 Caiophora madrequisa
W4019 Caiophora cf. buraeavii
W4011 Caiophora canarinoides
W4009 Caiophora coronata
W1343 Caiophora carduifolia
W4024 Caiophora cirsiifolia
W1425 Caiophora cinerea
W4027 Caiophora contorta
W4029 Caiophora grandiflora
W4520 Caiophora andina
W4517 Caiophora rusbyana
W1342 Caiophora pentlandii
W4518 Caiophora rosulata subsp. taraxacoides
W4022 Caiophora chuquitensis
W1435 Caiophora boliviana
W1422 Caiophora deserticola
W4013 Caiophora pulchella
W4015 Caiophora stenocarpa
W4014 Caiophora dederichiorum
W1346 Caiophora pterosperma
W4003 Caiophora arechavaletae
W4035 Scyphanthus stenocarpus
W1467 Scyphanthus elegans
W1354 Pinnasa pinnatifida
W4017 Pinnasa nana
W1284 Pinnasa bergii
W1350 Grausa lateritia
W1609 Grausa gayana
W1466 Grausa micrantha
W1360 Loasa insons
W1323 Loasa heterophylla
W1340 Loasa acerifolia
W4016 Loasa tricolor
W1339 Loasa nitida
W4511 Loasa triloba
W4132 Loasa sclareifolia
W1468 Loasa acanthifolia
W1393 Loasa floribunda
W1390 Loasa illapelina
W4034 Loasa pallida
W4512 Loasa elongata
W4686 Blumenbachia catharinensis
W2846 Blumenbachia hieronymi
W4513 Blumenbachia amana
W1383 Blumenbachia latifolia
W1622 Blumenbachia insignis
W5016 Blumenbachia scabra
W1621 Blumenbachia prietea
W4002 Blumenbachia sylvestris
W1607 Blumenbachia dissecta
W2350 Presliophytum arequipense
W1288 Presliophytum incanum
W1369 Presliophytum heucheraefolium
W1367 Presliophytum sessiliflorum
W1328 Presliophytum malesherbioides
W1289 Aosa rupestris
W4000 Aosa parviflora
W5017 Aosa plumieri
W4685 Aosa grandis
W4133 Aosa sigmoidea
W1376 Aosa rostrata
W4509 Aosa uleana
W1627 Nasa umbraculifera
W4599 Nasa rugosa subsp. llaqtacochaensis
W1377 Nasa jungiifolia
W4347 Nasa grandiflora
W1372 Nasa argemonoides
W1378 Nasa hornii
W4684 Nasa speciosa
W1332 Nasa basilica
W2262 Nasa ranunculifolia subsp. ranunculifolia
W1333 Nasa magnifica
W1337 Nasa weberbaueri
W1334 Nasa tulipadiaboli
W2839 Nasa contumazensis
W1568 Nasa otuzcensis
W1414 Nasa moroensis
W4375 Nasa formosissima
W4525 Nasa olmosiana
W1625 Nasa insignis
W4528 Nasa weigendii
W2257 Nasa bicornuta
W1237 Nasa pteridophylla subsp. pteridophylla
W2680 Nasa triphylla subsp. triphylla
W1375 Nasa aequatoriana
W1624 Nasa dyeri subsp. australis
W1283 Nasa humboldtiana subsp. roseoalba
W1366 Nasa venezuelensis
W1463 Nasa picta subsp. picta
W1413 Nasa dillonii
W4522 Nasa lambayequensis
W1235 Nasa solata
W1379 Nasa profundiserrata
W4008 Nasa urentivelutina
W1352 Nasa usquiliensis
W1329 Nasa macrothyrsa
W1232 Nasa laxa
W1380 Nasa trianae
W1245 Nasa sanchezii
W1460 Nasa victorii
W1239 Nasa lenta
W1238 Nasa driesslei
W1331 Nasa carnea
W1336 Nasa pongalamesa
W4404 Nasa auca
W1373 Nasa rubrastra
W1241 Nasa tingomariensis
W1240 Nasa loxensis
W1351 Nasa herzogii
W1353 Nasa ramirezii
W1242 Nasa ferruginea
W1243 Nasa poissoniana subsp. poissoniana
W1231 Nasa raimondii
W1415 Nasa chenopodiifolia
W1246 Nasa urens
W2261 Nasa vargasii
W2245 Nasa limata
W1381 Nasa pascoensis
W4691 Nasa carunculata
W4508 Klaprothia fasciculata
W4679 Klaprothia mentzelioides
W1290 Plakothira parviflora
W1287 Xylopodia klaprothioides
DB26956 Kissenia arabica
W1364 Kissenia capensis
W1325 Huidobria chilensis
W4690 Huidobria fruticosa
W1286 Mentzelia scabra subsp. chilensis
W4502 Mentzelia aspera
W4503 Mentzelia parvifolia
W1285 Mentzelia albescens
W4504 Mentzelia decapetala
W5034 Cevallia sinuata
W4677 Gronovia scandens
W5035 Petalonyx linearis
W4499 Eucnide urens
W1292 Deutzia rubens
W1293 Deutzia discolor
W4533 Kirengeshoma palmata
W4586 Fendlerella utahensis
W4536 Carpenteria californica
W1291 Philadelphus pekinensis
W4531 Hydrangea arborescens
W4681 Hydrangea oerstedii
W4529 Hydrangea ampla
W4584 Hydrangea caerulea
W1294 Jamesia americana
W4585 Fendlera rupicola
W4580 Cornus kousa var. chinensis
W4683 Cornus disciflora
W4579 Cornus canadensis
W4581 Cornus officinalis
W4534 Cornus mas
W4582 Cornus cf. walteri
W4578 Cornus alternifolia
W4680 Cornus peruviana
W4535 Alangium platanifolium var. trilobum
W4583 Curtisia dentata
W4126 Nyssa sylvatica
W4682 Nyssa talamancana
W4577 Camptotheca acuminata
W4532 Davidia involucrata var. vilmoriniana
W4530 Diplopanax stachyanthus
7
6
5
4
1
2
3
EARLY CRETACEOUS
LATE CRETACEOUS
PALEOCENE
EOCENE
OLIGOCENE
MIOCENE
PL
Q
120
100
80
60
40
20
0
Ma


(b)
W4030 Caiophora hibiscifolia
W4010 Caiophora aconquijae
W4028 Caiophora dumetorum
W4031 Caiophora lateritia
W4516 Caiophora clavata
W4021 Caiophora chuquisacana
W4007 Caiophora cernua
W1429 Caiophora madrequisa
W4019 Caiophora cf. buraeavii
W4011 Caiophora canarinoides
W4009 Caiophora coronata
W4024 Caiophora cirsiifolia
W1343 Caiophora carduifolia
W1425 Caiophora cinerea
W4029 Caiophora grandiflora
W4027 Caiophora contorta
W4517 Caiophora rusbyana
W4520 Caiophora andina
W4518 Caiophora rosulata subsp. taraxacoides
W1342 Caiophora pentlandii
W4022 Caiophora chuquitensis
W1435 Caiophora boliviana
W1422 Caiophora deserticola
W4013 Caiophora pulchella
W4015 Caiophora stenocarpa
W1346 Caiophora pterosperma
W4014 Caiophora dederichiorum
W4003 Caiophora arechavaletae
W1467 Scyphanthus elegans
W4035 Scyphanthus stenocarpus
W4017 Pinnasa nana
W1354 Pinnasa pinnatifida
W1284 Pinnasa bergii
W1350 Grausa lateritia
W1609 Grausa gayana
W1466 Grausa micrantha
W1323 Loasa heterophylla
W1360 Loasa insons
W1340 Loasa acerifolia
W4016 Loasa tricolor
W1339 Loasa nitida
W4511 Loasa triloba
W1468 Loasa acanthifolia
W4132 Loasa sclareifolia
W1390 Loasa illapelina
W1393 Loasa floribunda
W4034 Loasa pallida
W4512 Loasa elongata
W4686 Blumenbachia catharinensis
W2846 Blumenbachia hieronymi
W4513 Blumenbachia amana
W1383 Blumenbachia latifolia
W1622 Blumenbachia insignis
W5016 Blumenbachia scabra
W4002 Blumenbachia sylvestris
W1621 Blumenbachia prietea
W1607 Blumenbachia dissecta
W2350 Presliophytum arequipense
W1288 Presliophytum incanum
W1369 Presliophytum heucheraefolium
W1367 Presliophytum sessiliflorum
W1328 Presliophytum malesherbioides
W4685 Aosa grandis
W5017 Aosa plumieri
W4000 Aosa parviflora
W1289 Aosa rupestris
W4133 Aosa sigmoidea
W1376 Aosa rostrata
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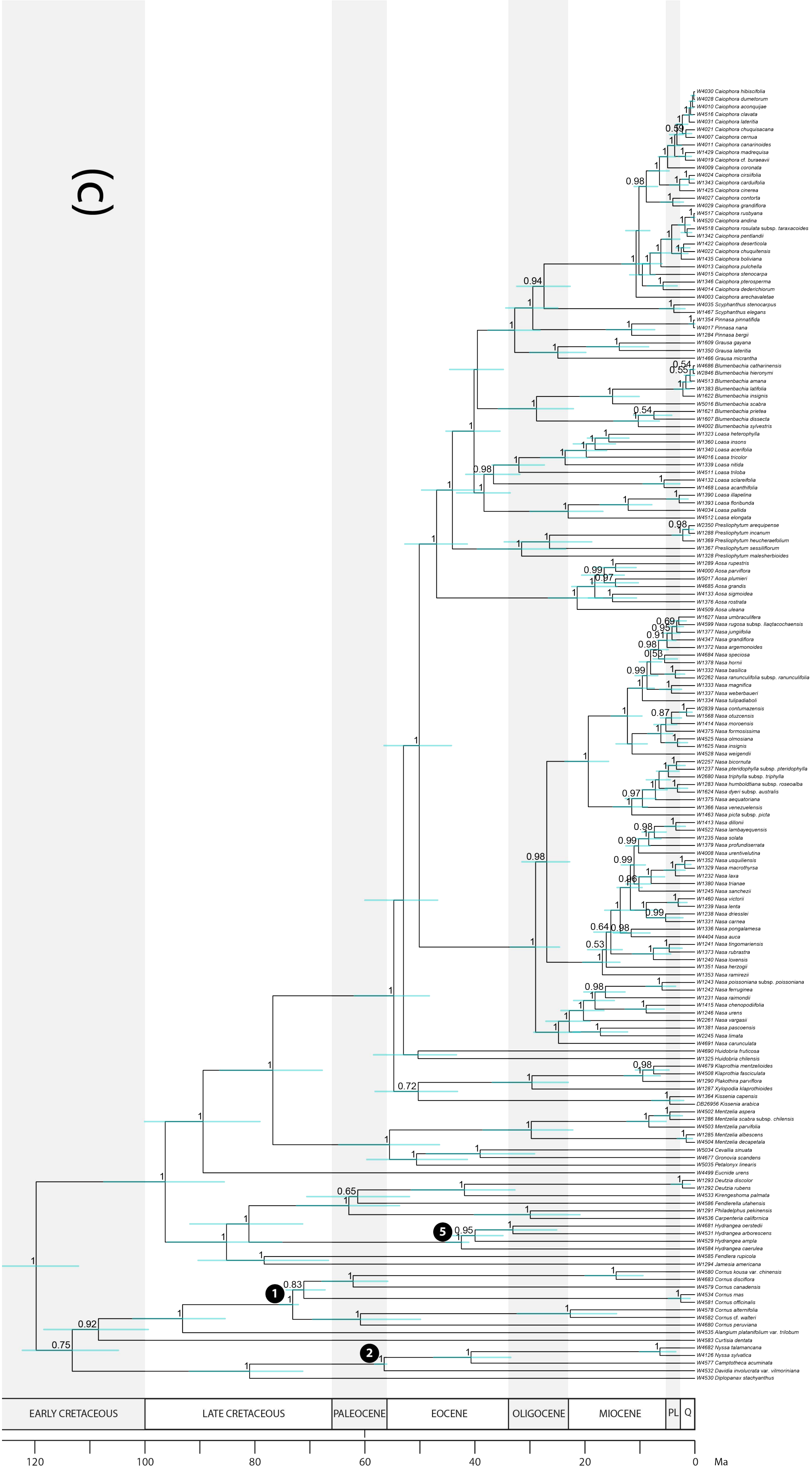
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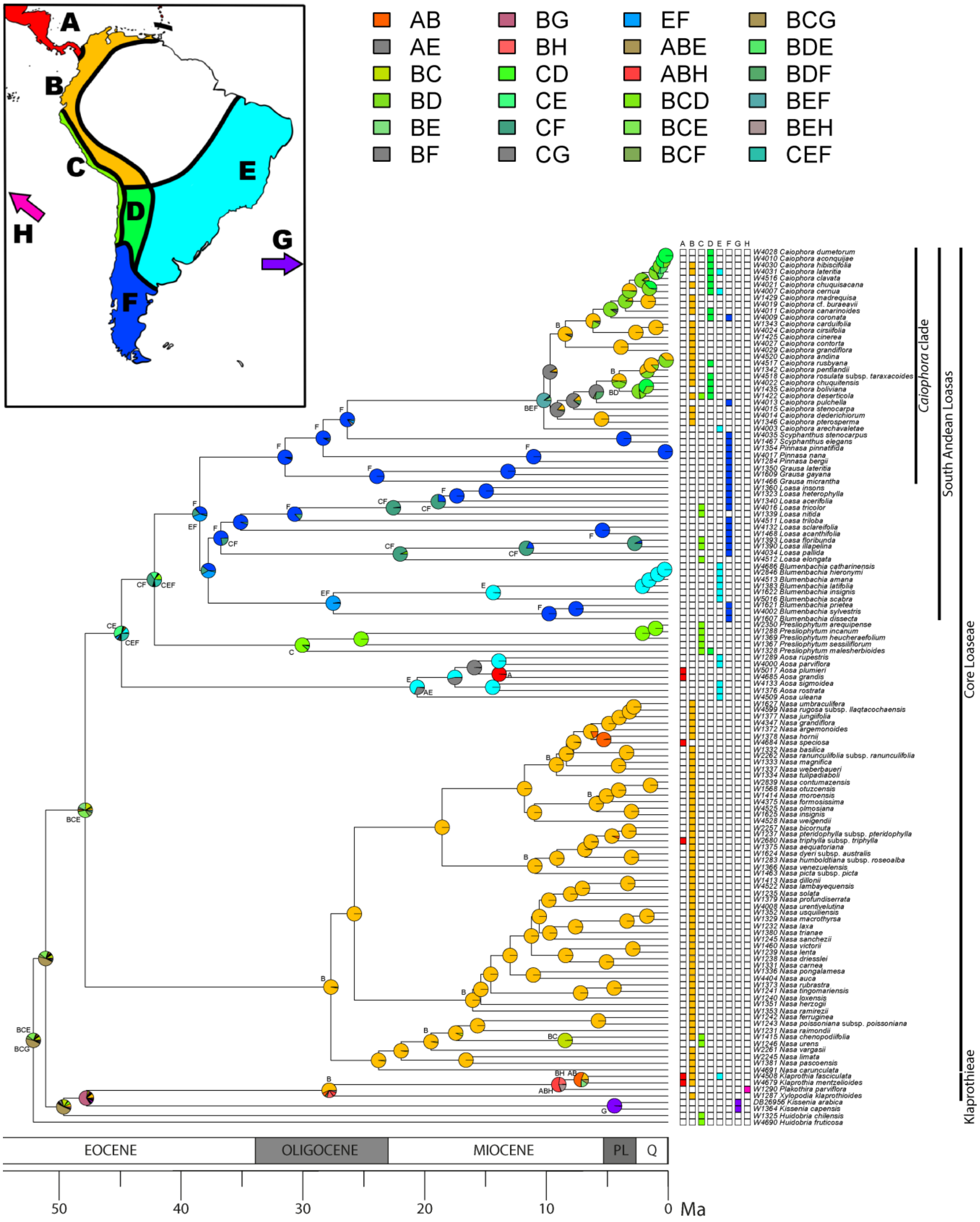
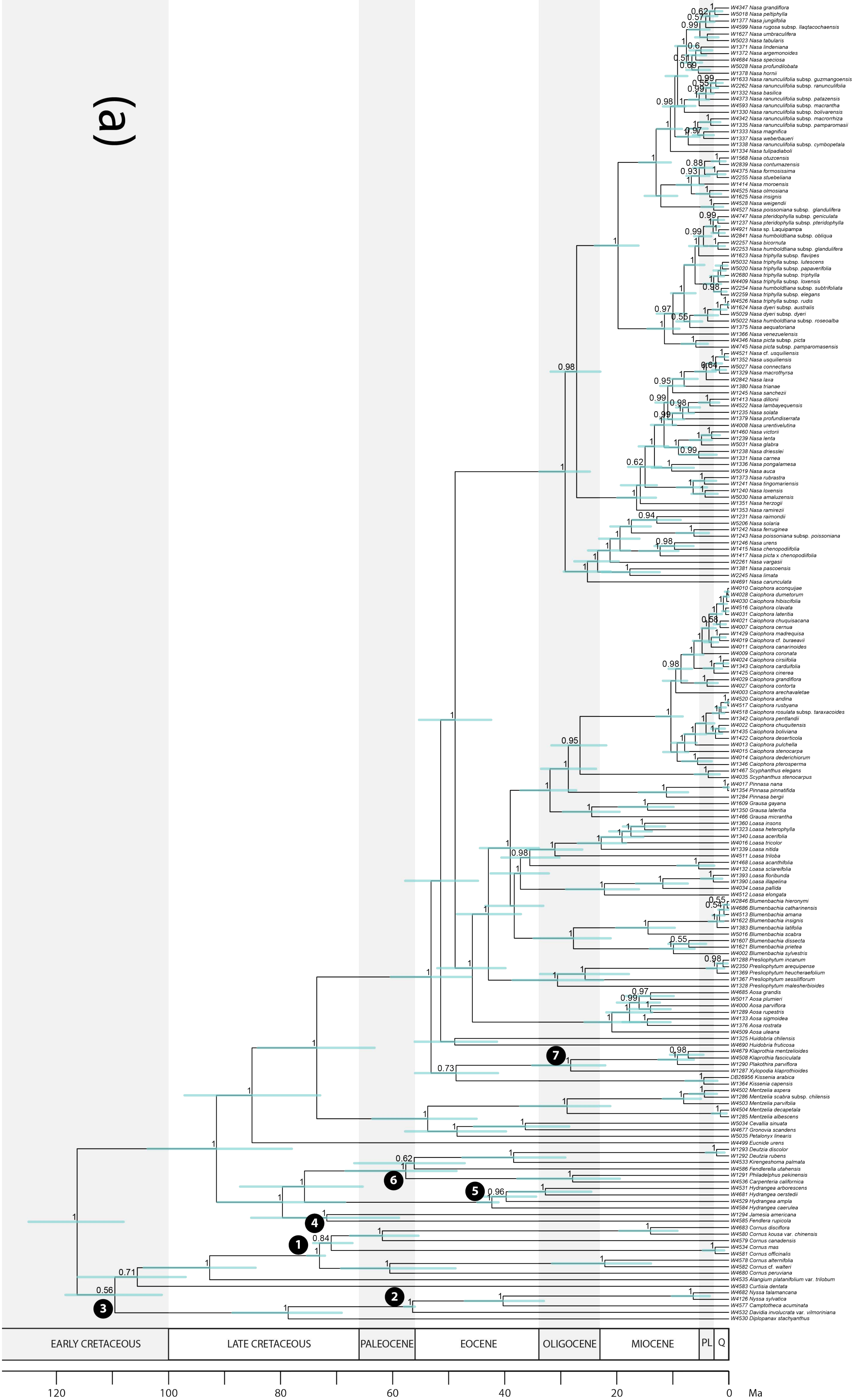


Fig. D.2. Divergence time estimates for the phylogeny of Loasoideae using calibration scheme (a) for the dispersal-unconstrained DEC analysis, with a maximum of 3 areas per node. Coloured grids to the right of the tips indicate the distribution assigned to the species. Colour codes correspond to the areas indicated on the maps and legends. The Pie charts at the nodes depict relative probabilities of areas as estimated from the DEC analyses. The letters next to the pies indicate areas with relative probabilities > 0.2. Only area combinations with relative probabilities > 0.05 are included in the legend. Scale bars in millions of years before present (Ma). PL= Pliocene, Q = Quaternary. Map outline based on Rivas-Martínez et al. (2011).

APPENDIX E (Chapter 7: Diversification patterns of *Nasa* (Loasaceae subfam. Loasoideae) in the Neotropics)

Fig. E.1. Chronograms of Cornales under calibration schemes (a) and (b). Posterior probability support values (above each branch, values under 0.5 not shown) and 95% highest posterior density intervals for the age of the respective node (grey bars) are shown. Scale bars in millions of years before present (Ma). . Black circles numbered 1–7 indicate the fossil calibration points as described in Materials and Methods: 1. *Cornus* cf. *piggae*, 2. *Davidia antiqua,* 3. *Obamacarpa edenensis*, 4. *Jamesia caplani*, 5. *Hydrangea knowltonii*, 6. *Philadelphus creedensis,* 7. *Klaprothiopsis dyscrita*. **A)** Calibration scheme including all seven calibration points. **B)** Calibration scheme including only points 1, 2 and 5. Scale bars in millions of years before present (Ma). PL= Pliocene, Q = Quaternary.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W1627 Nasa umbraculifera
W5023 Nasa tabularis
W1371 Nasa lindeniana
W1372 Nasa argemonoides
W4684 Nasa speciosa
W5028 Nasa profundilobata
W1378 Nasa hornii
W1633 Nasa ranunculifolia subsp. guzmangoensis
W2262 Nasa ranunculifolia subsp. ranunculifolia
W1332 Nasa basilica
W4373 Nasa ranunculifolia subsp. patazensis
W4593 Nasa ranunculifolia subsp. macrantha
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W1334 Nasa tulipadiaboli
W1568 Nasa otuzcensis
W2839 Nasa contumazensis
W4375 Nasa formosissima
W2255 Nasa stuebeliana
W1414 Nasa moroensis
W4525 Nasa olmosiana
W1625 Nasa insignis
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W4747 Nasa pteridophylla subsp. geniculata
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W4921 Nasa sp. Laquipampa
W2841 Nasa humboldtiana subsp. obliqua
W2257 Nasa bicornuta
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W1623 Nasa triphylla subsp. flavipes
W5032 Nasa triphylla subsp. lutescens
W5020 Nasa triphylla subsp. papaverifolia
W2680 Nasa triphylla subsp. triphylla
W4409 Nasa triphylla subsp. loxensis
W2254 Nasa humboldtiana subsp. subtrifoliata
W2259 Nasa triphylla subsp. elegans
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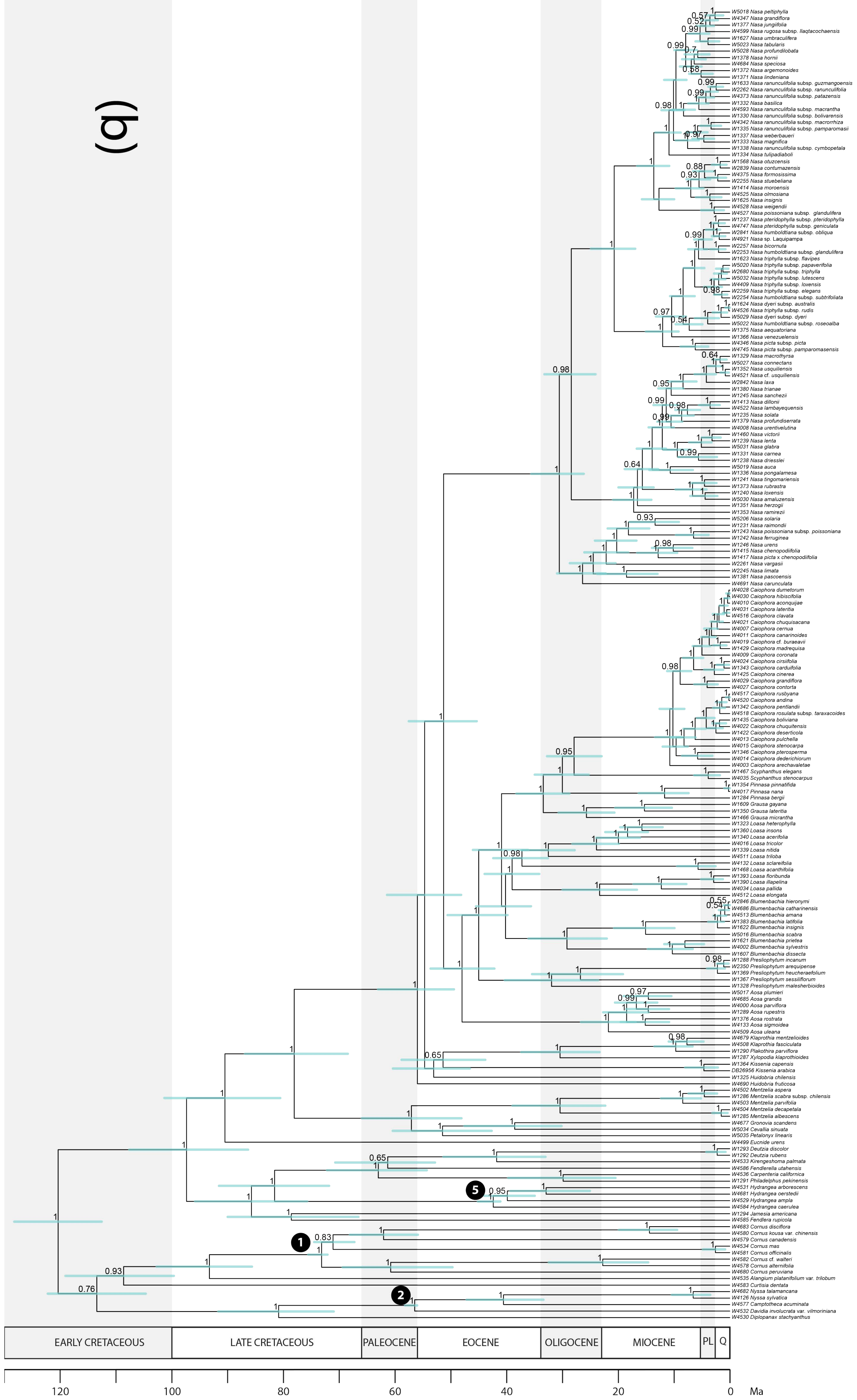
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W4518 Caiophora rosulata subsp. taraxacoides
W1435 Caiophora boliviana
W4022 Caiophora chuquitensis
W1422 Caiophora deserticola
W4013 Caiophora pulchella
W4015 Caiophora stenocarpa
W1346 Caiophora pterosperma
W4014 Caiophora dederichiorum
W4003 Caiophora arechavaletae
W1467 Scyphanthus elegans
W4035 Scyphanthus stenocarpus
W1354 Pinnasa pinnatifida
W4017 Pinnasa nana
W1284 Pinnasa bergii
W1609 Grausa gayana
W1350 Grausa lateritia
W1466 Grausa micrantha
W1323 Loasa heterophylla
W1360 Loasa insons
W1340 Loasa acerifolia
W4016 Loasa tricolor
W1339 Loasa nitida
W4511 Loasa triloba
W4132 Loasa sclareifolia
W1468 Loasa acanthifolia
W1393 Loasa floribunda
W1390 Loasa illapelina
W4034 Loasa pallida
W4512 Loasa elongata
W2846 Blumenbachia hieronymi
W4686 Blumenbachia catharinensis
W4513 Blumenbachia amana
W1383 Blumenbachia latifolia
W1622 Blumenbachia insignis
W5016 Blumenbachia scabra
W1621 Blumenbachia prietea
W4002 Blumenbachia sylvestris
W1607 Blumenbachia dissecta
W1288 Presliophytum incanum
W2350 Presliophytum arequipense
W1369 Presliophytum heucheraefolium
W1367 Presliophytum sessiliflorum
W1328 Presliophytum malesherbioides
W5017 Aosa plumieri
W4685 Aosa grandis
W4000 Aosa parviflora
W1289 Aosa rupestris
W1376 Aosa rostrata
W4133 Aosa sigmoidea
W4509 Aosa uleana
W4679 Klaprothia mentzelioides
W4508 Klaprothia fasciculata
W1290 Plakothira parviflora
W1287 Xylopodia klaprothioides
W1364 Kissenia capensis
DB26956 Kissenia arabica
W1325 Huidobria chilensis
W4690 Huidobria fruticosa
W4502 Mentzelia aspera
W1286 Mentzelia scabra subsp. chilensis
W4503 Mentzelia parvifolia
W4504 Mentzelia decapetala
W1285 Mentzelia albescens
W4677 Gronovia scandens
W5034 Cevallia sinuata
W5035 Petalonyx linearis
W4499 Eucnide urens
W1293 Deutzia discolor
W1292 Deutzia rubens
W4533 Kirengeshoma palmata
W4586 Fendlerella utahensis
W4536 Carpenteria californica
W1291 Philadelphus pekinensis
W4531 Hydrangea arborescens
W4681 Hydrangea oerstedii
W4529 Hydrangea ampla
W4584 Hydrangea caerulea
W1294 Jamesia americana
W4585 Fendlera rupicola
W4683 Cornus disciflora
W4580 Cornus kousa var. chinensis
W4579 Cornus canadensis
W4534 Cornus mas
W4581 Cornus officinalis
W4582 Cornus cf. walteri
W4578 Cornus alternifolia
W4680 Cornus peruviana
W4535 Alangium platanifolium var. trilobum
W4583 Curtisia dentata
W4682 Nyssa talamancana
W4126 Nyssa sylvatica
W4577 Camptotheca acuminata
W4532 Davidia involucrata var. vilmoriniana
W4530 Diplopanax stachyanthus
1
2
5
EARLY CRETACEOUS
LATE CRETACEOUS
PALEOCENE
EOCENE
OLIGOCENE
MIOCENE
PL
Q
120
100
80
60
40
20
0
Ma

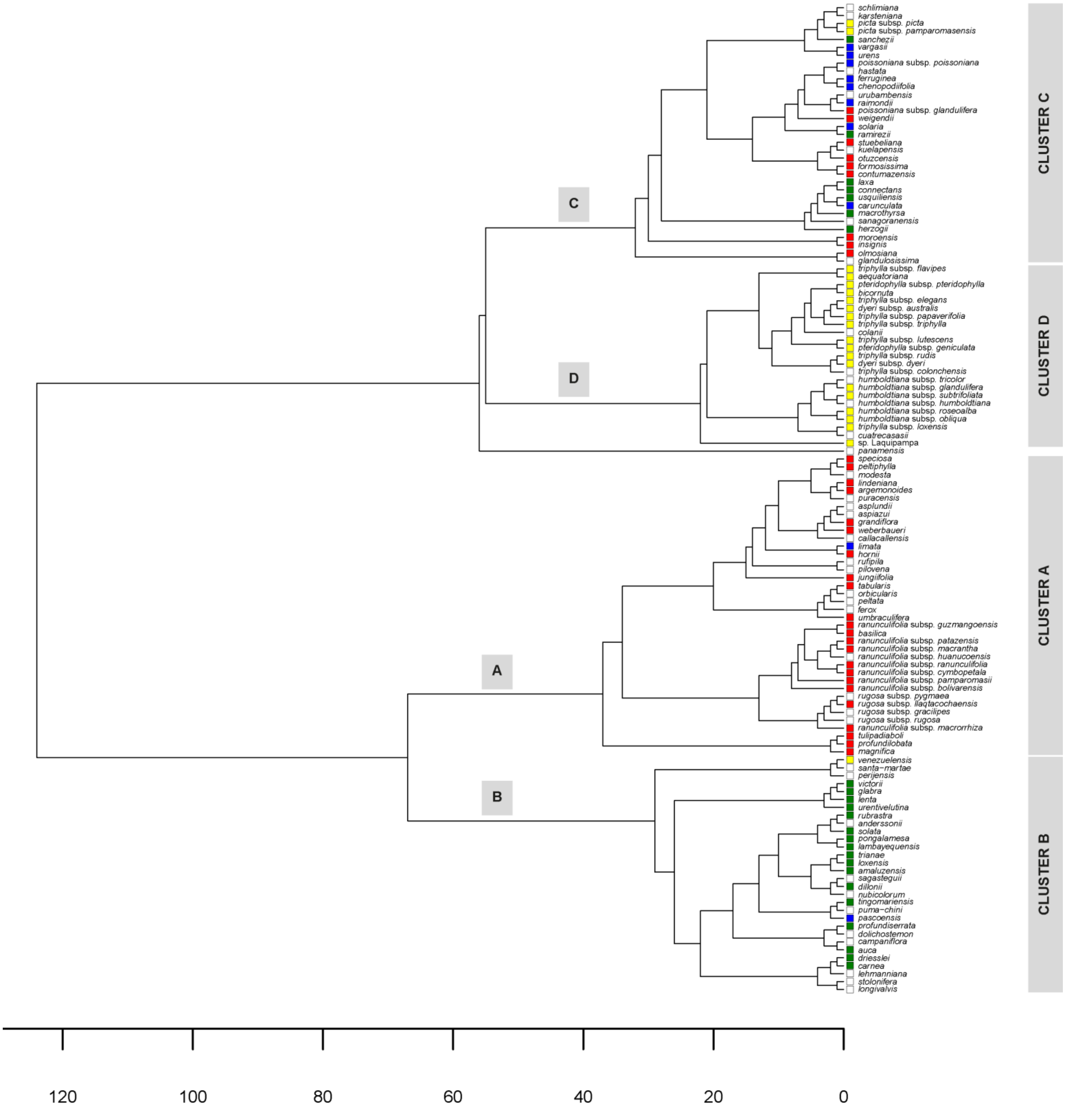

Fig. E.2. Dendrogram of the hierarchical cluster analysis obtained the observed-only dataset. The dissimilarity distances were calculated from data coming from 26 directly observed morphological traits. Clusters named according to the most similar clusters shown in Fig. 6. The colored grid to the left of each name represents the clade to which the respective species was retrieved in the phylogenetic analyses. Blue: Clade I, Green: Clade II, Yellow: Clade III, Red: Clade IV.

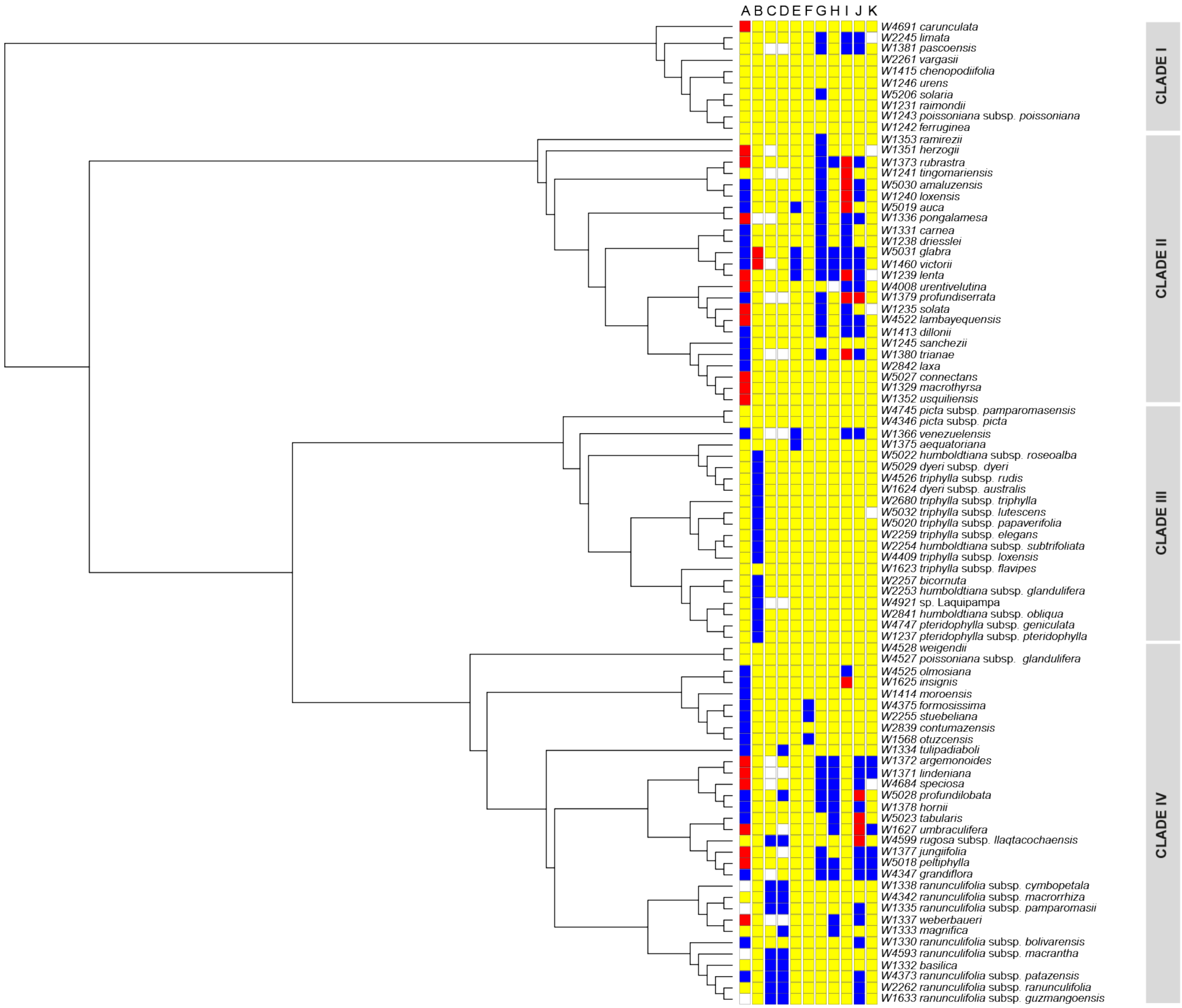

Fig. E.3. Cladogram obtained from the BI consensus phylogram of *Nasa,* plotting the character state distribution for 11 traits with strong phylogenetic signal. A) Shoot lignification: yellow = No or minimal lignification, blue = moderate lignification, red = strong lignification. B) Shoot shape in transversal section: yellow = terete, blue = sulcate, red = ridged. C) Presence or absence of rhizomes: yellow = absent, blue = present. D) Presence or absence of basal leaf rosettes: yellow = absent, blue = present. E) Growth form: yellow = self-supported, blue = subscandent. F) Presence or absence of pseudostipules: yellow = absent, blue = present. G) Distal phyllotaxy: yellow = alternate, blue = opposite. H) Petal texture: yellow = membranous, blue = carnose. I) Angle of the apical wing relative to back of the nectar scale: yellow = erect, blue = semierect, red = horizontal. J) Length apical wing relative to length of the back of the nectar scale: yellow = < 30%, blue = 30–50%, red = >50%. K) Type of capsule dehiscense: yellow = apical, blue = apical and longitudinal. White cells refer to unobserved traits.

PUBLICATION LIST

Related to my thesis research

Acuña R, Fließwasser S, Ackermann M, Henning T, Luebert F & Weigend M. 2017. Phylogenetic relationships and generic re-arrangements in "South Andean Loasas" (Loasaceae). *Taxon* **66**: 365--378.

Acuña R & Weigend M. 2017. A taxonomic revision of the western South American genus *Presliophytum* (Loasaceae). *Phytotaxa* **329**: 51--68.

Henning T, Acuña R, Weigend M & Mello-Silva R. 2017. Loasaceae. In Jardim Botânico do Rio de Janeiro (Ed.) *Flora do Brasil 2020 em construção*. Available from: http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB150

Acuña R, Chinchilla I & Weigend M. 2018. An unusual disjunction in Loasaceae: Central American *Chichicaste grandis* is nested in Brazilian *Aosa*. *Phytotaxa* **365**: 273--287.

Acuña R & Weigend M. 2018. The generic affinity of *Caiophora pulchella*. *Darwiniana nueva serie* **6**: 94--98.

Henning T, Mittelbach M, Ismail SA, Acuña R & Weigend, M. 2018. A case of behavioural diversification in male floral function – the evolution of thigmonastic pollen presentation. *Scientific Reports* **8**: 14018.

Other publications

Acuña R & Gómez LD. 2009. Selección de un lectotipo para *Zamia fairchildiana* (Zamiaceae). *Brenesia* **71--72**: 65-66.

Acuña R. 2010. *Dermophis* sp.: Predation. *Herpetological Review* **41**: 344.

Acuña R. 2010. A new species of *Zamia* (Zamiaceae) from Costa Rica. *Brenesia* **73--74**: 39-33.

Acuña R & Marín-Méndez W. 2012. Some aspects of the foliar anatomy of two *Zamia* species from Costa Rica: *Z.fairchildiana* L.D. Gómez and *Z. neurophyllidia* D.W. Stev. *Memoirs of the New York Botanical Garden* **196**: 192—203.

Acuña R & Marín-Méndez W. 2013. Comparative anatomy of the leaflets of *Zamia acuminata* and *Z. pseudomonticola* (Zamiaceae) in Costa Rica. *Revista de Biología Tropical* **61**: 539--546.

Acuña R & Marín-Méndez W. 2013. Leaflet anatomy of *Zamia disodon* D.W.Stev & Sabato and *Z. restrepoi* (D.W. Stev.) A. Lindstr. *Caldasia* **35**: 1--9.

Häger A, Fernández-Otárola M, Stuhlmacher M, Acuña R, & Contreras-Arias A. 2014. Effects of management and landscape composition on the diversity and structure of tree species assemblages in coffee agroforests. *Agriculture, Ecosystems and Environment* **199**: 43—51.

Acuña R. 2014. The Genus *Chamguava* L.R. Landrum (Myrtaceae) in Costa Rica. *Brenesia* **81-82**: 3--7.