Microevolutionary processes and taxonomic investigations of the Atacama Desert flora

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One of the most perfect deserts in the world lies between the equator and the Tropic of Capricorn, in Peru and northern Chile.

Th. Dobzhansky, 1950

Preface

It is Tuesday October 1st 2019. The fourth field trip in three years, and the fourth time that we are on our way to Alto Chipana, the site of the largest remaining population of *Eulychnia iquiquensis* north of Tocopilla. But also the only locality where *Eulychnia* shares the habitat with an extensive *Tillandsia landbeckii* population, and the rarest and least collected species of *Ophryosporus* in Chile, *Ophryosporus anomalus.* Alto Chipana lies on the western slopes of the Coastal Cordillera, north of the Río Loa, at about 900 metres elevation, facing the Pacific Ocean.

The problem is that Alto Chipana has to be approached from the eastern side, because access from the coast is extremely steep and thus limited to very few places. Coming from the east, however, means to face difficulties to remember the way through the absolutely barren landscape. Despite the extensive GPS tracking recorded on previous trips, the paths through the sandy and mountainous terrain often change according to the needs of the mining company that resides here. New tracks are made frequently, creating a confusing spider web-like network through the sand; they all remain visible for hundreds if not thousands of years because here, there is no rain to erase them – this is the driest place on Earth, the hyper arid core of the Atacama Desert of northern Chile.

I drive the pick-up, trying to reconstruct the way from my memory without having to glance at the GPS, but I fail and have to return twice. Still, I try to remain self-assured as to avoid being teased by my two companions. Respect and admiration for the "old-school" explorers like Rodolfo Amandus Philippi or Ivan Murray Johnston, who did not have the latest technology at their disposal, and still led the way of botanical exploration of the Atacama, always knowing where they were without a GPS. They travelled on horse-back, we by air-conditioned 4x4 cars. No plants grow here, barren land as far as we can see. Nothing but sand and glaring sun. To the east, we can make out the flickering air above the Salar Grande, and in the far east a faint line of snow covered peaks, the Andes, as if to frame this narrow belt of desert to which we have come to study the plants.

We pass a donkey skeleton, I remember this from previous visits here, and it tells me that we are on the right way. Soon there will be the llama, a pre-Columbian geoglyph covering a hill side and which is still visible after all this time. Suddenly a tiny lizard races across the path in front of the car. What does it live off here? And then we notice a green hue on the sandy plains. Lichens. Thin and ghostly streaks of cloud begin to hush past. We must be getting close. Soon after, small grey cushion-like structures appear on the ground, tucked between the rocks. We have reached our aim,

a large *Tillandsia* loma, a fog oasis where the only source of humidity, coastal fog known locally as camanchaca, allows for some vegetation to exist. These *Tillandsia* have no roots and can absorb fog moisture through specialised leaf trichomes.
As we pass the last curve around the hill in front of us, the dark blue Pacific Ocean appears below us, and above it, a thin but dense belt of clouds, capped at the top and bottom, and meeting the slopes immediately below us. As we descend approximately another 100 metres down into these sea-facing slopes, the fog becomes so dense, that the sunlight that has been burning mercilessly until now suddenly becomes enveloped into a shadow as if someone had dimmed the light, and with it, the temperature drops drastically. We have entered the fog zone, I can feel water droplets forming in my beard. Large columns arise here and there; they are *Eulychnia* – cacti endemic to northern Chile and southern Peru. Other plants emerge, herbaceous vegetation, mostly *Nolana*, a speciose group of recent radiation in the Atacama, and also here and there a small shrub with whitish inflorescences – *Ophryosporus anomalus*, a taxon with a complex taxonomic history.

We have come all the way here in order to make some last plant collections for our studies on the Atacama Desert flora. Three of four plant groups that I am studying occur here at Alto Chipana. These studies are part of the Collaborative Research Centre 1211, an interdisciplinary attempt to study the interplay of biotic and abiotic evolutionary processes in the Atacama from the perspectives of climate, landscape and biological evolution. Within these umbrella categories, my contributions come from the realm of botany, represented by the study of plant evolutionary processes, specifically the microevolutionary dynamics within and among populations, which are possibly isolated through (a-)biotic barriers to gene flow. In advancing the understanding of such complex processes, we can formulate new hypotheses concerning plant evolution in arid environments, and infer macroevolutionary processes such as biogeographic history, modes of speciation, or taxonomic concepts.

This dissertation is the result of my research as a Ph.D. student within the Collaborative Research Centre 1211, ”Earth – Evolution at the Dry Limit”.

Felix F. Merklinger
Aach, August 2020

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Contents

Chapter 1

Introduction

1 Introduction

This thesis is an investigation of plant evolutionary processes of four model organisms from the Atacama Desert of northern Chile. It includes two chapters that analyse the microevolutionary patterns based on changes in allele frequencies within individuals and populations, and that seek to understand the genetic connectivity between populations in response to geomorphology and past and current climate in the Atacama. These investigations are based on the analysis of single nucleotide polymorphism (SNP) data obtained from genotyping by sequencing (GBS). Further included is an analysis of biogeographic patterns and divergence time estimates of a genus endemic to the Peruvian and Atacama Desert systems. Two remaining chapters are taxonomic and nomenclatural revisions of two plant genera from northern Chile and southern Peru. These shall serve as a reference for future studies of the Atacama Desert flora.

1.1 Geographic location of the Atacama Desert

The Peruvian and Atacama Deserts form a narrow belt of arid environments along the west coast of South America, spanning a distance of more than 3500 km from northern Chile to northern Peru (Rundel *et al.*, 1991). The Atacama Desert has been defined as the austral part of these Pacific coastal deserts, with its southern limits near Coquimbo ∼30°S (Smith & Johnston, 1945), coinciding with the transitional zone from arid to hyper arid environments (Luebert & Pliscoff, 2017). It reaches a width of approximately 80–200 km from the shores of the Pacific ocean inland and up the west flank of the Andes (Rundel *et al.*, 1991; Smith & Johnston, 1945), and is thus situated at the heart of the South American dry diagonal, which stretches from ∼5°S on the west coast to ∼50°S on the east coast of the continent, and with a mean annual rain fall of less than 200 mm (Houston & Hartley, 2003). The northern boundary of the Atacama Desert is considered at about 18°S, coinciding with today's border between Chile and Peru (Rundel *et al.*, 1991; Smith & Johnston, 1945). This geographic and climatic definition of the Atacama Desert is further supported by shifts in vegetation cover. South of Coquimbo, a transition to a Mediterranean-type ecosystem dominated by sclerophyllous vegetation can be observed (Armesto *et al.*, 2007), while in the north, there is a reported abrupt floristic break along the coast between northern Chile and southern Peru (Pinto & Luebert, 2009; Ruhm *et al.*, 2020), albeit several disjunct distribution patterns in some plant families between northern Chile and southern Peru (Gengler–Nowak, 2002; Dillon *et al.*, 2009). The reason for the vegetation shift in northern Chile can be found in a drastic south-north gradient in decreasing precipitation, which becomes very obvious near the town of Copiapó with approximately 20 mm precipitation per year, to about 1 mm/year in Arica (Houston, 2006).

1.2 Causes of aridity

Several factors largely govern aridity in the Atacama, and these work in synergy to create one of the driest environments on Earth (McKay *et al.*, 2003). (1) The position of the Atacama at the eastern boundary of the subtropical Pacific, characterized by large-scale subsidence, which produces a year-round mild and arid climate, and supporting a Pacific anticyclone (Rodwell & Hoskins, 2001); (2) this anticyclone creates equatorward winds, and directs the movement of surface currents such as the Humbold or Peru-Chile currents that carry cold Antarctic waters northward in parallel to the Chilean and southern Peruvian coastline, resulting in the formation of persistent stratus clouds (Garreaud *et al.*, 2008, 2010); (3) the high Andes to the East, acting as a barrier to Amazonian moist air and creating a rain-shadow effect on the western slopes of the Andes, further intensifying aridity there (Houston & Hartley, 2003).

The Atacama has probably been arid since the late Jurassic due to the continentality effect (stable position of the South American continent) and the long-term existence of a proto-Humboldt current (Hartley *et al.*, 2005). The precise timing of the onset of hyper arid conditions, however, remains unclear (Hartley & Chong, 2002; Dunai *et al.*, 2005; Sillitoe & McKee, 1996; Ritter *et al.*, 2019). Newly emerging evidence points to the evolution of hyper aridity in a non-uniform way, yet with a long-term predominantly hyper arid core (Dunai *et al.*, 2005; Ritter *et al.*, 2019). Hyper aridity was punctuated by wetter phases, in particular during the Pleistocene, possibly associated with variable positions of the southern westerlies (Lamy *et al.*, 1999; Betancourt, 2000; Stuut & Lamy, 2004; Latorre *et al.*, 2002, 2006), with variability further intensified by cyclic events such as El Niño (Garreaud *et al.*, 2009).

According to Houston & Hartley (2003), hyper aridity is defined by the ratio of lack of rainfall (P) to potential evapotranspiration (PET) of less than 0.05. If this definition is followed, most of the area between 15°S and 30°S and from sea level to 3500 m elevation should be considered as hyper arid (Houston & Hartley, 2003). However, when examining the distribution of vegetation in this entire region, it becomes clear that the variation of aridity is much more complex and a mosaic of ombrotypes has therefore been defined (Luebert & Pliscoff, 2017). Although for the purpose of this thesis, the definition of the terms "arid", "hyper arid" and "ultra-hyper arid" follow Luebert & Pliscoff (2017), it is important to note, that a clear definition of concepts of aridity, which are to be broadly accepted, is still outstanding due to the perspectives on the matter arising from evidence obtained from various disciplines of study (see also Garreaud *et al.*, 2010). This holds particularly true when current conditions are placed in a broader temporal context, for example through the comparison of paleo-climatic studies.

Of particular interest for the purpose of this present study is how vegetation is distributed across the Atacama Desert, depending on the availability of water. There are two major ways that contribute to the delivery of water to the Atacama:

The first is the occurrence of occasional precipitation events, albeit rare and often very localized, but more noticeable during El Niño/La Niña cyclic events, in particular along the coast (Aceituno *et al.*, 2009; Dillon & Rundel, 1990). These precipitation events are caused by the impact of the westerlies (or western trade winds), which are particularly noticeable during the austral winter, and their geographic extent depends on the north-south fluctuation of the pacific anticyclone. In the high Andes, such precipitation events occur particularly during the summer monsoon and depend on the easterlies (eastern trade winds). Northwesterly jets deflect moisture of equatorial Atlantic origin southward (Orme, 2007) causing ”spill-overs” into the Altiplano and bringing moisture to the northeast of the Atacama during the austral summer (Garreaud *et al.*, 2009, 2010). This also leads to discharge into the lower elevations, usually through the numerous canyons (quebradas) that dissect the landscape.

Secondly, the persistent stratus clouds forming above the Pacific ocean meet the western slopes of the coastal cordillera at a more or less constant altitude due to a strong temperature inversion. They deliver moisture in the form of dense fog, locally known as camanchaca and particularly intense during the winter months (Cereceda *et al.*, 2008; Cereceda & Schemenauer, 1991). The influx of coastal fog confined to a particular altitudinal level below 1000 m has allowed for vegetation to evolve on the steep western slopes of the coastal cordillera, composed of a comparatively high number of plants with over 60% endemism (Dillon & Hoffmann, 1997). The resulting belt of vegetation has accordingly been termed the ”fog zone”, ”fertile belt” or ”fog oases” (Johnston, 1929; Muñoz-Schick *et al.*, 2001; Rundel *et al.*, 1991).

1.3 Plant evolution in the Atacama

The vegetation of the coastal fog zone has been characterized by Rundel *et al.* (1991) and Dillon & Hoffmann (1997) as so called lomas formations – isolated islands of floristic assemblages, which are separated from each other by barren, hyper arid habitat. These lomas can exist where fog (or occasional rain) meets the coastal cordillera, delivering the necessary humidity. Where, due to absent mountains or the lack of steep cliffs, fog dissipates inland, no such vegetation zone has evolved (Rundel *et al.*, 1991). Further isolation of these lomas is brought about by the hyper arid inland desert pampa into which the eastern slopes of the coastal cordillera descend, and which remains almost totally barren until the higher elevations of the western slopes of the Andean cordillera.

Considering the physical environment and long-term aridity of the Atacama Desert, the question arises how such a high degree of species diversity and endemism, as well as numerous taxa highly specialised to aridity have evolved. Are they the result of trans-Andean or southern/northern incursions followed by adaptive radiations? Or have they evolved autochthonously in parallel to the evolution of aridity? Numerous studies have addressed the macroevolutionary questions relating to larger-scale biogeographic patterns for Atacama lineages through a phylogenetic approach (e.g. Luebert *et al.*, 2009; Luebert, 2011; Gengler–Nowak, 2002; Böhnert *et al.*, 2019; Dillon *et al.*, 2009), and four floristic elements, the basal unit in the study of historical biogeography, have been identified for the Atacama flora by Luebert (2011): Central Chilean, trans-Andean, antitropical and neotropical. This means, that the Atacama Desert flora is composed of lineages with myriads of differing evolutionary histories, in particular over deep geological time. The high rate of endemism of taxa of the Atacama Desert flora further points to highly dynamic plant evolutionary processes. Lineages evolve in direct response to geological and climatological processes (Axelrod, 1967; Gillespie & Roderick, 2014). In long-term arid environments, such evolutionary processes are more profound than in more stable habitats (such as the wet tropics), because greater spatio-temporal species turnovers may lead to higher extinction rates (Gaston & Spicer, 2004; Stebbins, 1952). Through contraction and expansion of suitable habitats during climatic oscillations, populations may undergo phases of isolation, experience genetic drift, and reconnect to form new species and species assemblages (Stebbins, 1952). These dynamics can cause the evolution of endemic organisms, either as relicts of extinctions (paleo-endemics), or newly evolved taxa, (neo-endemics; Stebbins & Major, 1965). So far, only few studies have addressed this gap of knowledge for the Atacama Desert (e.g. Viruel *et al.*, 2012; Baranzelli *et al.*, 2014; Ossa *et al.*, 2013, 2017).

The aim of this thesis is to investigate the microevolutionary processes within and between geographically isolated popoulations of desert plants, and how these processes are influenced by abiotic factors. Biogeographic patterns and diversification events in relation to the climate history of the Atacama arid habitats are analysed, and a synopsis of two genera is provided that take into account morphological and phylogenetic discoveries, which arose from the studies presented here, or from the scrutiny of plant material that has become available through the course of this project. This work contributes to advancing our understanding of plant evolution in arid environments.

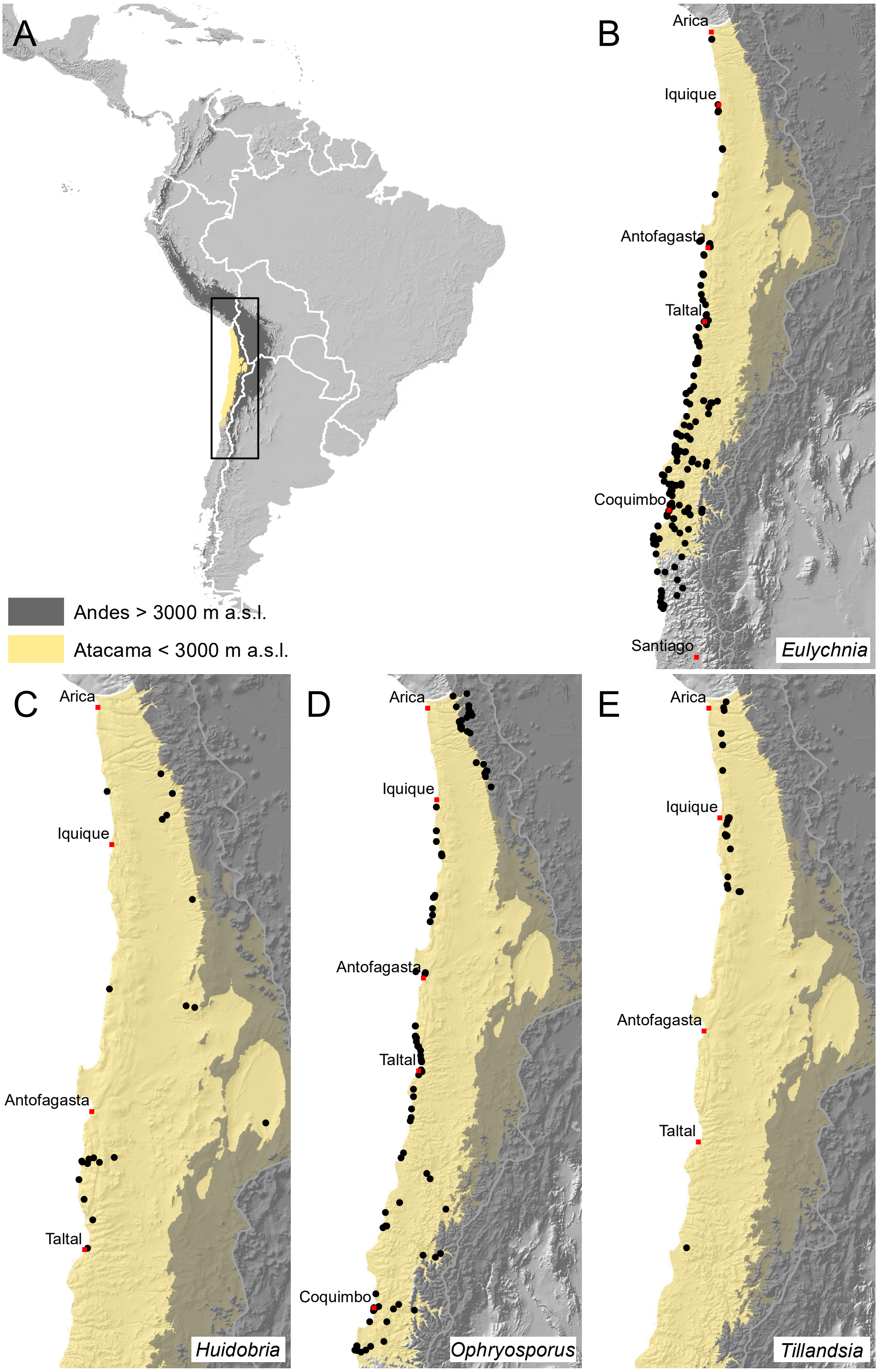

Figure 1: Location of the Atacama Desert in South America (A) and distribution of taxa studied in the present thesis (B–E). *Eulychnia* (B) and *Tillandsia* (E) occur in lomas formations along the coast, while *Huidobria* (C) and *Ophryosporus* (D) are distributed along the coast as well as along the western flank of the Andes, their populations separated by the hyper arid desert core.

1.4 Study system and organisms

The plants investigated in this dissertation belong to four genera: *Eulychnia* Phil. (Cactaceae), *Huidobria* Phil. (Loasaceae), *Ophryosporus* Meyen (Asteraceae) and *Tillandsia* L. (Bromeliaceae). *Eulychnia* and *Ophryosporus* were studied as species complexes. In the two remaining genera a single species each was under study. These are *Tillandsia landbeckii* Phil. and *Huidobria fruticosa* Phil. Selection of these organisms was based upon the following criteria:

(1) Their distribution begins with the transitional zone from Mediterranean vegetation into the arid, hyper arid and ultra-hyper arid environments (Luebert & Pliscoff, 2017). As such their centres of distribution are in the core Atacama, with one species, *Huidobria*, as an endemic lineage that only occurs here (Grau, 1997).

(2) The distribution of these taxa is either coastal (*Tillandsia* and *Eulychnia*) or both coastal and Andean (*Huidobria* and *Ophryosporus*), in which latter case the distribution range is separated by the hyper arid, barren landscape of the interior Atacama (Fig. 1), possibly acting as barrier to gene flow. Coastal populations of these species occur in geographically distinct lomas formations. The resulting isolated populations lend themselves perfectly for the study of genetic connectivity among them.

(3) The four genera have different life strategies. *Eulychnia* and *Tillandsa* are both perennial herbs/succulents, while *Huidobria* and *Ophryosporus* are shrubs. Their dispersal differs in their strategy, ranging from anemochory (*Huidobria*, *Ophryosporus* and *Tillandsia*) to zoochory (*Eulychnia*) (Weigend *et al.*, 2004; Cares *et al.*, 2018), and include the possiblity of dispersal via clonal offsetts (plantlets), particularly in *Tillandsia*.

The environmental requirements of these four genera have received little study, perhaps with the exception of *Tillandsia landbeckii* (Rundel *et al.*, 1997; Latorre *et al.*, 2011), and to a lessert extent *Eulychnia* (e.g. Larridon *et al.*, 2018), and still very little is known about the ecological factors which control the distribution of the species in their respective environments. Over the next pages follows, a summary of the current knowledge of these organisms and their habitats.

Eulychnia Phil., Cactaceae

This genus is principally distributed along the coast of the Atacama and Peruvian Deserts, with the southermost species, *E. castanea* occurring in Region IV Coquimbo, near the town of Los Molles 33°S. From here the distribution stretches along the coast of northern Chile all the way to Region XVI Arica y Parinacota and the town of Arica (Pinto, 2007; Fig. 1B). Some 500 kilometres from the Chilean border, near the town of Chala in southern Peru at 15°S, there is a disjunct occurrence of a single species, *E. ritteri.* Species diversity in this genus is highest in the hyper arid coastal regions of northern Chile, beginning north of Coquimbo, with very few inland incursions of taxa such as *E. acida* var. *elata* and *E. breviflora* var. *tenuis.* The different taxa seem to replace each other along a south-north gradient, with the exception of a single taxon, *E. breviflora*, which has a more or less continuous distribution along the coast from Coquimbo to Caldera. The current allopatric distribution of most taxa suggests that speciation may have taken place in geographic isolation, which is common in the Cactaceae family (e.g. Donati *et al.*, 2017; Larridon *et al.*, 2015). Adaptation to local vegetation zones depending on precipitation regimes have been suggested in previous studies (Larridon *et al.*, 2018). Preliminary divergence time estimates have been provided by Hernández-Hernández *et al.* (2014), but these studies only included very few taxa of this genus and to enlargen the sampling has been shown to increase the age of the clades (Linder & Rieseberg, 2004). Given this preliminary knowledge of the genus *Eulychnia* an increased age of the clade is to be expected, as well as a diversification in response to the evolution of climate in this region. The evolution of taxa in isolation further calls for an update of taxonomic concepts in the genus.

Huidobria fruticosa Phil., Loasaceae

Huidobria Gay is an ancient endemic lineage of the Atacama Desert, with two species, *H. fruticosa* and *H. chilensis* that have diverged between 40–60 mya (Acuña Castillo *et al.*, 2019). *Huidobria fruticosa* is distributed along the coast of northern Chile, as well as along the western flanks of the Andean cordillera to an elevation of about 3700 m (Grau, 1997; Fig. 1C). No plants have been found in the hyper arid inland core of the Atacama, although a botanical inventory of the canyons connecting the Andes with the coast is still needed. *Huidobria* very effectively disperses its seeds, which ripen in their thousands within capsules, are typical dust flyers, and as such are probably distributed considerable distances with the wind (Weigend *et al.*, 2004). As a result, populations of this species appear to "move" – where during one field work visit populations could be observed, during the next visit there would be none. Instead, new populations had emerged in previously apparently unoccupied places. This suggests a seed bank reservoir. Further, the plants seem capable of very fast growth following precipitation events, and are also able to re-grow from the rootstock of plants that had died back already. The plants appear where water accumulates following a precipitation event, including road side depressions, small run off channels on hill slopes or larger quebradas with a more constant flow of water. They possess a strong tap root (Acuña Castillo & Weigend, 2017), and one can observe populations of *Huidobria* to occupy dry river beds in places where no other plants seem to occur, likely because of their ability to access ground water (Jaeschke *et al.*, 2019). Given this patchy and irregular appearance of populations in an otherwise often barren landscape with few other floristic elements, this species is a very interesting candidate for population genomic studies, in particular considering the phylogenetic age estimates of this lineage which may have paralleled evolution of hyper aridity in the Atacama. In such a case one would expect the genetic signature to reveal long-term isolation of populations.

Ophryosporus Meyen, Asteraceae

The genus *Ophryosporus* contains approximately 40 species distributed along the Andes of northern Chile to southern Colombia, as well as the Atlantic rain forest of eastern Brazil (King & Robinson, 1972b). The coastal species of *Ophryosporus* in Chile form a morphologically distinct group (King & Robinson, 1972a), and preliminary data point to the possibility, that they are also forming a distinct phylogenetic clade, sister to the rest of the genus. Different Chilean species occur disjunct between the coast and the Andes (Fig. 1D), separated by the hyper arid and barren inland Atacama. Along the coast, different species replace each other on a south-north gradient, with some overlap and accordingly some apparent hybridisation (based on observations of morphology). The genus has a complex taxonomic history, and besides an unpublished thesis (Plos, 2012) and several lectotypifications (Plos & Sancho, 2013), a recent revision of the genus is so far lacking. This has led to the recognition of different species present in Chile, but so far no conclusive evidence based on thorough collecting and extensive herbarium research has been provided, in particular concerning several taxa that have only been collected very sporadically in the past and are only known from few locations. Nomenclatural clarification concerning several names in use is also required. Further, although not included in this dissertation, a population genomic study is currently in preparation.

Tillandsia landbeckii Phil., Bromeliaceae

Tillandsia landbeckii, subgenus *Diaphoranthema*, is one of the southernmost members of the genus *Tillandsia*. Molecular clock dating analyses suggest an origin of subgenus *Diaphoranthema* at the Miocene/Pliocene boundary, around 5 mya (Givnish *et al.*, 2011).

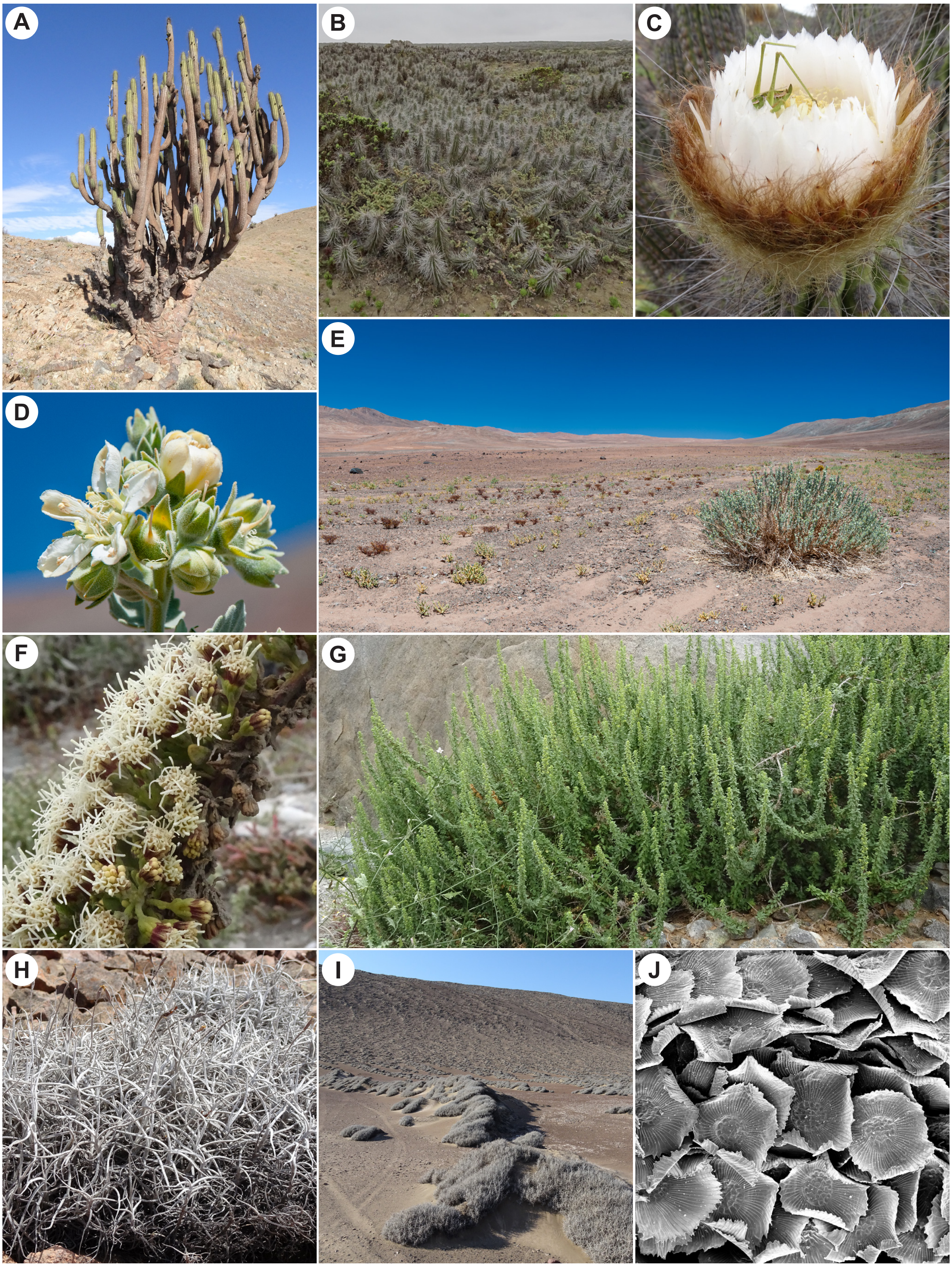

Figure 2: Overview of the four study genera. A) Tree-like habit of *Eulychnia acida* var. *elata* F.Ritter B) Procumbent habit of *Eulychnia acida* var. *procumbens* F.Ritter C) Flower of *Eulychnia breviflora* with the typical woolly hypanthium. D) Close-up of the flower of *Huidobria fruticosa*. E) *H. fruticosa* in its typical habitat. F) inflorescence of *Ophryosporus triangularis* Meyen. G) Habit of *O. triangularis*. H) Close-up of *Tillandsia landbeckii* Phil. I) View into a monospecific loma formation of *T. landbeckii*. J) SEM image of the plate-like, specialised leaf-trichomes of *T. landbeckii* which absorb fog moisture. Foto credit: F.F. Merklinger A–C & F–I; T. Böhnert D–E; J. Jeiter J.

Some species in this subgenus have invaded sandy soil habitats, with a shift from an epiphytic to epilithic life style (Rundel *et al.*, 1997). These functionally rootless plants form monospecific communities called "Tillandsiales" or simply *Tillandsia* lomas along the coast (Fig. 1E), in which the plants form a conspicuous banded growth pattern. They only occur in places where the influx of coastal fog provides enough humidity that can be absorbed, such as on the slopes of river canyons that allow for fog to penetrate through the coastal cordillera. *T. landbeckii* uses highly specialised leaf trichomes unique to the Bromeliaceae family to take up fog moisture. While species diversity in *Tillandsia* which form such "Tillandsiales" increases in Peru with about a dozen different taxa, (Rundel & Dillon, 1998), in Chile only three loma-forming species are known. Despite much important work on the establishment of these "Tillandsiales" (e.g. Latorre *et al.*, 2011; Borthagaray *et al.*, 2010; Rundel *et al.*, 1997), there is still a lack of understanding in the environmental variables other than fog, which control the distribution of this species. There is a conspicuous distribution gap between the southernmost known *Tillandsia* loma and the main concentrations of populations north of the Río Loa, and more research, in particular to model the abiotic factors including climate history of the region are needed in order to explain this phenomenon. Studies on loma forming *Tillandsia* in Peru revealed that the plants are capable of clonal reproduction (Masuzawa, 1986). Members of this subgenus further show varying types of reproductive strategies including cleistogamy (Gilmartin & Brown, 1985) and they produce seeds with a pseudo-pappus, predestined for wind dispersal (Till, 1992). The question arises if these isolated *Tillandsia* lomas maintain gene flow among each other through pollinaton or seed dispersal, whether the typical banded patterns of the lomas are the result of clonal reproduction, and how this phylogenetically young group has evolved under the recent climatic fluctuations in the Atacama, particularly during the Pleistocene.

1.5 Hypotheses

Current populations of the species under study are genetically isolated from each other, correlating to their geographically isolated distribution across the Atacama Desert. Genetic isolation is mitigated to some extend by the dispersibility of species, but gene flow is shaped by geographical barriers such as surface rivers, or patches of hyper arid absolute desert. Populations of groups of species that are currently understood as belonging to different taxonomic units maintain gene flow among each other, because they have evolved in or have arrived into the Atacama Desert recently, are in the process of speciation and have not yet reached complete reproductive isolation. The evolutionary split between extant Atacama lineages was triggered by the onset of hyper arid conditions.

Specific hypotheses regarding the four organisms studied here, and which have been addressed in this thesis are as follows:

(1) *Tillandsia* lomas are genetically isolated from each other in correlation to their geographic isolation, expressed in high levels of genetic differentiation and measured by F_{ST}. The species' ability to disperse effectively via seeds allows for limited gene flow between populations which are geographically close to each other. The banded patterns of *Tillandsia* lomas establish by clonal growth of the individuals. If so, the inbreeding coefficient F_{IS} should point to a low genetic diversity within populations.

(2) The genetic signature of current populations of *Huidobria fruticosa* reflects long term isolation in parallel with the long term aridity of the Atacama Desert. This is expressed by higher values of F_{ST}. Effective dispersal and seed bank effects are responsible for the establishment of current populations. Repeated colonisation events incurred from Andean populations toward the coast along desert canyons.

(3) *Eulychnia* has evolved in the coastal Atacama Desert and began to diversify in response to the onset of hyper aridity. If so, divergence time estimates should parallel the timing of major past climatic events in the context of development of aridity in the Atacama Desert.

1.6 Overview of the dissertation

This dissertation is a cumulative work of manuscripts, either published, already submitted, or yet to be submitted. Chapter 2 has been published and chapters 4 and 6 have been accepted for publication. Chapters 3 and 5 are included at manuscript stage and are in preparation to be submitted. Each chapter follows the basic structure of introduction, materials and methods, results and discussions, however these sections may vary in appearance, depending on the style of the journal where publication is envisaged. References are cited for each chapter individually to avoid duplication at the end with those already appearing in published articles; appendices for each chapter appear in summary at the end of the dissertation.

Chapter 2[i] explores the genetic structure within and between populations of *Tillandsia landbeckii*. Our sampling included over 300 individuals from 21 populations, and

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[i] Merklinger, F.F., Y. Zheng, F. Luebert, D. Harpke, T. Böhnert, A. Stoll, M.A. Koch, F.R. Blattner, T. Wiehe, D. Quandt 2020. Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert. *Global and Planetary Change* 184, doi: 10.1016/j.gloplacha.2019.103076.

the analyses were based on SNP data. The main findings are (1) *Tillandsia* employs a mixed-mating system including sexual and asexual reproduction, and population establishment is facilitated by the production of wind-dispersed seeds and the dispersal via plantlets; (2) genetic differentiation increases with geographic distance between the populations, indicating signatures of past migration and ancient gene flow. Current genetic patterns are maintained by geographical barriers that shape distribution patterns and colonisation success; (3) there is considerable genetic diversity within populations of *Tillandsia* belonging to a geographically central cluster, pointing to sexually reproducing individuals, but low genetic connectivity between clusters points to past dispersal of few founding individuals and subsequent isolation.

Chapter 3[ii] analyses the population dynamics of a palaeo-endemic species, *Huidobria fruticosa*. The sampling included over 350 individuals from 21 populations, and the analyses were based on SNP data. The main findings are (1) Pleistocene climatic events appear to be responsible for the observed population structure, which is at odds with the phylogenetic age of ∼60 Ma for this lineage. (2) Genetic differentiation between populations is higher than in the neo-endemic lineage *Tillandsia*, which may be correlated to its ephemeral habit promoting the formation of new populations in geographic and genetic isolation, and linked to a seed bank (3) Dispersibility and seed bank enable the species to re-colonise coastal and Andean habitats repeatedly, perhaps facilitated by Pleistocene refugia along the coast as well as the river canyons that cross the hyper arid inland Atacama.

Chapter 4[iii] is a phylogenetic and biogeographical study of the genus *Eulychnia* that includes material from the type localities of all previously published names in the genus. The study employs two methods, (1) plastid markers based on a family-wide phylogeny of the Cactaceae provided by Hernández-Hernández *et al.* (2014), into which our sampling of *Eulychnia* is incorporated, and (2) SNP data obtained from GBS. Phylogenies were calculated using sequence alignments obtained from both methods, and these phylogenies were molecular clock-dated in order to understand the diversification history of the genus in the Atacama Desert. *Eulychnia* split from its sister genus *Austrocactus* at the Miocene-Pliocene boundary approximately 6.70 mya and diversified into an already arid environment in a relatively short period beginning about 2 mya. This onset of diversification correlates well with recent findings of geological data (Rit-

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[ii] Merklinger, F.F., Y. Zheng, F. Luebert, T. Böhnert, D. Harpke, A. Stoll, T. Wiehe, M. Weigend & D. Quandt 2020. Genome-wide SNP data reveal recent population structure of an ancient endemic lineage in the Atacama Desert. To be submitted.

[iii] Merklinger, F.F., Böhnert, T., Weigend, M., Quandt, D., Luebert, F. Quaternary diversification of a columnar cactus in the driest place on Earth. Submitted to: *American Journal of Botany.*

ter *et al.*, 2018), which suggests oscillations of aridity during the Pleistocene. These oscillations led to contraction (and isolation) of populations, a process we interpret as the key driver for diversification in *Eulychnia*.

Chapter 5[iv] is a synopsis of the genus *Eulychnia*. The most important characters that have been used since Britton & Rose (1920) to differentiate between species of *Eulychnia* have been associated with the flower, because the hypanthium bears either dense wool, bristly spines or only very short, sparse and inconspicuous hairs. However, we are presented with a continuum of morphological variation throughout the range of the genus, and this is also reflected in the ca. 14 names that have been created in the past to describe various morphological variants. The species and subspecies of *Eulychnia* have a more or less distinctly allopatric distribution with some overlap at range limits. In this synopsis we present a summary of all currently available knowledge, resolve several nomenclatural issues and offer a key to the species of *Eulychnia*, following a taxonomic concept based on three species and nine subspecies.

Chapter 6[v] is a summary of the Chilean species of *Ophryosporus*, based on morphological and nomenclatural systematic studies, and including an overview of pappus morphology inferred from scanning electron microscopy (SEM). Several lectotypifications and an epitypification are provided to resolve the status of types associated with the genus. We conclude that six species of *Ophryosporus* naturally occur in Chile, with two further previously accepted species restricted to Peru. A key to the Chilean species of *Ophryosporus* is provided. This work serves as a basis for further, population-based studies in this genus.

Chapter 7 presents the overall conclusions of this dissertation and provides an outlook for further research questions that arose during the course of this study but could not be included here.

1.7 Contribution to chapters

Chapter 2: Merklinger, F.F., Y. Zheng, F. Luebert, D. Harpke, T. Böhnert, A. Stoll, M.A. Koch, F.R. Blattner, T. Wiehe, D. Quandt 2020. Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert. *Global and Planetary Change* 184, doi: 10.1016/j.gloplacha.2019.103076.

[iv] Merklinger, F.F. U. Eggli and F. Luebert. A synopsis of the genus *Eulychnia* (Cactaceae). To be submitted.

[v] Merklinger F.F. and Luebert, F. (*Phytokeys*). Notes on the genus *Ophryosporus* in Chile. In press.

Own contributions: Conducted field work together with Luebert, Böhnert, Quandt and Stoll. Designed the study and performed the analyses together with Zheng, Luebert, Harpke, Wiehe and Quandt. Wrote the paper, to which all others contributed.

Chapter 3: Merklinger, F.F., Y. Zheng, F. Luebert, T. Böhnert, D. Harpke, A. Stoll, T. Wiehe, M. Weigend & D. Quandt 2020 (manuscript). Genome-wide SNP data reveal recent population structure of an ancient endemic lineage in the Atacama Desert.
Own contributions: conducted field work together with Luebert, Böhnert, Quandt and Stoll. Designed the study, performed the analyses and wrote the paper together with Zheng, Luebert, Wiehe and Quandt. All authors contributed at the writing stage.

Chapter 4: Merklinger, F.F., T. Böhnert, M. Arakaki, M. Weigend, D. Quandt, F. Luebert (accepted manuscript). Quaternary diversification of a columnar cactus in the driest place on Earth. Submitted to: *American Journal of Botany.*
Own contibutions: Conceived the idea for the study, designed the research, conducted all analyses and wrote the paper together with Böhnert & Luebert. Arakaki, Weigend and Quandt contributed to the writing of the paper.

Chapter 5: Merklinger, F.F., U. Eggli and F. Luebert (manuscript). A synopsis of the genus *Eulychnia* (Cactaceae).
Own contributions: Conceived the idea in joint discussions with Luebert, with whom the study was designed and samples were collected in the field. All authors revised herbarium specimens and solved nomenclatural problems. Merklinger wrote the manuscript, which was commented on and improved by Luebert & Eggli.

Chapter 6: Merklinger, F.F. and F. Luebert (*Phytokeys*, in press). Notes on the genus *Ophryosporus* in Chile.
Own contributions: Both authors conceived the idea in joint discussions, designed the study, collected the samples in the field and revised herbarium material and nomenclatural issues. Merklinger wrote the manuscript, Luebert further contributed critical comments to the manuscript.

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Chapter 2

Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert

Merklinger, F.F., Y. Zheng, F. Luebert, D. Harpke, T. Böhnert, A. Stoll, M.A. Koch, F.R. Blattner, T. Wiehe, D. Quandt 2020. Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert. *Global and Planetary Change* 184, doi: 10.1016/j.gloplacha.2019.103076.

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Research article

Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert

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Population genetics

ABSTRACT

In hyper-arid habitats vegetation tends to be highly patchy with individual plant populations set widely apart from each other. In the Atacama Desert of northern Chile, rainfall is essentially absent, but fog occurring both at the coast and sometimes reaching inland areas supports patches of vegetation in an otherwise barren environment. *Tillandsia landbeckii* (Bromeliaceae), a epiarenic plant without functional roots, completely depends on fog as source for water, therefore it is found only in fog corridors. Here, we investigate the genetic connectivity within and between populations of *T. landbeckii*, using genome-wide single-nucleotide-polymorphisms (SNP) obtained through genotyping-by-sequencing (GBS). The 21 sampled populations from the Chilean Atacama Desert are distributed in three geographically ordered south to north clusters, with the southern cluster containing only one population that is genetically very distant from the others. From our study we obtained three genetic groups that corresponded to these three geographical clusters, with the exception of the two populations 16 and 18, where genetic affiliation lies at least in part with the central cluster. Further, our results show uneven distribution of genetic diversity among the populations with highest diversity in the central cluster. We found large amounts of shared heterozygous SNPs as well as negative values for the inbreeding coefficient F_{IS} in the populations of the north and south. They indicate that these populations are strongly affected by clonal reproduction, while the populations in the center are mostly reproducing sexually. We interpret these data as the result of genetic bottlenecks due to founder events involving few dispersing genotypes combined with strong geographical isolation for the northern and southern populations, following stepping stone dispersal of *Tillandsia* during more climatically favorable episodes.

1. Introduction

The Chilean Atacama Desert is regarded as one of the driest deserts on earth (Dunai et al., 2005). Where clouds from the Pacific Ocean meet the steep westward facing slopes of the coastal cordillera, they deliver fog as the only major and regular source of humidity. As a result, vegetation with a relatively high species diversity is restricted to these areas, called loma formations, “fertile belts” or “fog oases” (Johnston, 1929; Muñoz-Schick et al., 2001; Rundel et al., 1991). Corridors of increased humidity are found where gaps in the coastal cordillera allow for fog to permeate inland, which in turn allows for plant growth also in these areas (Muñoz-Schick et al., 2001; Pinto et al., 2006; Rundel et al., 1991). These fog corridors are very unevenly distributed and the lomas are often isolated from each other by large stretches of barren landscape.

Tillandsia landbeckii Phil. (Bromeliaceae; Tillandsioideae; *Tillandsia* subg. *Diaphoranthema*) is one of 14 species in the genus *Tillandsia* adapted to the extremely arid conditions of the Atacama and Peruvian Deserts (Rundel et al., 1997). It's geographic distribution stretches from the Coquimbo region in Chile (type locality Illapel, 31.65°S) to the north of Peru and possibly into Ecuador (Smith and Downs, 1977; Till, 1992a). It can grow epiphytically, but it is mostly known as an

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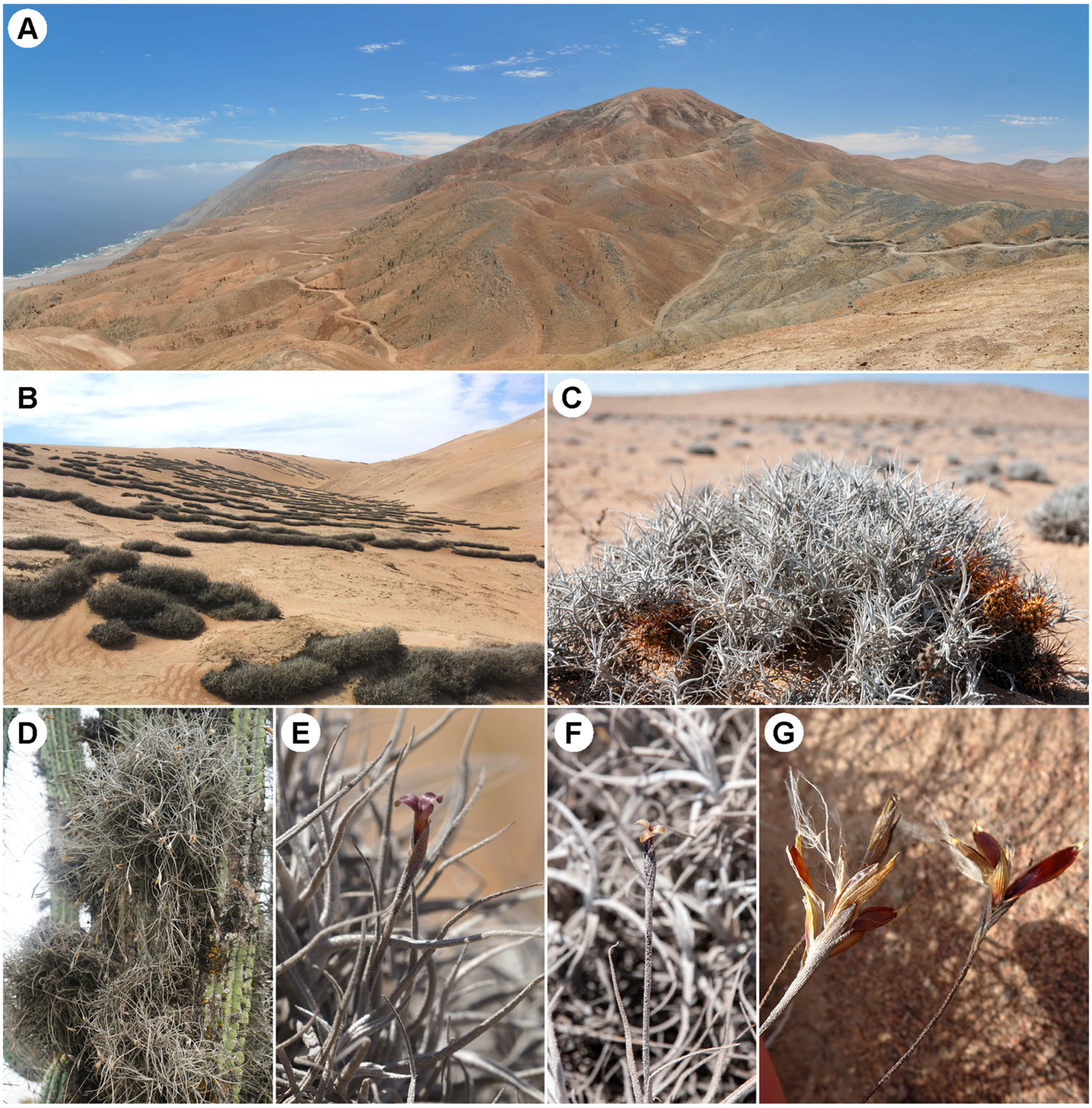

Fig. 1. Habitat (A-B) and details (C-G) of *Tillandsia landbeckii.* A) Panorama of a typical *T. landbeckii* loma at Alto Chipana (Pop 5), the gray-shaded slopes indicate the presence of dense *Tillandsia* stands. B) View into the Río Lluta population (Pop 21) showing the characteristic banded pattern. C) *T. landbeckii* individual from the Caldera population (Pop 1) growing together with *Cumulopuntia leucophaea.* D) *T. landbeckii* growing epiphytically on *Eulychnia acida* at the type locality of both species near the town of Illapel. E) 1-flowered inflorescence of *T. landbeckii* with purple flower at the Caldera locality (Pop 1). F) Inflorescence of *T. landbeckii* at the Salar Grande population (Pop 6). G) Seed shedding capsules of *T. landbeckii.* Note the pseudo-pappus on the seeds, predestined for wind-dispersal. Images: F. F. Merklinger (B, D &G) & T. Böhnert (A, C, E & F). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

epiarenic species from the arid coastal regions of Chile and Peru, where it forms near-mono-specific *Tillandsia* lomas, also referred to as “Tillandsiales” (Latorre et al., 2011; Pinto et al., 2006; Rundel et al., 1997; Rundel and Dillon, 1998; Fig. 1). These “Tillandsiales” form a characteristic banded landscape pattern that in some cases can even be recognized with remote sensing and satellite imagery (Castro Avaria et al., 2014; Wolf et al., 2016). While in Chile, *T. landbeckii* sometimes grows in sympatry with *T. virescens* or *T. marconae,* the majority of these xerophytic, lomas-forming species of *Tillandsia* is native to Peru. *T. landbeckii* is thus one of the southernmost representatives of the genus, alongside *T. minutiflora* and *T. usneoides.*

Tillandsia landbeckii is a physiological specialist: it traps and absorbs fog and nutrients through highly specialized leaf trichomes unique to this plant family and most complex in xerophytic *Tillandsia* species. In addition, the species employs Crassulacean acid metabolism (CAM) for photosynthesis (Benzing, 2000).

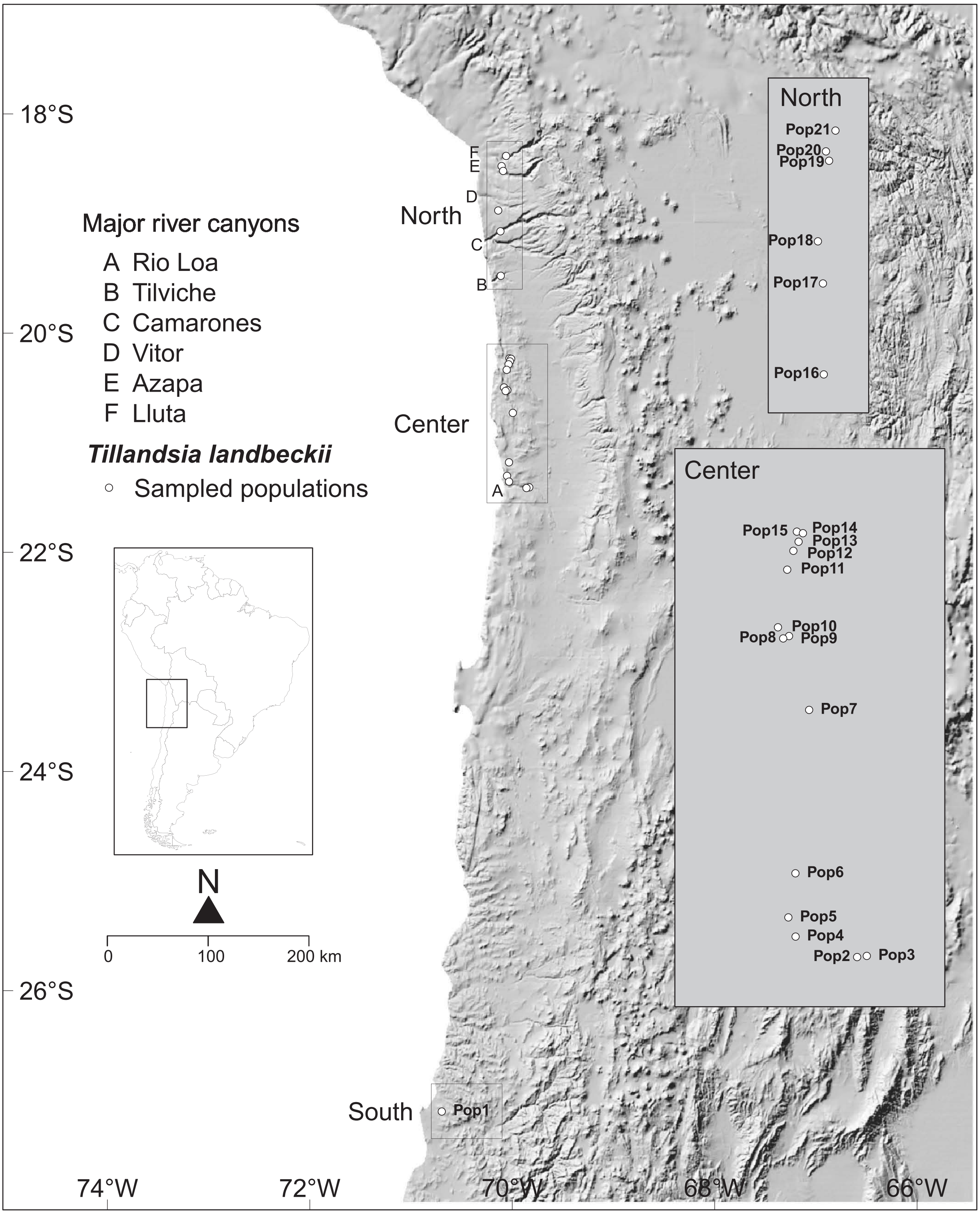

Fig. 2. The geographic location of sampling sites across the Atacama Desert, divided into three geographical clusters: north, center, south.

Tillandsia landbeckii plants trap airborne sand, leading to the formation of conspicuous dune systems (Borthagaray et al., 2010; Latorre et al., 2011; Pinto et al., 2006; Rundel et al., 1997; Westbeld et al., 2009). Attempts in dating subfossil remains of *Tillandsia* from within such dunes using radiocarbon methods showed that some dunes are at least 2500 to 3500 years old (Jaeschke et al., 2019; Latorre et al., 2011). Individual “Tillandsiales” often occupy several square kilometers, forming distinct, geographically isolated units (Fig. 1). These individual “Tillandsiales” are treated here as distinct populations. The *T. landbeckii* populations we studied are found between the town of Caldera in the Atacama region (27.07°S) and the town of Arica in the Arica and Parinacota region (18.48°S) stretching over a geographical distance of almost 1000 km (Fig. 2) and can be roughly grouped into three main geographical clusters. While each cluster comprises several populations in close proximity to each other, there are major gaps between these clusters, likely due to the lack of suitable habitats. The southernmost *Tillandsia* loma (Caldera, Pop 1) is particularly remote (600 km) from its closest known neighbor at the northern banks of the Río Loa (Pop 2; 21.41°S).

Studies of vegetation patterns in arid ecosystems indicate that abiotic factors (geomorphology and topography) are the main drivers of pattern formation because they regulate the water and nutrients available to plants (Borthagaray et al., 2010; Deblauwe et al., 2008; Rietkerk, 2004; von Hardenberg et al., 2001; Wolf et al., 2016). However, from a biotic perspective it is also important to take into account dispersibility and gene flow. If long-distance seed dispersal and pollen transfer are rare, the lomas in different areas might be effectively isolated and distant lomas could establish through propagule dispersal of few or single genets, thus consisting of one or very few genotypes only (Latorre et al., 2011; Rundel et al., 1997). The seeds of *T. landbeckii* are adapted to wind-dispersal with a plumose appendage called pseudopappus (Benzing, 2000; Magalhães and Mariath, 2012; Smith and Downs, 1977; Szidat, 1922). Although this indicates the possibility of long-distance dispersal, such a dispersal mode would be heavily influenced by prevailing wind patterns and subsequent germination success. In contrast, also clonal populations could play a role, as *T. landbeckii* is capable of vegetative reproduction. Colonizers may reproduce this way, forming mostly clonal populations if only few individuals or genotypes were able to arrive and establish at a new locality.

The breeding system of plant species can contribute importantly to gene flow and is crucial for the amount of genetic diversity within and between populations. In *Tillandsia* subgenus *Diaphoranthema* autogamy (including cleistogamy, an extreme form of selfing) has been reported (Donadío, 2013; Donadío et al., 2015; Gilmartin and Brown, 1985; Till, 1989, 1992b, 1992a). There are no published data on the breeding system of *T. landbeckii*, but Till (1992a, 1992b) suggested the occurrence of cleistogamy also in this species. This is supported by the reduced petal size and usually inconspicuous petal color, pointing to autogamy (as well as cleistogamy) rather than pollinator attraction. As far as we are aware, no pollinator has so far been reported for *T. landbeckii.* Further, the position of the anthers very close to the stigma, forming a hood above it to prevent cross-pollination strengthens the case for autogamous behavior in this species. Autogamy, combined with fast life cycle may be responsible for the successful colonization of extreme habitats (Till, 1992b).

To understand the genetic structure of *T. landbeckii* populations we investigate here (1) the genetic diversity within populations in order to test if reproduction within individual populations is predominantly sexual or asexual; (2) whether genetic distance and differentiation correlates with geographical distance or the presence of dispersal barriers; (3) whether the distribution pattern we see today is the result of long-distance dispersal events, or if the outlying populations are relics of an ancient, more widespread distribution.

To address these questions, we employed genotyping-by-sequencing (GBS), a method that has become widely used in population genetics because of its ability to screen large numbers of single-nucleotide polymorphisms (SNPs) across the genome. GBS has also been useful for the study of genetic diversity within populations, gene flow between populations, and of mating type and hybridization, without the need of a completely sequenced reference genome (Elshire et al., 2011; Narum et al., 2013; Nemati et al., 2019; Pannell, 2012; Pannell and Fields, 2014; Wendler et al., 2014).

2. Materials and methods

2.1. Study system

There is a large distribution gap of approximately 600 km without known *Tillandsia* lomas between the southernmost population situated in Caldera (Pop 1; 27.06°S) and the closest one to the north near the Río Loa (Pop 2; 21.41°S). Only individual epiphytic specimens rather than specimens belonging to extensive populations have been recorded in this intervening area (Johnston, 1929; Rundel et al., 1997; Smith and Downs, 1977). The northernmost population sampled for this study was the one at the Río Lluta valley (Pop 21;18.37°S), close to the town of Arica near the Chilean border with Peru. We were unable to confirm previous reports of populations at the edge of Quebrada Honda and Quebrada La Higuera (18.32°S and 18.68°S, Pinto et al., 2006). Because this study formed part of the Collaborative Research Center 1211 (sfb1211.uni-koeln.de) with focus on the mutual evolutionary relationships between Earth surface processes and biota in the Chilean Atacama Desert, sampling did not extend into Peru. In Chile, the highest population density is found between the Río Loa and the town of Iquique. According to Pinto et al. (2006) a total of over 30 individual populations are known of which 21 were sampled for the present study. These can be roughly assigned to three geographical clusters (Fig. 2): Cluster 1 (Caldera), cluster 2 (Río Loa to Iquique) and cluster 3 (Quebrada Tiliviche to Arica).

2.2. Population sampling

Stem and leaf tissue from a total of 21 populations and 307 individuals was sampled and silica-dried. An accession number was assigned to each population (Fig. 2, Table 1). All populations were sampled with 15 individuals each, apart from Pop 6 and Pop 2, which only consisted of 8 and 14 individual plants, respectively (Table 1).

Sampling was carried out along a transect crossing the population diagonally in order to gather plant material from different dunes and for the best possible coverage of the entire population. At least one voucher specimen from each population was deposited at the herbarium of the Nees Institute for Biodiversity of Plants, University of Bonn, Germany (BONN) and the herbarium of the La Serena University, Chile (ULS).

2.3. DNA extraction

DNA was isolated from silica-dried leaf and stem material, following the Macherey Nagel Nucleo Mag 96 protocol (Macherey Nagel, Düren, Germany). After homogenization and lysis, the samples were transferred into 96 Square Well Blocks to be processed by a Thermo Fisher Scientific KingFisher Flex benchtop system (Thermo Fisher Scientific, Waltham, MA, United States), binding DNA to NucleoMag C-beads and eluting at 55 °C into 150 µl MC6 elution buffer.

Extracted DNA was electrophoresed on 1% agarose gels using Lonza GelStar Nucleic Acid Gel Stain (100 ×) for testing the quantity and quality of the isolated products. A sample of 20 ng linear, double-stranded Lambda DNA (New England Biolabs, N3011S) was used to assess quality and quantity of the genomic DNA. Based on the agarose-gel images, Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, United States) measurements of selected individuals were taken, in order to assign a numerical value to the agarose-gel readings. Samples were then standardized to 20 ng/µl and a sample amount of 15 µl for

Table 1

Geographic and genetic summary for the 21 sampled populations. Geographic cluster (GC). Number of sampled individuals (n). Sum of haplotype diversity (Σπ). Individual level diversity (ID). Ratio individual level diversity/haplotype diversity (ID/π ratio). Number of SNPs (#SNPs). Observed haplotype diversity (Σπ /s). Expected haplotype diversity (expected Σπ/s). Normalized version of observed haplotype diversity – the expected haplotype diversity (Tajima's D). Total F_{IS} per population (Total F_{IS}). Mean F_{IS} per population (Mean F_{IS}).

Pop	Locality	Latitude	Longitude	Elev. (m)	GC	Voucher	n	Σπ	ID	ID/π ratio	# SNPs	Σπ/s	expected Σπ/s	Tajima's D	Total F_{IS}	Mean F_{IS}
1	Caldera	−27.10049	−70.675983	340	S	FL 3888 (BONN)	15	403.54	70.86	0.18	994	0.4060	0.2524	2.3793	−559.27	−0.56
2	Rio Loa 2	−21.41230	−69.837087	980	C	FFM 2017–11 (BONN)	14	245.85	97.88	0.40	751	0.3274	0.2570	1.0813	−201.94	−0.27
3	Rio Loa 1	−21.40911	−69.810407	1021	C	FFM 2017–10 (BONN)	15	243.93	208.10	0.85	846	0.2883	0.2524	0.5564	30.71	0.04
4	Alto Chipana - Rio Loa	−21.35518	−70.008463	950	C	FL 3429 (BONN)	15	281.39	286.04	1.02	1107	0.2542	0.2524	0.0277	61.30	0.06
5	Alto Chipana	−21.30233	−70.028094	1000	C	FL 3426 (BONN)	15	378.55	368.90	0.97	1539	0.2460	0.2524	−0.0997	22.92	0.01
6	Salar Grande	−21.17943	−70.008580	940	C	FL 3430 (BONN)	8	485.34	317.00	0.65	1263	0.3843	0.3014	1.2076	−250.07	−0.20
7	Cerro Pajonal	−20.72587	−69.970655	960	C	FL 3432 (BONN)	15	260.11	143.87	0.55	1018	0.2555	0.2524	0.0482	−108.99	−0.11
8	Salitrera San Lorenzo 2	−20.52704	−70.042713	1183	C	FFM 2017–13 (BONN)	15	317.43	327.90	1.03	1785	0.1778	0.2524	−1.1566	118.15	0.07
9	Salitrera San Lorenzo 1	−20.52079	−70.026417	1204	C	FFM 2017–14 (BONN)	15	421.46	372.12	0.88	1710	0.2465	0.2524	−0.0920	−15.10	−0.01
10	N Salitrera San Lorenzo	−20.49637	−70.057147	1206	C	FFM 2017–15 (BONN)	15	497.45	505.21	1.02	2180	0.2282	0.2524	−0.3757	12.98	0.01
11	Cerro Guanaco	−20.33597	−70.031656	1040	C	FL 3433 (BONN)	15	425.17	306.69	0.72	1433	0.2967	0.2524	0.6868	−162.42	−0.11
12	Cerro Carpas	−20.28434	−70.014092	1000	C	FL 3434 (BONN)	15	405.64	374.83	0.92	1525	0.2660	0.2524	0.2108	−16.03	−0.01
13	E of Alto Hospicio	−20.25889	−70.00000	1049	C	FFM 2017–18 (BONN)	15	307.55	333.75	1.09	1465	0.2099	0.2524	−0.6585	122.71	0.08
14	NE of Alto Hospicio 2	−20.23537	−69.987869	1049	C	FFM 2017–17 (BONN)	15	356.45	373.42	1.05	1619	0.2202	0.2524	−0.4999	101.46	0.06
15	NE of Alto Hospicio 1	−20.23059	−70.004541	1023	C	FFM 2017–16 (BONN)	15	419.83	390.81	0.93	1856	0.2262	0.2524	−0.4064	−9.73	−0.01
16	Quebrada Tilivice	−19.47631	−70.090870	1064	N	FFM 2017–66 (BONN)	15	382.19	317.72	0.83	1354	0.2823	0.2524	0.4631	−83.89	−0.06
17	N edge of Camarones	−19.07064	−70.094111	1112	N	FFM 2017–58 (BONN)	15	504.93	318.21	0.63	1629	0.3100	0.2524	0.8927	−311.21	−0.19
18	Quebrada Vitor	−18.88194	−70.116794	1030	N	FL 3442 (BONN)	15	264.53	65.84	0.25	669	0.3954	0.2524	2.2127	−331.94	−0.50
19	N edge of Azapa	−18.52052	−70.066858	1093	N	FFM 2017–60 (BONN)	15	524.84	426.12	0.81	1822	0.2881	0.2524	0.5531	−158.01	−0.09
20	Pampa 2 Cruces	−18.47623	−70.084514	1000	N	FL 4002 (BONN)	15	450.93	213.40	0.47	1321	0.3414	0.2524	1.3791	−375.69	−0.28
21	N edge of Rio Lluta	−18.38547	−70.038438	1145	N	FFM 2017–59 (BONN)	15	471.38	263.92	0.56	1476	0.3194	0.2524	1.0383	−362.13	−0.25

each individual was used for library preparation and sequencing.

2.4. Library preparation and genotyping-by-sequencing

For library preparation 200 ng of genomic DNA were used and digested with the restriction enzymes *Pst*I-HF (New England Biolabs, R3140S) and *Msp*I (New England Biolabs, R0106S). Library preparation, individual barcoding, and single-end sequencing on the Illumina HiSeq 2500 followed Wendler et al. (2014). The library was size-selected to a range of 200–600 bp with a SYBR gold stained electrophoresis gel. Fragment size distribution and DNA concentration were evaluated on an Agilent BioAnalyzer High Sensitivity DNA Chip and using the Qubit DNA Assay Kit in a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, United States). Finally, the DNA concentration of the library was checked by a quantitative PCR run. Cluster generation on Illumina cBot and sequencing (1 × 100 bp) on the Illumina HiSeq 2500 platform followed Illumina's recommendations and included 1% Illumina PhiX library as internal control.

Barcoded reads were de-multiplexed using the Casava pipeline 1.8 (Illumina, Inc.). The obtained raw sequence reads (0.5–4 million per individual) were adapter trimmed and quality trimmed (phred score > 25) with cutadapt v1.16 (Martin, 2011), and reads shorter than 65 bp after adapter removal were discarded. Sequence reads for the GBS Illumina runs were deposited in the European Nucleotide Archive under the study accession PRJEB35036.

2.5. Analysis of genotyping-by-sequencing data

A de novo assembly of the GBS data of 307 *Tillandsia* individuals was carried out using ipyRAD v0.7.19 (Eaton, 2014). The minimal number of samples per locus was set to 200, the maximum cluster depth within samples was set to 0.85. Based on data by Koch et al. (unpublished), *T. landbeckii* is assumed to be diploid, so the ploidy level was set to diploid. For the other parameters the default settings of parameter files generated by ipyRAD were used. VcfTools v0.1.14 (Danecek et al., 2011) was used to filter out the SNP positions with a depth below six and to produce a filtered vcf file for downstream analyses.

In order to document the intra-population genetic variability, we calculated the following statistics per population: (1) Haplotype diversity π, i.e., the average number of different nucleotides between two random haploid genomes. (2) average genotype diversity $\bar{g}$, a measure of genotype difference between two randomly chosen individuals, defined as:

$\bar{g} = \frac{2}{n(n-1)} \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} x_{ij}$, where n is the number of individuals sampled and

$$x_{ij} = \begin{Bmatrix} 0 \\ 0.5 \\ 2 \end{Bmatrix} \text{if genotypes } i \text{ and } j \text{ share} \begin{Bmatrix} 2 \\ 1 \\ 0 \end{Bmatrix} \text{alleles.}$$

(3) Tajima's D (Tajima, 1989). (4) The inbreeding coefficient $F_{IS.}$ (5) Number of nucleotides that are heterozygous in 1, 2, …, n individuals, where n is the sample size. (6) Number of positions that are homozygous for one allele in at least x individuals, and homozygous for a different allele in at least x other individuals; x equals 1, 2 or 3. For (5) and (6), a subset of SNPs was used so that every locus is represented only by its most (globally) variable site.

In a structured population, the expectations of π equals $4N\mu$, where N is the population size of the analyzed population plus other populations with which it has substantial gene flow, and μ is the mutation rate. Under Hardy-Weinberg Equilibrium and neutral evolution, individual level diversity is expected to equal π.

Population structure was examined through a sparse Non-Negative Matrix Factorization algorithm (SNMF) using the R-package LEA v2.4.0 (Frichot and François, 2015) in an initial run for K = 2 to K = 21 clusters and 20 repetitions. SNMF provides a STRUCTURE-like output.

In parallel, fastSTRUCTURE v1.0 (Raj et al., 2014) was employed to approximate ancestry estimates, i.e. to identify the proportion of ancestry contributed by different populations averaged across the entire genome (Padhukasahasram, 2014). A maximum likelihood analysis was conducted with RAxML v. 8 (Stamatakis, 2014) using the population consensus sequences under the GTRCAT+I model (GTR model, approximate Gamma rate heterogeneity and a proportion of invariable sites). Bootstrap values were calculated based on 100 replicates. Average F_{ST} across all loci was calculated based on pairwise comparisons between populations and correlated to geographical distance between each pair of populations using a Mantel test with the R package VEGAN v.2.5–4 (Oksanen et al., 2019). F_{ST} was also calculated between groups of populations defined by the LEA analysis with K = 3.

3. Results

3.1. Genetic diversity and inbreeding coefficient F_{IS}

The genetic diversity at haplotype level (π) and individual level (ID) are shown in Table 1. Here we see that while π has a 2.15-fold variation, individual level diversity has the highest (505.2) being 7.67 times as large as the lowest (65.8). The values obtained for Tajima's D are strongly positive in populations that have a more negative F_{IS}, indicating an excess of intermediate-frequency alleles (in the case of the all-heterozygous SNPs, the frequencies are 50%).

The inbreeding coefficient analysis (Table 1) shows the lowest mean F_{IS} for the southernmost population Pop 1 (Caldera) with a value of −0.56, followed by population 18 (Vitor) with a value of −0.50. Negative values were also obtained from other northern populations, including Pop 20 (Pampa dos Cruces), Pop 21 (Rio Lluta) and Pop 17 (Camarones), with values of −0.28, −0.25 and − 0.19 respectively. In the central cluster around Iquique and the Rio Loa, population Pop 2 (Rio Loa) showed the lowest negative value (−0.27), followed by Salar Grande (Pop 6) with −0.20, Cerro Pajonal (Pop 7) and Cerro Guanaco (Pop 11), both with a value of −0.11 and Salitrera San Lorenzo 1 (Pop 9), Cerro Carpas (Pop 12) and Alto Hospicio 1 (Pop 15) with a mean F_{IS} of −0.01. The other populations in this area generally indicated values close to zero. Populations with strongly negative F_{IS} are also likely to have a lower individual level diversity than π. The correlation between mean F_{IS} and individual-diversity-to-haplotype-diversity ratio is 0.94.

3.2. Heterozygosity analysis

Four populations showed excessively high numbers of shared heterozygous SNPs among all individuals (Table 2). In particular Pop 1 (Caldera) showed 439 heterozygous SNPs for all 15 individuals, followed by Pop 18 (Vitor) with 230 heterozygous SNPs for 15 individuals, Pop 21 (Rio Lluta) 127 heterozygous SNPs for 15 individuals and Pop 2 (Rio Loa 2) with 103 heterozygous SNPs for 14 individuals. Populations 20, 7 and 17 shared 59, 51 and 48 heterozygous SNPs. In summary, seven populations out of 21 shared a large number of heterozygous SNPs while the other 14 populations shared less than eight heterozygous SNPs. Additionally, some populations show atypical "peaks" in the number of heterozygotes shared by x and n-x individuals: for example, $x = 3$ for Pop 21, and $x = 2$ for Pop 20. The populations with an excessive number of shared heterozygous SNPs also have the most negative F_{IS} values and there is an inverse relationship between F_{IS} and shared heterozygous SNPs (Fig. S1).

3.3. Genetic structure among populations

Evaluation of population structure through a sparse Non-Negative Matrix Factorization algorithm (SNMF) in LEA resulted in a decreasing cross entropy curve as values for increasing K. LEA results for K = 2–9 are shown in Fig. 3. The analysis obtained from fastSTRUCTURE revealed an optimal K = 8.

The separations of the populations obtained at each value for K revealed a geographical pattern (Fig. 3). At K = 3 the three resulting genetic groups roughly correspond with the three geographical clusters with the exceptions of Pop 18 and Pop 16 that are geographically in the northern cluster (Fig. 2), but genetically fall into the central group. This grouping is also reflected in the ML-tree from the RaxML analysis (Fig. S2). At K = 8 populations from the central group at the Río Loa (Pop 3, Pop 2) and Cerro Pajonal (Pop 7) clearly separate from the rest. Within the northern group populations Vitor (Pop 18), Lluta and Azapa (Pop 21 and Pop 19) separate from the others.

Genetic distances expressed by F_{ST} were small to moderate (< 0.3), but a trend was observed between the three geographical clusters of populations (Fig. 4 and supplementary Table S1). Between populations belonging to the central genetic group, mean F_{ST} was comparatively low (e.g., between Pop 9 and Pop 10 = 0.025). Mean F_{ST} was also very low between populations from the northern geographic cluster (e.g. between Pop 20 and Pop 17 = 0.028), while the F_{ST} between populations of the central and northern geographic clusters was comparatively high (e.g., between Pop 7 and Pop 21 = 0.163). Consistently large mean F_{ST} was detected between the southernmost population and the northern geographic cluster (e.g., between Pop 1 and Pop 17 = 0.193; Pop 1 and Pop 20 = 0.222, etc.; see heat map Fig. 5 and Table S1). F_{ST} showed a positive correlation to geographical distance $r = 0.72$ (Fig. 4) supported by Mantel test ($P < .001$). When F_{ST} was compared between groups of populations resulting from the SNMF analysis with K = 3 (see above) similar trends were observed. Relatively low F_{ST} values were obtained between the central genetic group and both the northern and the southern genetic groups (0.031 and 0.037 respectively), while a greater F_{ST} value was obtained between the northern and southern genetic groups (0.103). An overall pattern of genetic separation in three geographic clusters is evident. However, we would like to emphasize that there are some incongruences between genetic groups and geographic clusters, particularly Pop 16 (Tiliviche) and Pop 18 (Vitor), are genetically more similar to the central genetic group, albeit geographically belonging to the northern cluster.

4. Discussion

4.1. Sexual reproduction versus clonality

A high number of shared heterozygous SNPs was found in several populations sampled in this study. In a sexually reproducing population, the probability of finding such shared heterozygous SNPs is extremely low (for each SNP, at most ~1/30,000 in 15 sampled individuals), independent of mating system. The very low F_{IS} values and the high differences between diversity at haplotype (π) and individual level show that the individuals of these seven populations are very similar to each other. In a sexually reproducing population, crossing of two individuals with a shared heterozygous SNP would result in 50% homozygous offspring. With 15 samples per population, we would therefore expect to find more homozygous individuals than we actually see in our results. The same holds true if we assumed reproduction purely by selfing (including cleistogamy), as the proportion of heterozygous loci would be halved in each successive generation. The only possible explanation for the presence of such high numbers of shared heterozygous SNPs in some populations is therefore that these populations predominantly reproduce vegetatively, i.e. are mostly clonal. In *Tillandsia,* the importance of vegetative reproduction has been reported for *T. latifolia,* a lomas-forming species from southern Peru (Masuzawa, 1986) and has been suggested for *T. landbeckii* at a local scale (Rundel et al., 1997). Observations by Till (1992a) suggested that populations in Chile produce flowers that do not open but instead self-pollinate. He further noted, that in the Peruvian coastal deserts many populations do not produce flowers at all anymore. However, our own observations do not confirm this, rather did we find several of the Chilean populations in full bloom. It may thus be that flowering occurs irregularly and

Table 2

Count of sites by heterozygosity based on one SNP per locus. Each column indicates the number of sites for which *x* individuals in a population share heterozygous SNPs. Populations with > 10 all-heterozygous sites are shown in bold. We also included count of sites that are homozygous for two different alleles, in at least one, two or three individuals. Column GC indicates the geographical cluster to which the populations belong.

			Homozygotes	Heterozygotes																
Population	GC	Count	1 + 1	2 + 2	3 + 3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	**S**	**15**	**83**	**20**	**7**	**29**	**13**	**7**	**4**	**1**	**1**	**1**	**0**	**2**	**2**	**1**	**3**	**6**	**21**	**439**
2	**C**	**14**	**104**	**37**	**9**	**102**	**5**	**7**	**6**	**3**	**2**	**0**	**2**	**3**	**2**	**5**	**38**	**130**	**103**	
3	C	15	170	64	33	59	52	21	10	10	2	2	1	96	131	26	10	7	5	0
4	C	15	219	98	59	29	26	25	392	29	37	23	35	21	17	6	3	1	0	1
5	C	15	268	115	60	89	221	56	31	212	50	122	57	30	18	7	2	0	1	0
6	C	8	184	48	2	206	18	29	43	74	285	51	0							
7	**C**	**15**	**153**	**33**	**10**	**209**	**50**	**5**	**5**	**6**	**1**	**1**	**4**	**5**	**2**	**4**	**13**	**118**	**81**	**51**
8	C	15	325	115	55	481	153	84	61	30	18	34	30	30	45	20	16	11	3	0
9	C	15	279	142	60	144	108	131	292	48	16	12	9	61	42	56	34	15	16	8
10	C	15	356	178	120	145	292	152	264	163	74	66	61	34	15	16	4	8	1	0
11	C	15	271	72	28	160	71	70	47	17	20	18	77	65	60	87	96	68	21	3
12	C	15	367	149	63	101	90	120	112	94	90	84	81	71	32	28	12	7	1	0
13	C	15	359	141	71	247	139	98	96	81	67	47	23	32	10	3	2	3	0	0
14	C	15	393	161	70	239	140	120	88	80	74	60	54	38	19	7	3	4	2	0
15	C	15	307	182	57	160	412	60	70	72	46	79	37	58	40	46	18	16	6	1
16	N	15	242	146	38	100	80	98	38	99	47	83	47	43	67	41	32	28	4	7
17	**N**	**15**	**175**	**92**	**25**	**36**	**306**	**15**	**104**	**7**	**3**	**1**	**6**	**6**	**20**	**313**	**7**	**106**	**2**	**48**
18	N	**15**	**42**	**20**	**13**	**28**	**5**	**3**	**6**	**1**	**3**	**5**	**1**	**4**	**4**	**4**	**11**	**24**	**49**	**230**
19	N	15	263	73	42	109	102	47	136	51	120	283	35	37	16	29	31	35	28	7
20	**N**	**15**	**148**	**89**	**11**	**26**	**220**	**8**	**8**	**6**	**5**	**5**	**4**	**4**	**3**	**4**	**23**	**382**	**13**	**59**
21	N	**15**	**127**	**56**	**39**	**33**	**22**	**359**	**2**	**2**	**3**	**0**	**2**	**2**	**0**	**13**	**314**	**2**	**5**	**127**

perhaps in response to certain climatic conditions only, which would mean that *T. landbeckii* may be capable of rapidly shifting its reproductive strategy. Interestingly, petal color varied between the southernmost population 1 (Caldera) and other populations further north. We do not know whether this can be related to reproductive strategies. Further studies would be needed to fully understand this species' breeding system including potential pollinators. In summary, *T. landbeckii* appears to maintain a mixed-mating system including clonal reproduction.

Seven out of 21 populations showed a high number of shared heterozygous SNPs, of which four exhibit an extremely high amount (Table 2). The four populations with the largest numbers of shared heterozygous SNPs and the lowest F_{IS} values are all geographically farthest away from the central cluster. However, populations 2 and 7 also have high numbers of shared heterozygous SNPs. These two populations occur in the central cluster, that is composed of a genetic group otherwise dominated by highly admixed populations reflected in high F_{IS} values and zero to maximum eight shared heterozygous SNPs in the whole of the sampled populations.

The reason why some populations appear to reproduce sexually and others almost exclusively by cloning remains unknown. There are, however, two possible processes that may serve as an explanation for this phenomenon, namely long-term isolated relic populations and founder effects, which in both cases result in strongly reduced diversity of the gene pool of a population. The first scenario implies a formerly continuous metapopulation that became fragmented, while the second scenario would be based on the colonization of outlier populations from a source gene pool.

4.2. Fragmentation versus founder effects

In a scenario of a former more continuous distribution range of *T. landbeckii* along the coastal Atacama Desert, one needs to look for mechanisms which could have led to fragmentation and subsequent isolation of populations such as (1) directional climatic changes causing expansions of arid regions leading to local extinction and thus contraction and isolation of their floras and (2) geological changes to the land surface leading to gene flow barriers.

The core Atacama has been predominantly hyper arid since the Miocene, possibly even the Oligocene/Eocene (Dunai et al., 2005; Ritter et al., 2018). However, aridity was subsequently interrupted repeatedly by wetter phases (Evenstar et al., 2017; Jordan et al., 2010). These climatic fluctuations during the Pleistocene (Stuut and Lamy, 2004) caused expansions and contractions of the arid regions and the associated floras, leading to alternating phases of isolation and secondary contact of populations. Molecular clock dating analyses suggest an origin of diversification within *Tillandsia* subg. *Diaphoranthema* at the Miocene/Pliocene boundary, around 5 Ma (Givnish et al., 2011). Since *T. landbeckii* is nested in subg. *Diaphoranthema* (Barfuss et al., 2016*;*Donadío, 2013*;*Donadío et al., 2015) the split from its sister species is certainly more recent and likely coincident with Pleistocene climatic fluctuations. The genetic signature left by this process may be indistinguishable from that left by continuous gene flow (Barton and Hewitt, 1985; Strasburg et al., 2012), which is at odds with the apparent limitation to gene flow between geographical clusters in *T. landbeckii.* However, divergence time estimations would need to be carried out, perhaps based on more recent studies by Donadío et al. (2015), Barfuss et al. (2016) or Granados Mendoza et al. (2017) which indicate *T. landbeckii* as sister to *T. usneoides* and *T. mollis.*

Habitat fragmentation and subsequent isolation of plant populations may also have been caused by geological changes to the land surface, including the evolution of surface rivers such as the Río Loa, with the maximum age of its incision dated to 274 ± 74 ka (Ritter et al., 2018). Such a major change in the landscape may pose a substantial barrier to gene flow by influencing the spread of wind-dispersed pollen and seed and/or vegetative propagules. However, Evenstar et al. (2017) also show, that the incision of the northern rivers such as Quebrada Camarones, Quebrada Azapa and Río Lluta are much older, dating back to ~15 Ma and ~11 Ma. Given the phylogenetic divergence age of *Tillandsia* subg. *Diaphoranthema* in northern Chile (< 5 Ma, Givnish et al., 2011), population fragmentation of *T. landbeckii* therefore must postdate landscape fragmentation, and dispersal across already extant barriers is the only explanation for its current range. This is also supported by population 16 (Quebrada Tiliviche), which is situated in the northern geographical cluster but genetically belongs to the central cluster. Quebrada Tiliviche is the youngest surface river of the northernmost river systems, with its incision dated at ~6.4 Ma (Evenstar et al., 2017). It is also the narrowest river valley of the four

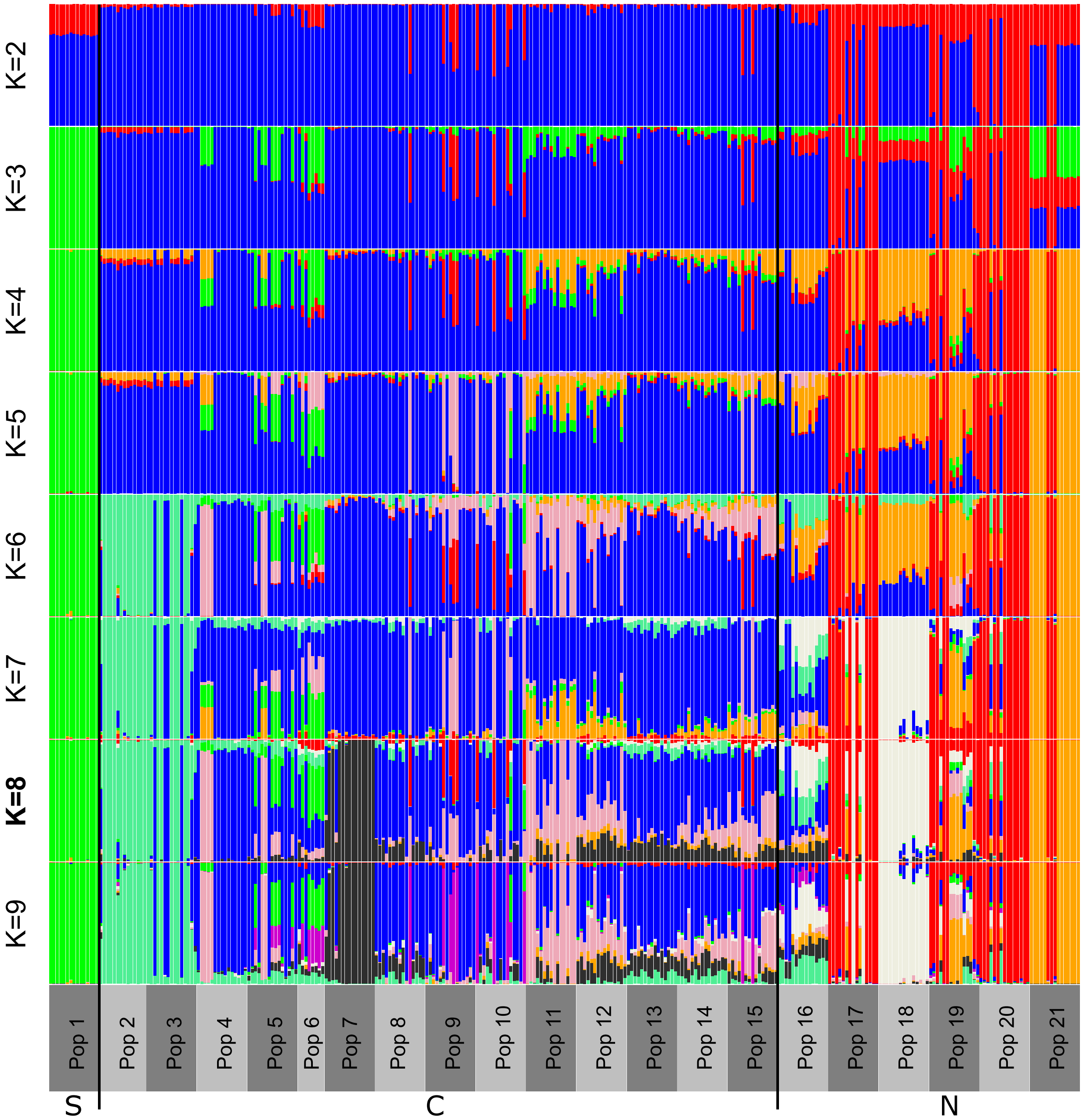

Fig. 3. Output of Non-Negative Matrix Factorization algorithm from the R package LEA. Number of clusters, K from 2 to 9 are shown. The fastSTRUCTURE result with an optimal K = 8 is shown in bold.

northernmost systems and dispersal across it may thus be more frequent than further north. However, for most populations that we see today, these large river valleys represent geographical barriers that limit gene flow between the "Tillandsiales" in all three geographical clusters. These physical barriers may act in combination with scarcity of suitable environmental conditions for establishment across the barrier, reducing the probability of successful colonization. While fog and topography are certainly important factors (Hesse, 2012; Rundel and Dillon, 1998), little is known about the exact combination of environmental conditions required for the establishment of "Tillandsiales".

Furthermore, in a scenario of fragmentation of a formerly continuous metapopulation one would expect to find a more balanced number of distinct ancient genetic lineages between geographical clusters than observed in our data. Additionally, genetic distance would tend to be lower within geographical clusters than between them. While this is indeed the case for most of the investigated populations, it did not apply to two of them (Pop 18 and Pop 21). The F_{ST} values of Pop 18 (Vitor, northern genetic group) are equally high between Pop 18 and other populations from the northern group, and between Pop 18 and populations from the central group. A similar situation is found with population Pop 21 (Río Lluta), another highly clonal population (see also Fig. 5). With the apparent limitation to gene flow between

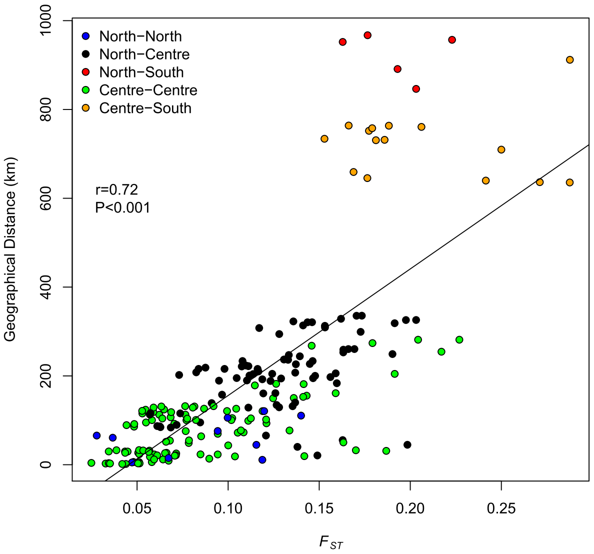

Fig. 4. Plot of genetic distance (F_{ST}) and geographic distance (km) between every population pair. The correlation is $r = 0.72$. The line is the best fitting linear model. Color codes represent the comparisons between and within geographical clusters. Mantel test significance $P < .001$.

geographical clusters, the question arises what led to the establishment of the geographically outlying populations. An explanation may be founder effects, a particular type of genetic bottleneck in which the founder(s) of a new population carry only a small subset of the genetic diversity of the source population (Freeland, 2007; Hewitt, 1996). The central genetic group, displaying the highest levels of admixture and the largest numbers of genetically distinct lineages, would represent the source population from which few founder individuals could have spread to the north and south, carrying only a fraction of the genetic diversity of their source. Founding individuals may have dispersed through a “stepping stone” dispersal process. The Caldera population (Pop 1) in particular appears to be the result of very few founding individuals, perhaps originating from a dispersal event. While it has the largest number of shared heterozygous SNPs pointing to almost exclusive clonal reproduction, it has very few distinct genetic lineages, which excludes the possibility of a single founding plant. The northern region may have been repeatedly colonized by individuals from the central cluster. At least two independent colonization events would be required to explain the genetic diversity and among-population differentiation found within the northern cluster. The isolated population Pop 18 and the populations of the Azapa/Lluta area, Pop 20 and Pop 21 represent two distinct genetic groups (Fig. 3), the latter two showing very low genetic differentiation with the Camarones population Pop 17 (Fig. 5), the southernmost population of the northern cluster.

The Chilean “Tillandsiales” are protected by the Chilean protected areas in only around 1% of their total area (Luebert and Pliscoff, 2017). Our results suggest that new protected areas would more effectively be located in the central cluster, because they concentrate the largest proportion of the genetic diversity of the species.

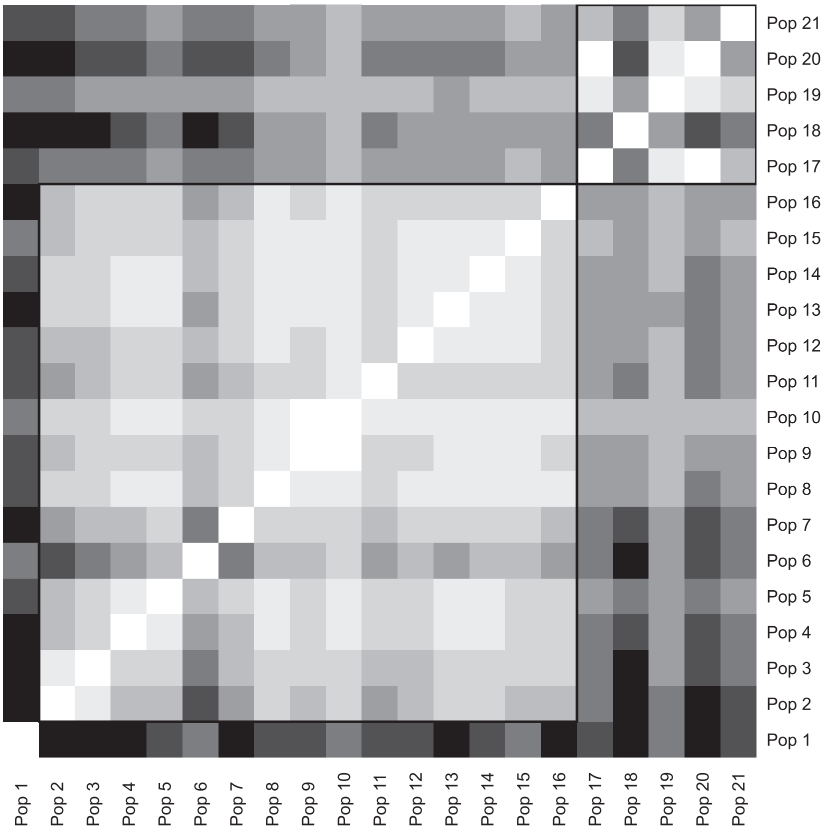

Fig. 5. Heat map of F_{ST} pairwise comparisons between populations. White cells are low F_{ST} values, darker cells are increasingly higher F_{ST}. The upper right (smaller) square represents the northern genetic group, including Pop 18 and the central (larger) square represents the central genetic group.

5. Conclusions

Twenty-one of the > 30 populations of *T. landbeckii* reported by Pinto et al. (2006) were sampled in this study. The sampling size of over 300 individuals and the subsequent analyses of historical genetic connectivity based on GBS data represent a hitherto unprecedented population-based study of any plant group in the core Atacama Desert of northern Chile. Our results show three main findings: (1) In addition to sexual reproduction, asexual (clonal) reproduction seems to be an important mechanism by which this species maintains its populations, particularly at the southern and northern range limits of the Chilean distribution of *T. landbeckii*. (2) In most cases, genetic differentiation increases with geographical distance between the populations as shown by our results of F_{ST} and Mantel test indicating signatures of past migration and ancient gene flow. Present-day geographical barriers appear to play an important role to preserve these genetic patterns, in particular between the northern and central clusters, so shaping current distribution patterns and colonization success of this species, resulting in an overall picture of unbalanced genetic diversity. (3) There is considerable genetic diversity within populations of *Tillandsia* belonging to the central geographical cluster, pointing to sexually reproducing individuals, which probably represent the most ancient populations in the Atacama Desert of Chile. Low genetic connectivity between geographical clusters probably resulted from few founding individuals and subsequent isolation. The northern and southern geographical clusters were probably colonized at least twice by long distance dispersal events from the center to the north, one from the center to the south as well as additional short distance colonization events within the central cluster. Maintaining a mixed-mating strategy, combined with varying degrees of clonal reproduction, as well as producing wind dispersed seeds with a pseudo-pappus enables this species to disperse over considerable distances, and facilitates the establishment of “Tillandsiales”. Further studies including populations of *Tillandsia* from Peru, a more explicit temporal framework and modeling of past and present climate may shed further light onto the population dynamics of this species.

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Declaration of Competing Interest

The authors declare that all research was conducted independently of any commercial or financial relationships that could be interpreted as a conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gloplacha.2019.103076.

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Chapter 3

Genome-wide SNP data reveal recent population structure of an ancient endemic lineage in the Atacama Desert of northern Chile

This chapter has been included at manuscript stage and is currently in preparation to be submitted.

Genome-wide SNP data reveal recent population structure of an ancient endemic lineage in the Atacama Desert

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Abstract

The Atacama Desert is a biodiversity hotspot of neo-endemic radiations for which long-term aridity and complex physiographic processes form a unique environmental setting. Current species assemblages are mainly concentrated in highly patchy lomas formations, and plant populations occurring in these are often geographically isolated from each other. Despite a common consensus on long-term aridity in the Atacama, climatological and geological evidence points to repeated changes in climate, making the Atacama Desert an ideal system for studying population genetic processes in highly unstable habitats. We analyzed the genetic structure within and among populations of *Huidobria fruticosa*, a paleo-endemic lineage of the Atacama Desert. We shed new light on its biogeographic history, broadening our understanding of the evolution of life in extreme aridity, and increasing our knowledge of plant evolution in response to a changing environment. We analyzed SNP data obtained from genotyping-by-sequencing of 354 individuals in 21 populations which represents the largest SNP data set so far analyzed for plant populations in the Atacama Desert. Our results suggest, that, despite being an ancient lineage, current populations of *Huidobria* have been strongly affected by abiotic processes acting over the last 2 million years. We interpret our data to indicate phases of aridity during the Pleistocene causing strong population substructure.

Keywords: *Huidobria*, Loasaceae, genotyping-by-sequencing, population genomics, aridity, landscape genetics
Short title: Population genomics of isolated desert plants

3.1 Introduction

On a deep geological timescale, patterns of biodiversity evolve in direct response to geological and climatological processes (e.g. Axelrod, 1967; Gillespie & Roderick, 2014). In extreme environments, such as those marked by prolonged and severe drought, these evolutionary processes are more severely affected than in more stable habitats (such as the wet tropics), because greater spatio-temporal species turnovers may lead to higher extinction rates (Gaston & Spicer, 2004; Stebbins, 1952). Through contraction and expansion of suitable habitats during climatic oscillations, populations may undergo phases of isolation, experience genetic drift, and reconnect to form new species and species assemblages (Stebbins, 1952). Environmental heterogeneity has direct consequences for plant adaptation and as such, is reflected in the genetic make-up of the organisms studied.

The Atacama Desert of northern Chile is an extremely arid environment with a hyper arid core (between 19°S and 22°S) receiving less than 1 mm/yr precipitation (Houston, 2006b). Nevertheless, precipitation events do occur occasionally, delivering moisture in two major ways: (1) through precipitation events in the Andes leading to occasional discharge into the desert core, and, on a more continuous level, feed the few surface rivers that cross the Atacama on their way towards the Pacific Ocean; (2) regular advection fog that meets the westward facing slopes of the coastal cordillera at a more or less constant altitude due to a temperature inversion (Garreaud *et al.*, 2008). As a result, the Atacama Desert, despite being considered one of the driest places on earth (McKay *et al.*, 2003), harbours a comparatively large number of plant species with a high rate of endemism (Dillon & Hoffmann, 1997). Vegetation is confined to those areas that experience periods of moisture availability, while in contrast, vast stretches of the Atacama Desert are almost totally barren, leaving the populations of many species in geographical isolation over long periods of time. In addition, the Atacama is an ancient desert with arid conditions persisting at least since the early Miocene (Dunai *et al.*, 2005) and possibly even earlier (Hartley & Chong, 2002; Ritter *et al.*, 2018b). For these reasons, the Atacama Desert is an ideal study area for the evolution of life under hyper arid conditions.

While a number of botanical and biogeographical studies on the Atacama Desert has recently been published (Böhnert *et al.*, 2019; Dillon *et al.*, 2009; Luebert *et al.*, 2011), very few studies have so far focused on population genetics and plant population structure in relation to geological and climatological events (e.g. Baranzelli *et al.*, 2014; Luebert *et al.*, 2014; Ossa *et al.*, 2013, 2017; Viruel *et al.*, 2012, and even less have studied population dynamics of plants that are found across the hyper arid core of the Atacama Desert (Merklinger *et al.*, 2020).

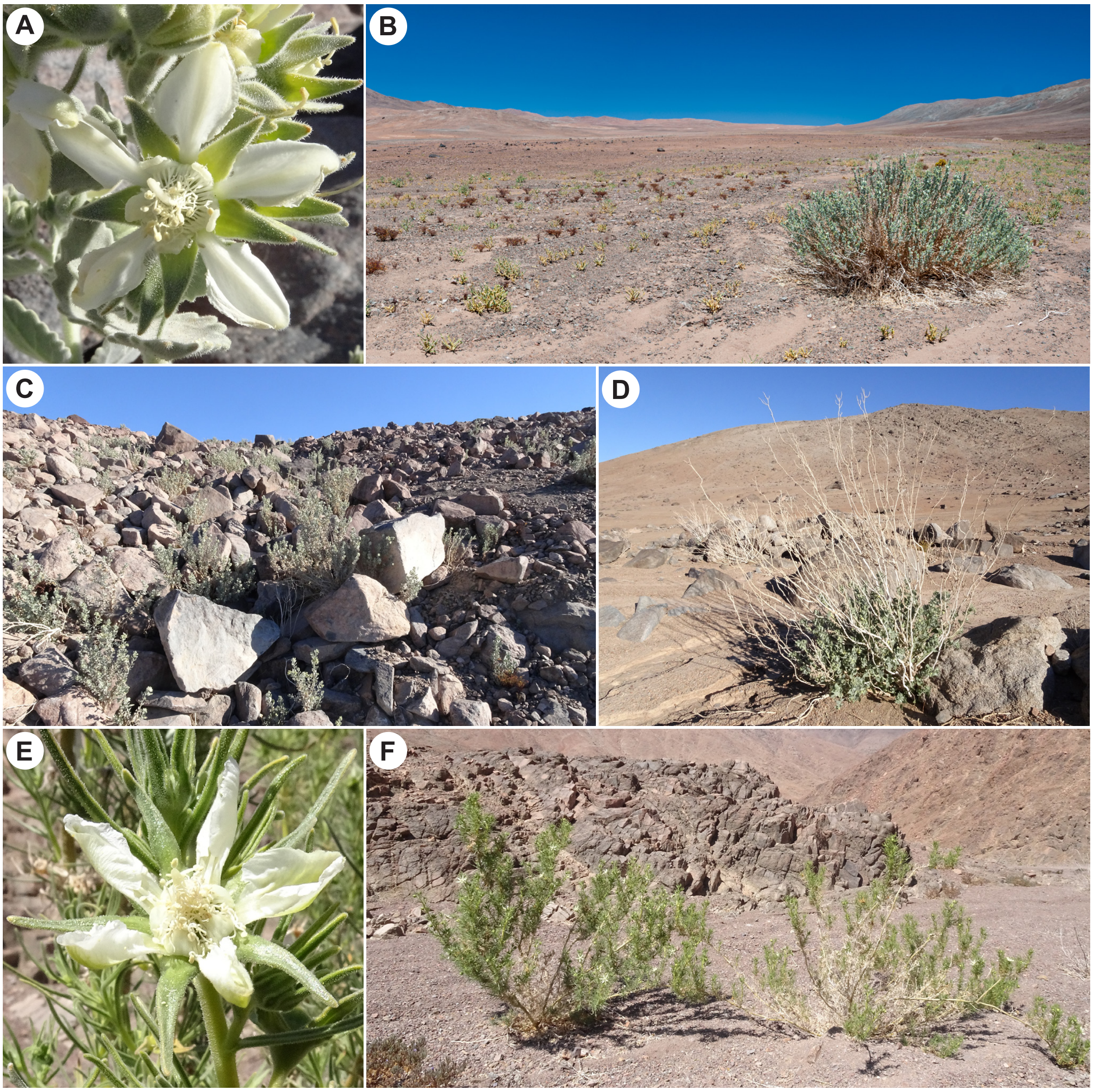

Figure 1: Overview of *Huidobria fruticosa* (A–D) and *H. chilensis* (E–F). A) Flower. B–C) Habitat and typical compact shrubby habit of the plant. D) Re-growth in response to precipitation after previous die-back. E) Flower of *H. chilensis*. F) Lax shrubby habit of *H. chilensis*.

The genus *Huidobria* Gay (Loasaceae, subfamily Loasoideae) is endemic to the Chilean Atacama and currently consists of two taxonomically recognised species, *H. fruticosa* and *H. chilensis* (Acuña Castillo *et al.*, 2017; Grau, 1997). Both species occupy the hyper arid zones of the Atacama (the definition of hyper aridity follows Luebert & Pliscoff, 2017). The distribution of the species in focus here, *H. fruticosa*, encompasses the coastal cordillera as well as the western slopes of the Andes. Its populations are seemingly separated from each other by the barren desert pampa that stretches from the eastern slopes of the coastal cordillera east toward the Andes. Because they are found at either end of the canyons (quebradas) that cross the Atacama from the Andes to the Pacific coast, it seems plausible that these (usually dry) rivers act as dispersal corridors for *H. fruticosa.* An ecological peculiarity of this species is its ability to occupy extremely dry habitats where often only few other plants are found. Based on our own field observations, the plants die back periodically above ground, but re-grow to flowering size in the course of a few months or even weeks (Fig. 1D). Depending on the availability of water, plants may persist for longer periods of time, whereas elsewhere they are more ephemeral. We also observed areas covered by many hundreds of individuals where a few months before there was barren land. This mass-occurrence may be originating from a seed bank, which in turn can facilitate populations to re-connect from isolation and to allow for gene flow after precipitation events.

Based on morphological traits, the evolutionary split of *Huidobria* from its nearest relatives in the core Loasoideae has been suggested to have taken place at an early stage in their evolutionary history (Grau, 1997). This has recently been confirmed by molecular clock dating studies, suggesting a divergence between *H. fruticosa* and *H. chilensis* during the early Eocene, around 40–60 Ma (Acuña Castillo *et al.*, 2019). Both species could thus be classified as a paleo-endemics (Stebbins & Major, 1965), which contrasts several other Atacama groups studied for this region, the neo-endemics – groups that originated more recently in the Atacama Desert, such as *Nolana* (Solanaceae, Dillon *et al.*, 2009), *Heliotropium* (Heliotropiaceae, Luebert *et al.*, 2011), *Malesherbia* (Malesherbiaceae, Gengler–Nowak, 2002) or *Cristaria* (Malvaceae, Böhnert *et al.*, 2019). Interestingly, the presence of ancient endemic lineages is rare in the Atacama Desert, where predominantly neo-endemic lineages have been found (Scherson *et al.*, 2017), a feature also documented in other hot deserts (Thornhill *et al.*, 2016, 2017).

Considering *Huidobria fruticosa* as an ancient endemic lineage of the Atacama Desert, we aimed to understand the population dynamics of this species in the light of climatological processes. We ask, if populations have undergone long-term isolation in parallel to the long-lasting aridity of the Atacama Desert. In such a case, we would expect a comparatively high genetic differentiation among populations with respect to other, neo-endemic plant groups. Alternatively, regular precipitation events, accompanied by seed bank effects and/or dispersal events may lead to a situation with very little genetic differentiation among subpopulations. To obtain a clearer picture about demographic and genetic substructure, we employed genotyping-by-sequencing (GBS) with a total of 354 samples from 21 accessions and analysed single-nucleotide-polymorphism (SNP) data. We suggest that the genetics of *H. fruticosa* populations has been impacted by prolonged phases of aridity during the last 2 Ma leading to fragmentation of former metapopulations, and with increasing genetic isolation of subpopulations. Extinction risk of these subpopulations is mitigated by dispersal.

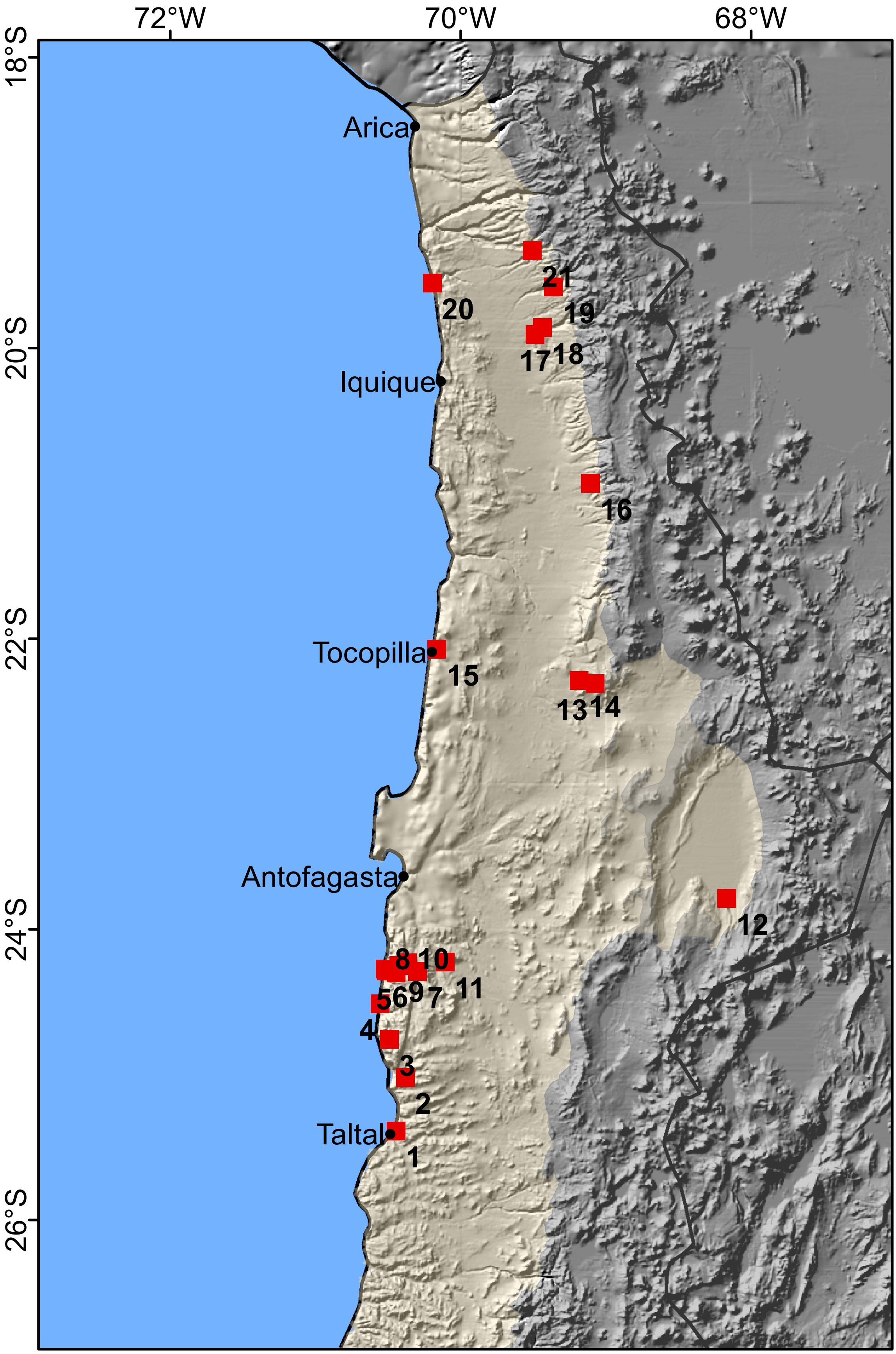

Figure 2: Distribution map of the sampled populations of *Huidobria fruticosa*, which are distributed along the coast as well as the west flank of the Andes, separated by the hyper arid desert pampa. Grey-shaded areas corespond to the Andes >3000 m elevation, beige coloured area corresponds to the Atacama Desert <3000 m elevation.

3.2 Materials and Methods

Study system

Huidobria fruticosa is known to occur in northern Chile from 25.4°S to about 18.2°S. Locality information was taken from Grau (1997) and ground-thruthed during four field studies between 2017 and 2019. In the present study, we sampled 21 populations between quebrada San Ramón near Taltal (25.4°S) and quebrada Tiliviche near the town of Pisagua (19.5°S). To the north of Pisagua, we could not find the species despite previous reports of it (Grau, 1997). Populations are usually confined to the extremities in the East (Andes) and West (coastal cordillera) of the dry river beds that cross the Atacama, with populations at either end of these river beds separated by the barren desert pampa. The plants are shrubs of usually about one to two, rarely three metres in height. They produce white flowers that contain small amounts of highly concentrated nectar, pointing to insect pollination (Ackermann & Weigend, 2006). Masses of tiny seeds are released from small capsules, and as typical dust-flyers are distributed by wind (Weigend *et al.*, 2004).

Population sampling

Within our study area, 21 populations were found and leaf tissue of 15 individuals per population was sampled and silica gel-dried. Occasionally populations consisted of fewer plants in which case all plants were sampled (Table 1). Sampling was carried out along a transect crossing the entire population in order to obtain the best possible coverage. At least one voucher specimen from each population was deposited at the herbarium of the Nees Institute for Biodiversity of Plants, University of Bonn, Germany (BONN) and the herbarium of the La Serena University, Chile (ULS).

DNA extraction, library preparation and genotyping-by-sequencing

DNA extraction followed Merklinger *et al.* (2020). In brief, DNA was isolated from silica-dried leaf material, following the Macherey Nagel Nucleo Mag 96 protocol (Macherey Nagel, Düren, Germany) and processed by a Thermo Fisher Scientific KingFisher Flex benchtop system (Thermo Fisher Scientific, Waltham, MA, United States), binding DNA to NucleoMag C-beads. Quantity and quality of extracted DNA was tested by gel electrophoresis using Lonza GelStar Nucleic Acid Gel Stain (100x) including a sample of 20 ng linear, double-stranded Lambda DNA (New England Biolabs, N3011S). Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, United States) measurements of selected individuals were taken and samples were standardized to 20 ng/µl. An aliquot of 15 µl for each individual was used for library preparation and sequencing. For library preparation 200 ng of genomic DNA were digested with the restriction enzymes PstI-HF (New England Biolabs, R3140S) and MspI (New England Biolabs, R0106S).

Library preparation, individual barcoding, and single-end sequencing on the Illumina HiSeq 2500 followed Merklinger *et al.* (2020). Barcoded reads were de-multiplexed using the CASAVA pipeline 1.8 (Illumina, Inc.). The obtained raw sequence reads (0.5–4 million per individual) were adapter trimmed and quality trimmed (phred score >25) with CUTADAPT V1.16 (Martin, 2011), and reads shorter than 65 bp after adapter removal were discarded. Sequence reads for the GBS Illumina runs were deposited in the European Nucleotide Archive under the study accession XXXXXX.

Assembly parameters

A de novo assembly of the GBS data was carried out using IPYRAD v0.9.40 (Eaton & Overcast, 2020). This assembly contained 354 individuals of *H. fruticosa* and three individuals of *H. chilensis* as an outgroup for phylogenetic analyses (see below). Two different sets of output files were generated through the IPYRAD branching option, one with the complete set of 357 individuals, another without the outgroup samples, to be used for population structure analyses. The minimal sample number per locus was set to 150, the maximum cluster depth within samples was set to 0.9. *H. fruticosa* is assumed to be diploid (2n=36, Grau, 1997), so the ploidy level was set to diploid. For the other parameters the default settings of parameter files generated by IPYRAD were used.

Genetic structure

Based on the assembly output which included the outgroup *H. chilensis*, we ran a maximum likelihood analysis with RAXML v8.2.9 (Stamatakis, 2014) on the CIPRES gateway (Miller *et al.*, 2010). We used population consensus sequences created in PhyDe v0.9971 (Müller *et al.*, 2005) and ran the analysis under the GTR+Γ substitution model with 20 tree searches and 1000 bootstrap replicates to calculate node support.

Population structure was further explored with the second data set (min sample locus 150) without the outgroup. We used the program STRUCTURE v2.3.4 (Pritchard *et al.*, 2000) as implemented in IPYRAD v0.9.40, to cluster all sampled individuals into K-distinct populations, and using the imap dictionary to group individuals into populations. The minmap command was specified at 0.5, requiring that 50 % of samples have data. Values for K were tested for K = 2–6 with 20 replicates run per test. Each replicated was run for 800 000 Markov chain Monte Carlo (MCMC) steps with a burn-in period of 200 000.

From the initial SNP matrix, we excluded all sites that contained a gap in any individual (insertions or deletions), and then excluded all individuals with more than 6,000 “N” (unresolved) nucleotides. The cleaned dataset (hereafter referred to as “the SNP matrix”) contained 315 individuals and 21,086 SNP sites, and was used for all analyses below. For an initial visualization of our dataset, excluding the outgroup, we conducted a principal component analysis (PCA) using 4,118 SNP sites that did not contain any “N” and had only two possible nucleotide states. Homozygotes of the two states were coded “0” and “1” and heterozygotes were coded “0.5” for the input matrix. The first four principle components were plotted (Fig. 3 & 4; Fig. S2 & S3) and interpreted in the light of population subdivision.

***F_{ST}* and isolation by distance** Average F_{ST} across all loci was calculated based on pairwise comparisons between populations and visualized in a heat map (Fig. 6). Further, we tested for isolation by distance by correlating the average F_{ST} to geographical distance between each pair of populations using a Mantel test with the R package VEGAN v.2.5-4. (Oksanen *et al.*, 2019).

Genetic diversity, past population sizes and sequence divergence
Based on the SNP matrix, we calculated three estimates of the population-scaled mutation rate θ, a measure of genetic diversity that is independent of sample size. $\theta\pi$ is the mean number of differences between two haploid genomes in the population. θ_{w} is the total number of variable sites in the population divided by the harmonic number of 2n-1 (Watterson’s estimator). Finally, θ_{s} is the number (raw count) of singletons, polymorphic sites where one allele is present only in one genomic copy in the entire genome. The values are presented as the total amount of diversity in the SNP matrix.

The program Stairway plot v2.1 (Liu & Fu, 2015; Liu, 2020) was used to estimate the past population sizes from the allele frequency spectra. Because no known estimates of generation time and unscaled mutation rates are available, the results are displayed as if the mutation rate is 1.2e-8 mutations per site per generation, and generation time is one year. Only sites that are not “N”s in any individual in that population are counted for both the total site number and the site frequency spectrum.

We also estimated the time of sequence divergence between population clusters using a “triple comparison method” . The loci were first aligned to an outgroup (OG), *H. chilensis*. To estimate the divergence time between two clusters, we took one individual from each cluster (H1 and H2) and counted the number of sites that were non-ambiguous in both individuals and the OG. Then we counted the number of transitions and transversions between H1 and H2, H1 and OG, as well as H2 and OG. Heterozygotes were counted as half a mutation. Following this, we calculated the Kimura-2-Parameter distance (d) between each pair, and the divergence time between H1 and H2 was estimated as $t_{\mathrm{H1\text{-}H2}} = t_0 \times 2 \times d_{\mathrm{H1\text{-}H2}} / (d_{\mathrm{H1\text{-}OG}}+d_{\mathrm{H2\text{-}OG}})$, where t_0 is the divergence time between *H. fruticosa* and *H. chilensis*. Four individuals from four different groups of populations were chosen for each cluster based on the least number of “N”s, except for the mid-coastal population (pop 15), where the four individuals were from the same (only) population. Therefore, for each pair of clusters the triple comparison was repeated 16 times and the mean estimate was used.

3.3 Results

Assembly statistics

The assembled data set including the H. chilensis outgroup and with 29.07% missing data consisted of 13 400 loci with 40 753 SNPs, of which 31 045 were parsimony informative sites. The second data set ("SNP matrix") excluding the outgroup samples had 31.66% missing data and contained 13 405 loci with 35 253 SNPs of which 26 252 were parsimony informative sites. After filtering, 315 individuals and 21,086 SNP sites remained for further analyses.

Genetic structure

The RAxML analysis based on consensus sequences per population retrieved a tree with three fully supported clades, one containing the Andean and mid-coastal populations (pops 12–16), one including all northern populations (pops 17–21) and one with southern coastal populations. The latter was recovered in two subclades, pops 1–3 with full support in one subclade, pops 4–11 in another, both subclades with good support (97%) despite weaker support values within the larger subclade (55–83%, pops 4–11; see Fig. S1). Zero support was obtained in the RAxML tree for the split between the northern cluster and southern cluster, which we attribute to the long divergence time between the two *Huidobria* species. The PCA as well as the structure analysis retrieved a similar grouping of populations as in the RAxML analysis. PCA 1–2 together explained X% of the total variability (Fig. 3). A first split separates the northern cluster from the rest along the first PCA axis. Along the second axis, a further split between the southern coastal populations and the mid-coastal + Andean populations was observed, while the southern coastal populations appeared close to the northern populations. PCA axis 3, with explanatory power of X% retrieved the mid-coastal population farthest away from the rest. To further clearly distinguish the four distinct geographical clusters, they are from here on referred to as NC (northern cluster), AC (Andean cluster), SC (southern cluster) and TC (Tocopilla-coast). Results of the structure analysis were congruent with the results from the PCA. The delta K value was identified to be optimal for K = 4. At K = 2 the C including all populations north of Iquique at 20°S (populations 17–21) separated from the rest. At K = 3 the AC separated from the NC and SC, containing populations 12–16. At K = 4, the TC (population 15) separated from the other populations and became increasingly distinct with increasing K (K = 5–6) but retaining a weak connectivity to its closest Andean neighbour, population 16 near Huatacondo.

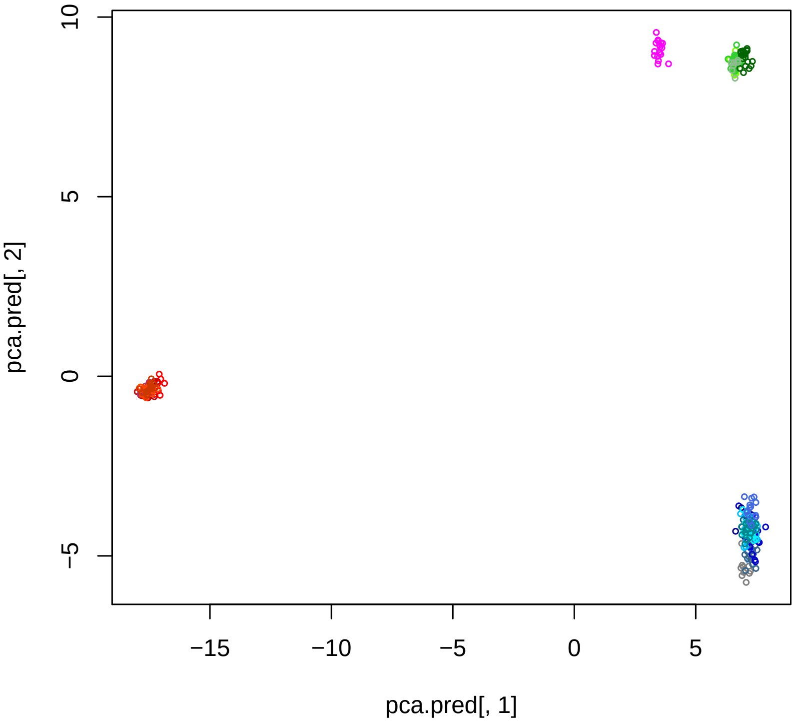

Figure 3: Principal Component Analysis axes 1–2

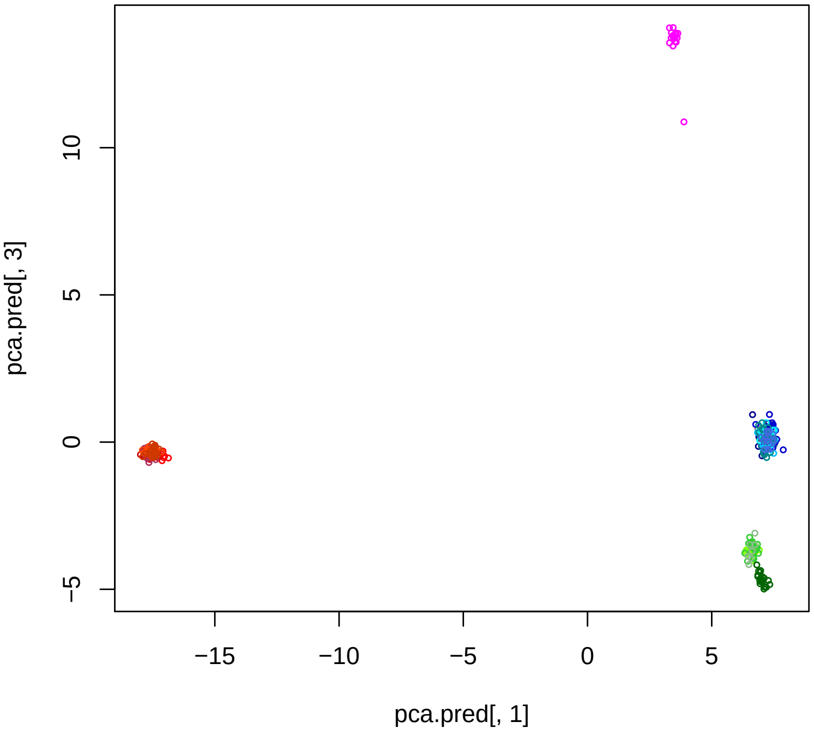

Figure 4: Principal Component Analysis axes 1–3

Figure 5: Analysis of genetic structure to cluster all sampled individuals into K-distinct populations (K=2–6). The most likely number of populations as suggested by *delta* K is K=4

F_{ST}, Mantel test and isolation by distance

Genetic distances between populations were small to moderate (0.05–0.35), however, some distinct tendencies could be observed between the populations belonging to the four geographical clusters (Fig. 6 heat map). The populations belonging to the NC all shared a very low F_{ST} among each other ($\leq$0.1), as did the populations of the SC. The two Andean populations (pop 13 and 14) equally shared a very low F_{ST} value (0.05) although this increased with geographical distance toward the other populations belonging to the AC further north (pops 12 and 16, ~0.1). The largest F_{ST} values were found between the Andean population 16 near Huatacondo and the populations of the NC (0.35). Further, between two of the southernmost populations (1 and 3) and the NC (0.3–0.35), and between the TC (15) and the rest (0.3–0.35).

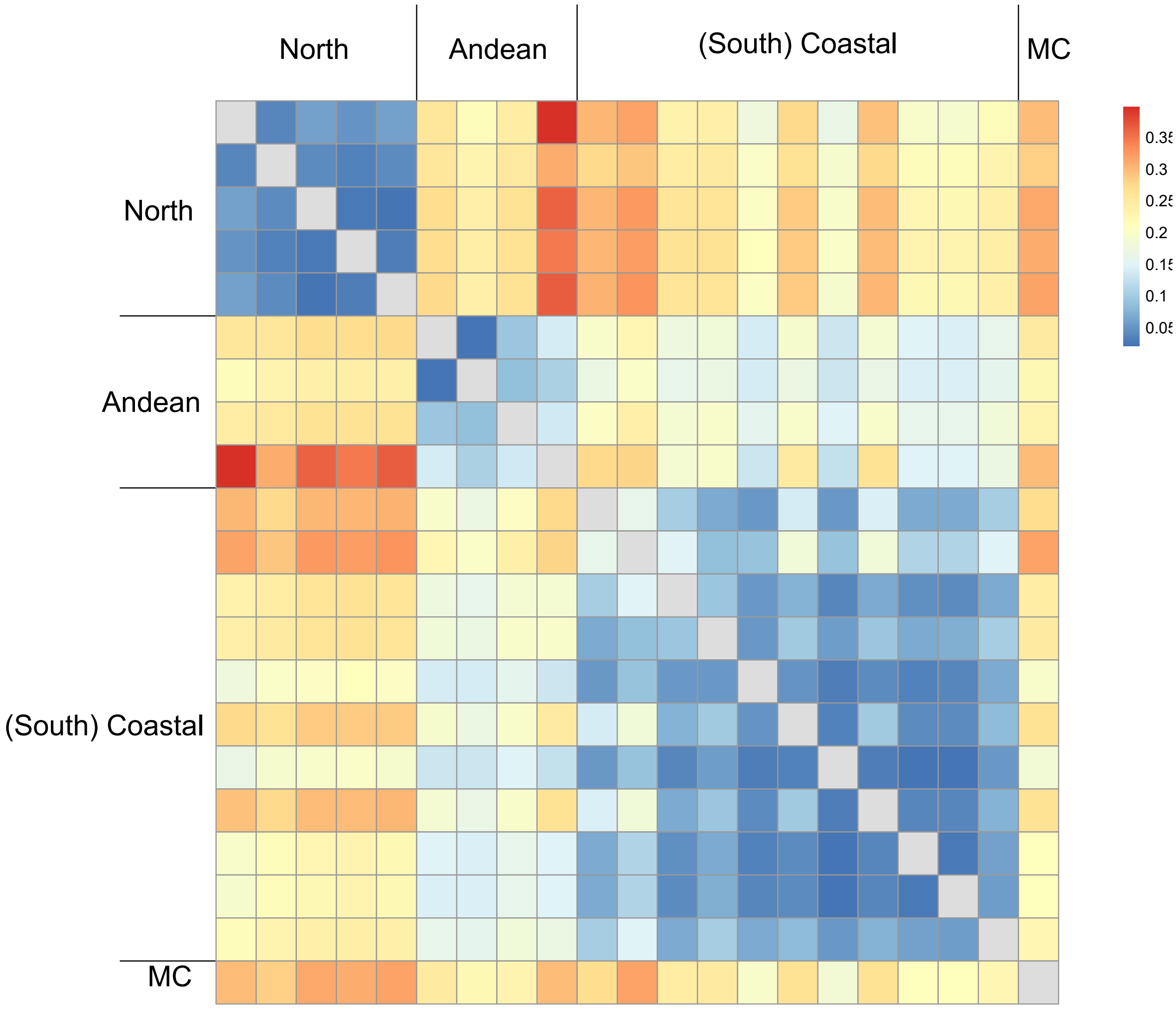

Figure 6: Heat map based on a pairwise comparison of genetic differentiation between all populations and expressed by F_{ST}

Genetic diversity, past population sizes and sequence divergence

Populations belonging to the NC showed relatively homogeneous estimates of θ, both among subpopulations and among different estimators (θ_{w}, θ_{π} and θ_1), supporting the notion of populations being in, or close to, mutation-drift equilibrum. If all three estimators of θ agree, this indicates mutation drift equilibrium of a population without the influence of selection and demographic changes. Such a population could be called stable. However, if there are discrepancies between the three estimators, this may be indicative of recent demographic events/absence of neutrality/being far away from equilibrium. The only exception is population 18, which has an excess of singletons. They are not concentrated in a single individual. Therefore, an initial suspicion that this may be due to a sequence artifact or miss-classification was not confirmed. Since the estimated effective population size is very small in this cluster (NC), the excess of singletons could be due to a very recent expansion after a strong bottleneck. Generally, AC has a larger population size than NC, and appears to be slightly expanding. The only exception here is population 16, which has a small $\mathrm{N_e}$, but very even estimates of θ, indicating again that it is close to mutation drift equilibrium, and has stably persisted as a small subpopulation. SC has the largest populations, but most of them, with the exception of populations 4, 5 and 11, appear to be shrinking. Lastly, the TC population is intermediate in size and stable.

The result of the stairway analysis was generally similar to the interpretation of the values of θ [explain]. There was a noticeable decline in all populations belonging to the SC at $\sim$ 2000 time units, but only a few of these populations showed a subsequent rebound. Also the TC population appeared to have experienced a bottle neck around the same time.

Based on the The models from our "triple comparison method" suggest a divergence time of approximately ~2 Ma for the first split between the NC and the remaining populations (Fig. S2). This also corresponds to the first PC in the PCA graph. A next three-way split was at 1.2~1.4 Ma between AC, SC and TC. It is to note, however, that this timescale is probably much older than anything that can be retrieved from the stairway or θ analyses. The Stairway timescale is always relative to generation time, which means that the absolute time scale may vary considerably according to what the generation time actually is.

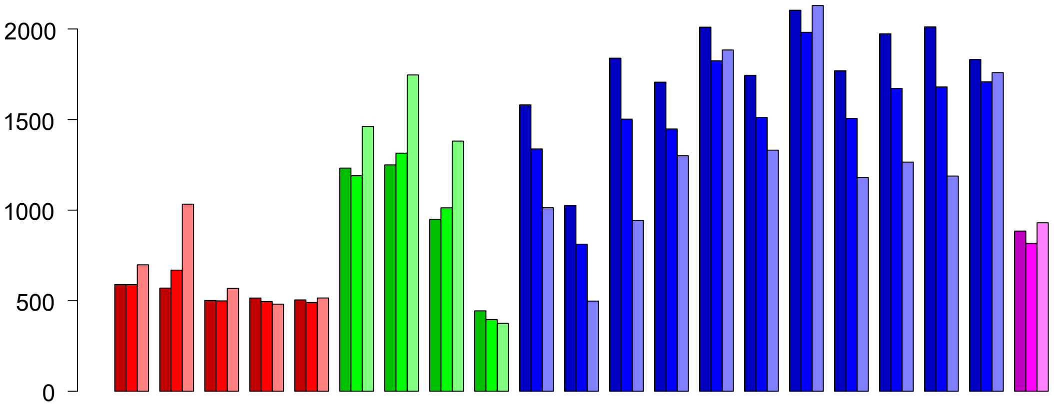

Figure 7: Estimations of θ, expressed in θ_π, θ_w and θ_s for the NC (red), AC (green), SC (blue) and TC (pink).

3.4 Discussion

Huidobria fruticosa is an ancient plant species of the Atacama Desert that originated around 50 MYA (Acuña Castillo *et al.*, 2019) and as such predates most other plants of the Atacama for which dated phylogenies are available (e.g. *Cristaria*, *Malesherbia*, *Oxalis*, *Heliotropium*, *Nolana*). The Atacama Desert has been predominantly arid possibly since the Jurassic (Hartley *et al.*, 2005) with a hyper arid core since at least since the Oligocene (Dunai *et al.*, 2005; Hartley *et al.*, 2005). At the outset of this study, we expected that the long-term arid climate might have impacted the genetic signature and past population structure of this species. However, our results suggest that the history of extant populations is rather recent and has spread only in the last 2 MY. As such, our analyses do not provide any insights into the pre-Pleistocene history of *Huidobria* in the Atacama Desert.

According to our analyses, the sampled *Huidobria* populations are characterized by a well-defined genetic structure that corresponds to four geographically distinguished population clusters, with a first split between the Northern Cluster (NC) and the other ones. The results from our triple output analysis suggest these clusters diverged approximately 2.0 MYA. Within the non-Northern clusters, a second split between the Andean Cluster (AC) and Southern Coastal Cluster (SC) took place approximately 1.5 Ma ago, and a third between the AC and the Tocopilla Cluster (TC) approximately 1.2 Ma. Our structure analysis points to continuous gene flow between coastal and inland populations within NC, while SC is genetically isolated from AC, as is, at least to some extent, MC. This could imply, that gene flow between the northern coast and the northern Andes has been more continuous than further south.

The question arises as to what may have caused this divergence of population clusters as well as varying levels of gene flow. *Huidobria* very effectively disperses its seeds, so it appears plausible to infer dispersal events and founder effects at various periods in time as a cause for current population distribution. Effective dispersal and colonisation have been shown for other lineages in the Atacama such as e.g. *Tillandsia* (Merklinger *et al.*, 2020), *Hoffmannseggia* (Simpson *et al.*, 2005) or members of the Boraginaceae (Simpson *et al.*, 2017). However, because *Huidobria* is an endemic lineage of the Atacama, this would imply the presence of a source pool, or at least the continuity of populations over time in some areas of the Atacama, from where repeated colonisations into adjacent areas could have taken place. In general, population differentiation (F_{ST}) is relatively high compared to that of neo-endemic lineages such as *Tillandsia* (Merklinger *et al.*, 2020), which supports the idea, that populations of *Huidobria* are genetically isolated from each other, either mirroring long-term isolation caused by geographic location and aridity, but possibly also resulting from the species' ephemeral life-style and short generation times.

The southern coastal cluster (SC) has on average higher genetic diversity (θ) than other clusters. Interestingly, with the exception of populations 4, 5, 11, the coastal populations have $\theta_\mathrm{s} < \theta_\mathrm{w} < \theta_\pi$ as well as steep drops in the stairway plot line, possibly indicative of contracting population sizes. A possible explanation may be that, given the highest genetic diversity in the SC, that *Huidobria* populations have managed to persist at coastal localities throughout their long-term evolutionary history, and from here, repeated colonization events over time have taken place to different regions of the Atacama. The hypothesis that SC populations derived from repeated short-distance colonisation events may be supported by the buildup of a seed bank of the species' wind dispersed seeds. Further, the extinction risk of newly founded small subpopulations, which is particularly high in arid environments (Stebbins, 1952), may have been mitigated by the dispersibility of *Huidobria* (Den Boer, 1968; Pisa *et al.*, 2019).

Despite very effective dispersal of seeds, however, *H. fruticosa* has remained an endemic of the extreme north of Chile and has apparently not managed to disperse into adjacent regions such as southern Peru, despite similar levels of aridity there. Although there is a floristic break between southern Peru and northern Chile, other taxa which have a coastal as well as Andean distribution have dispersed across this barrier along the Andean cordillera (Dillon *et al.*, 2009; Gengler–Nowak, 2002), which has been shown to function as a dispersal corridor for taxa (Luebert & Weigend, 2014). The absence of *Huidobria* in southern Peru is thus surprising and it is likely that *Huidobria* currently disperses effectively only within its extent of occurrence. The occurrence in Peru of another Loasoid lineage (*Presliophytum* (Urb. & Gilg) Weigend), which shows very similar ecological and physiological traits as *Huidobria* in terms of habitat preference (Acuña Castillo & Weigend, 2017) and seed dispersibility (Weigend *et al.*, 2004) may further act as an effective competitor preventing *Huidobria* to successfully colonise habitats in southern Peru.

In the extreme north of Chile, the coastal cordillera decreases in elevation (and is finally absent, Madella *et al.*, 2018). Further, several large canyons dissect the inland pampa and connect the Andes with the coast (Evenstar *et al.*, 2017; Binnie *et al.*, 2020). These may have acted as dispersal corridors, for example through directional winds or flash floods from the Andes into the desert pampa along such river canyons following cyclic weather extremes (Houston, 2006a), so maintaining gene flow among the populations of *Huidobria.* Although we did not find *Huidobria* in several of the larger northern canyons, they might have been present here in the past, before intensive agricultural use in e.g. the Río Azapa or Río Camarones altered the habitat requirements in such a way as to become unsuitable for this species. In contrast, further south, the coastal terrain is more strongly fragmented, and the resulting patchy loma vegetation is mostly concentrated on the western slopes of the coastal cordillera (Dillon & Hoffmann, 1997; Rundel *et al.*, 1991). This fragmentation as well as the hyper arid pampa del Tamarugal without the Andean-coastal canyon connection may present the species with much stronger geographical barriers (Ruhm *et al.*, 2020) and so hamper *Huidobria* dispersal, resulting in the Andes-Coast genetic differentiation between populations observed here. The only exception to this is the mid-coastal population, which shows a slight Andean genetic influence. In fact, this population seems more closely related to the Andean population near Huatacondo (pop 16) than to any other, geographically closer Andean populations (pops 13 & 14) in our sampling. This suggests that gene flow not only existed across the hyper arid central desert core, but also across the Río Loa canyon, which has been interpreted as a geographical barrier maintaining current patterns of gene flow in previous studies (Merklinger *et al.*, 2020).

The north-south and Andes-coast fragmentation of population clusters may further be explained by the increase of hyper arid conditions in the central pampa del Tamarugal and in locations along the coastal cordillera. Ritter *et al.* (2018a) suggested the maximum age of the Río Loa canyon incision to be about 274 ka +/- 74 ka, based on cosmogenic nuclide exposure dating of ancient shoreline terraces, originating from a large water body (endohereic drainage system) that streched from Quillagua to the Monton de Gloria pass near todays Salar Grande (Ritter *et al.*, 2018a). A lake of this size would have accounted for continuous stretches of vegetation in this area, allowing for gene flow between the west flank of the Andes and the coast. The incision of the Río Loa was undoubtedly a process of major geological impact in the region, due to the relatively sudden change from an endohereic to an exohereic drainage system so terminating the existence of lakes in this region (Ritter *et al.*, 2018a). This would have impacted the distribution of organisms, and further, the evolution of this major canyon may have also meant the development of a barrier to gene flow among populations of taxa previously connected. During the course of evolution of this endohereic drainage system, Ritter *et al.* (2018a) demonstrated the presence of several pronounced arid phases when no lakes existed, a finding similar to that obtained by Jordan *et al.* (2014). The dates obtained by Ritter *et al.* (2018a) for these phases of prolonged aridity, 2.65 ± 0.15 Ma and 1.27 ± 0.47 Ma, fit remarkably well with the divergence time estimations obtained in this present study for the split between our *Huidobria* clusters. These prolonged phases of aridity would favor the hypothesis of a climate-driven habitat and population fragmentation, leading to an isolation of populations geographically, and consequently to the genetic structured populations that we see today. Similar patterns of population fragmentation have been proposed for other ancient lineages in other parts of the world, such as *Welwitschia* Hook. from Namibia and Angola, which also show strong signals of population structure, and which has also been interpreted as the result of aridification (Jacobson & Lester, 2003).

In summary, populations of *Huidobria* likely became increasingly fragmented with the onset of hyper aridity and the cessation of fluvial landscape modification about 2–3 Ma (Ritter *et al.*, 2018a). The species' ability to disperse effectively would prevent fragmented sub-populations from going extinct and so enable it to maintain populations in genetically and geographically isolated clusters across the hyper arid core of the Atacama Desert.

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Chapter 4

Quaternary diversification of a columnar cactus in the driest place on Earth

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Quaternary diversification of a columnar cactus in the driest place on Earth

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Short title – Diversification of *Eulychnia* in the Atacama Desert

Abstract

The cactus family (Cactaceae) is a major lineage of succulent plants that is thought to have diversified during the Miocene expansion of New World arid environments. In southern South America, the Bolivian and northern Argentinian South Central Andes as well as the Atacama and Peruvian deserts are considered as centers of diversity for Cactaceae. The Atacama and Peruvian deserts are among the driest places on earth, yet a surprisingly high number of plant species and a high percentage of endemic lineages have been recorded from these regions. The genus *Eulychnia* has eight currently recognized species and ca. nine synonyms and is endemic to the Atacama and Peruvian deserts. In the present study we investigate the phylogeny of this group based on material from the type localities of all published names in order to shed new light on species delimitation and biogeographic history. We employ a multi-step approach including a family-wide Bayesian molecular clock dating based on plastid sequence data in order to estimate the age of the genus and date the split from its sister genus *Austrocactus*. We present a comprehensive genotyping-by-sequencing (GBS) dataset analyzed with maximum likelihood and coalescent based phylogenetic reconstructions, using the family-wide age estimate as a secondary calibration to date the GBS phylogeny. We also infer ancestral ranges, employing the dispersal extinction cladogenesis approach. Our GBS phylogeny of *Eulychnia* is fully resolved with high support values nearly throughout the phylogeny. Our data indicate that the split from *Austrocactus* took place in the late Miocene, with a subsequent diversification of *Eulychnia* during the early Quaternary, coinciding with the onset of hyper aridity in the Atacama and Peruvian deserts. Three lineages are retrieved: *Eulychnia ritteri* from Peru is sister to all Chilean species, which in turn fall into two sister clades of three and four species respectively. Diversification in the Chilean clades started in the middle Pleistocene.

Keywords: Cactaceae, *Eulychnia*, genotyping-by-sequencing, arid environments

4.1 Introduction

The cactus family (Cactaceae) is a prominent and species rich lineage of succulent plants with an almost entirely New World distribution (Barthlott & Hunt, 1993). The family is thought to have originated shortly after the Eocene-Oligocene global drop in CO_2 levels and had a subsequent diversification during the Miocene in parallel to the expansion of arid environments (Arakaki *et al.*, 2011). Cacti have colonized a broad range of semi- to hyper arid habitats from Patagonia to southern Canada. In southern South America, the Bolivian and northern Argentinian South Central Andes as well as the Atacama and Peruvian deserts are considered as centers of diversity (Barthlott *et al.*, 2015). The predominantly Chilean genus *Eulychnia* Phil. has a checkered taxonomic history - the most recent taxonomic account of the genus was produced by Ritter (1980, 1981), but many of the taxa he described are currently considered synonyms

(Hunt, 2016), and the genus is in urgent need of revision. *Eulychnia* is a columnar cactus, usually attaining a shrubby, small tree-like or sometimes decumbent habit. Its species are most easily identified by their broadly campanulate flowers and a pericarpel often covered in dense wool or even spines (Fig. 1). The distribution of the genus is largely coastal, in particular in the northern half of its range, where the taxa are confined to the fog zone of the coastal cordillera. Taxa in the southern half of the distribution range occur in a more Mediterranean-like environment and are sometimes found further inland. Overall, the majority of taxa are found in the transition from arid to hyper arid regions of northern Chile (Luebert & Pliscoff, 2017). Only a single species of *Eulychnia* is known from southern Peru, with its populations separated from its Chilean relatives by ca. 1000 km (Fig. 2). In contrast, *Austrocactus* Britton & Rose, the sister genus of *Eulychnia* (Hernández-Hernández *et al.*, 2011, 2014), is found mainly east of the Andes in Patagonia, with only few records from the Andes of central Chile (Sarnes & Sarnes, 2012). The geographical center of distribution of *Eulychnia* is found in the coastal cordillera of the Atacama Desert and it represents one of 17 genera of Cactaceae occurring in this region (Lembcke & Weisser, 1979; Hunt, 2016).

The Atacama Desert of northern Chile is generally considered as one of the driest places on earth (Dunai *et al.*, 2005), with a modern hyper arid core receiving less than 10 mm of precipitation per year (Houston & Hartley, 2003). This extreme aridity is caused by (1) the desert's position at the subtropical high pressure belt which has been stable since the late Jurassic (Hartley *et al.*, 2005), (2) the cold Humboldt current along the Pacific coast restricting moisture uptake by onshore winds and (3) the Andes preventing the entry of moist Amazonian air-masses (Rundel *et al.*, 1991).

While the precise timing of the onset of aridity in the Atacama Desert remains a matter of debate, recent studies of climate evolution and aridity in this region support the notion that aridity has not developed uniformly and, as such, generalizations for the entire Atacama Desert should be avoided (e.g. Sillitoe & McKee, 1996; Hartley & Chong, 2002; Dunai *et al.*, 2005; Latorre *et al.*, 2006; Rech *et al.*, 2006; Evenstar *et al.*, 2017. However, the stable position of the South American continent for the last 150 million years (Hartley *et al.*, 2005) in combination with the establishment of the Peru-Chile Current system (PCC) approximately 50 Ma (Cristini *et al.*, 2012) has led to the generally accepted conclusion that the Atacama Desert is an ancient desert, with a hyper arid core since at least the Miocene (Dunai *et al.*, 2005) or even earlier (Hartley *et al.*, 2005). This long-lasting aridity was, however, repeatedly interrupted by wetter (though still semi-arid) phases largely coinciding with globally warmer periods as shown by evidence obtained from ^{14}C dated vegetation fragments from rodent middens (Betancourt, 2000; Latorre *et al.*, 2006), radiocarbon dates of fossil vegetation from the hyper arid core (Nester *et al.*, 2007; Gayo *et al.*, 2012), cosmogenic nuclide exposure

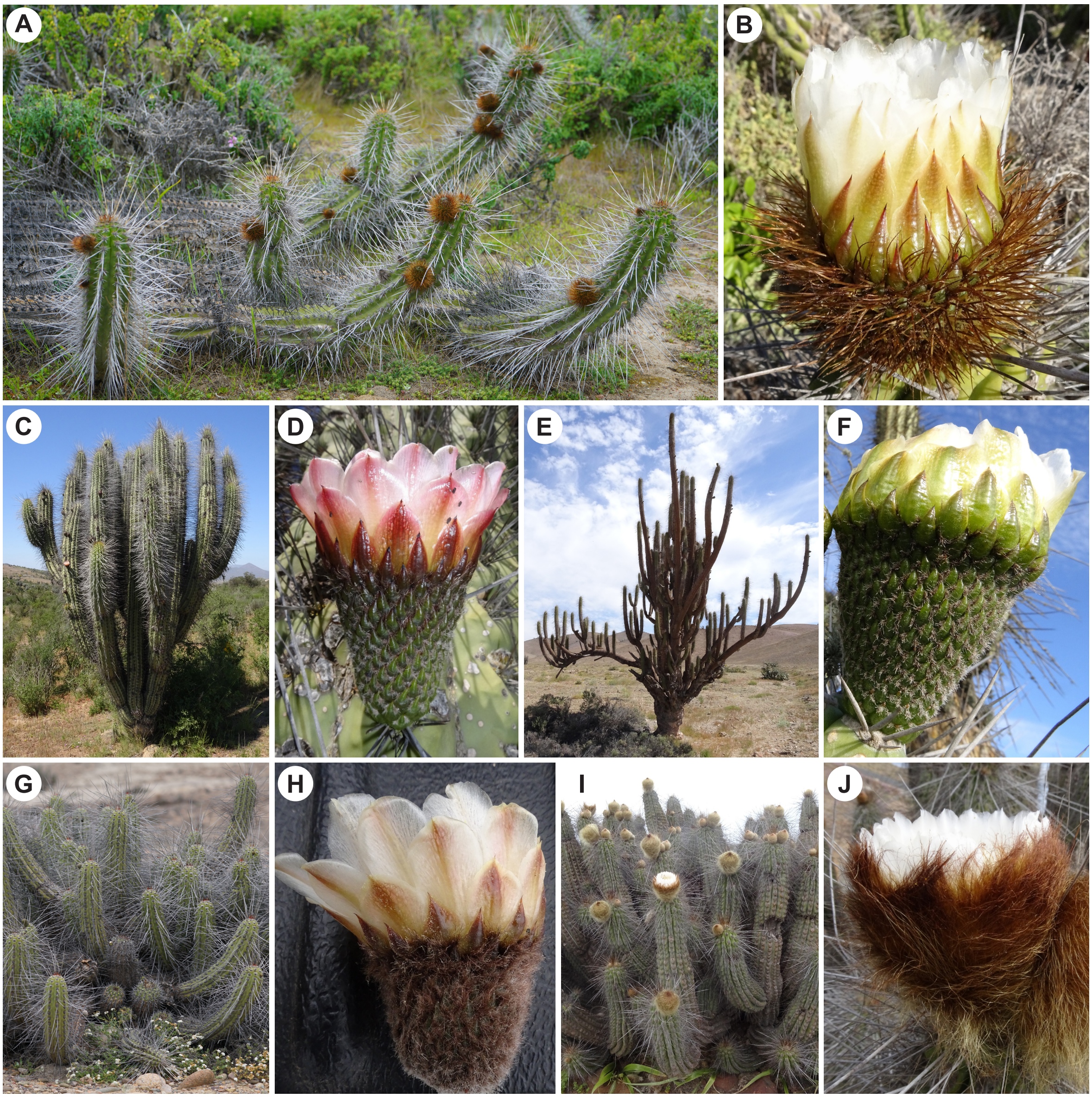

Figure 1: Morphological diversity within *Eulychnia.* A) *E. castanea*, decumbent habit. B) Flower of *E. castanea* with typical spiny pericarpel. C) *E. acida* near the type locality in Illapel. D) Flower of *E. acida* with the typical pericarpel lacking a conspicuous indumentum. Flower color varies from white to light pink. E) *E. acida* var. *elata*, massive form of *E. acida* at the type locality Estancia Castilla. F) Flower of *E. acida* var. *elata* lacking the conspicuous indument on the pericarpel. G) *E. acida* var. *procumbens*, decumbent habit. H) Flower of *E. acida* var. *procumbens* with short, dark wool on the pericarpel. I) Typical shrubby habit of *E. breviflora.* J) Typical, densely woolly pericarpel of *E. breviflora.*

dating (Ritter *et al.*, 2018b,a) and palaeoclimatic reconstructions based on a drill core (Ritter *et al.*, 2019).

Present-day Atacama vegetation is restricted to the coastal cordillera that benefits from occasional winter precipitation and the influence of coastal fog (Rundel *et al.*, 1991; Schulz *et al.*, 2011) and the Andean foothills, receiving summer rain. These vegetation zones are separated by the hyper arid core, an area virtually devoid of plant life (Rundel *et al.*, 1991; Luebert & Pliscoff, 2017). In spite of the overall hyper arid conditions, the northern coastal Atacama harbors a surprisingly high number of vascular plants (∼550 species), with a high percentage of endemism of >60% (Dillon & Hoffmann, 1997). Regular advection fog that meets the westward facing slopes of the coastal cordillera facilitates the establishment of fog oases (lomas), in which the majority of species occur (Rundel *et al.*, 1991). Few molecular phylogenetic studies have been conducted to investigate the origin, divergence times and causes for diversification of Atacama Desert lineages. Individual studies have been able to demonstrate a correlation between Andean uplift, the onset of hyper aridity in the Atacama and diversification in parallel to aridification (Gengler–Nowak, 2002; Luebert & Wen, 2008; Dillon *et al.*, 2009; Heibl & Renner, 2012; Böhnert *et al.*, 2019). Despite these studies, our knowledge of the evolution of the Atacama flora and, in particular, associated abiotic drivers of plant diversification in this extremely arid climate remains fragmentary.

In a recent evolutionary study based on thirteen samples of *Eulychnia*, including seven accepted species, Larridon *et al.* (2018) obtained a phylogeny that defined two well supported clades, with the sampled taxa falling into a northern and southern group corresponding to the main morphological characters used to distinguish between *Eulychnia* species. Unfortunately, support within these clades was weak. Here we generated a comprehensive dated phylogeny of *Eulychnia* to investigate its biogeographic history and diversification. Our dataset includes sequences from plants collected at the type localities for all previously published names, some of which are placed in synonymy of currently eight accepted species (Philippi, 1860; Cullmann, 1958; Ritter, 1964, 1980, 1981; Eggli *et al.*, 1995; Hoxey & Klaassen, 2011; Guerrero & Walter, 2019). We attempt to resolve the evolutionary and biogeographic history of *Eulychnia*, specifically to estimate (1) the divergence time between *Eulychnia* and *Austrocactus*, (2) correlate this divergence to the onset of aridity of the Atacama Desert, (3) elucidate the processes of diversification within *Eulychnia* and (4) infer its ancestral ranges. The analysis of data obtained from genotyping-by-sequencing is a relatively new approach toward the study of the cactus family and also to the study of the Atacama Desert flora in general.

4.2 Methods

Taxon sampling–A complete taxon sampling of the genus *Eulychnia* was gathered for the present study, based on our own collections realized between 2016 and 2019 in Chile and Peru.

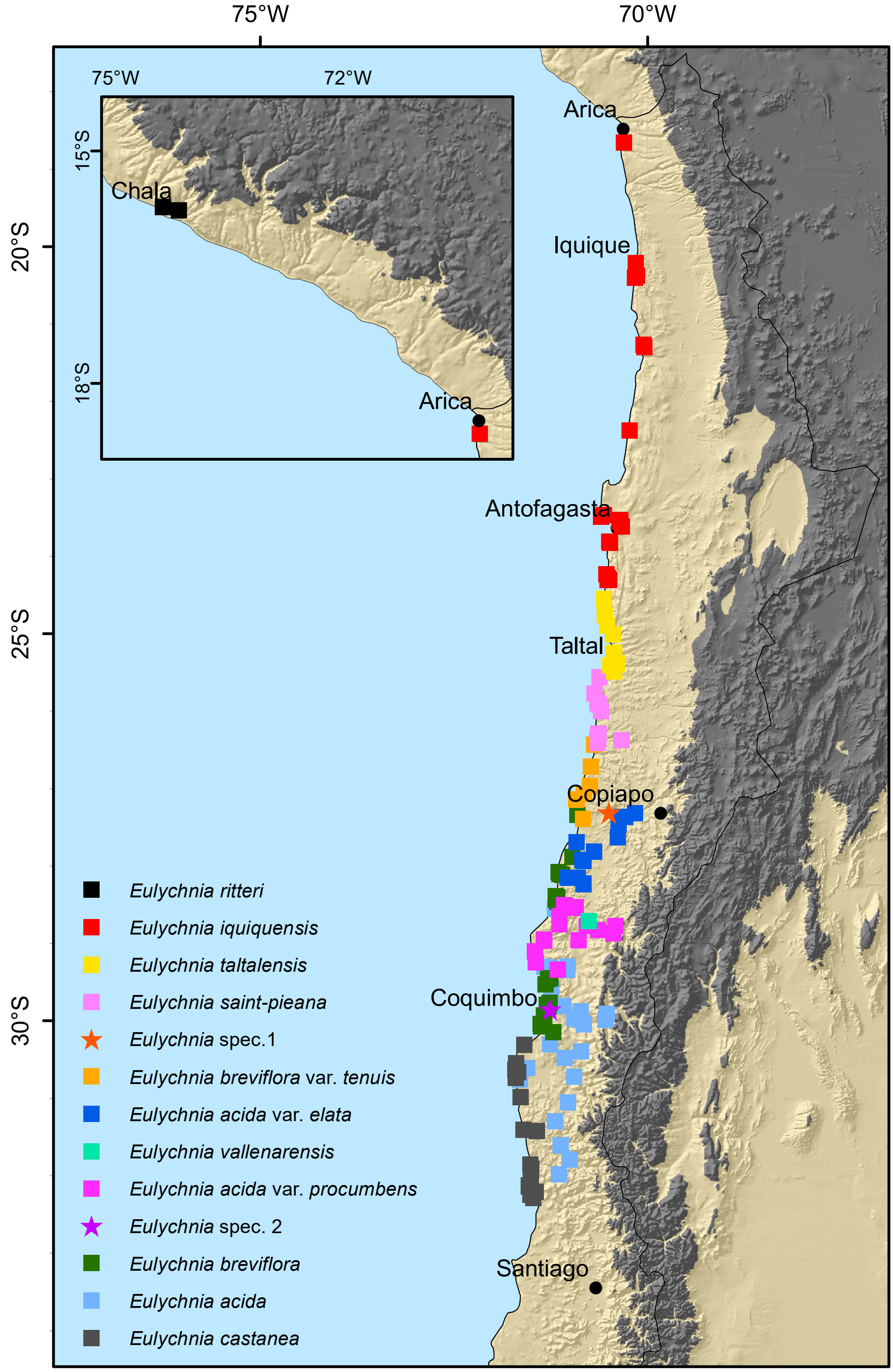

Figure 2: Distribution of *Eulychnia* in Chile and Peru. Note the restriction of taxa to the coast north of Copiapó. Most populations of *E. iquiquensis* between Antofagasta and Iquique have disappeared, also most likely those in Arica, increasing the distance to the disjunct taxon *E. ritteri* in Peru to approximately 1000 km. Distribution of taxa based on herbarium vouchers (Table S1).

The type localities of all but one previously published taxon were visited and sampled (Philippi, 1860, 1864; Cullmann, 1958; Ritter, 1964, 1980, 1981; Eggli *et al.*, 1995; Hoxey & Klaassen, 2011; Guerrero & Walter, 2019). The only population not sampled by us in the field was that of *E. aricensis* F.Ritter from Cerro Camaraca South of Arica. This population is likely extinct and although an attempt was made to re-collect material of this locality, this was not possible, and cultivated material from this locality introduced by Ritter was used instead. Vouchers were deposited in the herbaria BONN, EIF, K and ULS (Table S1). Collections from the type localities (locotypes) are here labelled with the oldest available name at the species or variety level. This decision does not reflect a taxonomic judgment. An additional subset of several species of *Austrocactus* was obtained from the living collection of E. & N. Sarnes (Eschweiler, Germany) grown from seed material. Previous studies recovered *Austrocactus* as the sister genus of *Eulychnia* (Hernández-Hernández *et al.*, 2011, 2014), and we included these samples in order to obtain age estimates for the split between these two genera. A further sample of *Corryocactus* Britton & Rose was included as outgroup since this genus was retrieved as sister to the PHB clade (Pachycereeae, Hylocereeae and three genera formerly included in Browningieae: *Castellanosia*, *Neoraimondia* and *Armatocereus*; Hernández-Hernández *et al.*, 2011). A two-step analysis was conducted using (1) a three plastid-marker alignment of the whole Cactaceae based on the work of Hernández-Hernández *et al.* (2014), in order to obtain age estimates for the split between *Eulychnia* and *Austrocactus* and (2) a dataset of *Eulychnia*, *Austrocactus* and two *Corryocactus* samples obtained from genotyping-by-sequencing (GBS), in order to produce a fully resolved phylogeny based on genome-wide single-nucleotide-polymorphism (SNP) data. A comprehensive list of all taxa used for this study, including GenBank accession and voucher information are provided in the supplementary material (Table S1).

Our definitions of the terms “arid”, “hyper arid”, etc. follows that of the spatial distribution of ombrotypes provided by Luebert & Pliscoff (2017).

Molecular methods–For both datasets, genomic DNA was extracted from silica-dried stem tissue using the NucleoSpin Plant II kit (Macherey-Nagel, Germany) following the manufacturer’s protocol with an increased incubation time of 90 min and an increased volume of lysis and binding buffers to reduce viscosity of the lysate. Following the work of Hernández-Hernández *et al.* (2014), three plastid DNA regions (*trn*K-*mat*K region, *trn*L-*trn*F region, *rpl*16 group I intron) were amplified using the primer combination and PCR cycling conditions given in supplementary material (Tables S2–S5). In order to obtain high-quality reads, PCR products were purified using gel extraction

and NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. Sequencing was performed at a sequencing service on a 3730XL DNA Analyzer (Applied Biosciences) and DNA sequences were manually edited using PhyDe v. 0.9971 (Müller *et al.*, 2005).
As higher quality and purity are needed for DNA used for high throughput sequencing, extracted DNA was electrophoresed on 1% agarose gels using Lonza GelStar Nucleic Acid Gel Stain (100x) and a sample of 20 ng linear, double-stranded Lambda DNA (New England Biolabs, N3011S) was added for testing the quantity and quality of the genomic DNA. Qubit Fluorometer (Life Technologies, Carlsbad, CA, United States) measurements were taken in order to assign a numerical value to the agarose-gel readings. Samples were standardized to 20 ng/µl and aliquots of 15 /µl per sample were used for library preparation and sequencing.

Library preparation and genotyping-by-sequencing protocols followed those of Merklinger *et al.* (2020). 200 ng of genomic DNA were digested with the restriction enzymes *PstI*-HF (New England Biolabs, R3140S) and *MspI* (New England Biolabs, R0106S), followed by a size selection, individual barcoding and finally single-end sequencing on the Illumina HiSeq 2500 (Illumina, Inc., San Diego). Barcoded reads were de-multiplexed using the CASAVA pipeline v. 1.8 (Illumina, Inc.). The obtained raw sequence reads (0.6 – 3 million per individual) were adapter and quality trimmed with a phred score of >25 using CUTADAPT v. 1.12 (Martin, 2011). Reads shorter than 50 bp after adapter removal were discarded. Sequence reads for the GBS Illumina runs were deposited in the European Nucleotide Archive under the study accession PRJEB39114.

A de novo assembly of the GBS data of initially 47 taxa belonging to *Eulychnia*, *Austrocactus* and the outgroup *Corryocactus* was carried out using ipyrad v. 0.9.17 (Eaton & Overcast, 2020). Different output files were generated with a minimal number of samples per locus set to 8, 12, 16 and 20 respectively, the maximum cluster depth within samples set to 0.9, and the ploidy level set to diploid. For all other parameters, the default settings of ipyrad were used.

Analytical methods–(1) The complete taxon sampling of Hernández-Hernández *et al.* (2014) was downloaded from GenBank and complemented with our original sequence data obtained from Sanger sequencing for 23 individuals of *Eulychnia* and *Austrocactus*. The final dataset was manually aligned using PhyDe v. 0.9971 (Müller *et al.*, 2005). Five hairpin associated inversions of 109 bp were detected in the whole alignment (two in *trn*K-*mat*K, two in *trn*L-*trn*F and one in *rpl*16) and reverse-complemented and aligned for analysis following Quandt *et al.* (2003). Additionally, 18 hotspots (Borsch *et al.*, 2003) with a total of 228 bp were detected in all three DNA regions and excluded from analysis. Positions and sizes are documented in the supplementary files available on the CRC1211-database (see data availability). Tree topology was tested for congruence with those presented by Hernández-Hernández *et al.* (2014) applying maximum likelihood (ML) analyses using RAxML v. 8.2.9. (Stamatakis, 2014). The GTRCAT substitution model was specified and the analyses were run with 1000 rapid bootstrap replicates, treating each gene region as a single partition. The final tree was visualized using the python package toytree (Eaton, 2019) and is available in the supplementary files on the CRC1211-database (see data availability).

(2) RAxML was used to infer maximum likelihood trees based on concatenated supermatrices of each of the most inclusive datasets (min12) obtained from the GBS assembly in ipyrad. The GTR+Γ substitution model was used with 20 tree searches and 1000 bootstrap replicates to calculate node support. A species tree based on SVDQUARTETS (Chifman & Kubatko, 2014) under multispecies coalescence was estimated using TETRAD, as implemented in ipyrad with 1000 bootstrap replicates.

Structure v. 2.3.4 (Pritchard *et al.*, 2000), also implemented in ipyrad was used to cluster individuals into K distinct populations based on the min12 SNP dataset (30,100 sites after filtering), and using the imap dictionary to group individuals into populations. The minmap command was specified at 0.5, requiring that 50% of samples have data in each group. Multiple values for K (2–6) were tested and 20 replicates were run per test. Each replicate was run for 500K Markov Chain Monte Carlo (MCMC) steps. A burn-in of 100K was applied. Results were visualized and exported using toyplot v. 0.18.0 (Shead, 2014).

Molecular clock dating– (1) Following the work of Hernández-Hernández *et al.* (2014) a Bayesian relaxed clock analyses using BEAST 2.5.1 (Bouckaert *et al.*, 2014) was conducted for the whole Cactaceae plastid dataset in order to estimate divergence times of the separation of *Eulychnia* and *Austrocactus*. BEAUTI 2.5 (Bouckaert *et al.*, 2014) was used to set up an XML file. The partitions of the three cpDNA regions were unlinked with respect to site model, but linked with respect to clock and tree models. Further, we specified a birth-death model as tree prior and applied a relaxed lognormal clock with an estimated clock rate (Drummond *et al.*, 2006; Gernhard, 2008). Since there are no fossil records within Cactaceae, we used a secondary calibration following Hernández-Hernández *et al.* (2014) with a uniform prior distribution and a lower value of 22.71 and an upper value of 42.43 for the Cactaceae crown node. The MCMC was run for 75 million generations, sampling every 7500 generations. The log file was checked in Tracer v. 1.71 (Rambaut *et al.*, 2018) and TreeAnnotator produced a Maximum Clade Credibility Tree (MCCT) using mean heights, a burn-in of 10% and a posterior probability limit of 0.95. Finally, the R-packages ape v. 5.0 (Paradis & Schliep, 2019), phyloch v. 1.5-5 (Heibl, 2008 onwards), strap v. 1.4 (Bell & Lloyd, 2015) and geoscale v. 2.0 (Bell, 2015) were used in R v. 3.5.1 (R Core Team, 2018) to plot and annotate the dated tree. Phylogenetic and dating analyses were conducted on the CIPRES Gateway (Miller *et al.*, 2010).

(2) Penalized Likelihood Analysis for GBS Data

To estimate divergence times within *Eulychnia*, we first tested the molecular clock hypothesis of the dataset using the R-package treedater v. 0.3.0 (Volz, 2019). Based on the GBS-based ML phylogeny of *Eulychnia* and *Austrocactus*, we used the penalized likelihood (PL) approach (Sanderson, 2002) as implemented in the R-package ape. We conducted a cross validation (Sanderson, 2002; Paradis, 2012) to determine the best value of the smoothing parameter lambda and used that value to obtain divergence time estimates with the PL method. Two outgroup samples of *Corryocactus* were removed from the analysis. We calibrated the stem node of *Eulychnia* and *Austrocactus* and the crown nodes of *Eulychnia* and *Austrocactus*, respectively, setting three calibration points as minimum ages corresponding to the median ages obtained from our BEAST analysis (see above).

Ancestral area reconstruction– To reconstruct the range evolution of *Eulychnia* we used the dispersal extinction cladogenesis (DEC) approach (Ree & Smith, 2008) as implemented in the R package BioGeoBEARS v. 1.1.1 (Matzke, 2013). The DEC+j model was not employed due to reported statistical problems (Ree & Sanmartín, 2018). Two analyses were run with differing assumptions in the number of maximum areas, which were set to two and three, without dispersal constraints over time, as their effect on the results is negligible (Chacón & Renner, 2014). We used the time calibrated ML tree based on the GBS dataset and removed most of the 44 accessions, to be left with 14 samples, one per well-supported clade, each representing an operational taxonomic unit (OTU), which were designated based on our locotype collections using the oldest available name at the species or variety levels. The analysis was conducted for these taxa, including one sample of *Austrocactus* to assess the directionality of this mostly trans-Andean distribution and a lineage through time (LTT) plot was generated with the R-package ape. Every sample in the phylogeny was assigned to one of six areas based on the distribution patterns of *Eulychnia* in Chile and Peru and *Austrocactus* in Patagonia. These geographic units corresponded to: (A) Patagonia, (B) Southern Atacama - inland, (C) Southern Atacama - coastal, (D) Northern Atacama - inland, (E) Northern Atacama - coastal and (F) Peru. The assignment of OTUs to geographical areas was based on information obtained from our own collections as well as herbarium vouchers revised at the herbaria of the Zürich Succulent Collection (ZSS), the Natural History Museum in Santiago de Chile (SGO) and the Naturalis Biodiversity Center in Leiden (L). Additional voucher images were consulted online at the herbaria in Kew (K), Halle (HAL) and Concepción (CONC). Our first division into geographic areas was based on the distribution of *Austrocactus* in Patagonia and of *Eulychnia* in Chile and Peru. Secondly, we separated "North" and "South", with the border between this separation set at the Huasco river (Coquimbo region), reflecting the transition from arid to hyper arid climate of the Atacama Desert, coinciding with the N-S limit of several OTUs. Further we differentiated between "coastal" and "inland", because some taxa such as *E. castanea* and *E. breviflora* have a strictly coastal distribution, while others occur further inland. Lastly, we defined "Peru" as its own geographical area reflecting the disjunct distribution of *E. ritteri.* The geographical distribution of each taxon for the two analyses is documented in the supplementary Table S6.

4.3 Results

cpDNA phylogeny and divergence times in Cactaceae–The final alignment of the three chloroplast DNA regions encompassed 248 taxa and had a length of 6065 bp after exclusion of 18 hotspots with a length of 228 bp and reverse-complementing five hairpin associated inversions. The BEAST ML analyses of whole Cactaceae (Fig. 3; Figs. S1–S2) confirmed the phylogenetic relationships of *Eulychnia* within the Core Cactoideae and as sister to *Austrocactus* with high Branch support (BS = 100%, PP = 1). Within *Eulychnia*, two well-supported clades were retrieved (BS = 100%, PP = 1). The first clade includes *E. breviflora* as well as the other Chilean taxa *E. iquiquensis* and *E. taltalensis* (BS = 97%, PP = 1). The Peruvian taxon *E. ritteri* is sister to this *E. breviflora*-clade with high support (BS = 82%, PP = 0.97). A second clade was retrieved for the *E. acida*-group including *E. castanea*, *E. acida* var. *procumbens* and *E. vallenarensis*, with *E. castanea* as sister to the other taxa in this clade, and with *E. acida* (type locality sample ED3110) as sister to *E. acida* var. *procumbens*, *E. vallenarensis* and *E. acida* var. *elata* (BS = 83%, PP = 1).

While the branch support for the major clades separating morphologically distinct groups is high, the support values within the respective clades are low (BS <70%, PP <0.95). The last common ancestor of *Eulychnia* and *Austrocactus* was dated to the Miocene-Pliocene transition at 6.70 Ma (95% HPD: 3.28–10.51). The crown node age for *Eulychnia* was retrieved for the early Pleistocene (2.19 Ma, 95% HPD: 0.88–3.84). The split between Peruvian *E. ritteri* and the Chilean *E. breviflora* clade is dated to the middle to upper Pleistocene (1.49 Ma, 95% HPD: 0.55–2.72). Diversification within the two Chilean *Eulychnia* clades was dated to the middle Pleistocene for both the *E. breviflora* clade (0.68 Ma, 95% HPD: 0.23–1.29) and the *E. acida* clade (0.82 Ma, 95% HPD: 0.22–1.62).

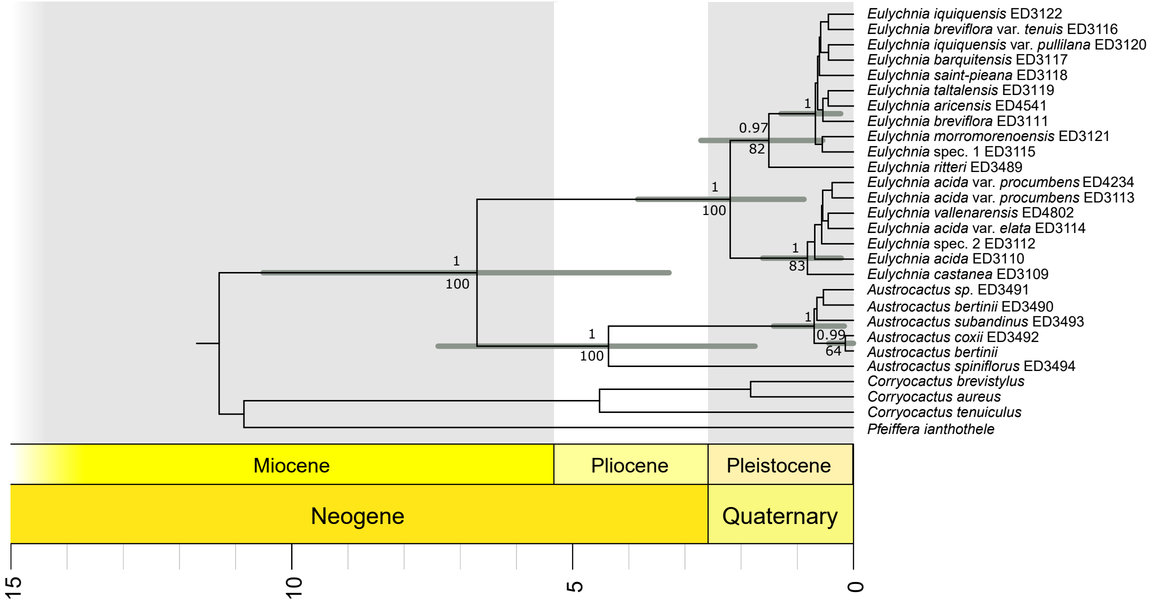

Figure 3: Section of the BEAST-dated plastid phylogeny based on the sampling of Hernández-Hernández *et al.* (2014) and including our additional samples, showing the relationships and age estimates within *Eulychnia* and *Austrocactus*. Numbers above nodes represent posterior probabilities, those below nodes represent bootstrap values. The full phylogeny is available in the supplementary materials, Fig. S1.

Genotyping-by-sequencing phylogeny and biogeography–A Maximum Likelihood analysis based on SNP data for *Eulychnia* and *Austrocactus* including *Corryocactus* as an outgroup retrieved a tree with the split between *Eulychnia* and *Austrocactus* fully supported (Fig. 4). *Eulychnia* itself is resolved with Peruvian *E. ritteri* sister to all other (Chilean) taxa. The Chilean clade is further resolved into two sister clades, clade A and clade B with full support (BS = 100%). Clade A includes *E. breviflora*, *E. iquiquensis*, *E. taltalensis*, *E. breviflora* var. *tenuis*, *E.* spec. 1 and *E. saint-pieana*/*barquitensis*. Clade B includes *E. acida*, *E. acida* var. *elata*, *E. acida* var. *procumbens*, *E. vallenarensis* and *E. castanea*. Taxa in both clades roughly follow a geographical order from South to North. The support for clade A is significant (BS = 97%), with most of the subclades showing full support (BS = 100%). Clade B receives maximum statistical support of consistently BS = 100% with the exception of the *E.* spec. 2, *E. castanea* and *E. acida* subclade (BS = 61%) and the *E. vallenarensis*, *E.*

acida var. *elata* subclade (BS = 66%).
The tree obtained from the TETRAD analysis is congruent in the major clades, but with some differences in the relationships between sister taxa. *Eulychnia* is again monophyletic and retrieved as sister to *Austrocactus*. *Eulychnia ritteri* is sister to both Chilean clades, also fully supported *E. acida* var. *procumbens* is sister to *E. vallenarensis* in the northern *E. acida*-clade, while in the RAxML analysis, *E. vallenarensis* is sister to *E. acida* var. *elata*. Overall, statistical support in the TETRAD phylogeny is lower than in the RAxML tree.

The structure analysis (Fig. 5) revealed three distinct genetic groups for the various values of K = 2–6, with these groups generally corresponding to the three major clades retrieved in the RAxML analysis. There is a clear separation of taxa belonging to the *E. acida* and *E. breviflora* groups at K = 2, with *E. ritteri* genetically predominantly part of the *E. breviflora* group. At K = 3, *E. ritteri* has started to separate into its own genetic group. At K = 4–6, *E. ritteri* is distinct, as are the *E. acida* and the *E. breviflora* groups. Noteworthy is the position of *E. acida* from Cuesta Buenos Aires as well as *E. castanea* from Tongoy, both of which retain a mixed genetic signal from the *E. acida* and *E. breviflora* groups respectively.

The test of the molecular clock hypothesis failed to reject the strict clock (coefficient of variation of rates: ≈ 0). Accordingly, the cross validation resulted in a best smoothing parameter lambda = 100. The penalized-likelihood dating of the RAxML tree (Fig. 6) provided congruent results to the dated plastid phylogeny in terms of divergence times: the split between *Eulychnia* and *Austrocactus* is retrieved for the Miocene-Pliocene transition at 6.70 Ma. For the crown node of *Eulychnia* an age of 2.20 Ma is inferred, separating the Peruvian taxon *E. ritteri* from the Chilean congeners in the early Pleistocene. The crown node age of the Chilean taxa is dated to the early Pleistocene at 2.16 Ma when they separated into the *E. acida* and *E. breviflora* clades. The crown node age for the *E. breviflora* clade was retrieved for the early Pleistocene at 2.11 Ma, when it further separates into a southern and a northern clade, with the northern clade of *E. breviflora* and relatives separating into four subclades at 2.03 Ma, still during the early Pleistocene, with these subclades in geographical order from north to south. The crown node age for *E. acida* and relatives was dated to 2.07 Ma in the early Pleistocene, with the samples again following a south to north order in two subclades separating at 1.97 Ma, the southern one including *E. castanea* and *E. acida*, the northern subclade including an *E. acida* sample as sister to all other taxa northwards, including *E. acida* var. *procumbens*, *E. vallenarensis* and *E. acida* var. *elata*.

Ancestral area reconstruction– The two analyses with two and three maximum

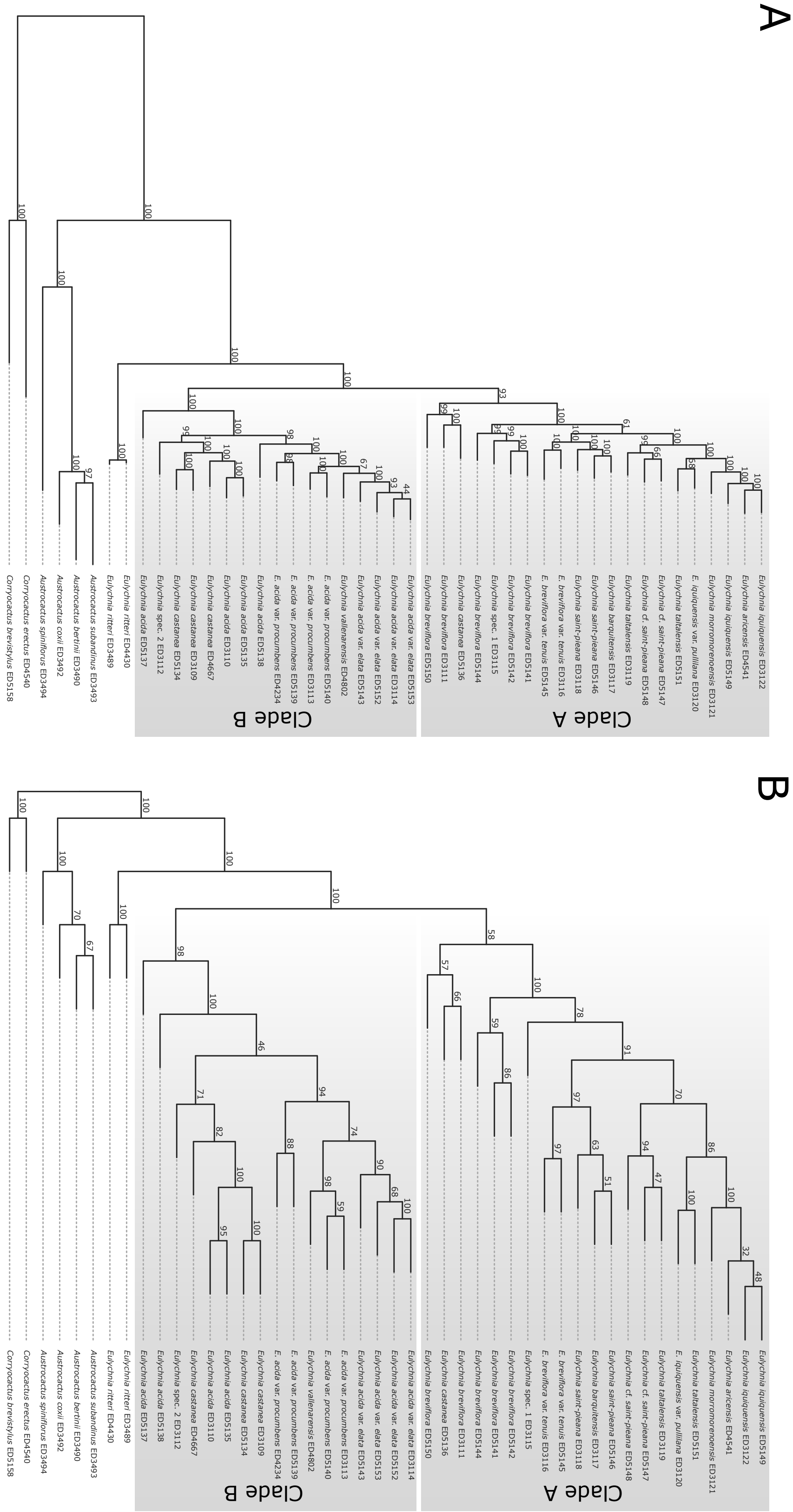

Figure 4: Maximum-Likelihood phylogeny (A) and TETRAD species tree (B) based on the GBS dataset. Numbers at nodes represent bootstrap values.

areas resulted in a likelihood difference of 0.93 (LnL = -32.35 and LnL = -31.42, respectively; Fig. 6). The ancestral area reconstruction with maximum areas of two indicates a separation of an ancestral taxon of *Austrocactus* and *Eulychnia* between Patagonia and the south coastal Atacama during the Miocene-Pliocene transition, though no highly likely ancestral area was recovered. During the Pliocene, the coastal Atacama range of *Eulychnia* separated from Patagonia. Peru was colonized in the early Pleistocene from the northern coastal Atacama. With ancestral taxa widely distributed along the Atacama coast from south to north, ancestors of the *E. acida* clade apparently disappeared over most of the range, surviving only at the southern coast. The origin of the *E. acida* clade is the southern coastal Atacama, with two independent inland colonizations in the South (*E. acida* and *E. acida* var. *procumbens*) and one inland colonization in the North (*E. acida* var. *elata*). The origin of the *E. breviflora* clade in the early Pleistocene was estimated at the coast, with most extant members restricted to the northern coastal Atacama, but with one inland colonization in the northern Atacama (*E.* spec. 1). In contrast, the maximum area setting of three indicates a higher probability of a coastal-Peruvian origin of *Eulychnia*. Vicariance between Peru and Chile led to the divergence of *E. ritteri* and a colonization along the Chilean coast by an ancestral *Eulychnia* during the early Pleistocene. From here the processes are congruent with those of the first analysis.

4.4 Discussion

Our results from the plastid phylogeny, the GBS RAxML phylogeny and the TETRAD analysis provide a largely congruent tree topology. In all three analyses, two clades of Chilean *Eulychnia* were retrieved, one designated as *Eulychnia breviflora* clade, and a second clade including *E. acida* and relatives. These two groups are supported by general morphology, with individuals of the *E. breviflora* group sharing a densely woolly pericarpel and a higher number of softer spines, and those of the *E. acida* group with a non-hairy (but sometimes spiny) pericarpel and fewer but coarser spines (Fig. 1). Pericarpel indumentum has been used as the most reliable diagnostic character in *Eulychnia* since Britton & Rose (1920). The fact that several species appear non-monophyletic may be explained by several reasons. First, there is a difference between species and names.

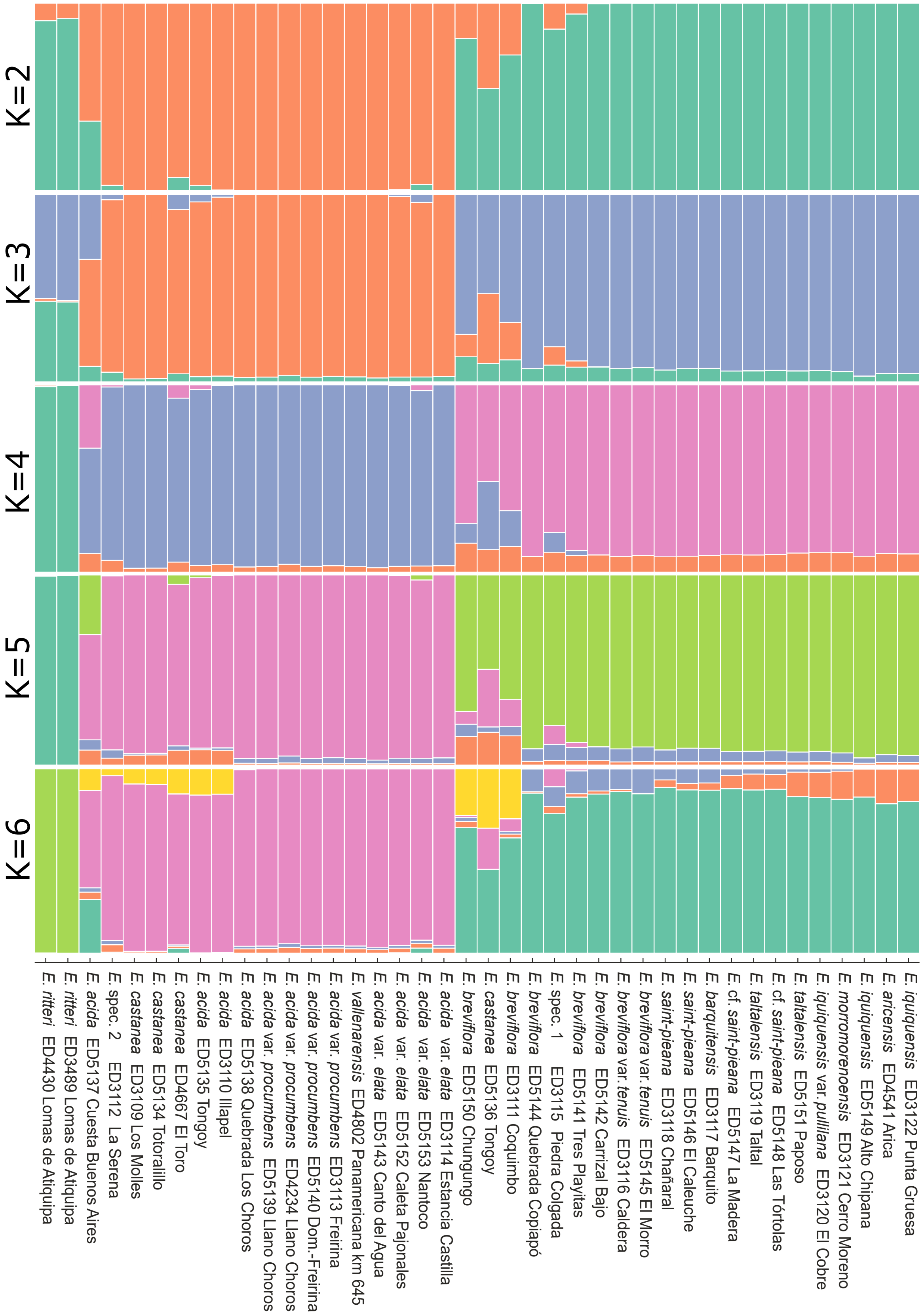

Figure 5: Structure analysis showing the clustering of individuals into K = 2–6 distinct populations based on the min12 SNP dataset.

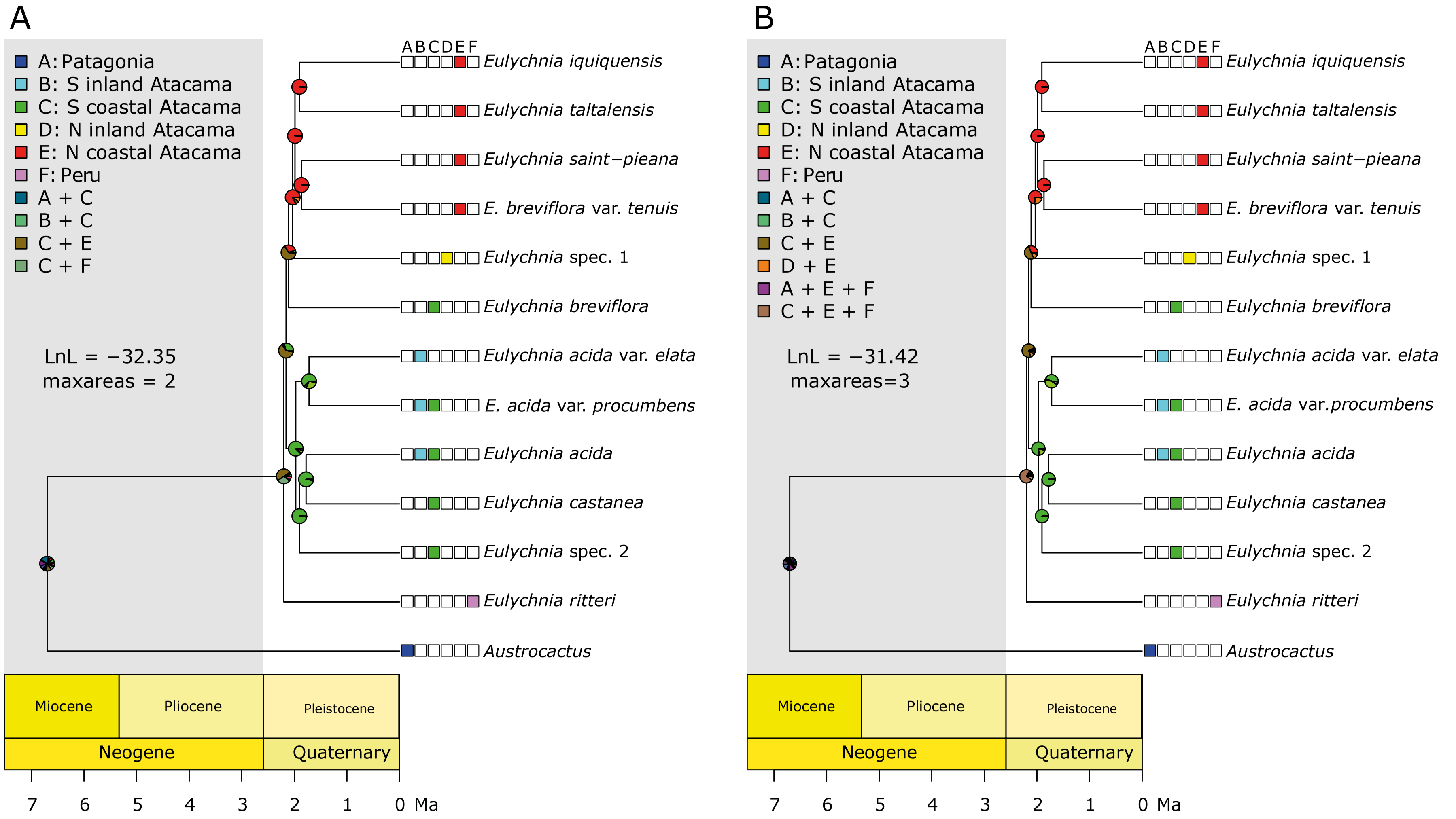

Figure 6: Penalized-likelihood phylogeny and ancestral area reconstruction based on the GBS dataset and tested for two different settings, maximum areas = 2 and maximum areas = 3.

The names we have applied to different populations are those available in the literature, but they do not necessarily reflect real taxa. That is likely the case of the infraspecific taxa in *E. breviflora* and *E. acida.* In these cases we may need to recognize that there is cryptic diversity – morphological characters that have not yet been assessed, and that current diagnostic characters are perhaps insufficient to separate these infraspecific taxa as different species. Alternatively, using broader species concepts in *Eulychnia* would also eliminate the problem of non-monophyly. Further, hybridization between taxa has been suggested between e.g. *E. acida* and *E. castanea* (Eggli & Leuenberger, 1998), pointing to gene flow between these taxa and supporting our idea of incomplete speciation. An updated taxonomic revision of the genus should address these issues.

The position of *E. ritteri*, which in the plastid phylogeny is sister to the *E. breviflora* group, but in the GBS RAxML tree and Tetrad analysis is sister to all Chilean *Eulychnia* is interesting. This taxon was in the past considered a subspecies of *E. iquiquensis* (*E. breviflora* clade; Hunt, 2006), or alternatively, as a species in its own right (Hunt, 2016; Larridon *et al.*, 2018). The incongruence between the cp phylogeny and the GBS and Tetrad phylogenies may have several reasons. Firstly, it may be due to the different phylogenetic signals from the different data sets. While the cp regions are of maternal inheritance, the loci obtained from the genotyping by sequencing data are distributed across the whole genome (Davey & Blaxter, 2010). The clades with polyphyletic relationships in our cp phylogeny were moderately to highly supported (e.g. *E. ritteri* as sister to the *E. breviflora* clade; *E. morromorenoensis* as sister clade to all other northern taxa), which points to both recent and ancestral interspecific hybridization/introgression as likely reasons. While hybridization/introgression is difficult to be distinguished from incomplete lineage sorting (Joly *et al.*, 2009), introgression among *Eulychnia* taxa is likely to occur, even with the current scenario of allopatric distribution of taxa and hybrid zones have been observed at the periphery of various taxa (e.g. Ritter, 1980; Eggli & Leuenberger, 1998; Hoxey & Klaassen, 2011. Individuals that did not form a clade in the cp phylogeny formed clades with other conspecific members in the GBS tree (with the exception of two individuals that had been collected as of hybrid origin due to morphological features, such as *E. castanea* ED5136 and *E. acida* ED5137, two samples which also showed great admixture in our Structure analysis). Chloroplast captures stemming from introgression events are common in plants (Rieseberg & Soltis, 1991; Acosta & Premoli, 2010) and the incongruence we see in our cp phylogeny may also be due to the heterospecific origin of the chloroplast genome. In the GBS tree, the two Chilean clades further separate into a southern and northern clade, comprising taxa that fall into a clear south-north geographical area, with the exception of a sample of *E. castanea* from Tongoy, which was retrieved as sister to a sample of *E. breviflora* from Coquimbo, a few kilometers north of Tongoy. Based on the results of the structure analysis and its close geographic position to the distribution range of *E. breviflora*, we interpret this sample as the result of introgression between *E. castanea* and *E. breviflora.* The retrieved split into two major clades of *Eulychnia* into a southern and northern clade corroborates the results of Larridon *et al.* (2018) and we favor the hypothesis of a climatic transition into a hyper arid environment as an explanation. Further studies in Chilean Cactaceae (Guerrero *et al.*, 2019b) also found such a north-south division, although unfortunately this latter study does neither provide age estimates, nor an explanatory hypothesis. The transition between the two clades seems to be the region around Copiapó. Here the southern species' distribution ends and the distribution of the northern species begins, with the exception of *E. breviflora* along the coast. The coast was perhaps better connected in the past, possibly through the distribution of the guanaco, which may explain the continuous distribution of *E. breviflora* there. It is also possible that refugia existed where, due to more humid conditions, taxa persisted even during arid phases. Based on populations from these refugia, an expansion southward from northern populations and a parallel expansion northward from southern populations with subsequent colonizations inland (as shown by our ancestral area reconstruction) seems plausible. Similar expansion events have been reported for e.g. *Nolana* (Ossa *et al.*, 2013, 2017). A scenario of isolated refugia could be understood as a more extreme form of the present-day isolated lomas separated by hyper arid habitat (Rundel *et al.*, 1991), which reconnect to become more continuous during climatically favorable periods, and the diversification processes of species under these circumstances seems compatible with the extremely short time periods of diversification which we obtained from our study. The study by Larridon *et al.* (2018) was based on six chloroplast markers and *E. ritteri* was retrieved as sister to the *E. breviflora* clade, albeit with moderate support. By including almost twice as many samples in the plastid phylogeny and expanding our methods to include a GBS phylogeny with more than three times as many samples than in the previous study by Larridon *et al.* (2018), we were able to study the diversification processes within *Eulychnia* in much more detail, and also expand more precisely on the age estimates previously provided (Hernández-Hernández *et al.*, 2014).

Evolution of Eulychnia at the Miocene-Pliocene boundary– Our results identify a last common ancestor of *Eulychnia* and *Austrocactus* at the Miocene-Pliocene boundary, with a minimum crown node age of 6.70 Ma. Previous studies provided age estimates for the crown node of *Eulychnia* and *Austrocactus* at 4.9 Ma and a stem age of divergence at 9.17 Ma (Hernández-Hernández *et al.*, 2014). Although our estimates overlap and lie within the error rates of this study, the discrepancy in ages obtained may be traced back to our increased taxon sampling. While Hernández-Hernández *et al.* (2014) only included a single species of *Austrocactus* and two species of *Eulychnia*, our taxon sampling for *Eulychnia* is the most comprehensive to date and we also included several samples of *Austrocactus*. It is well known that increasing the number of species tends to increase the age of the clades (Linder & Rieseberg, 2004). A further source of these differences may lie in the alignment, which we conducted anew and excluded hotspots (see Methods) that may have impacted the substitution regime. In any case, our age estimates agree with previous studies, postulating the origin of the majority of species-rich clades in the Cactaceae as well as the emergence of other succulent lineages in North America and Africa at the Miocene-Pliocene boundary, ca. 10 – 5 Ma (Arakaki *et al.*, 2011; Hernández-Hernández *et al.*, 2014). This simultaneous global emergence of succulent lineages suggests a common trigger, most likely a shift in global climate. While the evolution of novel pollination syndromes or local adaptation to edaphic factors may play an important role in driving speciation of succulent lineages (Ellis *et al.*, 2006; Good-Avila *et al.*, 2006; Kellner *et al.*, 2011), the underlying processes that have been used to explain the genetic differentiation and thus species diversification of these lineages are linked to shifts in climate (Trejo *et al.*, 2016; Scheinvar *et al.*, 2017) and as such, biotic and abiotic processes are linked with each other. The Miocene-Pliocene boundary is characterized by gradual cooling and an expansion of the ice-sheets on West-Antarctica (Alpers & Brimhall, 1988; Zachos, 2001). The Antarctic ice sheet expansion led to the cooling of the Peru-Chile Current system (PCC) as well as deep Pacific waters and took place in parallel with a global cooling trend (Lamb & Davis, 2003), resulting in the establishment of (semi-) arid conditions in Africa (Klak *et al.*, 2004; Horn *et al.*, 2014) and North America (Moore & Jansen, 2006), as well as an overall expansion of grasslands dominated by C_4 photosynthetic species (Edwards *et al.*, 2010). According to Lamb & Davis (2003), the cooling of the PCC correlates with the most rapid phase of central Andean uplift and the Plio-Pleistocene epoch, when the Andes for the first time exceeded an average elevation of $\sim$3 km (Gregory-Wodzicki, 2000). This would have caused an additional shift from long-term aridity (Dunai *et al.*, 2005) to hyper aridity, due to the cooling of the PCC and the now increasing orographic barrier to moist air from the Amazon basin imposed by the Andes. The split of the two genera *Eulychnia* and *Austrocactus* coincides with this transition, and can possibly be attributed to changes in vegetation triggered by this increased aridity. Since the distribution of these genera is separated by the current spread of the Mediterranean sclerophyllous woodlands of Central Chile (Luebert & Pliscoff, 2017), this split might be related to the emergence of these woodlands. Paleoecological studies have suggested that the sclerophyllous vegetation of central Chile may have originated as a consequence of the development of the South American arid diagonal during the late Miocene resulting from the major phase of Andean uplift (Hinojosa & Villagrán, 1997; Armesto *et al.*, 2007), roughly coinciding with the split between *Austrocactus* and *Eulychnia*. It must be emphasized here, that although in the present study we follow Luebert & Pliscoff (2017) for our definition of hyper aridity, there are discrepancies in the scientific community pertaining to the different levels of aridity in the past and the present. So far there is no consistent definition of hyper aridity as well as no common consensus of the timing of onset of hyper aridity (see also Garreaud *et al.*, 2010 for a review of this matter).

Quaternary diversification of Eulychnia in the Atacama Desert– Our biogeographic analysis finds a high probability for a coastal origin of *Eulychnia* with a coastal range evolution and several independent inland colonizations (*Eulychnia* spec. 1 and *Eulychnia acida*, *E. acida* var. *elata* and *E. acida* var. *procumbens*). Ritter (1980) proposed a southern coastal Atacama origin of *Eulychnia* based on the assumption that *E. castanea* should be regarded as the most basal representative of the genus. Although Ritter does not elaborate on this statement, the current distribution of *E. castanea* at the southernmost edge of *Eulychnia* (Los Molles, 32°S) is geographically the closest to the northernmost locality of *Austrocactus* (Farellones, 33°S) in Chile. A possible explanation of the divergence of *E. castanea* and *E. acida*, the only two taxa at the southern end of *Eulychnia* distribution, may be found in peripatric speciation processes (Losos & Glor, 2003; Eduardo Palma *et al.*, 2005), with *E. castanea* forming divergent populations at the south-western edge of the geographical range of *E. acida*. This process could also be used to interpret the speciation of other taxa further north, and a process that again, might ultimately be driven by climatic variables. Our biogeographical analysis is unable to confidently identify a southern or northern coastal origin. Similar studies in the cactus genus *Copiapoa* (Larridon *et al.*, 2015), proposed a northern origin with a subsequent southward range evolution. However, considering the current disjunct distribution of several non-Andean desert cacti that are restricted to either Peru or Chile (Rundel *et al.*, 1991; Pinto & Luebert, 2009), generalizing statements on geographic origins should best be avoided until more studies have become available.

The Peruvian *E. ritteri* is clearly retrieved as an isolated, early lineage of *Eulychnia* and cannot go back to recent long-distance dispersal. However, an early dispersal event cannot be ruled out, in particular considering the large, juicy fruits of some *Eulychnia* taxa, which may in the past have been dispersed by now extinct herbivore mammals (Cares *et al.*, 2018). The guanaco (*Lama guanacoe*) for example, once widespread from northern Peru to Tierra del Fuego, but now distributed in less than 30% of its range at the time of arrival of Europeans to South America (Marin *et al.*, 2013 references therein), may have acted as an important disperser of *Eulychnia* seeds. While this may explain the geographical extension of *Eulychnia* as a whole, it does not shed light into the current geographical differentiation within *Eulychnia*, because the past distribution of the guanaco may have remained continuous in the Atacama Desert throughout the Quaternary (Politis *et al.*, 2011; González *et al.*, 2013; Marin *et al.*, 2013). Rather, the present-day isolated populations of guanaco in Chile and adjacent countries appear to be a human-induced phenomenon based on the introduction of livestock, hunting, habitat loss and altering climatic requirements (Baldi *et al.*, 2001; Castillo *et al.*, 2018). Under a scenario of climate-induced vegetation shifts in the past, populations of *Eulychnia* would have experienced isolation and secondary contact: *Eulychnia* originated in the Pliocene and diversified in a very short time span around 2 Ma (Figs. 3, 6, Fig. S4) into an already arid environment. The Pliocene-Pleistocene experienced several pluvial phases that were interrupted by stages of marked aridity (Jordan *et al.*, 2014; Ritter *et al.*, 2018a), particularly between approximately 2.65 to 1.27 Ma, which agrees well with our crown node age for *Eulychnia* dated to 2.2 Ma. Pluvial phases might have enabled *Eulychnia* populations to expand, forming extensive meta-populations ranging into Peru, perhaps similar to the wide-ranging *E. acida* populations of the southern Atacama we see today (Merklinger, 2018). The onset of hyper arid conditions during the early Pleistocene would have led to habitat fragmentation, promoted speciation, and possibly caused the disjunction of the Peruvian *E. ritteri* from the Chilean congeners. Despite a reported floristic break along the coast between northern Chile and southern Peru (Ruhm *et al.*, 2020), several studies have reported a similar pattern of disjunct species distribution in other plant groups, such as *Malesherbia*, *Nolana* and *Cristaria* (Gengler–Nowak, 2002; Dillon *et al.*, 2009; Böhnert *et al.*, 2019). Although we favor here the contraction-expansion model of populations during climatic oscillations as the ultimate driver for speciation, we do not dismiss the importance of newly emerging pollination syndromes or local adaptation to edaphic factors as other important reasons for *Eulychnia* diversification. These aspects could be explored in further studies based on the analyses of population-level data. Pollination syndromes in cacti are considered not stable (Nyffeler & Eggli, 2010) and other Chilean cacti have been shown to fit into this concept (Guerrero *et al.*, 2019a), however, *Eulychnia* flowers are functionally conserved across taxa and throughout the genus' range. Larridon *et al.* (2018) provided interesting insights into the possible causes of the current allopatric distribution patterns of the different *Eulychnia* species, based on climatic gradients and precipitation regimes linked with geographic changes and associated bioclimates, ombrotypes and vegetation zones (Larridon *et al.*, 2018 and references therein). These ideas are congruent with our hypothesis, that ultimately, climatic oscillations shaped the habitat occupied by and the diversification of *Eulychnia*. In the study by Larridon *et al.* (2018) as well as in our own results presented here, taxa clustered into groups that largely correspond to geographical areas. For example, *E. breviflora* var. *tenuis* and *E. saint-pieana* clustered together, rather than with *E. breviflora* (to which they were originally thought to be most closely related), and these two taxa occur only between the Río Copiapó and Chañaral. Further north, *E. taltalensis* clustered with *E. iquiquensis*. The former is distributed only between Taltal and El Cobre, an area that may well have acted as a refugium during arid phases, an idea that is supported also by other taxa present only here, such as *Tillandsia tragophoba* (Bromeliaceae; Zizka *et al.*, 2009) and for other coastal areas of the northern Atacama Desert (Ossa *et al.*, 2013). The second clade obtained by Larridon *et al.* (2018) including *E. acida* and relatives also shows a similar picture of allopatric distribution patterns in *Eulychnia*. Ritter (1980) already stated that the Huasco river forms a natural barrier between *E. acida* to the south and *E. acida* var. *elata* to the north. This observation is confirmed by the results of Larridon *et al.* (2018) that retrieved *E. acida* var. *elata* as sister to the southern *E. acida* samples. Further, *E. chorosensis* is restricted to the Llano Choros and the Huasco valley, and may be the result of speciation in this area due to more favorable conditions, even during persistent aridity in the surrounding areas. Overall, these results are in line with our hypothesis, that climatic oscillations are ultimately responsible for driving speciation in *Eulychnia*, supported by repeated isolation of populations with subsequent expansions and reconnections to form new species assemblages.

It is likely that during predominantly arid conditions, *Eulychnia* was restricted to the more humid coastal cordillera, as shown by our ancestral area reconstruction, with bursts of range expansion during the later Pleistocene pluvial phases. Range expansions would have promoted secondary contact of populations not yet fully genetically isolated from each other and so explain the current distribution of *Eulychnia*, as well as the potential hybridization that appears to take place in areas of contact between putative, morphologically different species. According to Ritter *et al.* (2018a), there were very long periods of aridity during the Pleistocene, periods long enough to allow species to evolve (Levin, 2019). Considering also the numerous potential hybrids that have been observed (Ritter, 1980; Eggli & Leuenberger, 1998), also hybrid speciation (Levin, 2019) must be considered a relevant process, even for a potentially long-lived species with long generation times as is the case in *Eulychnia*. The mosaic of local climates and variable precipitation regime we see today is in line with our hypothesized oscillation of aridity particularly during the Pleistocene as the main driver for *Eulychnia* diversification, and it also explains the present-day distribution of taxa in southern Peru and northern Chile.

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Author contributions

FFM, TB, MA, DQ & FL undertook fieldwork. FFM generated the sequence data. FFM, TB & FL designed the study and did the analyses. All authors contributed to writing and critical revision of the manuscript draft.

Declaration of interest

The authors declare that all research was conducted independently of any commercial or financial relationships that could be interpreted as a conflict of interest.

Data availability

Raw sequence reads for the GBS Illumina runs were deposited in the European Nucleotide Archive under the study accession PRJEB39114. Additional data including alignments and results are available from the CRC1211-database (https://crc1211-db.uni-koeln.de; DOI: https://xxxxxxxx)

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Chapter 5

A synopsis of the genus *Eulychnia* (Cactaceae)

This chapter has been included at manuscript stage and is currently in preparation to be submitted.

A synopsis of the genus *Eulychnia* (Cactaceae)

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Abstract

A taxonomic account of the genus *Eulychnia* (Cactaceae), endemic to Chile and Peru, is here presented, based on extensive field studies as well as herbarium and literature research. In the light of current knowledge, a taxonomic concept for the genus is proposed and discussed. Three species and nine subspecies are recognized. Two new taxa at the rank of subspecies are described and nine rank changes and new combinations are made, and a key to the species and subspecies is provided. *Eulychnia acida* is neo-typified and *E. ritteri* is lectotypified.

Resumen

Se ofrece una sinopsis taxonómica del género *Eulychnia* (Cactaceae) para Chile y Perú basada en estudios de campo y la revisión de ejemplared de herbario y la literatura. A la luz del conocimiento existente se propone y discute un concepto taxonómico para el grupo. Se reconocen tres especies y nueve subespecies. Se describen dos nuevas subespecies y se efectúan nueve cambios de rango taxonómico. Se neotipifica *Eulychnia acida* y se lectotipifica *E. ritteri.*

Keywords: Arid environments—Atacama Desert—nomenclature—speciation—taxonomy

5.1 Introduction

Eulychnia Phil. is a genus of columnar cacti predominantly distributed along the coast from about 32°S near Los Molles in central Chile to 15°S near the town of Chala in southern Peru (Fig. 1). Historically, there was a geographical distance of approximately 400–500 km separating the Peruvian taxon from its nearest relatives in northern Chile (Ritter, 1981; Hunt, 2002), however, in recent decades, this distance has arguably increased to almost 1000 km due to the disappearance of *Eulychnia* populations in Chile north of Iquique because of increasing drought (Pinto, 2007; Schulz *et al.*, 2011). At the southern end of the distribution range, *Eulychnia* species receive notably more precipitation (ca. 200 mm) than their northern congeners (<1 mm) (Houston, 2006). Here, parts of the landscape are often dominated by *E. acida* Phil., reaching into the interior of the country. Further north, with the transition from a more Mediterranean-like climate and vegetation into the hyper arid core Atacama Desert (Luebert & Pliscoff, 2017), *Eulychnia* becomes restricted to the coastal mountains and quebradas (canyons) where regular fog from the Pacific coast and the occasional rain fall provide some humidity for vegetation to persist. As a result, populations are less extensive and more isolated from each other (Lembcke & Weisser, 1979). The plants are here restricted to the typical loma vegetation of the fog zone or fertile belt (Johnston, 1929; Muñoz-Schick *et al.*, 2001), which stretches along the western flanks of the coastal cordillera and has been shown to be surprisingly rich in plant species (∼550 spp.) and with a high percentage of endemism of >60% (Dillon & Hoffmann, 1997).

Morphologically, *Eulychnia* is easily distinguishable from the other columnar cacti of the region, *Leucostele* Backeb. (formerly under *Trichocereus* (A.Berger) Riccob.), as long as the plants are fertile, because the main diagnostic characters to differentiate *Eulychnia* from these are found in the flowers and fruits (Fig. 2). *Eulychnia* flowers are short and stout and lack the long flower tube typically found in *Leucostele.* The *Eulychnia* pericarpel is usually covered in spines (*E. acida* subsp. *castanea* (Phil.) Merkl.), or dense golden-brown trichomes, often of considerable length (e.g. *E. breviflora* Phil.), that arise from the base of small scales. Ritter (1980) observed that the outer cortical layer at the distal end of the pericarpel folds inwards after flowering together with the tepal remains and covers the inner cortical layer with its appendages, a behaviour similar to that seen in *Opuntia* (L.) Mill. Vegetative material of *Eulychnia* is almost impossible to separate from *Trichocereus*, especially when specimens grow in mixed stands. Nyffeler *et al.* (1997) noted, however, that the stems of *Eulychnia* have a parenchymatic cortex with two distinct regions, containing idioblastic sclereids that are unique to this genus.

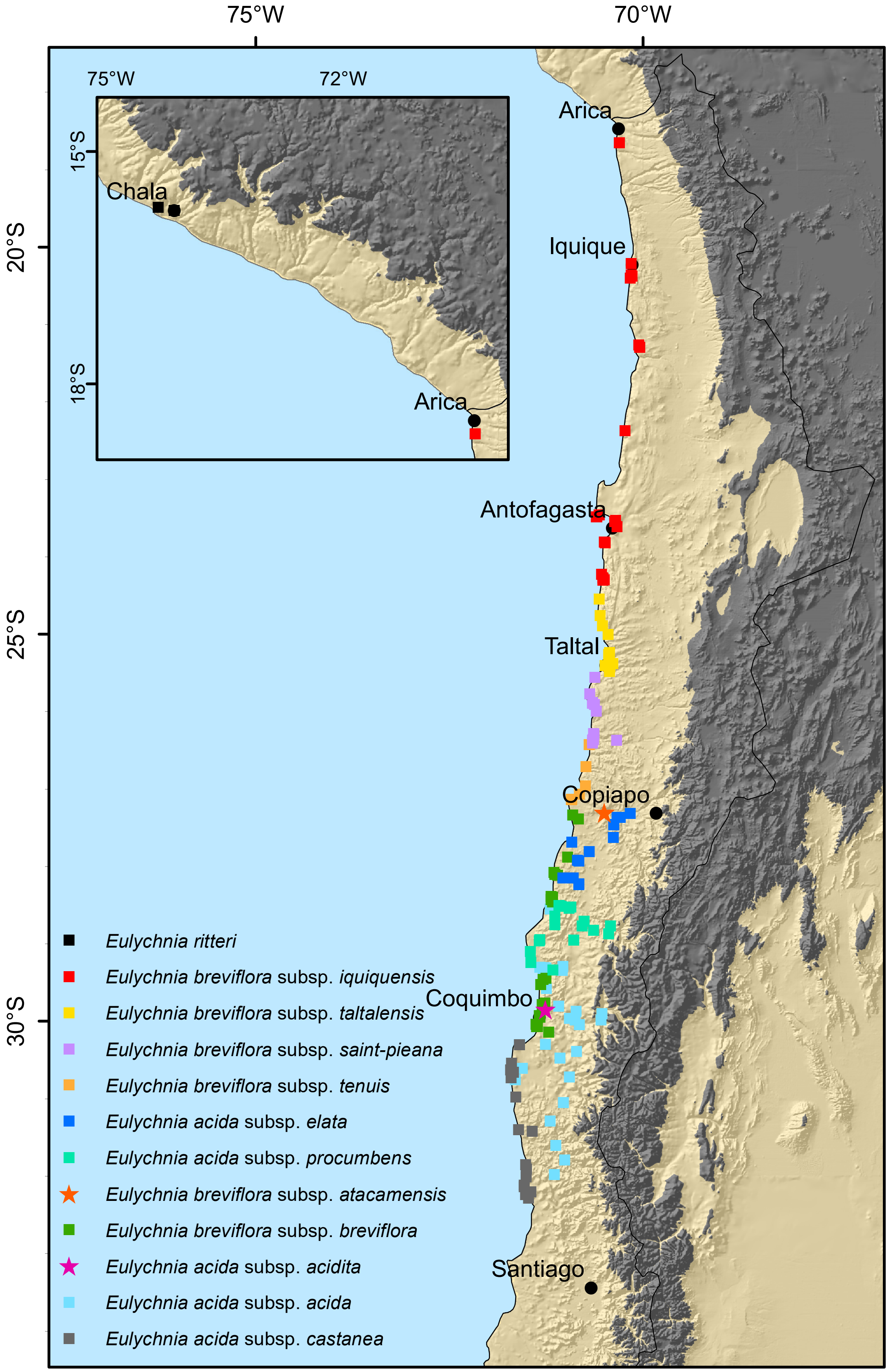

Figure 1: Geographical distribution of the twelve taxa of *Eulychnia* in northern Chile and southern Peru. Areas shaded in dark gray represent the Andean Cordillera >3000 m elevation, areas in beige <3000 m elevation. The black line represents the political border of Chile.

Barthlott & Hunt (1993) considered *Eulychnia* Phil., *Austrocactus* Britton & Rose and *Corryocactus* Britton & Rose as early diverging members of the Notocacteae. More recent phylogenetic studies showed that *Eulychnia* falls into the core Cactoideae I as sister to *Austrocactus* and forms a subclade of a clade including *Corryocactus* and the tribes Hylocereeae, Leptocereeae and Pachycereeae (ACHLP clade; Nyffeler, 2002) or as a clade in the core Cactoideae I as sister to a clade containing Pachycereeae, Hylocereeae and Browningieae members (PHB clade; Hernández-Hernández *et al.*, 2011).

Although *Eulychnia* is a conspicuous element of coastal central and northern Chilean vegetation, botanical interest in the genus has at best been sporadic (Merklinger, 2018). This may in part be due to the slow growth and rather large size of adult plants, thus not being favored for ex-situ collections. The most detailed taxonomic account following extensive field work was presented by Ritter (1980, 1981) who described several new taxa. He developed a taxonomic concept based on morphological characters and geographic entities, but he provided no key to the species, which has made it rather difficult to distinguish some taxa based on his diagnoses. The *Eulychnia* names described by Philippi remained untypified until the study of Leuenberger & Eggli (2000), causing some inconsistencies in the literature; as a consequence, the number of accepted taxa in the genus has varied from 14 taxa accepted by Ritter (1980, 1981) to four taxa accepted for Chile by Hoffmann & Walter (2004) and five taxa in total accepted by Hunt (2006).

The need for a taxonomy based on available molecular phylogenetic evidence and clearly defined diagnostic morphological discontinuities was realised by the authors of the present work during four extensive field periods where populations of *Eulychnia* were sampled in order to conduct molecular studies.

Philippi (1860) originally stated that the genus *Eulychnia* bears long, dense wool on its pericarpel. Schumann (1903) doubted *Eulychnia* to be a natural genus, because the species *E. acida* and *E. castanea*, added later by Philippi (1864) did not possess this character. The presence of wool on the pericarpel can thus be regarded as the most obvious character to identify the species of *Eulychnia*, and has in fact been treated as such since Britton & Rose (1920). In addition, rib shape, areolar felt and spine-characters have been used for species delineation (Hoxey & Klaassen, 2011; Larridon *et al.*, 2018). During our field work, however, we observed that plants presented a continuum of morphological variation along the range of the genus, in particular concerning areolar felt and spine morphology. While areolar felt varied in length and density within presumed species, spine characters, with spine number, length and differences between juvenile and adult spination proved equally inconsistent (Merklinger, 2019).

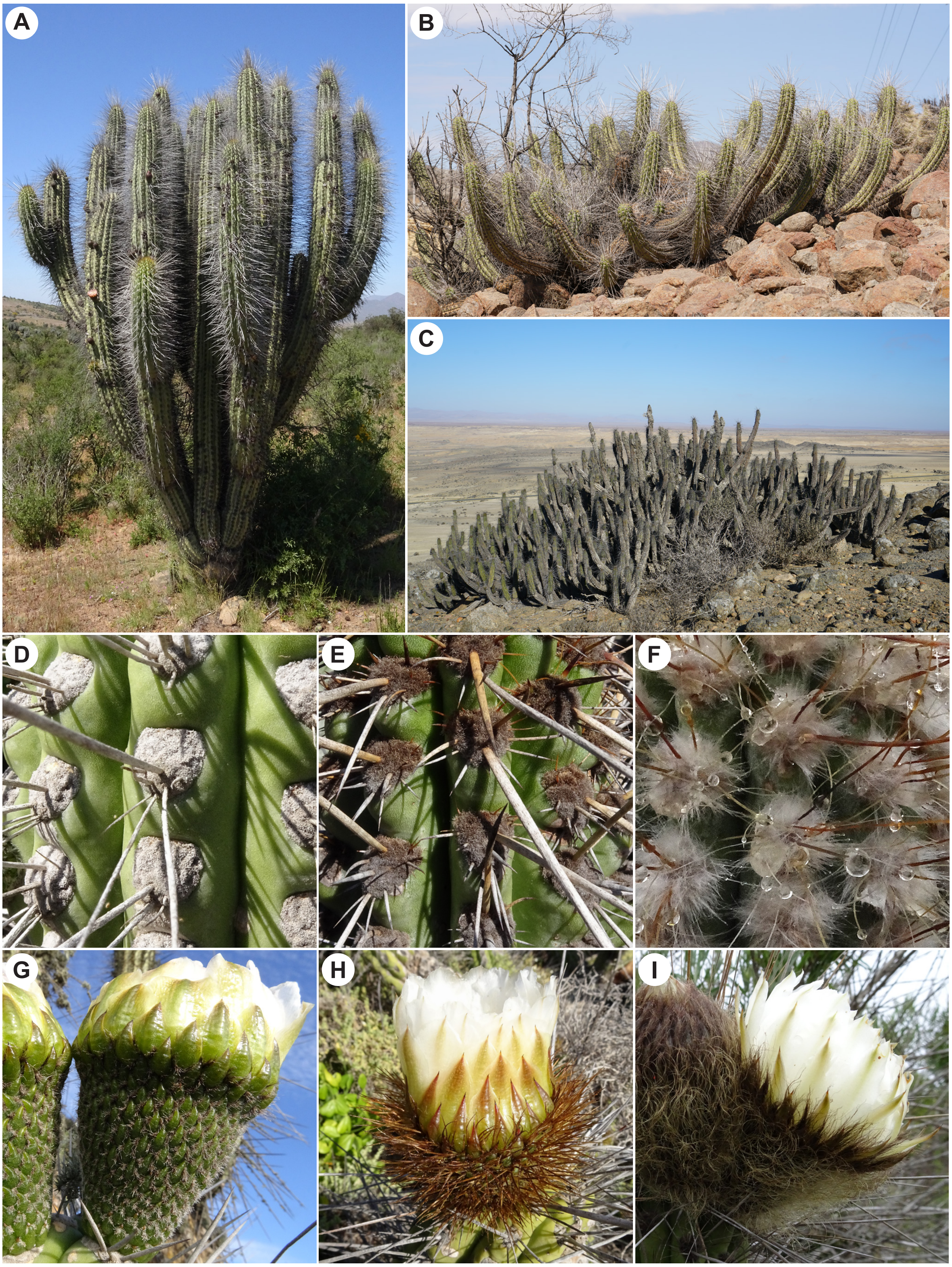

Figure 2: Overview of the morphological diversity found in *Eulychnia*. A–C) habit: A) *E. acida* subsp. *acida* at the type locality Illapel, B) typical habit of *E. acida* subsp. *procumbens* near Freirina, C) *E. breviflora* subsp. *tenuis* near Caldera. D–E) overview of areolar felt: D) *E. acida* subsp. *acida*, E) *E. breviflora* subsp. *taltalensis*, F) *E. breviflora* subsp. *saint-pieana*. G–I overview of *Eulychnia* flowers: G) *E. acida* subsp. *elata*, H) *E. acida* subsp. *castanea*, I) *E. breviflora* subsp. *breviflora*.

Pericarpel morphology thus remained the most reliable character to assign plants at least to a relationship to either *E. breviflora* (hairy pericarpel) or *E. acida* (non-hairy pericarpel) groups (with the inclusion of *E. castanea* into *E. acida*). These groups were also retrieved as clades in molecular studies by Larridon *et al.* (2018) and Merklinger *et al.* (in prep.). The latest additions of species to the genus (Hoxey & Klaassen, 2011; Guerrero & Walter, 2019) as well as published molecular phylogenetic analyses (Hernández-Hernández *et al.*, 2011, 2014; Larridon *et al.*, 2018) showed, that a detailed review of *Eulychnia* is needed.

The authors were able to visit the type localities of all previously published names, except one (*E. aricensis* F.Ritter), and gained a detailed overview of the plants throughout the entire range of the genus. Based on observations and collections made, and in combination with the study of specimens at various herbaria, including types, a species concept of the genus was devised, which aims at reflecting, as well as possible, the variability within *Eulychnia*, while considering geographic distribution and phylogenetic results from previous works (Larridon *et al.*, 2018). Here we provide a complete taxonomy of the genus *Eulychnia*.

Taxonomic history

The genus *Eulychnia* Phil. was established by Rodolfo Amando Philippi in 1860 based on *E. breviflora* Phil., which he found in Coquimbo, and which he drew for his publication Florula Atacamensis (Philippi, 1860). In 1864, he added two further species to the genus, *Eulychnia acida* Phil. and *E. castanea* Phil. (Philippi, 1864). Although the genus *Eulychnia* was accepted by most authors, Schumann (1899, 1903) included *Eulychnia* in *Cereus* Mill., stating that the genus was “completely unnatural. . . whose main character, the woolly-hairy ovary, is not even present in the two later described species” [*E. acida* & *E. castanea*; translated by the authors from the German original] (Schumann, 1903). Britton & Rose accepted the genus *Eulychnia* and recognized *E. acida*, *E. castanea*, *E. iquiquensis* (K.Schum.) Britton & Rose and *E. spinibarbis* Britton & Rose (Britton & Rose, 1920). Backeberg (1942) created the monotypic genus *Philippicereus* Backeb. for *Eulychnia castanea* based on the flowers and fruits, which differ from other *Eulychnia* flowers in that they are covered in bristly spines similar to those of the sweet chestnut *Castanea sativa* Mill.. *Philippicereus* was, however, not accepted by subsequent authors, because the plant is a typical *Eulychnia* in all other characters.

The most comprehensive work on the genus so far was prepared by Friedrich Ritter during his extensive field work in Chile (Ritter, 1964, 1980, 1981). He described five new species, five new varieties and made one new combination, totalling 14 taxa (Ritter, 1980). However, Ritter provided no keys, and although his descriptions are the most detailed, they are of limited diagnostic value in order to reliably identify the species in the field. While in a first edition of a book on the cacti of Chile six species of *Eulychnia* are accepted (Hoffmann, 1989), the second edition of the same book only accepts four species (Hoffmann & Walter, 2004). Hunt (1992) lists five species and two species as provisionally accepted. The New Cactus Lexicon (Hunt, 2006) lists four species, one of which, *E. iquiquensis* was divided into two subspecies.

An important summary of *Eulychnia* was provided by Leuenberger & Eggli (2000), who collected material of *Eulychnia* extensively in the 1990s. In this paper, the confusion associated with several alleged type specimens was largely resolved, and the necessary lectotypifications were made for *E. breviflora*, *E. castanea* and *E. iquiquensis*. In 2011, Hoxey and Klaassen reexamined two taxa from the Llanos de Choros (south of the Huasco River) and Taltal, previously described by Ritter as *E. acida* Phil. var. *procumbens* F.Ritter and *E. breviflora* Phil. var. *taltalensis* F.Ritter, respectively. Both taxa were raised to species level, as *E. chorosensis* P.Klaassen and *E. taltalensis* (F.Ritter) Hoxey, resulting in a total number of seven species accepted for the genus (Hoxey & Klaassen, 2011; Hunt, 2016).

Larridon *et al.* (2018) produced the first molecular phylogeny of the genus using six chloroplast markers. Subsequently, Guerrero & Walter (2019) published a new name, *E. vallenarensis* P.C.Guerrero & Helmut Walter for a plant from a small area between Vallenar and Freirina, in the Atacama province, from the type locality of *E. chorosensis* P.Klaassen ($\equiv$ *E. acida* var. *procumbens* F.Ritter). The discussion of this species lacks any reference to the type locality of *E. chorosensis*, which is in the same geographical area, and collections of which were cited by Ritter from the region around Vallenar. Further, the type material does not include fertile structures, yet the authors used fruit size among other characters in a key to the species of *Eulychnia* (Larridon *et al.*, 2018) to differentiate *E. vallenarensis* (*E.* aff. *acida* “Vallenar”; (Larridon *et al.*, 2018)) from other *Eulychnia* species.

Since no serious attempt at revising this genus has been made since Ritter (1980, 1981), and newly described species were apparently based purely on phylogenetic trees rather than a modern taxonomic concept (see introduction in Guerrero & Walter, 2019), the acceptance or rejection of some names by some authors seems rather arbitrary, and not based on well-informed decisions, emphasizing the need for this present work.

5.2 Materials and methods

Four field trips to the Atacama between 2017 and 2019 were carried out in order to visit the type localities of all but one published names for *Eulychnia* (Cullmann, 1958; Ritter, 1964, 1980, 1981; Eggli *et al.*, 1995; Leuenberger & Eggli, 2000; Hoxey & Klaassen, 2011; Guerrero & Walter, 2019). 74 voucher specimens were collected and deposited at the herbarium of the Nees Institute for Biodiversity of Plants, University of Bonn, Germany (BONN), with duplicates at the herbaria of La Serena University, Chile (ULS) and the University of Chile in Santiago (EIF), as well as the herbarium at the Royal Botanic Gardens, Kew, U.K. (K). In addition, 135 specimens were studied at the herbaria of Santiago, Chile (SGO), Leiden, Netherlands (L [U]) and the Succulent Plant Collection Zürich (ZSS). Online databases and digital images online were also consulted.

Special difficulties are associated with the typification of the names published by Friedrich Ritter (Ritter, 1964, 1980, 1981). The numbers used by Ritter appear to be ordinary field numbers or collection numbers, but are in fact more something like "taxon numbers" (Eggli *et al.*, 1995). For material from different localities (i.e. different gatherings in the terminology of the current Art. 8.2 of the ICN (Turland *et al.*, 2018)) that were interpreted as representing the same taxon, the same "collection" number was used, and to complicate the issue, material collected at later dates (sometimes years after the first gathering was made) also received the very same "collection" number. A full synopsis of the Ritter collections was provided by Eggli *et al.* (1995), and including differentiation of multiple gatherings under the same number. Ritter (1963) states that his holotypes were usually deposited at Utrecht (U), and, following the definition of "gathering" according to the code of botanical nomenclature (Art. 8.2), all other specimens with the same "collection" number should be treated as paratypes and are thus listed in the specimens examined section.

5.3 Taxonomic concept

Throughout its range, *Eulychnia* presents a continuum of morphological characters, yet populations with distinct phenotypes occupy discrete geographic ranges with little or no overlap, but with the occasional formation of apparent hybrid zones along their range limits (Ritter, 1980; Hoxey & Klaassen, 2011). We must therefore decide on (1) a taxonomic concept that allows for practical taxon delineation based on distinct morphological characters whilst attempting to understand speciation processes in the genus, and (2) the naming of this variety of forms – a formality for taxonomic convenience. The genus *Eulychnia* as a group evolved comparatively recently and the split from its sister genus *Austrocactus* is dated at a mere 6–7 Ma (Hernández-Hernández *et al.*, 2014; Merklinger *et al.*, in prep.). *Eulychnia* can therefore be considered a "neo-endemic" of the Atacama and Peruvian deserts (Badgley *et al.*, 2017), and is likely a lineage in the process of speciation. Molecular phylogenetic reconstructions have retrieved two major clades for all Chilean taxa, one consisting of *E. acida* and allies, and the other consisting of *E. breviflora* and relatives, while the Peruvian species *E. ritteri* falls outside of these clades ((Larridon *et al.*, 2018); Merklinger *et al.*, in prep.). Two possible taxonomic concepts could thus be envisioned, namely splitting of the genus into all morphologically recognizable forms, or grouping the same into names, mirroring the evolutionary hisotry as retrieved by the molecular phylogenetic analyses. We thus propose the recognition of three morphologically well-defined species based on pericarpel morphology and geographic range, while the local variants, each with distinct phenotypic traits shall be treated as taxonomic entities at subspecies level, following the geographical speciation concept (for a review see Grant, 1971; Mallet, 2013).

In this present work, we accept three species and nine subspecies of *Eulychnia*. This approach is in line with morphological variation, geographic distribution and phylogenetic results, while providing names that can be considered operational taxonomic units, and which are easily adapted according to potential future research. We have raised several previously published varieties to the rank of subspecies – we consider the rank of subspecies more appropriate because these taxa form geographically and morphologically distinct populations.

5.4 Taxonomy

Eulychnia Phil., Fl. Atac. 1860.
Type: *Eulychnia breviflora* Phil.

incl. *Philippicereus* Backeb. Jahrb. der Deutsch. Kakteen-Ges. 1941, pt. 2: 29, 1942.
Type: *Philippicereus castaneus* (Phil.) Backeb.

Description. Shrubby to arborescent cacti with erect, ascending, narrowly to broadly candelabriform branched stems or decumbent-sprawling stems; parenchymatic cortex with two distinct regions, containing idioblastic sclereids unique to this genus; stems 4–16 cm diameter, with 9–20 ribs; areoles with short whitish-grey to brown-grey or brown felt, felt in some species becoming long, dense and bushy (*E. breviflora* subsp. *saint-pieana*). Spination with usually easily distinguishable central and radial spines, porrect or deflexed, central spines often strong, sometimes needle -or bristle-like, or becoming hair-like, to 20 cm long; radiating spines few to many, short and strong, sometimes bristle -to hair-like; flowers usually close to the apex of the stem, sometimes also from further down along the shoot, broadly campanulate, hypanthium shorter than the pericarpel, covered in imbricate scale-like bracts, thick hypanthium and pericarpel walls, the distal part of the outer wall folding inwards after flowering rather than withering; flowering areoles with woolly hairs, sometimes barely visible, sometimes becoming very long and dense (*E. breviflora*), sometimes with additional bristle-like spines (*E. acida* subsp. *castanea*); perianth erect to spreading, tepals short, white to light pink; stamens many; style short and thick; stigma with up to 35 lobes; fruits globose, depressed-globose or pyriform, fleshy, usually with dense areolar wool or sometimes spiny; perianth remnant persistent, leaving a hard apex during fruit maturation; seeds broadly oval, ca. 1.5–2 × 1.2–1.5 mm, brown to black; hilum, oblique or basal, impressed.

5.4.1 Key to the species and subspecies of *Eulychnia*

1 Flowers 2.5–8.5 cm long, pericarpel densely woolly, spiny, very sparsely hairy or hairs absent, areolar felt dense bushy or short, whitish-grey or brown; plants columnar, arborescent, decumbent or clustering, to 6 m tall; endemic to Chile **2**

- Flowers to 2.5 cm long, pericarpel densely woolly; areoles with dense bushy whitish-grey felt; arborescent plants to 3 m tall; restricted to southern Peru . . . . **(12) *E. ritteri***

2 Pericarpel without spines but with long and dense golden-brown wool, with dense short wool, sparsely hairy or hairs seemingly absent; plants columnar, arborescent, decumbent or clustering, coastal or inland **3**

- Pericarpel with chestnut-coloured bristly spines, plants decumbent, strictly coastal **(3) *E. acida subsp. castanea***

3 Pericarpel with hairs usually absent or with sparse silvery-grey hairs; plants arborescent, 0.6–4–(6) m tall; coastal and/or inland **4**

- Pericarpel with short brown wool; decumbent; coastal and inland from the Llano de Choros to Vallenar . . . . **(5) *E. acida subsp. procumbens***

4 Pericarpel with hairs usually absent, plants arborescent, 4–6 m tall; mostly inland **5**

- Pericarpel with very sparse silery-grey hairs; plants arborescent, 0.6–1 m tall; restricted to coastal sand dunes N of La Serena . . . . **(2) *E. acida subsp. acidita***

5 Flowers to 7.5 cm long, pericarpel with hairs seemingly absent; plants arborescent to 4 m tall; south of the Río Huasco . . . . **(1) *E. acida subsp. acida***

- Flowers to 8.5 cm long, pericarpel with hairs seemingly absent; arborescent to 6 m tall; north of the Río Huasco . . . . **(4) *E. acida subsp. elata***

6 Pericarpel long and densely woolly; plants arborescent, branches 6–10 cm diameter; coastal and inland distribution **7**

- Pericarpel long and densely woolly; plants clustering with narrow parallel-erect shoots 4–7 cm diameter; restricted to the coast between the Río Copiapó and El Barquito **(11) *E. breviflora subsp. tenuis***

7 Pericarpel densely woolly; areolar felt long and dense whitish-grey or short and brown; plants arborescent, 1–4 m tall; coastal distribution **8**

- Pericarpel densely woolly; plants arborescent, 1–3 m tall; restricted inland at Piedra Colgada . . . . **(7) *E. breviflora subsp. atacamensis***

8 Pericarpel densely woolly; areolar felt dense bushy and whitish-grey; restricted to the coastal cordillera between Chañaral and Taltal **(9) *E. breviflora subsp. saint-pieana***

- Pericarpel densely wolly; areolar felt short brown; restricted between Taltal and El Cobre . . . . **(10) *E. breviflora subsp. taltalensis***

9 Pericarpel densely wolly; plants arborescent to 4 m tall, distributed from El Cobre northward . . . . **(8) *E. breviflora subsp. iquiquensis***

- Pericarpel densely wolly; plants arborescent, typically 1.5–3 m tall, rarely reaching 4 m, distributed from the Lengua da Vaca peninsula S of Coquimbo to the Río Copiapó **(6) *E. breviflora subsp. breviflora***

Eulychnia acida Phil., Linnaea 33 (17): 80, 1864.
≡ *Cereus acidus* (Phil.) K.Schum., Gesamtb. Kakt. Nachtr.: 22, 1903.
Type (neotype, designated here): Chile. Region IV Coquimbo. Prov. Choapa, NE of Illapel along D-705 towards Combarbalá on hill slopes, 450 m elev. 22 Sept. 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3767* (ULS); isoneotype (BONN).

Description. Arborescent or decumbent columnar cacti, 0.5–6 m tall; branching low, erect or decumbent; branches 5–12 cm diameter; ribs 8–16; areoles 5–14 mm diameter and at 4–15 mm distance to each other, with short grey-brown, dark brown or grey felt; spines emerging dark brown or black at tips with light brown to yellowish or yellowish-green or reddish-brown base, centrals well differentiated from radials; central spines 1–3, robust, up to 20 cm long; radial spines up to 12, a few mm to several cm long, robust to needle like; flowers close to the apex of the shoot and frequently appearing down the side of the shoot to about 1 m, 2–6 cm wide when open and ca. 3–8.5 cm long including pericarpel; pericarpel with narrow scales with sparse, very short to short silvery-grey or dark brown hairs at their base or with dense, short grey-brown wool or with bristly chestnut-coloured spines to 2 cm long; tepals white, sometimes pink, or often with prominent pink central stripe, with rounded or acute apex; style ca. 4–6 mm thick and 15–25 mm long; stigma with 15–20 lobes; fruits globose to pyriform, brownish-green or yellowish-green, ca. 4–6 cm diameter and 5–6 cm long, covered in sparse hairs or short dark brown wool or bristly spines, pulp white, becoming translucent when ripe, sour tasting; seed ca. 1.5–2 mm long, testa dark brown or black, keeled or not keeled, hilum small, oval, white.

Distribution. Chile. Regions III Atacama and IV Coquimbo, both at coastal and interior localities.

Discussion. *Eulychnia acida* Phil. is here redefined as containing five allopatric subspecies.

Leuenberger & Eggli (2000) concluded that there is a confusion of type specimens of *E. breviflora* and *E. acida* at the Santiago herbarium (SGO). Because none of the several specimens present could reliably be identified to represent original type material of *E. acida* (Leuenberger & Eggli, 2000), these authors did not designate a lectotype. A specimen at SGO annotated by Hutchinson (SGO 052680) as the type contains a fruit of *Trichocereus* (see also Leuenberger & Eggli, 2000). According to the protologue a drawing by Landbeck should exist, but this has so far not been found. No further vouchers that may be part of the type collection could be located in other herbaria. In the absence of original material, a neotype needs to be designated. We propose *Luebert 3767* (BONN) as neotype for *E. acida*, because that specimen is in full agreement with the protologue and has the same provenance ('frequens prope Illapel et Choapa' Philippi, 1864).

1. ***Eulychnia acida*** Phil., subsp. ***acida***

Type: As for *E. acida.*

Description. Arborescent, columnar cacti, 2–4 m tall; branching low, erect; branches 9–12 cm diameter; ribs 10–16; areoles 7–14 mm diameter and at 7–15 mm distance to each other, with short grey-brown to grey felt; spines emerging dark brown at tips with light brown to yellowish-green base, centrals well differentiated from radials; central spines 1–3, robust, 10–20 cm long; radial spines up to 12, ca. 1 to several cm long, robust to needle like; flowers close to the apex of the shoot and frequently appearing down the side of the shoot, 4–6 cm wide when open and ca. 5–7.5 cm long including pericarpel; pericarpel with narrow scales with sparse, very short to short dark brown hairs at their base; tepals white, sometimes pink, or often with prominent pink central stripe; style ca. 5 mm thick and 20 mm long; stigma with 15–20 lobes; fruits globose to pyriform, yellowish-green, ca. 5 cm diameter and 5–6 cm long, pulp white, sour tasting;

seed ca. 1.5 mm long, testa black, keeled, hilum small, oval, white.

Distribution. Chile. Regions III Atacama and IV Coquimbo, both at coastal and interior localities. Its northern range is limited by the Río Huasco. *Eulychnia acida* subsp. *acida*is widespread throughout its range. It typically inhabits the inland plains of the Coquimbo and Atacama regions, where it can form extensive communities. In some localities, where its distribution overlaps with that of *E. acida* subsp. *castanea*, it has been observed to hybridize with this latter taxon (Eggli & Leuenberger, 1998).

Discussion. The type subspecies if this taxon is closely related to *E. acida* subsp. *elata*, which occurs at inland locations north of the Río Huasco. The fruits of *E. acida* subsp. *acida* are locally known as "Copao", have a sour taste and are favored by the locals for the use in beverages and ice cream. Its omnipresence in Region IV Coquimbo is taken advantage of by the locals to construct effective fences around properties or pastures.

Specimens examined. **Chile**. **Region III Atacama**. Prov. Huasco, Quebrada Agua Grande, road Huasco to Caleta Tongoy, Nov 2007, *F.Kattermann 1242* (SGO 160944); Vallenar, 99 km S of Vallenar along the Panamericana (8 km S of Incahuasi), 25 Oct 1997, *Eggli & Leuenberger 3034* (ZSS 18451, SGO 145973). **Region IV Coquimbo**. Prov. Elqui, 17 km W of the Panamericana at Trapiche following the road to Los Choros, 19 Oct 1997, *Eggli & Leuenberger 2960* (ZSS 18422); 3 km SW of Los Choros, 19 Oct 1997, *Eggli & Leuenberger 2968* (ZSS 18440); 0.5–1 km N of Punta Teatinos, 2 Dec 1991, *Eggli & Leuenberger 1846* (ZSS 12916, SGO 145688); quebrada Los Choros along road to Punta Choros, 17 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-6* (EIF, BONN, K); La Higuera, 4 km NW of Punta Alta (El Trapiche) at the Panamericana towards Choros Bajos, Quebrada de los Choros, 19 Dec 1994, *Eggli & Leuenberger 2672* (ZSS 17459, SGO 144143); along road from Panamericana to Los Choros, 27 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3834* (ULS, BONN); along road from Panamericana to Los Choros, 27 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3834A* (ULS, BONN); 104 km S of Vallenar along Panamericana towards La Serena, 1 Dec 1991, *Eggli & Leuenberger 1840* (ZSS 12920, SGO 145683); La Higuera, 2 km SE of Chungungo / Cruz Grande towards the Panamericana, 25 Oct 1997, *Eggli & Leuenberger 3039* (ZSS 18288); N of La Serena, Cuesta Buenos Aires along Panamericana, 17 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-5* (EIF, BONN, K); N of La Serena, Punta Teatinos, 9 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-86* (ULS, BONN); La Serena, Quebrada de Santa Gracia, 19 km N of El Islón towards Almirante Latorre, 30 Oct 1997, *Eggli & Leuenberger 3081* (ZSS 18496, SGO 144234); road from Marquesa to Viñita Baja, 26 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3826* (ULS, BONN); Vicuña, 18 km W of Guanta on road CH41 towards Vicuña, 27 Oct 1997, *Eggli & Leuenberger 3053* (ZSS 18374, SGO 144112); Coquimbo, Sep 1885, *Philippi s.n.* (SGO 052702, as type of *E. breviflora* but see comment by Leuenberger & Eggli (2000); 2 km W of El Molle, Feb 1996, *F.Kattermann 1013* (SGO, photo); D-485 towards Paiguano, 8 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-84* (ULS, BONN); 37 km E of La Serena, s.a., *F.Ritter 232* (SGO 125184, loc.2); 2 km S of main road La Serena - Vicuña towards Observatory Tololo at entrance gate, 26 Oct 1997, *Eggli & Leuenberger 3041* (ZSS 18373, SGO 144111); La Serena, 1 km E of El Peñon, 4 Dec 1994, *Eggli & Leuenberger 2577* (ZSS 17511, SGO 144131); N of La Serena, Cuesta Las Cardas, 7 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-79A* (ULS, BONN); Coquimbo, 22 km W of Tongoy towards Puerto Aldea, 31 Oct 1997, *Eggli & Leuenberger 3085* (ZSS 18466, SGO 144249); Puerto Aldea, W of Tongoy, 16 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-2* (EIF, BONN, K). Prov. Limarí, Ovalle, Pichasca, 2 May 1980, *A. Troncoso A. s.n.* (SGO 128139); Ovalle, 13 km from asphalt road Ovalle-La Serena, 3 Dec 1994, *Eggli & Leuenberger 2566* (ZSS 17442, SGO 144123); 5 km W of the Panamericana following the road to the Fray Jorge NP, 3 Dec 1991, *Eggli & Leuenberger 1852* (ZSS 12915); road from Los Loros to Caleta El Toro, 23 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3782* (ULS, BONN); between Monte Patria and El Palqui above a gravel producing plant along D-55, 14 Sep 2018, *F.F.Merklinger & A.Kozok 2018-97* (ULS, BONN); 10 km N of Cogotí beyond La Isla towards La Ligua, ca. 1 km S of La Ligua, 2 Dec 1994, *Eggli & Leuenberger 2564* (ZSS 17443, SGO 145768). Prov. Choapa, S of Combarbalá near Reserva Nacional Las Chinchillas, 13 Sep 2018, *F.F.Merklinger & A.Kozok 2018-93* (ULS, BONN); road from Illapel to Combarbalá, near Illapel, 22 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3767* (ULS, BONN); forest reserve El Tambo near the town of Salamanca, 12 Sep 2018, *F.F.Merklinger & A.Kozok 2018-90* (ULS, BONN); Los Vilos, between Tilama and Caimanes, 13 km N of Tilama, 30 Nov 1994, *Eggli & Leuenberger 2550* (ZSS 17514, SGO 145789).

2. ***Eulychnia acida*** Phil. subsp. ***acidita*** Merkl., **subsp. nov.**

Type: Chile. Region IV Coquimbo. Prov. Elqui, N of La Serena along Panamericana, Fundo Juan Soldado, 25 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3814* (Holotype ULS!, isotype BONN!).

Diagnosis. *Eulychnia acida* subsp. *acidita* is similar to *E. acida* subsp. *acida*, from which it differs mainly in size, reaching 0.6–1 m in height only (vs. up to 4 m in height). It has more slender stems (5–8 cm vs. 9–12 cm diameter), smaller flowers of only 3–3.5 cm in length (vs. up to 7.5 cm in *E. acida*) and 2–2.5 cm in diameter (vs. 4–6 cm in *E. acida*).

Description. Shrubby, columnar cacti, 0.6–1 m tall; branching low, erect; branches

5–8 cm diameter; ribs 9–13; areoles 5–10 mm diameter and at 4–6 mm distance to each other, with very short, light-grey felt; spines emerging grey to light brown with light green base, soon turning silvery-grey, centrals well differentiated from radials; central spines 2–3, robust to needle-like, 5–10 cm long, the upper ones slightly porrect, the lower ones slightly deflexed; radial spines 2–5, 0.5–3 cm long, robust; flowers close to the apex of the shoot to about 15 cm downward along the shoot, 2–2.5 cm wide when open and ca. 3–3.5 cm long including pericarpel; pericarpel with narrow scales and very short, sparse, silvery-grey hairs; tepals white with pink central stripe; style thick and ca. 15 mm long; stigma with 15–20 lobes; fruits not seen; seed not seen.

Distribution. Chile. Region IV (Coquimbo). Found on sand dunes north of La Serena.

Discussion. Our attention was drawn to this plant by Nigel Taylor (see also the report in Hunt, 2012), who had found it at this locality and was unable to put a name to it, due to its morphological differences from other taxa in this area. After careful examination we decided to describe it as a new subspecies of *E. acida.* As Taylor points out in Hunt (2012), the population consists of very few plants only, which grow on the sea-facing slopes of a sand dune system on private land. It may thus be an ecological variant of *E. acida* adapted to poorer soil conditions of the sand dunes. Subsequent genetic analysis based on genotyping by sequencing (Merklinger *et al.*, in prep.) retrieved this taxon as sister to a subclade containing *E. acida* and *E. castanea.*

Etymology. The subspecific epithet refers to its close morphological resemblance to *E. acida*, of which it is a miniature version.

Specimens examined. **Chile. Region IV Coquimbo**. Prov. Elqui, N of La Serena along Panamericana, Fundo Juan Soldado, 25 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3814* (ULS, BONN); N of La Serena, Fundo Juan Soldado along Panamericana, 17 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-4* (EIF, BONN, K).

3. *Eulychnia acida* Phil. subsp. ***castanea*** (Phil.) Merkl. **comb. et stat. nov.**

Basionym: *Eulychnia castanea* Phil., Linnaea 33: 80–81, 1864.

≡ *Cereus castaneus* (Phil.) K.Schum. Gesamtb. Kakt. Nachtr.: 22–23, 1903.

≡ *Philippicereus castaneus* (Phil.) Backeb., Jahrb. Deutsch. Kakteen-Ges. 1941, pt. 2: 29, 1942.

Type (lectotype, designated by Leuenberger & Eggli (2000)): Chile. Region IV Coquimbo. Prov. Valparaíso, Los Molles, *Landbeck s.n.* (SGO 052701!); isolectotype (US [536085, photo!]).

Description. Shrubby, columnar cacti, 0.5–1 m tall; branching low, decumbent, the shoot apices curving upwards; branches 6–8 cm diameter; ribs 9–12; areoles ca. 5 mm diameter and at ca. 10 mm distance to each other, with short dark grey to dark brown felt; spines emerging with dark brown to black tips and yellowish base, centrals well differentiated from radials; central spines 1–2 to several shorter ones, 3–10 cm long; radial spines 6–10, 5–10 mm long, robust; flowers near the apex of the shoot and reaching to about 50 cm downward, broadly campanulate, 4–5 cm wide when open and ca. 5–5.5 cm long including pericarpel; pericarpel with narrow scales and covered in redish-brown to pale-brown bristly spines; tepals short, white, with rounded tips; style ca. 4 mm thick and 20 mm long; stigma with 15–20 lobes; fruits slightly pyriform, ca. 4 cm diameter and 5 cm long, covered in bristly spines as flowers, pulp white; seed ca. 1.5 mm long, testa black, not keeled, hilum small, oval, white.

Distribution. Chile. Regions IV (Coquimbo) and V (Valparaíso). *E. castanea* is the southernmost subspecies of *Eulychnia* and a strictly coastal taxon that occurs often in extensive colonies between Los Molles (~31.91°S) in the South and the Lengua de Vaca peninsula, W of Tongoy in the North (Ritter, 1980; Eggli & Leuenberger, 1998).

Discussion. *E. castanea* was described in 1864 by Philippi, based on a collection by Landbeck from near Los Molles, Petorca Province (formerly Aconcagua Province). It is one of the procumbent taxa and the most easily identified taxon among all *Eulychnia*, because of the spines produced from the areoles on the pericarpel. These bear strong similarity to those of the chestnut (*Castanea sativa*). Backeberg (1942) separated this species from *Eulychnia* and created the monotypic genus *Philippicereus* based on the spiny flowers and fruit, however, this genus was not accepted by subsequent authors. In some localities, introgression from *E. acida* has been observed by Eggli & Leuenberger (1998), where the distribution of the two taxa overlaps. At the southern end of the Fray Jorge National Park, near Caleta El Toro, for example, plants were found by the authors, which are not of the typical decumbent habit as normally observed in *E. castanea*, but are more erect and reaching an overall height of 1.5 m or more. Pericarpels from this locality sometimes bear the typical spines but these vary occasionally and become sparsely-hairy.

Specimens examined. **Chile**. **Region IV Coquimbo**. Prov. Elqui, Coquimbo, 22 km W of Tongoy towards Puerto Aldea, 31 Oct 1997, *Eggli & Leuenberger 3083* (ZSS 18342, SGO 144236); Puerto Aldea, W of Tongoy, 16 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-3* (EIF, BONN, K). Prov. Limarí, Parque Eolico de Arrayán, 10 Sep 2018, *F.F.Merklinger & A.Kozok 2018-88* (ULS, BONN); Ovalle, Parque Nacional Fray Jorge, 1 Nov 1997, *Eggli & Leuenberger 3086d* (ZSS 18340); Ovalle, Parque Nacional Fray Jorge, 18 Nov 1991, *Kraus s.n.* (ZSS 17978, photos); N of Caleta El Toro, 23 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3791* (ULS, BONN);

Ovalle, first hill N of the mouth of the Quebrada San Pedro, 18 Oct 1997, *Eggli & Leuenberger 2945* (ZSS 18447, SGO 144113). Prov. Choapa, 2 km N of Caleta Oscuro, 13 Sep 1995, *F.F.Merklinger & A.Kozok 2018-95* (ULS, BONN); Mincha, Caleta Oscuro, 3 km W of Puerto Oscuro, 2 Nov 1997, *Eggli & Leuenberger 3088* (ZSS 18358, SGO 144241); 7.3 km on Panamericana N of turnoff from Panamericana to Los Vilos, 10 Nov 1991, *Eggli & Leuenberger 1669* (ZSS 08010, SGO 144223); Los Vilos, May 1954, *F.Ritter 241* (SGO 125187); N of Totoralillo below Panamericana and Trattoria del Mar "L'Encuentro", 16 Sep 2019, *F.F.Merklinger,F.Luebert & J.Ruhm 2019-1* (EIF, BONN, K); between Los Vilos and Totoralillo along Panamericana, 11 Sep 2018, *F.F.Merklinger & A.Kozok 2018-89* (ULS, BONN); Los Vilos, Pichidangui, 17 Oct 1997, *Eggli & Leuenberger 2933* (ZSS 18483, SGO 144100); Pichidangui, s.a., *F.Ritter 241* (SGO 125186, loc.2); **Region V Valparaíso**. Prov. Petorca, La Ligua, 2 km S of turnoff of access road to Los Molles, just N of Puente El Chivato, 21 Dec 1994, *Eggli & Leuenberger 2678* (ZSS 17494, SGO 145769); 21 km N along Panamericana from turnoff to Papudo towards Los Vilos, 10 Nov 1991, *Eggli & Leuenberger 1656* (ZSS 07989, SGO 117503); Los Molles, 21 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3757* (ULS, BONN); La Ligua, Los Molles, extreme SW tip of peninsula at Virgin statuette, 17 Oct 1997, *Eggli & Leuenberger 2932a* (ZSS 18250, photos); La Ballena, 10 Oct 2019, *F.Luebert 4116* (EIF, BONN, K).

4. *Eulychnia acida* Phil. subsp. ***elata*** (F.Ritter) Merkl., **stat. nov.**
Basionym: *Eulychnia acida* var. *elata* F.Ritter, Kakteen Südamer. 3: 896, 1980.
Holotype: Chile. Region III Atacama. Prov. Copiapó, 1958, westlich Castillo [Hacienda Castilla]. Grenze der Dept Freirina und Copiapó, F.Ritter 651 (U [U 1601972!, coll. no. inferred]).

Description. Arborescent, columnar cacti, 4–6 m tall; branching low and high, erect; branches 8–10 cm diameter; ribs 9–13; areoles 7–12 mm diameter and at 5–10 mm distance to each other, with short grey felt; spines emerging with dark grey to black tips and reddish-brown base, centrals well differentiated from radials; central spines 1–2, one of which often strongly deflexed, robust, thicker than in *E. acida* subsp. *acida*, 10–20 cm long; radial spines up to 12, 1–several cm long, robust; flowers near the apex of the shoot, larger than in *E. acida* subsp. *acida*, 4–5 cm wide when open and ca. 6.5–8.5 cm long including pericarpel; pericarpel with narrow scales and with only sparse, short grey-brown hairs; tepals short, white to light pink or with pink central stripe; style ca. 20–25 mm long and 6 mm thick; stigma with 15–20 lobes; fruits globose to pyriform, ca. 5–6 cm diameter, with sparse, short grey-brown hairs, pulp white; seed ca. 2 mm long, testa dark-brown to black, keel less pronounced than in *E. acida* subsp.

acida, hilum small, white.

Distribution. Chile. Region III (Atacama). It forms large populations at interior localities on plains and hillsides between Vallenar and Copiapó.

Discussion. Ritter (1980) described this taxon as a variety of *E. acida* which he observed to be growing north of the Río Huasco, while the typical *E. acida* is distributed south of the Río Huasco. The plants differ principally in a more massive appearance; they produce a pronounced basal stem of sometimes exceeding 60 cm in diameter, become truly tree-like and produce flowers that are larger than those of any other *Eulychnia*, although they are morphologically similar to those of *E. acida* subsp. *acida*, the pericarpel being covered in very few short, silvery-grey hairs at most. Ritter (1980) stated as type locality ”westl. Castillo” [west of Castillo], referring to a locality west of Hacienda Castillo. As holotype, however, he indicated a specimen from Copiapó. Both these specimens are in Utrecht (U) and following his protologue, we treat his specimen from Hacienda Castilla as holotype, those from the other localities becoming paratypes.

Specimens examined. **Chile. Region III Atacama**. Prov. Copiapó, a 400 m cruce Paipote-Diego de Almagro, 25 Oct 1984, *M.Muñoz 1988* (SGO 108213); Berg nordöstlich Copiapó [mountain northeast of Copiapó], s.a., *F.Ritter 651* (SGO [SGO 125196, loc. 5, paratype of *E. acida* var. *elata*]); Copiapó, 1963, *F.Ritter 651* (U[U 160674B, barcode: 0007883, paratype (indicated as holotype by Ritter, see discussion]); along Panamericana S of Copiapó, 3 Oct 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-26* (EIF, BONN, K); 11 km S of Copiapó following the Panamericana towards Santiago, 29 Nov 1991, *Eggli & Leuenberger 1812* (ZSS 12908, as *E. acida*, SGO 145705); road from Panamericana to Nantoco, ca. 5 km past Panamericana, 29 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3881* (ULS, BONN); N of Caleta Pajonales along road, 3 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-71* (ULS, BONN); Estancia Castilla, road from Totoral to Panamericana, 28 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3866* (ULS, BONN); zwischen Castillo und Totoral [between Castillo and Totoral], s.a., *F.Ritter 651* (SGO [SGO 125197, loc. 6, paratype of *E. acida* var. *elata*]). Prov. Huasco, Chorillos nach El Chapote [Chorillos toward El Chapote], s.a., *F.Ritter 651* (SGO [SGO 125195, loc. 4, paratype of *E. acida* var. elata]); Carrizal, Dec 1957, *F.Ritter 651* (ZSS [SR13521, loc. 7, paratype of *E. acida* var. *elata*, sem.]); road from Carrizal Bajo to Canto de Agua, 4 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-74* (ULS, BONN); road from Carrizal Bajo to Canto de Agua a few km before the Panamericana, 18 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-11* (EIF, BONN, K); Huasco, 16 km SE of Canto del Agua towards Panamericana, 22 Oct 1997, *Eggli & Leuenberger 3015a* (ZSS 18326).

5. *Eulychnia acida* Phil. subsp. ***procumbens*** (F.Ritter) Merkl., **comb. et stat. nov.**

Basionym: *Eulychnia acida* Phil. var. *procumbens* F.Ritter, Kakteen Südamer. 3: 895–896, figs. 746–747, 1980.
≡ *Eulychnia chorosensis* P.Klaassen, Cact. Succ. J. (Los Angeles) 83 (4): 172, 2011.
Holotype: Chile. Region III Atacama. Prov. Huasco, Municipality of Freirina, 1963, F. Ritter 650 U ([U 160433B!, barcode: 0007884]); isotype: U [U 1601973, coll. no. inferred].

incl. *Eulychnia vallenarensis* P.C.Guerrero & H.Walter, Phytotaxa 392(1): 89, 2019.
Holotype: Chile. Region III Atacama. Prov. Huasco, 20 km south of Vallenar, Panamericana Road km 645, 28.71338°S, 70.76097°W, 741 m.a.s.l., *Guerrero 1258*, CONC [CONC 185660, photo!].

Description. Shrubby, columnar cacti, 0.5–1.5 m tall; branching low, decumbent; branches 5–8 cm diameter; ribs 8–12; areoles ca. 10 mm diameter and at 7–10 mm distance to each other, with short grey-brown to dark grey felt; spines emerging with dark grey to black tips and reddish-brown base, centrals well differentiated from radials; central spines 1–3, 4–15 cm long and porrect (upper) to slightly deflexed (lower); radial spines 3–15, to 0.5 cm long, robust; flowers not confined to the apical region and appearing downward to about 1 m along the shoot, broadly campanulate, 4–6 cm wide when open and ca. 6–6.5 cm long including pericarpel; pericarpel with narrow scales and covered in short, grey-brown woolly hairs; tepals white, often with pink central stripe; style ca. 6 mm thick and 20 mm long; stigma with 15–20 lobes; fruits globose, brownish-green, ca. 5 cm diameter, with dark brown wool, pulp translucent-white; seed ca. 2 mm long, testa dark-brown to black, keeled, hilum small, oval, white.
Distribution. Chile. Regions III (Atacama) and IV (Coquimbo). This species is found on a relatively short stretch of approximately 90 km between the northern Coquimbo region and the southern Atacama regions. Its narrow distribution centers around the Llano de Choros coastal plain inland and is bordered by the Huasco river valley at its northern limit (Hoxey & Klaassen, 2011).
Discussion. Morphologically, the plants fit into a wider concept of *Eulychnia acida*, apart from their procumbent habit and their woollier pericarpel. Hoxey & Klaassen (2011) raised this taxon to species level, however, changing the epithet to *chorosensis* in order to avoid confusion with Backeberg's *Eulychnia procumbens*, (a presumed *E. breviflora*). Hoxey & Klaassen (2011) state that this taxon separates the range of *E. breviflora* into a southern and a northern range and replacing it along this coastal stretch. Although presumed hybrids between *E. acida* subsp. *procumbens* and *E.*

breviflora have been found by the authors north of the Huasco river valley (F. Luebert 3852A, BONN, ULS), we agree with Hoxey & Klaassen (2011), that the Huasco river may be seen as a barrier between the two taxa.

Recently, a new name, *E. vallenarensis*, was created by Guerrero & Walter (2019) following a phylogenetic study of *Eulychnia* (Larridon *et al.*, 2018) in which a sample of this taxon clustered in an unexpected position in the cladogram. These authors argue, that rather than clustering with *E. acida*, this specimen clustered with *E. acida* subsp. *procumbens* and *E. castanea.* Their diagnosis also compares their proposed name *E. vallenarensis* to *E. acida* subsp. *acida* rather than to *E. acida* subsp. *procumbens.* In addition, they produced a key to the species of *Eulychnia* in Larridon *et al.* (2018), where they used thicker branch diameter, areolar felt and fruit size as distinguishing characters. Their type material, Guerrero 1258 (CONC) does not include flowers or fruits. The fact that Ritter had collected five specimens (*FR650, type and paratypes*) for his *E. acida* var. *procumbens*, including a collection from near Vallenar (Eggli *et al.*, 1995), was not discussed. The authors of this present paper have visited the type locality of *E. vallenarensis* (as well as that of *E. acida* subsp. *procumbens*) and conclude that *E. vallenarensis* fits undoubtedly into the concept of *E. acida* var. *procumbens.* We thus treat *E. vallenarensis* as a synonym of *E. acida* subsp. *procumbens.*
Specimens examined. **Chile**. **Region III Atacama**. Prov. Huasco, hills above Freirina, 27 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3848* (ULS, BONN); above Freirina, s.a., 900 m, *F.Ritter 650* (SGO 125194 [loc. 4, paratype]); hills above Freirina, 4 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-72* (ULS, BONN); north of Nicolasa, s.a., *F.Ritter 650* (ZSS SR13520 [loc. 5, paratype of *E. acida* var. *procumbens*, sem., paratype]); Freirina, ca. 17 km W of Vallenar towards Huasco, 22 Oct 1997, *Eggli & Leuenberger 2994* (ZSS 18299, SGO 146016); 20 km W of Vallenar, s.a., *F.Ritter 650* (SGO 125193 [loc. 3, paratype]); Freirina, 24 km S of Freirina, on gravel road towards Torres de Labrar, Quebrada Cuesta La Arena, 23 Oct 1997, *Eggli & Leuenberger 3016* (ZSS 18333, SGO 146145); 20 km S of Vallenar, Panamericana road km 645, 9 Aug 2018, *P.Guerrero 1258* (CONC); 20 km S of Vallenar, Panamericana road km 645, 10 May 2019, *F.Luebert & A.Sandoval 4041* (BONN, EIF); along road Domeyko-Freirina, 17 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-8* (EIF, BONN, K); 34 km N of Domeyko following the Panamericana towards Vallenar, 20 Oct 1997, *Eggli & Leuenberger 2970* (ZSS 18450, SGO 146029); Alto del Carmen, 8 km E of Alto del Carmen, 24 Oct 1997, *Eggli & Leuenberger 3032* (ZSS 18293, SGO 145964); Vallenar, 22 km E of Vallenar towards Alto del Carmen, 24 Oct 1997, *Eggli & Leuenberger 3033* (ZSS 18324, SGO 145972); Alto del Carmen, 17 km from Alto del Carmen towards San Felix, 21 Oct 1997, *Eggli & Leuenberger 2993* (ZSS 18442, SGO 146014); 25 km después de Chañar de Aceituna, 24 Sep 1977, *M.Muñoz S. 1160* (SGO

108702); Freirina, 45 km W of Domeyko, 6 Dec 1994, *Eggli & Leuenberger 2583* (ZSS 17452 [as *E. acida*, SGO 145796); below Domeyko, s.a., *F.Ritter 232* (SGO 125185, loc.3 as *E. acida*); along road to Carrizalillo on sandy plains of the Llano de Choros, 17 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-7* (EIF, BONN, K); E of Punta Choros, 27 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3841* (ULS, BONN); road Trapiche to Choros, Nov 2007, *F.Kattermann 1232* (SGO 160941).

Eulychnia breviflora Phil., Fl. Atac. 24, 1860.
≡ *Cereus breviflorus* (Phil.) K.Schum., Gesamtb. Kakt. Nachtr.: 22–23, 1903.
Type (lectotype, designated by Leuenberger & Eggli (2000)): Chile. Region IV Coquimbo. *Philippi, s.n.* Plate 2, fig. A 1–4, in Fl. Atac. of Phil. 1860.

≡ *Eulychnia procumbens* Backeb. (nom. inval., Art. 40.1 & 40.2, no type provided), Descr. Cact. Nov. 3: 6, 1963.

Description. Shrubby to arborescent or clustering, columnar cacti, 1–3–(5) m tall; branching low, erect, the lower branches occasionally somewhat decumbent; branches 4–10 cm diameter; ribs 9–14; areoles 3–14 mm diameter and at 3–15 mm distance to each other, with short grey-brown to dark grey felt or long, dense light-grey to dark grey felt; spines emerging black or dark brown with a black, light brown, reddish-brown or yellowish base, sometimes with a yellowish banded pattern, centrals well to sometimes not differentiated from radials, often a clear difference between juvenile and flowering shoots; central spines 1–5 of varying length, dark brown, soon turning grey, needle-like, sometimes hair-like, 2.5–15 cm long and erect; radial spines 2–22, a few mm to ca. 6 cm long, robust or sometimes bristl-like or hair-like; flowers confined to the apex of the shoot or very close to it, broadly campanulate, 3.5–5.5 cm wide when open and 4.5–7.5 cm long including pericarpel; pericarpel with narrow scales and densely covered in long, light grey, golden-brown or pale-golden, soft -to coarse woolly hairs; tepals short, narrow with acute apex to broad with rounded apex, white to light pink; style 3–6 mm thick and 5–25 mm long; stigma with up to 35 lobes; fruits globular, globose to pyriform, to 6.5 cm diameter and 8 cm in length, covered in long or short woolly hairs as the flowers, pulp translucent or white to light orange, tasteless to acidic; seed 1.5–2 mm long, testa dark-brown to greyish-black or black, sightly keeled to keeled, hilum white.

Distribution. Chile. Regions III (Atacama) and IV (Coquimbo). Plants usually occupy a coastal habitat, but one subspecies, *E. breviflora* subsp. *atacamensis* is found ca. 40 km inland near the town of Copiapó. *E. breviflora* in the wide sense occupies almost the whole of the northern Chilean coast from south of Coquimbo to Arica, although the northernmost populations have probably disappeared (Pinto, 2007).

Discussion. *Eulychnia breviflora* Phil. is here newly circumscribed as containing six allopatric subspecies.

E. breviflora was described by Philippi in 1860 as the only species of the genus *Eulychnia.* Schumann (1899) illustrated a flower of an *Eulychnia* with a woolly hypanthium but called the plant "*Cereus coquimbanus*", an interpretation he later realized and amended (Schumann, 1903). However, as he maintained a concept of *Cereus* including *Eulychnia*, he treated *E. breviflora* as *Cereus breviflorus.*

6. ***Eulychnia breviflora*** Phil., subsp. ***breviflora***

Type: As for *E. breviflora.*

Description. Shrubby to arborescent, columnar cacti, 1–3–(5) m tall; branching low, erect, the lower branches occasionally spreading-ascending; branches 6–10 cm diameter; ribs 10–13; areoles 7–10 mm diameter and at 10–15 mm distance to each other, with short grey-brown to dark grey felt; spines emerging dark brown with yellowish base, centrals well differentiated from radials, particularly on younger shoots; central spines 2–5 of varying length, dark brown, soon turning grey, needle-like, sometimes hair-like, 5–15 cm long and erect; radial spines 10–22, a few mm to ca. 3 cm long, robust or sometimes bristle-like or hair-like; flowers confined to the apex of the shoot, broadly campanulate, 4–5 cm wide when open and ca. 6 cm long including pericarpel; pericarpel with narrow scales and densely covered in long, golden-brown woolly hairs; tepals short, white to light pink; style ca. 6 mm thick and 25 mm long; stigma with ca. 25 lobes; fruits globose to pyriform, ca. 6 cm diameter, covered in woolly similar to the flowers, pulp white; seed ca. 2 mm long, testa dark-brown to black, slightly keeled, hilum narrow, white.

Distribution. Chile. Regions III (Atacama) and IV (Coquimbo). At the type locality of *E. breviflora* in Coquimbo, plants occupy a coastal habitat, growing directly on coastal rocks. This taxon occurs to the south of Coquimbo, roughly to Tongoy, inhabiting a coastal habitat together with *Puya chilensis* and *Eriosyce subgibbosa.* To the north of Coquimbo, the range of *E. breviflora* extends beyond the Llanos de Chaille National Park, Huasco Province, and just into Copiapó Province to the southern edge of the Copiapó river. Hoxey & Klaassen (2011) state that the distribution of *E. breviflora* subsp. *breviflora* is broken up into a southern and northern range, separated by a stretch of approximately 90 km. The southern end of the northern range is marked by the Huasco river.

Discussion. At the type locality, the plants are usually of a shrubby habit, attaining an overall height of 1–1.5 meters only, branching often profusely close to the ground from a rather short stem. South of Coquimbo the plants become more tree-like, reaching a height of up to 4 m. On the northern edge of the Copiapó river, subsp. *breviflora* is replaced by *E. breviflora* subsp. *tenuis*, which is found from here northward to Chañaral. In this area, however, the two taxa *E. breviflora* subsp. *breviflora* and *E. breviflora* subsp. *tenuis* grow sympatrically and intermediate individuals are common (Ritter, 1980).

Some kilometers inland, along the road from Carrizal Bajo to Canto de Agua, Huasco Province, plants were found with the arborescent habit similar to *E. acida* subsp. *elata*, reaching some 5 m in height and with a massive stem, some 30–40 cm in diameter. From the distance, these plants could not be differentiated from *E. acida* subsp. *elata*, yet when inspecting the plants closely, the woolly pericarpels as well as the softer and denser spination revealed their identity as part of the variable *E. breviflora* subsp. *breviflora*.

Specimens examined. **Chile. Region III Atacama**. Prov. Copiapó, S edge of Río Copiapó, 18 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-12* (EIF, BONN, K). Prov. Huasco, below Totoral, s.a., *F.Ritter 214a* (SGO 125180, loc.1; 125181, loc.2); Carrizal Bajo, s.a., *W.Maechler s.n.* (ZSS [T08450]); shortly after turnoff from Carrizal Bajo to Canto de Agua, 18 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-10* (EIF, BONN, K); road from Carrizal Bajo to Canto de Agua, 4 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-73* (ULS, BONN); Huasco, 8 km E of Carrizal Bajo towards Panamericana, 8 Dec 1994, *Eggli & Leuenberger 2600* (ZSS17469); road from Carrizal Bajo to Canto de Agua, 4 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-75* (ULS, BONN); 11 km N of Huasco Bajo towards Carrizal Bajo, 22 Oct 1997, *Eggli & Leuenberger 3000* (ZSS 18297, SGO 145949); N of Huasco Bajo above Tres Playitas, 18 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-9* (EIF, BONN, K); N of Huasco Bajo above Tres Playitas, 27 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3852* (ULS, BONN); 6 km from Huasco Bajo on the coastal road towards Carrizal Bajo, 30 Nov 1991, *Eggli & Leuenberger 1833* (ZSS 12902, SGO 145700); Tres Playitas, 3 miles N of Huasco, Feb. 1996, *F.Kattermann 1026* (SGO, photo); 2 km al poniente, fundo El Pino, hacia Huasco Bajo, 22 Oct 1984, *M.Muñoz S. 1934* (SGO 108214); **Region IV Coquimbo**. Prov. Elqui, 10 km W of the Panamericana on gravel road to El Tofo and Chungungo, 25 Oct 1997, *Eggli & Leuenberger 3036* (ZSS 18289, SGO 144116); N of Cuesta Buenos Aires, past old mine El Tofo, near Chungungo, 4 Oct 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-27* (EIF, BONN, K); La Higuera, 14 km SW of Chungungo – Cruz Grande towards the Panamericana, 25 Oct 1997, *Eggli & Leuenberger 3040a* (ZSS 18454); Camino del Romeral a 6 km de Punta de Teatinos, 15 Sep 1957, *C.Muñoz P. 4304* (SGO 118499); N of La Serena, Cuesta Los Porotitos, 9 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-85* (ULS, BONN); 0.5–1 km N of Punta Teatinos, 2 Dec 1991, *Eggli & Leuenberger 1844* (ZSS 12918); Coquimbo, 24 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3808* (ULS, BONN);

Coquimbo, 1851, *Philippi s.n.* (HAL 076126, possibly part of the type); Coquimbo, s.a., unknown s.n. (SGO 052683, halved flower of *E. breviflora*, possibly the other half of the flower of the isotype at K); Coquimbo, 1861, *Philippi s.n.* (K 000251165, halved flower of *E. breviflora*); Coquimbo, 1869, *Philippi s.n.* (MO 1760029, flower fragments of *E. breviflora*, probably part of isotype); 18 km N of northern access road to Guanaqueros/Tongoy, 31 Oct 1997, *Eggli & Leuenberger 3082a* (ZSS 18341, SGO 144232); S of La Serena, Playa Chica de Totoralillo, 10 Sep 2018, *F.F.Merklinger & A.Kozok 2018-87* (ULS, BONN); Coquimbo, 15 km S of Coquimbo along Panamericana just S of Totoralillo, 20 Dec 1994, *Eggli & Leuenberger 2676* (ZSS 17497, SGO 144139); La Serena, 1 km E of El Peñon, 4 Dec 1994, *Eggli & Leuenberger 2575* (ZSS 17509, SGO 144138).
Uncertain specimen. **Region III Atacama**. Prov. Huasco, N of Huasco Bajo above Tres Playitas, 27 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3852A* (ULS, BONN, possibly a hybrid with *E. acida* subsp. *procumbens*).

7. *Eulychnia breviflora* Phil. subsp. ***atacamensis*** Merkl., subsp. nov.
Type: Chile. Region III Atacama. Prov. Copiapó, Piedra Colgada, besides Panamericana on rocky slopes, ca. 300 m elev., 30 Sep 2017, *F.Luebert, T.Böhnert & F.F. Merklinger 3884* (holotype: ULS!; isotype: BONN).

Diagnosis. This subspecies differs morphologically from *E. breviflora* var. *tenuis* by an overall more massive habit with branches emerging from a short, stout trunk, and not the typical clustering habit with many slender stems growing narrowly parallel. Generally, its branch diameter is larger, central spines thicker and more numerous, and its pericarpel wool resembles that of *E. iquiquensis* in being shorter, softer, and less "messy" than in *E. breviflora.*

Description. Arborescent, columnar cacti, 1.5–3 m tall; branching low, erect; branches 5–10 cm diameter; ribs 9–11; areoles 5–10 mm diameter and at 5–10 mm distance to each other, with long, dense grey to dark grey felt; spines emerging black, light brown at the base, soon turning dark grey, central spines not clearly differentiated from radial spines; central spines 3–5, thin, needle-like, 2.5–12 cm long, the upper ones porrect, the lower ones deflexed; radial spines 5–many, ca. 1–several cm long, robust or bristle-like to hair-like; flowers at apex or very close to it, broadly campanulate, 4.6–5.5 cm wide when open and 4.5–6.5 cm long including pericarpel; pericarpel with narrow scales and densely covered in long, golden-brown woolly hairs; tepals short, broad, with slightly pointed to rounded apex, white; style ca. 3–5 mm thick and 5–15 mm long; stigma with 15–25 lobes; fruits not seen; seed not seen.

Distribution. Chile. Region III Atacama. The locality at Piedra Colgada is geographically separated from *E. breviflora* var. *tenuis* near Caldera by about 40 km and this subspecies occupies a distinct geographical unit. *E. breviflora* subsp. *atacamensis* is locally restricted to the area around Piedra Colgada, NW of Copiapó where it is only known from a few hillsides.

Discussion. As for many taxa described by Ritter, material with the type number was collected from several different localities as long as Ritter was of the opinion that the material belonged to the same taxon (Eggli *et al.*, 1995). Ritter visited the locality at Piedra Colgada and collected this taxon as part of his *E. breviflora* var. *tenuis*. Morphologically it appears, however, to be more closely related to the typical *E. breviflora* subsp. *breviflora*, mainly based on its more massive habit and branching from a pronounced basal stem as opposed to the multiple, slender stems that form large clusters in *E. breviflora* subsp. *tenuis*. Biogeographic analyses have shown, that this taxon represents one of few inland colonizations in the genus, and the locality at Piedra Colgada, separated from the nearest coastal taxon by approximately 40 km, may thus be an isolated relict of a once more widespread *Eulychnia* (Merklinger *et al.*, in prep.). This is further supported by molecular phylogenetic studies that retrieved this taxon as sister to *E. breviflora* subsp. *breviflora*, and not to *E. breviflora* subsp. *tenuis*, this latter taxon being more closely related to *E. breviflora* subsp. *saint-pieana* (Merklinger *et al.*, in prep.).

Specimens examined. **Chile. Region III Atacama**. Prov. Copiapó, Piedra Colgada, 30 Sep 2017, *F.Luebert, T.Böhnert, F.F.Merklinger 3884* (ULS, BONN); Piedra Colgada, 2 Sep 2018, *F.F.Merklinger, A.Kozok, D.Quandt 2018-68* (ULS, BONN); Piedra Colgada, besides Panamericana on rocky slopes, 3 Oct 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-25* (EIF!, BONN!, K!); Piedra Colgada, s.a., *F.Ritter 215a* (SGO 125183, loc.2, as part of type of *E. breviflora* var. *tenuis*).

Etymology. This species has a very narrow distribution close to the Atacama-region's capital of Copiapó. It seems therefore fitting to name it after the region from where it has been described.

8. ***Eulychnia breviflora*** Phil. subsp. ***iquiquensis*** (K.Schum.) D.R.Hunt, Cactaceae Syst. Init. 14: 16 (2002).

Basionym: *Cereus iquiquensis* K.Schum., Monatsschr. Kakteenk. 14: 99, 1904.

≡ *Eulychnia iquiquensis* (K.Schum.) Britton & Rose, Cactaceae 2: 83, 1920.

Type (lectotype, designated by Leuenberger & Eggli (2000)): Chile. Region I Tarapacá. Prov. Iquique, *Reiche s.n.* (B, alc.).

incl. *Eulychnia aricensis* F.Ritter, Taxon 13 (3): 115, 1964.

Type: Chile. Region XVI Arica y Parinacota. Prov. Arica, steep coast S of Arica, Camaraca, 21 Jun 1954, *F.Ritter 197* ZSS [T4672!].

incl. *Eulychnia iquiquensis* var. *pullilana* F.Ritter, Kakteen Südamer. 3: 901, 1980.
Type: Chile. Region II Antofagasta. Prov. Antofagasta, El Cobre, Mar 1963, *F.Ritter 479b* U [U 0007882!]; isotypes: SGO [SGO 125192!]; ZSS [SR 13375].

incl. *Eulychnia morromorenoensis* (nom. inval. Art. 40.1) F.Ritter, Kakteen Südamer. 3: 901–902, 1980.
Type (two syntypes): Chile. Region II Antofagasta. Prov. Antofagasta, Morro Moreno bei Antofagasta, 1954, *F.Ritter 202* ZSS [S10090, T4711, T4712, T4713, T4715], Chañaral, ZSS [T4714]; *F.Ritter 479* ZSS [SR13373]; SGO[125189], ZSS[S12562, sem.].

Description. Arborescent, columnar cacti, 2–4 m tall; branching from the base to about 1.5 m, erect; branches 7–10 cm diameter; ribs 10–13; areoles 8 mm diameter and at 4–8 mm distance to each other, with short grey-brown to dark grey felt; spines emerging black with a redish-brown base, centrals not clearly differentiated from radials but 2–4 of greater length reaching 3–10 cm, golden-brown in color when fresh, soon turning dark brown to blackish or dark grey, erect, on older shoots becoming thinner and bristle-like; radial spines 10–20, to ca. 1 cm long, robust or sometimes bristle-like; flowers near the apex of the shoot, 5–6 cm wide when open and ca. 5.5–6.5 cm long including pericarpel; pericarpel with narrow scales and covered in dense pale-yellow to golden woolly hairs, shorter than in *E. breviflora* subsp. *breviflora*; tepals short, white; style ca. 20 mm long and 6 mm thick at base; stigma with 27–35 lobes; fruits globose, ca. 5–6.5 cm diameter, covered in woolly hairs similar to the flowers, pulp white to light pink, sour; seed ca. 1.7 mm long, testa black, keeled, hilum whitish.

Distribution. Chile. Regions I Tarapacá, II Antofagasta and XVI Arica y Parinacota.

Discussion. Schumann described this species in 1904 as *Cereus iquiquensis* based on collection made by Dr. Reiche near Iquique. While it shares the long woolly pericarpel with *E. breviflora* subsp. *breviflora*, its distinguishing character is, according to Schumann, the much shorter spines. Ritter applied this name to plants growing between Iquique and Antofagasta, and created a new species, *E. aricensis*, for plants growing between the Quebrada Camarones and Arica (Ritter, 1964). Later he separated *E. morromorenoensis* from the Cerro Moreno near Antofagasta, as well as *E. iquiquensis* var. *pullilana* from S of Antofagasta to El Cobre (Ritter, 1980). Subsequent authors such as Hunt (2006) or Larridon *et al.* (2018) synonymized *E. aricensis*, *E. morromorenoensis* and *E. iquiquensis* var. *pullilana* under *E. iquiquensis*, an approach we are following here. In the protologue of *E. morromorenoensis*, Ritter (1980) cites two different syntypes. According to Art. 40.1 this name is thus invalid (Eggli *et al.*, 1995).

The type locality of Ritter's *E. aricensis* on Cerro Camaraca near the town of Arica was the only type locality not visited by the authors. The site is today a military site requiring permits to enter. Pinto (2007) counted 244 plants at this site, of which only 17 were alive. A trend of population die-back has been noted along the coast of northern Chile, affecting *E. breviflora* subsp. *iquiquensis* in particular, because of increasing drought over the past few decades (Schulz *et al.*, 2011). It is thus possible, that the plants have by now become extinct. Our studies of *E. aricensis* are based on herbarium records and cultivated material.

Specimens examined. **Chile. Region XVI Arica y Parinacota**. Prov. Arica, steep coast south of Arica, Dec 1953, *F.Ritter 197* (ZSS [T4673, T4674, loc. 2, paratypes of *E. aricensis*]); steep coast south of Arica, Dec 1953, *F.Ritter 197* (ZSS [S10054, SR13208, loc. 4, paratypes of *E. aricensis*, [sem.]); steep coast south of Arica, 5 Mar 1957, *F.Ritter 197* (SGO [SGO 125177, in two parts, loc. 3, paratype of *E. aricensis*]); Alto Camaraca, 2 Jan 2001, *W.Krahn 945* (ZSS 19996, photos). **Region I Tarapacá**. Prov. Iquique, Iquique, Jan 1954, *F.Ritter 202* (SGO 125178); Alto Punta Gruesa, 14 Dec 1997, *R.Pinto s.n.* (SGO, photo); Alto Punta Gruesa, 16 Oct 2017, *F.Luebert, T.Böhnert & F.Merklinger 4016* (ULS, BONN); ca. 20 km S of Iquique on ruta 1 towards airport above Playa Lobito, 6 Mar 1997, *Eggli & Leuenberger 2835* (ZSS 18103, SGO 146063); Alto Chipana, 31 Jan 1998, *R.Pinto s.n.* (SGO, photo); Punta Chipana, N de la desembocadura del Río Loa, 6 Mar 1998, *M.A.Trivelli s.n.* (SGO 142913); Alto Chipana, 1 Oct 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-19* (EIF, BONN, K). **Region II Antofagasta**. Prov. Tocopilla, ca. 38 km S of Tocopilla on ruta 1 towards Antofagasta, Mina Rincón, 7 Mar 1997, *Eggli & Leuenberger 2843* (ZSS 18115, SGO 146052). Prov. Antofagasta, Cerro Moreno, Jan 1954, *F.Ritter 202a* ((ZSS [T04711, T04712, T04713, T04714, T04715]); Cerro Moreno, s.a., *F.Ritter 479* (ZSS 911411/0, ex cult.); Peninsula Moreno, SW flank of Morro Moreno above Caleta Errázuriz, 18 Feb 1997, *Eggli & Leuenberger 2694* (ZSS 18015, SGO 145911); Cerro Moreno, 8 Oct 2017, *F.Luebert, T.Böhnert & F.Merklinger 3983* (ULS, BONN); Quebrada La Chimba, 29 Feb 1996, *F.Kattermann 1076* (SGO 117477, photo); Quebrada La Chimba, 11 Nov 1996, *R.Rodríguez 3092* (SGO 152967); La Chimba, quebrada Guanaco, 27 Aug 2018, *F.F.Merklinger & A.Kozok 2018-56* (ULS, BONN); Quebrada Caracoles E of Antofagasta on ruta 26, 5 km E of town edge, 18 Feb 1997, *Eggli & Leuenberger 2701* (ZSS 18025, SGO 145914); hills S of Caleta Coloso, 28 Aug 2018, *F.F.Merklinger & A.Kozok 2018-57* (ULS, BONN); hills S of Caleta Coloso, 7 Oct 2017, *F.Luebert, T.Böhnert & F.Merklinger 3976* (ULS, BONN); Cerro Coloso, s.a., *F.Ritter 479b* (SGO [SGO 125234, loc. 2, paratype of *E. iquiquensis* var. *pullilana*]); hills above El Cobre, 6 Oct 2017, *F.Luebert, T.Böhnert & F.Merklinger 3967* (ULS, BONN); 50

km SW of the Panamericana towards El Cobre, 8 Mar 1997, *Eggli & Leuenberger 2850* (ZSS 18122, SGO 146059); hills above El Cobre, 29 Aug 2018, *F.F.Merklinger & A.Kozok 2018-59* (ULS, BONN).

9. *Eulychnia breviflora* Phil. subsp. ***saint-pieana*** (F.Ritter) Merkl., **comb. et stat. nov.**

Basionym: *Eulychnia saint-pieana* F.Ritter, Taxon 13 (3): 115, 1964.

Holotype: Chile. Region II Antofagasta. Prov. Chañaral, N of Chañaral, Dec 1957, *Ritter 479a*, U [not found]; isotypes: SGO [SGO 125191!, in 3 parts]; ZSS [S10282].

incl. *Eulychnia barquitensis* F.Ritter, Kakteen Südamer. 3: 899, 1980.

≡ *Eulychnia saint-pieana* var. *barquitensis* (F.Ritter) A.E.Hoffm., Cact. Fl. Silvestre Chile 142 (1989).

Holotype: Chile. Region III Atacama. Prov. Chañaral, Barquito, 1956, *F.Ritter 215*, U [U 0007881!].

Description. Arborescent, columnar cacti, 2–4 m tall; branching low, erect; branches 7–10 cm diameter; ribs 10–15; areoles 6–8 mm in diameter and at 3–8 mm distance to each other, with long and dense light grey felt; spines emerging dark brown to blackish, soon turning grey, centrals not clearly differentiated from radials; central spines several but 1–2 of 5–10 cm length, the others short and often hidden in the areolar felt; the stronger spines dark brown, soon turning yellowish-brown; radial spines 8–12, from 0.5 cm to ca. 2 cm in long, robust or sometimes bristle-like or becoming very thin and hair-like, often twisted; flowers near the apex of the shoot, ca. 5.5 cm wide when open and ca. 6–7.5 cm long including pericarpel; pericarpel with narrow scales and densely covered in long, golden-brown woolly hairs; tepals short, white to light pink; style ca. 3–5 mm thick and 15–20 mm long; stigma with 23–31 lobes; fruits slightly pyriform, to ca. 8 cm long, covered in woolly hairs as flowers, pulp light orange and tasteless; seed ca. 1.4 mm long, testa greyish-black, keeled, hilum oval, white.

Distribution. Chile. Region II Antofagasta. It occurs at coastal localities between the towns of Chañaral and Taltal.

Discussion. Ritter (1964) described *E. saint-pieana* as a new species based on several morphological differences to *E. breviflora* subsp. *breviflora* and *E. breviflora* subsp. *iquiquensis*, most notably the long and dense areolar felt which differentiates it from *E. breviflora* subsp. *breviflora* and its distribution between Chañaral and Taltal, which separates it geographically a considerable distance from *E. breviflora* subsp. *iquiquensis*. Subsequent authors included *E. saint-pieana* as a synonym of *E. iquiquensis* (Hunt, 2006, 2016; Hoxey & Klaassen, 2011) but in addition to its morphological differences compared to this subspecies, it also shows genetic affiliation with *E. breviflora* subsp. *tenuis* (Merklinger *et al.*, in prep.), and was retrieved sister to a plant from the same area that Ritter (1980) had named *E. barquitensis* and which we include here into the concept of *E. breviflora* subsp. *saint-pieana*. The geographic distribution of *E. breviflora* subsp. *saint-pieana* does not overlap with that of any other subspecies.

Ritter (1963) states that his holotype specimens have been deposited at Utrecht (U, now in Leiden) unless otherwise specified, which also holds true for his further diagnoses (Ritter, 1964). Neither Eggli *et al.* (1995), nor the present authors were able to locate the holotype of *E. saint-pieana* there. Therefore, a lectotypification of this name may be required after further investigations.

Specimens examined. **Chile. Region II Antofagasta**. Prov. Antofagasta, Playa Las Tórtolas along road, 23 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-16* (EIF, BONN, K); Caleta La Madera, 19 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-15* (EIF, BONN, K); ca. 3 km N of Planta Esmeralda, Sierra Esmeralda, 2 Feb 1952, *P.C.Hutchinson 421* (ZSS 00326); road to Caleta Esmeralda, 1 Oct 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3914* (ULS, BONN); Esmeralda, s.a., *F.Ritter 479a* (SGO [SGO 125190], loc. 2, paratype of *E. saint-pieana*); Quebrada Cachina, Nov 2007, *F.Kattermann 1274* (SGO 160953); Pan de Azúcar, 25 Feb 1996, *F.Kattermann 1054* (SGO, photo). **Region III Atacama**. Prov. Chañaral, Falda Verde, i.e. slopes of Cerro Chañaral, 4–5 km N of Chañaral, 5 Oct 1997, *Eggli 2887* (ZSS 18409, SGO 146111); Falda Verde, 1 Oct 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3910* (ULS, BONN); Chañaral, Jan 1956, *F.Ritter 479a* (ZSS [SR13374, loc. 4, paratype of *E. saint-pieana*, sem.]); Chañaral, s.a., *F.Ritter 215* (ZSS [10070, T5091, loc. 2, paratypes of *E. barquitensis*]); SW of Chañaral, s.a., *F.Ritter 215* (SGO [SGO 125182, loc. 3, paratype of *E. barquitensis*]); Chañaral, Jan 1954, *F.Ritter 215* (ZSS [SR13243, loc. 4, paratype of *E. barquitensis*]); quebrada above Barquito, 30 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3898* (ULS, BONN); peak adjacent to the W of Cerro Paso Malo ca. 5 km S of Chañaral and immediately S of El Barquito, 7 Oct 1997, *Eggli 2906* (ZSS 18408, SGO 146112); quebrada behind Playa el Caleuche, 19 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-14* (EIF, BONN, K).

10. *Eulychnia breviflora* Phil. subsp. ***taltalensis*** (F.Ritter) Merkl., **stat. nov.**

Basionym: *Eulychnia breviflora* var. *taltalensis* F.Ritter, Kakteen Südamer. 3: 898–899, 1980.

≡ *Eulychnia taltalensis* Hoxey, Cact. Succ. J. 83(4): 169, 2011.

Holotype: Chile. Region II Antofagasta. Prov. Antofagasta, the town of Taltal, *Ritter 214*, 1956, U [U 0007885!]; isotype: ZSS [S10069!, sem.].

Description. Arborescent, columnar cacti, 2–4 m tall; branching low, erect; branches 6–10 cm diameter; ribs 9–14; areoles 8–14 mm diameter and at 5–10 mm distance to each other, with short grey to dark brown felt; spines emerging dark grey with a black base and yellowish banding, centrals well differentiated from radials, particularly on younger shoots; central spines 3–5, robust on young shoots with a basal thickness of about 2 mm, becoming thinner to bristle-like on older shoots with a basal thickness of about 1 mm, 8–12 cm long and erect; radial spines 10–15, a few mm to ca. 6 cm long, robust or sometimes bristle-like or hair-like; flowers at the apex of the shoot, 3.5–4 cm wide when open and ca. 5–6.5 cm long including pericarpel; pericarpel with narrow scales and covered in light grey to golden-brown hairs; tepals short, broad and with mucronate apex, white; style 15–18 mm long, ca. 5 mm thick; stigma with 19–24 lobes; fruits globose, ca. 6 cm diameter, covered in hairs as flowers but somewhat sparser, pulp white, translucent when ripe, acidic; seed ca. 1.5 mm long, testa black, slightly keeled, hilum narrow, white.

Distribution. Chile. Region II Antofagasta. According to Hoxey & Klaassen (2011), this taxon occupies a mere 100 km stretch between the town of Taltal and Caleta Botija, just S of Antofagasta.

Discussion. Johnston (1929) included material of this taxon into *E. breviflora* subsp. *breviflora*, pointing out differences in flower and areoles compared to *E. iquiquensis*. Ritter (1980) formally described the taxon as *E. breviflora* var. *taltalensis*, considering it to be more closely related to *E. breviflora* rather than *E. iquiquensis*. Hoxey & Klaassen (2011) elevated this taxon to species rank, and postulate a closer relationship to *E. iquiquensis* based on the shared gross body morphology. As distinguishing character, they used the distinct spination between juvenile and mature flowering stems, which turns from strong and robust to soft and flexible, sometimes almost hair-like. According to Hoxey & Klaassen (2011), this character is only shared with *E. iquiquensis* and *E. ritteri*, but no other *Eulychnia* species (Hoxey & Klaassen, 2011). We could not confirm the diagnostic character based on our own observations in the field. A distinct difference between juvenile and mature spination has indeed been observed in nearly all *Eulychnia* species. Rather, this taxon is identified by its sparsely hairy pericarpels (as opposed to densely hairy in *E. iquiquensis* and *E. breviflora*) and the short and brown areolar felt and absence of wool from the areoles (as opposed to grey-white and long in *E. iquiquensis* and *E. saint-pieana*).

Specimens examined. **Chile. Region II Antofagasta**. Prov. Antofagasta, N of Paposo, Miguel Díaz, 5 Oct 2017, *F.Luebert, T.Böhnert, F.F.Merklinger 3954* (ULS, BONN); 32 km N of Paposo along road, 30 Aug 2018, *F.F.Merklinger & A.Kozok 2018-60* (ULS, BONN); 17 km N of Taltal towards Paposo along coastal road, 25 Nov 1991, *Eggli & Leuenberger 1755* (ZSS 07971, as *E. iquiquensis*, SGO 141282); hills above

Paposo near CONAF station, 4 Oct 2017, *F.Luebert, T.Böhnert, F.F.Merklinger 3933* (ULS, BONN); 20 km N of Taltal, 31 Aug 2018, *F.F.Merklinger & A.Kozok 2018-62* (ULS, BONN); 17 km N of Paposo along road, 30 Aug 2018, *F.F.Merklinger & A.Kozok 2018-61* (ULS, BONN); Taltal, quebrada San Ramón just N of Taltal, ca. 12 km inland from the mouth of the quebrada, 16 Dec 1994, *Eggli & Leuenberger 2661* (ZSS 17540); Taltal, Oct 1925, *Werdermann 869* (BM 001008542, stem parts only); Taltal, Jan 1954, *F.Ritter 214* (ZSS [SR13241, loc. 3, paratype of *E. breviflora var. taltalensis*, sem.]); SW of Taltal, s.a. *F.Ritter 214* (SGO [SGO 125179], loc. 2, paratype of *E. breviflora* var. *taltalensis*); Cerro Perales immediately E of Taltal, 10 Oct 1997, *Eggli 2915a* (ZSS 18809, photos); Cerro Perales, 3 Oct 2017, *F.Luebert, T.Böhnert, F.F.Merklinger 3921* (ULS, BONN); Taltal, quebrada Taltal, 13 km NNW of Panamericana coming from Chañaral towards Taltal, 13 Dec 1994, *Eggli & Leuenberger 2639a* (ZSS 17521).

11. ***Eulychnia breviflora*** Phil. subsp. ***tenuis*** (F.Ritter) Merkl., **stat. nov.**

Basionym: *Eulychnia breviflora* Phil. var. *tenuis* F.Ritter, Kakteen Südamer. 3: 898, 1980.

Holotype: Chile. Region IV Coquimbo. Prov. Copiapó, Caldera, *Ritter 215a*, Jan 1956, U [0007886!].

Description. Shrubby, columnar cacti, 1–2 m tall; branching profusely from the base, branches arching outwards, becoming erect and almost parallel to each other; branches 4–7 cm diameter; ribs 10–13; areoles 3–8 mm diameter and at 5–10 mm distance to each other, with long, dense grey felt; spines emerging black, light brown at the base, soon turning dark grey, erect, centrals well differentiated from radials; central spines 2–5, thin, needle-like, 2–10 cm long; radial spines 3–8 plus several hair-like ones, a few mm to ca. 3.5 cm long; flowers near the apex of the shoot to about 30 cm downward along the shoot, 3.5–6 cm wide when open and 4.5–6 cm long including pericarpel; pericarpel with narrow scales and densely covered in dense, golden-brown wool; tepals narrow with pointed apex, white; style ca. 3–5 mm thick and 7–20 mm long; stigma with 15–25 lobes; fruits with long, golden-yellow to grey wool (Ritter, 1980); seeds without observed difference to *E. breviflora* subsp. *breviflora* (Ritter, 1980), fruits and seeds not seen by the present authors.

Distribution. Chile. Region III Atacama. From the northern side of the Río Copiapó valley along the coast to Barquito.

Discussion. Ritter (1980) described this taxon as a variety of *E. breviflora* sensu stricto, separated from the latter by the Río Copiapó and reaching from here northward to El Barquito. Although he observed a hybrid zone with intermediate forms, it differs morphologically from the typical *E. breviflora* mainly by its habit. *E. breviflora* subsp. *tenuis* forms clusters of dense, erect, shoots which are narrower than in *E. breviflora* subsp. *breviflora* (4–7 vs. 6–10 cm). While Ritter (1980) had not seen the flowers of this taxon, we were able to make further observations of these, and noted differences in e.g. the tepal shape (narrow with acute apex vs. slightly more broad with a more rounded apex). Ritter (1980) had also included the plants from Piedra Colgada into his concept of *E. breviflora* var. *tenuis*, while we separate the Piedra Colgada plants as *E. breviflora* subsp. *atacamanensis* due to morphological features as well as distinct geographic occurrence (see discussion for the latter taxon).

Specimens examined. **Chile. Region III Atacama**. Prov. Chañaral, 12 km S of Chañaral along the Panamericana, 12 Dec 1994, *Eggli & Leuenberger 2630c* (ZSS 17477); 17 km S of Chañaral along Panamericana (near km 955), 27 Nov 1991, *Eggli & Leuenberger 1790* (ZSS 07977); 46 km S of Chañaral along Panamericana towards Caldera, 9 Mar 1997, *Eggli & Leuenberger 2868* (ZSS 18139, SGO 146062); Caldera, quebrada El León, 30 Sep 2017, *F.Luebert, T.Böhnert & F.F.Merklinger 3894* (ULS, BONN); coast near Caldera, 1957, *F.Ritter 215a* (ZSS [S1028!, SR13244], loc. 1, paratypes of *E. breviflora var. tenuis*, sem.); S of Caldera, El Morro, 2 Sep 2018, *F.F.Merklinger & A.Kozok 2018-69* (ULS, BONN); ca. 10 km S of Bahia Inglesa towards Bahia Cisne at foot of E slopes of Morro de Copiapó, 28 Nov 1991, *Eggli & Leuenberger 1795* (ZSS 07970, SGO 145695); S of Caldera on E facing slopes of El Morro, 19 Sep 2019, *F.F.Merklinger, F.Luebert & J.Ruhm 2019-13* (EIF, BONN, K).

Uncertain specimen. Region III Atacama. Prov. Copiapó, ca. 5 km N of Puerto Viejo, 3 Sep 2018, *F.F.Merklinger, A.Kozok & D.Quandt 2018-70* (ULS, BONN).

12. *Eulychnia ritteri* Cullm., Kakteen And. Sukk. 9 (8): 121, 1958.

≡ *Eulychnia iquiquensis* K.Schum. subsp. *ritteri* (Cullm.) D.R. Hunt, Cactaceae Syst. Init. 19: 13 (2005).

Type (lectotype, designated here): Peru. Dept. Arequipa. Prov. Caravelí, coastal region of Chala, 400 km N of Chilean border, *Ritter 276*, Jul 1954, ZSS [T4862]; isolectotype: ZSS [T4863!], [S10245, SR13958, sem.], SGO [SGO 125188!, in 2 parts].

Description. Arborescent, columnar cacti, 1.5–4 m tall; branching low, erect; branches 6–8 cm diameter; ribs 13–20; areoles 5–7 mm diameter and at 0.3–10 mm distance to each other, with very long, white-grey felt; spines emerging dark brown to black, soon turning grey, centrals well differentiated from radials; central spines 2–5 of varying length, emerging dark brown to black, soon turning grey, needle-like, sometimes hair-like, 3–8 cm long, the upper ones porrect, the lower ones deflexed; radial spines 8–12, 0.5–2 cm long, robust or sometimes bristle-like or hair-like; flowers near the apex of the shoot, sometimes downwards half the length of the shoot, globular to slightly oblong, campanulate, 1.5 cm wide when open and ca. 2–2.5 cm long including pericarpel; pericarpel with narrow scales and densely covered in golden-grey woolly hairs; tepals ca. 15 in number, short, narrow with rounded apex and held erect when open, white to light pink; style 3–5 mm thick and ca. 10 mm long; stigma with 10–15 lobes; fruits globose to pyriform, 2.5–3 cm diameter, covered in sparse, short, grey-brown hairs at maturity, pulp translucent, acid; seeds ca. 1.5 mm long, dark-brown, keeled, hilum oval, white.

Distribution. Peru. Dept. Arequipa. Prov. Caravelí, near the town of Chala, as well as on the lomas de Atiquipa near the village of Tanaca.

Discussion. This taxon is the only *Eulychnia* known to occur outside of Chile. It is separated by its nearest relatives, *E. breviflora* subsp. *iquiquensis* by at least 400–500 km (Cullmann, 1958; Ritter, 1981), and appears morphologically close to this latter taxon. Hunt (2006) placed this taxon as a subspecies of *E. iquiquensis* but it differs from these by the smaller flowers, which reach only to 2.5 cm in length (Cullmann, 1958). Based on our own observations, *E. ritteri* has areoles covered in dense long felt, much like *E. breviflora* subsp. *saint-pieana* and not like *E. breviflora* subsp. *iquiquensis* from the type locality near Iquique (although Ritter's *E. iquiquensis* var. *pullilana* and his *E. morromorenoensis*, both of which we include into *E. breviflora* subsp. *iquiquensis*, also share this character).

Specimens examined. **Peru. Dept. Arequipa**. Prov. Caravelí, Lomas de Atiquipa, behind the village of Tacana, 7 Oct 2018, *F.F. Merklinger, F.J. Merklinger & U. Merklinger 2018-151* (USM, BONN); N part of dept. Arequipa, steep slopes above (NE) of Chala, Jul 1976, *E.Markus s.n.* (ZSS 19657, photo).

5.4.2 Names excluded from *Eulychnia*

Eulychnia eburnea Phil. (nom. nud.) = *Trichocereus chiloensis* (Colla) Britton & Rose subsp. *eburneus* (Phil. ex K.Schum.) Albesiano (2012).

Eulychnia coquimbana (Molina) Albesiano: The name *Cactus coquimbanus* has been proposed to be rejected by Eggli & Walter (2013) and rejection was recommended (Applequist, 2013).

Eulychnia clavata Phil. ex K.Schum. = *Austrocactus spiniflorus* (Phil.) F.Ritter.

Eulychnia spinibarbis (Pfeiff.) Britton & Rose. The name *C. spinibarbis* has been rejected due to uncertainty in application of the name (Eggli & Walter, 2012; Wilson, 2016).

Eulychnia longispina (Salm-Dyck) F.Ritter.

Otto & Dietrich (1845) published a description of *Cereus longispinus.* Ritter (1965) originally used this name for *E. breviflora* Phil., but later (Ritter, 1980) changed his interpretation because he interpreted the protologue to not match the morphology of

Eulychnia breviflora. The application of the name *C. longispinus* remains unresolved. If it should turn out to represent a taxon of *Eulychnia*, it would have priority over any *Eulychnia* name at species rank, necessitating a proposal for rejection to conserve the existing familiar nomenclature.

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5.5 References

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Chapter 6

Notes on the genus *Ophryosporus* (Asteraceae: Eupatorieae) in Chile

This chapter has been accepted for publication in the journal *Phytokeys* and is currently in press.

Notes on the genus *Ophryosporus* (Asteraceae: Eupatorieae) in Chile

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Abstract

Ophryosporus Meyen is reviewed for Chile and an updated species list for the country based on herbarium records and literature review are presented. A key to the Chilean species is provided and a distribution range of taxa is indicated based on herbarium records and own collections. We include several lectotypifications as well as an epitypification of *Ophryosporus hoppii.* The presence of two species, *O. hoppii* and *O. floribundus*, formerly accepted for Chile is questioned and their actual distribution discussed.

Resumen

Se revisa el género *Ophryosporus* para Chile y se presenta una lista actualizada de especies para el país basada en registros de herbario y una revisión de la literatura. Se proporciona una clave para las especies chilenas y se indica el rango de distribución de los taxones basado en registros de herbario. Se cuestiona la presencia de dos especies, *O. hoppii* y *O. floribundus*, anteriormente aceptadas para Chile, y se discute su distribución real.

Keywords: Atacama Desert, Compositae, endemism, lomas vegetation, Peru, species distribution, taxonomy

6.1 Introduction

During field work and specimens determination as part of the collaborative research centre 1211 (http://crc1211.uni-koeln.de) – “Earth, Evolution at the Dry Limit”, we noted inconsistencies in the literature regarding the taxonomy and nomenclature of *Ophryosporus* Meyen. *Ophryosporus*, currently with 41 accepted species, is distributed in South America and is disjunct between the Andes from Colombia to Chile and the Atlantic Forest in southern Brazil and northern Argentina (King & Robinson, 1972a). The plants are (sometimes scandent) shrubs, with usually opposite secondary branching and opposite or alternate leaves, often in fascicles. The inflorescences are corymbose or thyrsoid (Hind, D.J. & Robinson, [2006] 2007). The genus is further characterized by reduced anther appendages, clavate style branches, a pronounced carpopodium and distinct asymmetrical cypsela base (King & Robinson, 1972b). In Chile, eight shrubby species are currently recognized (Rodriguez *et al.*, 2018; Zuloaga *et al.*, 2008) and they are distributed along the coast of northern Chile as well as along the Andean Cordillera, separated by the hyper arid absolute desert (Fig. 1).

An important revision of the Eupatorieae, including the genus *Ophryosporus* was published by Robinson (1906). This work maintained two sections in the genus, section *Euophryosporus* including *O. triangularis* and *O. paradoxus* (Hook. & Arn.) B.D.Jacks., also a Chilean species with short internodes and thyrsoid panicles, and section *Ophryochaeta* B.L. Rob., including 15 species with opposite leaves, well developed internodes and capitula largely in panicles or axillary cymes. Subsequent works included some new combinations and descriptions of new species, enlarging the genus to 29 species (King & Robinson, 1972b), and further additions from the genus *Piqueria* Cav. brought the total to 38 species (King & Robinson, 1972a). Apart from an unpublished thesis by Plos (2012), and several lectotypifications by Plos & Sancho (2013), no recent revision for the group exists.

We were able to make extensive collections of *Ophryosporus* in Chile over a period of three years. Our survey confirmed six of the eight species reported for the country by Rodriguez *et al.* (2018), Luebert *et al.* (2007) and Zuloaga *et al.* (2008). We present here an updated species list of *Ophryosporus* in Chile with a detailed account of their nomenclature and distribution, and include a key for species identification.

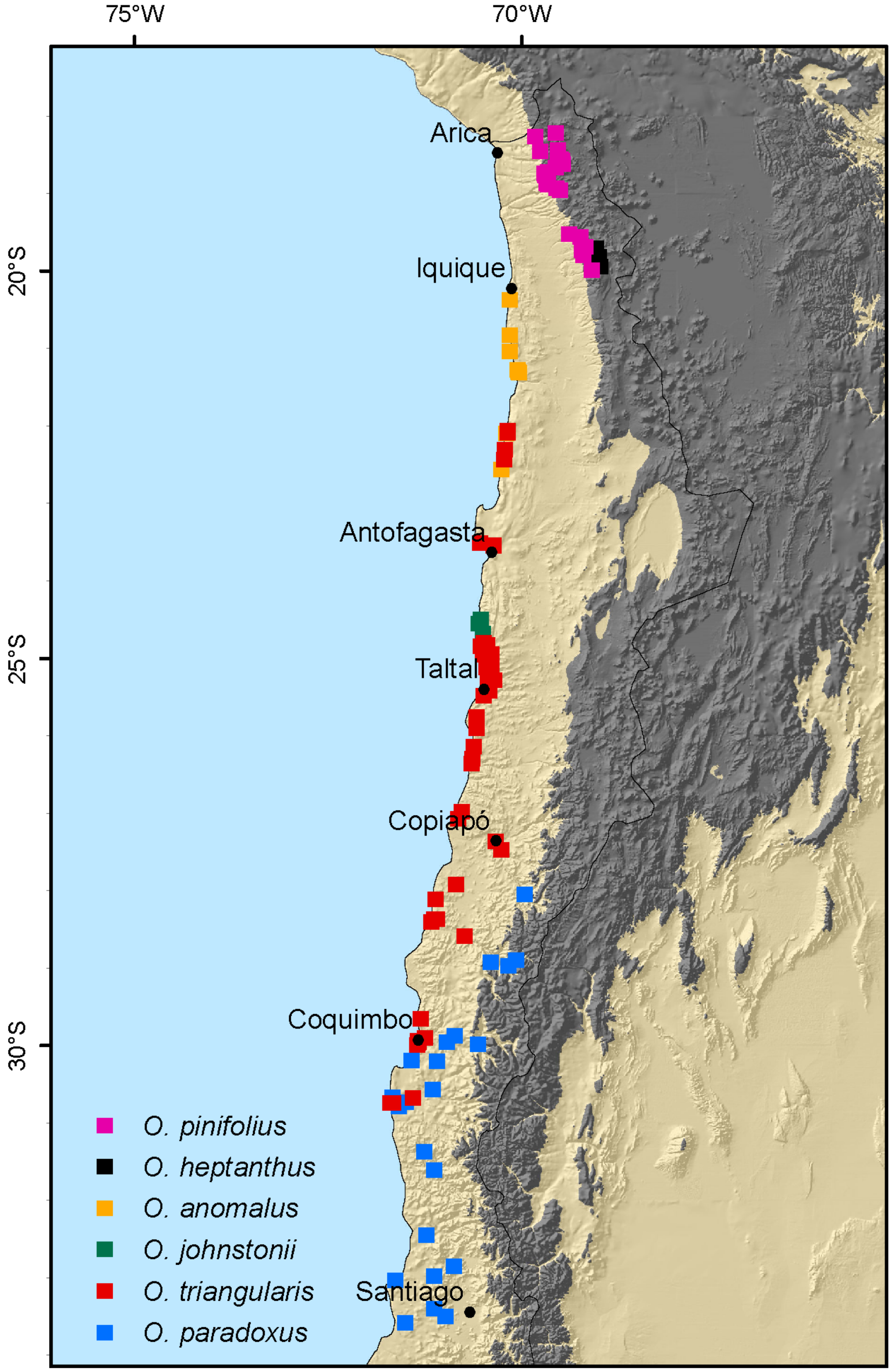

Figure 1: Geographical distribution of the six accepted species of *Ophryosporus* in northern Chile. Areas shaded in dark gray represent the Andean Cordillera >3000 m elevation, areas in beige <3000 m elevation. The black line represents the political border of Chile.

6.2 Methods

Field work was carried out in northern Chile and southern Peru between October 2016 and September 2019. The principal area of distribution of the Chilean species of *Ophryosporus* was covered, ranging from Valparaíso (33.05°S, type locality of *O. paradoxus*) to Arica (18.45°S) along the coast, and corresponding latitudes in the Andean cordillera of Chile. In Peru, sporadic collecting took place between Azangaro (14.92°S, type locality of *O. heptanthus* (Schultz-Bip. ex Wedd.) R.M.King & H.Rob.), Ollantaytambo (13.25°S), and in the vicinity of Lima (12°S). A total of 82 herbarium numbers were collected (Table S1). Vouchers were deposited at the herbaria of Bonn, Germany (BONN), the Universidad de La Serena, Chile (ULS), the Universidad de Chile, Santiago (EIF), and the University of San Marcos, Lima (USM).

Specimens from the herbaria at Santiago (SGO), Leiden (L), Field Museum of Natural History (F), and Stockholm (S) were critically revised and geo-referenced to create a distribution map (Fig. 1, Table S1). In addition, we used virtual herbaria to locate type material and online images of these were consulted where available.

Scanning Electron Microscope (SEM) images were taken of the cypselae and pappus of all taxa in question (Fig. 2). Cypselae obtained from herbarium specimens were mounted on aluminium stubs using conductive carbon cement (Leit-C, PLANO, Wetzlar, Germany) and sputter coated with gold in a sputter-coater (SCD 040, Balzers Union, Liechtenstein) for 3 minutes. Images were taken with a Stereoscan 200 electron microscope (Cambridge, England) at 15 kV.

6.3 Results

Our results confirm six species of *Ophryosporus* in Chile. These are *O. triangularis*, *O. paradoxus*, *O. johnstonii* B.L.Rob., *O. pinifolius* (Phil.) R.M.King & H.Rob., *O. heptanthus* and *O. anomalus* R.M.King & H.Rob.; *Ophryosporus hoppii* and *O. floribundus* do not seem to occur in Chile and are in need of further taxonomic investigation.

Figure 2: Scanning Electron Microscope (SEM) images of the apical part of the cypsela showing pappus setae/squamellae of *Ophryosporus* species. (A–F) Chilean species. (G–H) Peruvian species previously referred to Chile (pappus absent in *O. floribundus*). A) *O. anomalus* (FL 4102) with few spreading squamellae (arrowed); B) *O. heptanthus* (FFM 2019-20) with conspicuous pappus setae; C) *O. johnstonii* (Johnston 5259) with long, erect pappus setae; D) *O. paradoxus* (Zöllner 6827) with long, erect pappus setae; E) *O. pinifolius* (FL 3452) with small, erect squamellae (arrowed); F) *O. triangularis* (FFM 2018-63) with long, erect pappus setae; G) *O. floribundus* (Asplund 11079) with no visible pappus; H) *O. hoppii* (Dillon 3926) with squamellate pappus setae fused at the base and rather more conspicuous than in *O. pinifolius*. Scale bars represent 100 µm.

6.3.1 A key to the Chilean species of *Ophryosporus*

1 Compact shrubs, erect branching to about 1 m overall height; predominantly coastal **2**

- Loose shrubs, branching erect to spreading, regularly exceeding 1 m in height; Andean **5**

2 Pappus inconspicuous, formed by spreading squamellae . . . . ***O. anomalus***

- Pappus formed by conspicuous setae **3**

3 Leaves linear-lanceolate . . . . ***O. paradoxus***

- Leaves triangular **4**

4 Leaves rarely entire, usually regularly lobed or dentate, >3 × 5 mm; capitula pedunculate; widely distributed along the coast of northern Chile . . . ***O. triangularis***

- Leaves usually entire, rarely irregularly dentate, <3 × 5 mm; capitula sessile; known from the area between Aguada Cardón and Miguel Díaz . . . . ***O. johnstonii***

5 Leaves linear-lanceolate, pappus formed by minute 0.2 – 1 mm long, irregular squamellae . . . . ***O. pinifolius***

- Leaves triangular-lanceolate, pappus formed by conspicuous setae of 3 – 4 mm in length . . . . ***O. heptanthus***

Ophryosporus anomalus R.M.King & H.Rob., Phytologia 25: 66. 1972.
Typonym. *Piqueria cumingii* B.L.Rob., Proc. Amer. Acad. Arts, 42: 11. 1906, non *Ophryosporus cumingii* Benth. ex Baker (1895: 188, based on Mandon 264 from Bolivia).
Type. CHILE. Region I Tarapacá and Region II Antofagasta: ”Peruvia meridionalis: Cobija, Iquiqui et Arica”, H.Cuming 953 (lectotype, selected by Plos and Sancho (2013: 338): K [K000486684, photo!]; isolectotypes: E [E00322766, photo!], GH [GH00010778!], K [K000486685, photo!]), P [P02673192, photo!]; remaining syntypes: *Gaudichaud s.n.* (B [probably destroyed, could not be found], F [F1012247, photo!]).

Ophryosporus anomalus has been cited for Peru (Brako & Zarucchi, 1993; King & Robinson, 1972a), likely due to the type collection label (”Peruvia meridionalis...”). However, the localities mentioned there (”...Cobija, Iquiqui et Arica”) are now situated in Chile, and all other reports originate from the coastal zone around Tocopilla (Jaffuel, 1936; Johnston, 1932) and therefore Luebert *et al.* (2007) considered it a Chilean endemic. We hereby extend the distribution of the species to include the populations from the coastal area between Rio Loa and Iquique, previously referred to as *O. floribundus* (Muñoz-Schick *et al.*, 2001; Pinto & Luebert, 2009). See discussion below under the latter species.

Specimens examined. CHILE. Region I Tarapacà: Prov. Iquique, Alto Punta Gruesa, 20°22'S 70°09'W, 14 Dec 1997, *R. Pinto s.n.* (SGO142948); Alto Punta Patache, 20°49'S 70°09'W, 6 Dec 1997, *R. Pinto s.n.* (SGO142949); Alto Punta Patache, 22 Jan 2000, *R. Pinto s.n.* (SGO [photo]); Alto Punta Lobos, 21°02'S 70°09'W, 14 Jan 1998, *R. Pinto s.n.* (SGO142950); Alto Chipana, 21°16'S 70°03'W, 15 Oct 1997, *W. Sielfeld 7* (SGO143038); Alto Chipana, 21.304528°S 70.03204°W, 990 m, 21 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3427A* (BONN, ULS); Alto Chipana, 21.292633°S 70.042234°W, 840 m, 1 Oct 2019, *F. Luebert, F.F. Merklinger & J. Ruhm 4102* (BONN, EIF, K). **Region II Antofagasta**: Prov. Tocopilla, Tocopilla, 27 Oct 1930, *F. Jaffuel 1026* (GH); Tocopilla, Cerro Rosario, 1 Nov 1941, *M.R. Espinosa s.n.* (SGO143254); Cobija, s.a., *C. Gaudichaud s.n.* (F1012247); Cobija, quebrada Aguada Cañas, 4 Apr 1949, *W. Biese 3088* (SGO096693).

Ophryosporus heptanthus (Schultz-Bip.) R.M.King & H.Rob., Phytologia 58: 528. 1985.
Basionym: *Eupatorium heptanthum* Schultz-Bip., Bonplandia 4: 50 and 54. 1856. **Type**. PERU. Dept. Puno: "Pérou, sur les montagnes, aux environs de la ville de d'Azangaro", *W. Lechler 1751* (lectotype, selected by Plos and Sancho (2013: 336): P [P00742426, photo!]; isolectotypes: GOET [GOET001506, photo!], K [K000542525, photo!], P [P00742425, P00742428, photo!], W [W0018472, photo!]).

Eupatorium origanoides Meyen & Walp., Nov. Actorum Acad. Caes. Leop.-Carol. Nat. Cur. 19(Suppl. 1): 257. 1843, nom. illeg., non Kunth (1818: 89) [*Cronquistianthus origanoides* (Kunth) R.M.King & H. Rob., Phytologia 23: 411. 1972.].

Ophryosporus origanoides Hieron., Bot. Jahrb. Syst. 22: 707. 1897. Replacement name for *Eupatorium origanoides* Meyen & Walp.
Type. PERU. Dept. Tacna: In planitie circa Tacoram, Apr. 1831, F.J.F. *Meyen s.n.* (B, [probably destroyed]; F neg. 14714!).

Ophryosporus origanoides var. *microcephala* [as *microcephalus*] Hieron., Bot. Jahrb. Syst. 22: 708. 1897.
Type. BOLIVIA. Cochabamba: 4000 m, 26 Mar 1892, C.E.O. *Kuntze s.n.* (lectotype, selected by Plos and Sancho (2013: 338): NY [NY00230826]; isolectotype GH [GH00010786, photo!]).

This species is confirmed for Chile and has been collected by the authors near the village of Chusmiza, at the same locality as Zöllner 2997 (L, LP) and Gardner & Knees 6534 (SGO, E). The pappus setae of this species are formed by conspicuous yellowish-white setae of c. 4 mm in length. At this locality, *O. heptanthus* grows in local sympatry with *O. pinifolius*. However, the pappi of the latter are formed by minute squamellae and the two taxa are thus readily distinguishable. Plos (2012) cited specimen Zöllner 2997 twice, once for *O. heptanthus* (LP) and a second time for *O. hoppii* (CONC). It is possible, that having collected both specimens at the same locality where they occur sympatrically, Zöllner mistook them for a single species and only later at the two herbaria they were identified as belonging to two distinct taxa. We could not have access to the latter material at CONC, but we assume that it corresponds either to *O. heptanthus* or *O. pinifolius*.

Specimens examined. BOLIVIA. La Paz: Prov. Bautista Saavedra, Chajaya, a few km from Charazani, 15°13'S 69°01'W, 3500 m, 30 Mar 1985, *J.C. Solomon 13294* (U [U1145280]). Prov. Omasuyos, Viciniis Ochachache [Achacachi], 4000 m, Jan–Apr 1859, *G. Mandon 260* (S). Prov. Murillo, 4 km up the Río Achumani from Calacoto (La Paz), 16°30'S 68°02'W, 3600 m, 11 Apr 1986, *J.C. Solomon 15271* (U [U1145281]); 1 km NW of Ovejuyo, 16°32'S 68°03'W, 3700–3900 m, 2 Apr 1982, *J.C. Solomon 7453* (U [U1145279]). **CHILE. Region I Tarapacá**: Prov. Tamarugal, Chusmiza, 3200 m, 10 Jan 1969, *O. Zöllner 2997* (L125727); quebrada de Chusmiza, 19°41'4.9"S 69°11'01.9"W, 3350 m, 18 Feb 2003, *M.F. Gardner & S.G. Knees 6534* (E, SGO); Chusmiza, 19°40'48.2"S 69°10'49.5"W, 3380 m, 27 Apr 2008, *M. Muñoz & A. Moreira 4940* (SGO157269); at the entrance of the Andean village Chusmiza, 19.67880°S 69.17956°W, 3392 m, 2 Oct 2019, *F.F. Merklinger, F. Luebert & J. Ruhm 2019-20* (BONN, EIF, K). **PERU. Dept. Ayacucho**: Prov. Lucanas, a few km from Puente Toro Muerte, 14°42'55.1"S 74°32'44.7"W, 3589 m, 21 Mar 2019, *M. Weigend 9841/19–32* (BONN). **Dept. Cusco**: Prov. Cusco, alrededore Cusco, 17 May 1958, *A.L. Cabrera & H.A. Fabris 13536* (S); Río Blanco, 1500 ft [450 m], 8–19 May 1922, *J.F. Macbride & W. Featherstone 718* (S). **Dept. Puno**: Prov. Puno, Checayani, NE of Azángaro, 3980 m, 28 Mar 1957, *H. Ellenberg 461* (U [U1145288]); Huerta N of Puno, 3840 m, 22 Mar 1957, *H. Ellenberg 238A* (U [U1145284]); Huerta N of Puno, 4100 m, 22 Mar 1957, *H. Ellenberg 292* (U [U1145286]); Huerta N of Puno, 3840 m, 22 Mar 1957, *H. Ellenberg 238* (U [U1145285]). Prov. Lampa, Pucará, 3900 m, 22 Aug 1957, *H. Ellenberg 2753A* (U [U1145287]). **Dept. Moquegua**: Prov. de Mariscal Nieto, Carumas, 3200 m, 21 Feb–6 Mar 1925, *A. Weberbauer 7333* (S); Prov. General Sánchez Cerro, Puquina, outside Puquina towards Arequipa, 16°36'39.4"S 70°11'30.8"W, 3174 m, 29 Mar 2019, *M. Weigend 9994/19–182* (BONN).

Ophryosporus johnstonii B.L.Rob., Contr. Gray Herb. 77: 4. 1926.
Type. CHILE. Region II Antofagasta: Prov. Antofagasta, dept. Taltal, Aguada del Panul, *I.M. Johnston 5424* (holotype: GH [GH00010781, photo!]; isotypes: S [S-R-3810!, S 10-19704!], SGO [SGO-59043!]).

This peculiar species is known only from three localities north of the town of Paposo (Johnston, 1929). It is distributed very narrowly within the range of *Ophryosporus triangularis* and is sympatric with this latter species. Morphologically, *O. johnstonii* is very similar to *O. triangularis*.

Specimens examined. CHILE. Region II Antofagasta: Prov. Antofagasta, vicinity of Miguel Díaz, directly N of quebrada Iscuña, c. 55 km N of Paposo, 24°33'S 70°33'W, 100–300 m, 15 Dec 1987, *M.O. Dillon & S. Teillier 5292* (BONN); vicinity of Aguada de Miguel Díaz, 24°35'S, 1–4 Dec 1925, *I.M. Johnston 5310* (SGO059042); vicinity of Aguada Cardón, 24°45'S, 30 Nov 1925, *I.M. Johnston 5259* (S); rocky slopes of Aguada Cardón, 24.74173°S 70.54385°W, 210 m, 15 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3384* (BONN, ULS); Aguada Cardón, 24.741717°S 70.542687°W, 210 m, 5 Oct 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3950* (BONN, ULS).

Ophryosporus paradoxus (Hook. & Arn.) B.D.Jacks., Index Kew. 2 (1): 354. 1894.
Basionym: *Eupatorium paradoxum* Hook.& Arn., Compan. Bot. Mag. 1: 240. 1835.
Type. CHILE. Region V Valparaíso: *T.C. Bridges 52* (lectotype, selected by Plos and Sancho (2013: 336), E [00249901, photo!]; isolectotypes: K [K486667, photo!], W [W18468, photo!]); remaining syntypes: Valparaíso, *H. Cuming 337*, K [K486668, photo!], E [E249900, E249902, photo!]; W [W0018467, photo!]. Valparaíso, *J. Gillies* (not seen).

Nothites baccharidea DC., Prodr. 5: 187. 1836.
Stevia baccharoides (DC.) Meigen, Bot. Jahrb. Syst. 17: 283. 1893.
Type. CHILE. Region V Valparaíso: *C.L.G. Bertero 837* (lectotype, designated here: G-DC [G00495730, photo!]; isolectotypes: G-DC [G00495717, mounted onto the same sheet as lectotype]; L [L3661664!]).

This is the southernmost species of *Ophryosporus* in Chile, distributed from the region Metropolitana de Santiago northward to the region Atacama. This species is not strictly limited to the coast but also occurs further inland, for example in the Cuesta

Las Chilcas or Andacollo. *Ophryosporus paradoxus* is a very distinct species that can be differentiated from *O. triangularis* by its larger, linear-lanceolate and rather papery leaves with strongly lobed margins, as opposed to the small, triangular, slightly fleshy leaves with revolute margins in *O. triangularis.* The secondary inflorescences are thyrsoid, emerge terminally and produce florets with white corollas and a pappus of white setae up to c. 3 mm long.

Specimens examined. CHILE. Region III Atacama: Prov. Copiapó, Jorquera valley, 12 Jan 1970, *O. Zöllner 4682* (L3661656). Prov. Huasco, below El Chivato, 28°54'S 70°04'W, 1800 m, 4 Jan 1926, *I.M. Johnston 5870* (S); Resguardo, 28°58'S 70°10'W, 1530 m, 4 Jan 1926, *I.M. Johnston 5863* (S); valley San Félix, 1180 m, 16 Dec 1941, *E. Pisano V. & R. Bravo F. 1089* (SGO). **Region IV Coquimbo**: Prov. Elqui, valley of Río Turbio between Rivadavia and Guanta, 900 m, 18–19 Jan 1926, *I.M. Johnston 6271* (S); near Guanaqueros, 24 Jul 1973, *O. Zöllner 6827* (L3661655); road from Marquesa to Viñita Baja, 29.954529°S 70.964978°W, 340 m, 26 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3822* (BONN, ULS); road from Marquesa to Viñita Baja, 29.873778°S 70.860812°W, 700 m, 26 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3825* (BONN, ULS); road to Andacollo, c. 6 km before Andacollo, 30.201205°S 71.092169°W, 900 m, 24 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3807* (BONN, ULS). Prov. Limarí, Ovalle, Villaseca near Huamalata, 30.568064°S 71.150966°W, 270 m, 24 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3799* (BONN, ULS); Fray Jorge, 215 m, 13 Aug 1917, *C. & I. Skottsberg 746* (S); Ovalle, Fray Jorge, 200 m, Nov 1925, *E. Werdermann 892* (U [U1145306], S); Ovalle, Fray Jorge, Oct 1947, *B. Sparre 3061* (S); hotel Termas de Socos, 30.732502°S 71.493507°W, 80 m, 23 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3777* (BONN, ULS); road from Los Loros to Caleta El Toro, 30.741021°S 71.65348°W, 50 m, 23 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3790* (BONN, ULS); road from Alcones to Los Loros, 30.78262°S 71.587161°W, 330 m, 23 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3781* (BONN, ULS). Prov. Choapa, road from Combarbalá to Canela Baja, few km after Los Pozos, 31.363888°S 71.260395°W, 500 m, 22 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3775* (BONN, ULS); road from Illapel to Combarbalá, near Illapel, 31.604095°S 71.125953°W, 450 m, 22 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3772* (BONN, ULS). **Region V Valparaíso**: Prov. Petorca, La Ligua, 5 km from Petorca on road from Pedequa, 550 m, 27 Nov 1938, *C.R. Worth & J.L. Morrison 16704* (S). Prov. San Felipe de Aconcagua, Cuesta Las Chilcas, 560 m, 19 Jul 2003, *F. Luebert & L. Kritzner 1757* (EIF); Cuesta Las Chilcas, 32.851797°S 70.875068°W, 380 m, 20 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3741* (BONN, ULS); Cuesta Las Chilcas, 32.851797°S 70.875068°W, 380 m, 20 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3741A* (BONN, ULS). Prov. Valparaíso, 14 Jan 1947, *E. Wall & B. Sparre 45* (S); rocks near the sea, 17 Nov 1895, *O. Buchtien s.n.* (S). Prov. Quillota, Parque Nacional La Campana, Cerro La Campana, 32°58.777"S 71°7.670"W, 480 m, 30 Dec 2000, *F. Luebert 1398* (EIF). Prov. San Antonio, Nov. 1927, *O. Buchtien 3450* (L3661663). Prov. Melipilla, Curacaví, Dec 1967, *O. Zöllner 2245* (L3661657); La Barriga, Oct 1964, *O. Zöllner 1765* (L3661658).

Ophryosporus pinifolius (Phil.) R.M.King & H.Rob., Phytologia 25: 66. 1972.
Basionym: *Stevia pinifolia* Phil., Anales Mus. Nac. Santiago de Chile, sec. 2 (bot.) 1891: 37.
Piqueria pinifolia (Phil.) Hieron. ex B.L.Rob., Proc. Amer. Acad. Arts 42: 11 (1906).
Type. CHILE. Region I Tarapacá: Usmagama, 15 Mar 1885, *R.A. Philippi s.n.* (lectotype, selected by Plos and Sancho (2013: 339): SGO [SGO044738!]; isolectotype: K [K000486664, photo!]).

Ophryosporus pinifolius has an inconspicuous pappus that consists of minute irregular squamellae. Its leaves are extremely variable and range from linear-lanceolate with entire margins to irregularly dentate ones. It is one of two Andean species in the genus known to occur in Chile. Based on herbarium records its distribution is centered in the northern regions of Tarapacá and Arica y Parinacota where it is widespread (Fig. 1).

Specimens examined. CHILE. Region XV Arica y Parinacota: Prov. Arica, Timar, between Timar and Tignamar, 18.717336°S 69.663483°W, 2840 m, 29 Mar 2017, *F.F. Merklinger & A. Stoll 2017-51* (BONN, ULS); shortly after Timar, 18.736382°S 69.706777°W, 2447 m, 29 Mar 2017, *F.F. Merklinger & A. Stoll 2017-54* (BONN, ULS); shortly after Timar, 18.747992°S 69.699137°W, 2307 m, 29 Mar 2017, *F.F. Merklinger & A. Stoll 2017-47* (BONN, ULS); Codpa, between Codpa and Timar, 18.762747°S 69.69828°W, 2393 m, 29 Mar 2017, *F.F. Merklinger & A. Stoll 2017-46* (BONN, ULS); quebrada de Vitor NW of Palca, 18.827041°S 69.677724°W, 2085 m, 28 Mar 2017, *F.F. Merklinger & A. Stoll s.n.* (BONN, ULS); quebrada Chokaya, from Codpa into Camarones valley toward Pachica, 18.86473°S 69.68034°W, 2235 m, 28 Mar 2017, *F.F. Merklinger & A. Stoll 2017-30* (BONN, ULS); quebrada Chokaya, from Codpa into Camarones valley toward Pachica, 18.88289°S 69.664972°W, 2373 m, 28 Mar 2017, *F.F. Merklinger & A. Stoll 2017-40* (BONN, ULS); between Esquina and Pachica, 18.927444°S 69.552944°W, 2298 m, 28 Mar 2017, *F.F. Merklinger & A. Stoll 2017-43* (BONN, ULS); along road through Illapata, 18.94831°S 69.50272°W, 2300 m, 28 Mar 2017, *F.F. Merklinger & A. Stoll 2017-41* (BONN, ULS); road A-135 from Panamericana to Puquios, 18.254661°S 69.828852°W, 3100 m, 14 Oct 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 4008* (BONN, ULS). Prov. Parinacota, along road Putre–Arica, 18.21033°S 69.56082°W, 3500 m, 1 Apr 2017, *F.F. Merklinger & A. Stoll 2017-65* (BONN, ULS); Paychama [Pachama], 3600 m, 10 Mar 1927, *C. Troll 3244* (B, M); along road Putre–Arica, 18.45314°S 69.76415°W, 3102 m, 1 Apr 2017, *F.F. Merklinger & A. Stoll 2017-62* (BONN, ULS); along A-31 near Belen, 18.48545°S 69.52782°W, 3500 m, 29 Mar 2017, *F.F. Merklinger & A. Stoll 2017-56* (BONN, ULS); NE of Saxamar, 18.55108°S 69.50015°W, 3500 m, 29 Mar 2017, *F.F. Merklinger & A. Stoll 2017-57* (BONN, ULS); Saxamar, 18.56667°S 69.48333°W, c. 3000 m, 20 Mar 2015, *A. Moreira & F. Luebert 2456* (BONN); W of Tignamar along road, 18.57918°S 69.52785°W, 3300 m, 29 Mar 2017, *F.F. Merklinger & A. Stoll 2017-55* (BONN, ULS); Tignamar, 18°37'S 69°28'W, 3100 m, 11 Sep 1963, *F. Schlegel 4879* (F, CONC, [photo; as *Piqueria floribunda*]); between Timar and Tignamar, shortly before Tignamar, 18.663898°S 69.560963°W, 3354 m, 29 Mar 2017, *F.F. Merklinger & A. Stoll 2017-53* (BONN, ULS). Prov. Arica, Palca, 18°50'S 69°40'W, 2200 m, 30 Oct 1964, *F. Schlegel 5092* (CONC [photo, as *Piqueria floribunda*], F). **Region I Tarapacá**: Prov. Tamarugal, quebrada de Soga, 19.514361°S 69.381274°W, 2400 m, 16 Mar 2017, *F. Luebert, T. Böhnert & F.F. Merklinger, A. Stoll & D. Quandt 3455* (BONN, ULS); quebrada Aroma, 19.514361°S 69.381274°W, 2300 m, 16 Mar 2017, *F.F. Merklinger 2017-6* (BONN, ULS); above Jaiña, 19.551255°S 69.242848°W, 2750 m, 21 Mar 2017, *F. Luebert & T. Böhnert 3631* (BONN, ULS); at entrance of Andean village Chusmiza, 19.67830°S 69.17930°W, 3393 m, 2 Oct 2019, *F.F. Merklinger, F. Luebert & J. Ruhm 2019-21* (BONN, EIF, K); Chusmiza, above town, 19.683025°S 69.183376°W, 3400 m, 21 Mar 2017, *F. Luebert & T. Böhnert 3642* (BONN, ULS); 2 km above the village of Chusmiza in quebrada de Chusmiza at km 76 alongside the new road to Colchane, 19°40'53.7"S 69°11'11.0"W, 3406 m, 18 Feb 2003, *M.F. Gardner & S.G. Knees 6512* (SGO150393); road to Usmagama, turnoff ruta CH-15, 19.730253°S 69.218684°W, 2956 m, 26 Mar 2017, *F.F. Merklinger & A. Stoll 2017-26* (BONN, ULS); road to Usmagama, turnoff ruta CH-15, 19.730253°S 69.218684°W, 2956 m, 26 Mar 2017, *F.F. Merklinger & A. Stoll 2017-19* (BONN, ULS); cuesta Usmagama, km 3.9, 19.730154°S 69.217046°W, 3050 m, 28 Oct 2016, *F. Luebert & T. Böhnert 3452* (BONN, ULS); Usmagama, road from Usmagama to Limacsina, 19.78771°S 69.207368°W, 2434 m, 26 Mar 2017, *F.F. Merklinger & A. Stoll 2017-22* (BONN, ULS); quebrada de Parca, 19.985261°S 69.098117°W, 3261 m, 22 Mar 2017, *F.F. Merklinger & A. Stoll 2017-12* (BONN, ULS).

Ophryosporus triangularis Meyen, Reise Erde 1: 402. 1834.
Type. CHILE. Region III Atacama: Copiapó, *F.J.F. Meyen s.n.* (holotype: B [probably destroyed]; F neg. 14718!).

Eupatorium decipiens Hook. & Arn. in Hook. Compan. Bot. Mag. 1: 240. 1835.
Type. CHILE. Region IV Coquimbo: *H. Cuming 907* (lectotype, designated here, K [K000486661, photo!]; isolectotype, E [E00249908, photo!]); remaining syntypes: CHILE. Region IV Coquimbo: *Macrae s.n.* E [E00249907, photo!, *Macrae s.n.*, mounted together with lectotype].

Eupatorium foliolosum DC., Prodr. 5: 174. 1836.
Ophryosporus foliolosus (DC.) Reiche, Anales Univ. Chile 109: 9. 1901. Type: CHILE. Region IV Coquimbo: *Macrae s.n.* (lectotype, designated here: G-DC [G00130591, photo!]; remaining syntypes: Gaudichaud 100 (G-DC [G00130590, photo!]; P [P02673049 & P02673151, photo!]; K [K000486660 & K000486663, photo!]).

Kuhnia multiramea Turcz., Bull. Soc. Imp. Naturalistes Moscou 24: 168. 1851.
Type. CHILE. Region IV Coquimbo: *Bridges 1412* (holotype, KW [144506, photo!]; isotypes: KW [144505, photo!]; E [E00249909, photo!]; P [P02673149, photo!]).

Eupatorium volckmannii Phil., Anales Univ. Chile 18: 51. 1861.
Type. CHILE. Vallenar. *Volckmann s.n.*, 1860 (holotype: SGO [SGO065417!]; isotype: GH [GH00014290, photo!]).

This species is widespread and more or less continuously distributed along the coast of northern Chile. The southernmost localities where it was observed during our study was at the southern edge of the Río Limarí, where its range overlaps with that of *Ophryosporus paradoxus*. The northern end of its distribution appears to be the R'io Loa. North of Paposo, at Aguada del Panul, it grows sympatrically with *O. johnstonii*, to which it bears close morphological resemblance. However, *O. triangularis* is identified by its slightly larger leaves, which are grouped in alternating fascicles, are shortly petiolate, triangular with a cuneate base and an acute apex, and reaching about 3–5 × 5–15 mm as opposed to much smaller leaves in *O. johnstonii*, which reach only c. 1–3 × 3–5 mm. Leaf-size is, however, extremely variable, and plants that grow in more humid conditions often possess larger leaves. The leaf margins of *O. triangularis* are regularly lobed to dentate and revolute. The inflorescences are spike-like, and the capitula are pedunculate. The spike-like inflorescences appear somewhat denser than in *O. johnstonii*, and, in this latter species, the capitula are sessile. Its florets have a white corolla, sometimes with a violet taint. The pappus is formed by whitish-brown setae, c. 2.5–3.5 mm long. In the area around Cobija, both, *O. triangularis* and *O. anomalus* have been collected in the past.

Specimens examined. CHILE. Region II Antofagasta: Prov. Tocopilla, quebrada 2–3 km N of Tocopilla above old Caleta Duendes, 150–200 m, 18 Oct 1988, *M.O. Dillon & D. Dillon 5718* (BONN); Tercera quebrada Tocopilla, 22.0558°S 70.17662°W, 300 m, 18 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3413* (BONN, ULS); Quebrada La Higuera, S Tocopilla, 22°18'7"S 70°12'58"W, 30 Sep 2005, *F. Luebert, N. Garcia & N. Schulz 2569/963* (EIF); Quebrada above Caleta Buena, S Mantos de la Luna, 22.43075°S 70.22186°W, 640 m, 10 Oct 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3989* (BONN, ULS). Prov. Antofagasta, near Cobija, 19 Dec 1971, O. Zöllner 4593 (L153863); Juan López, 23.51205°S 70.53365°W, 150 m, 19 Jul 2003, *M. Antonissen 7* (EIF); Quebrada La Chimba, 17 Dec 1987, *M.O. Dillon & J.T.S. Teillier 5321* (BONN); Quebrada La Chimba, 23°33'S 70°22'W, 380–480 m, 11 Nov 1988, *M.O. Dillon & D. Dillon 5881* (BONN); Quebrada La Chimba, 23.53567°S 70.35887°W, 460 m, 17 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3408* (BONN, ULS); Quebrada Cardón, 24.741717°S 70.542687°W, 210 m, 5 Oct 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3949* (BONN, ULS); Aguada Cardón, 24.74173°S 70.54385°W, 210 m, 15 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3389* (BONN, ULS); Quebrada Panul, 24.773468°S 70.533915°W, 180 m, 4 Oct 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3939* (BONN, ULS); Quebrada Panul, 24.777263°S 70.531618°W, 190 m, 5 Oct 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3944* (BONN, ULS); Quebrada Panul, 24.777263°S 70.531618°W, 190 m, 5 Oct 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3944A* (BONN, ULS); c. 7 km N of Paposo, 24°57'S 70°29'W, 40 m, 14 Dec 1987, *M.O. Dillon & J.T.S. Teillier 5262* (BONN); El Rincón, al N de Paposo, 17 Sep 1941, *C. Muñoz P. & G.T. Johnson 2902* (SGO118350); Paposo, base Cerro Carneros, 24°56'24"S 70°28'44.6"W, 160 m, 23 Oct 2009, *A. Moreira & F. Luebert 1200* (SGO158780); Quebrada Portezuelo, 25.0124°S 70.446467°W, 550 m, 13 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3357* (BONN, ULS); Quebrada Matancilla, c. 5 km S of Punta Grande, 25°07'S 70°27'W, 170–350 m, 27 Oct 1988, *M.O. Dillon, D. Dillon, V. Asencio & M. Villarroel O. 5750* (BONN); Cachinalcito, 25°10', 28 Nov 1925, *I.M. Johnston 5173* (S); c. 20 km N of Taltal, quebrada Anchuña, 25.23543°S 70.42594°W, 183 m, 31 Aug 2018, *F.F. Merklinger, A. Kozok & D. Quandt 2018-63* (BONN, ULS); c. 20 km N of Taltal, quebrada Anchuña, 25.23543°S 70.42594°W, 183 m, 31 Aug 2018, *F.F. Merklinger, A. Kozok & D. Quandt 2018-64* (BONN, ULS); c. 20 km N of Taltal, quebrada Anchuña, 25.23543°S 70.42594°W, 183 m, 31 Aug 2018, *F.F. Merklinger, A. Kozok & D. Quandt 2018-65* (BONN, ULS); c. 20 km N of Taltal, quebrada Anchuña, 25.23543°S 70.42594°W, 183 m, 31 Aug 2018, *F.F. Merklinger, A. Kozok & D. Quandt 2018-66* (BONN, ULS); Quebrada El Médano, 300 m, 8 Oct 1941, *E. Pisano V. & R. Bravo F. 398* (SGO); Quebrada San Ramón, 25.38578°S 70.43658°W, 120 m, 11 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3346* (BONN, ULS); Quebrada San Ramón, 17 Sep 1968, *O. Zöllner 2851* (U [U3661650]); Cerro Perales, c. 5 km E of Taltal, 25°25'S 70°25'W, 550 m, 21 Nov 1988, *M.O. Dillon & D. Dillon 6002* (BONN); Hills SE of Taltal, 25°29'S, 25 Nov 1925, *I.M. Johnston 5080* (S); Posado Hidalgo, 25°45'S 70°35'W, 13 Dec 1925, *I.M. Johnston 5661* (S); along road Panamericana toward Caleta Esmeralda, 25.895921°S 70.581052°W, 500 m, 9 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3308* (BONN, ULS). Region III Atacama: Prov. Chañaral, 21 km W of Panamericana on northern route to Pan de Azúcar NP, 26°08'S 70°37'W, 85 m, 30 Sep 1988, *M.O. Dillon, D. Dillon & V. Pobleto 5609* (BONN); Falda Verde, hills N of Chañaral, 26.296721°S 70.631252°W, 75–600 m, 1 Oct 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3905* (BONN); Hills back of El Barquito, 26°23'S, 28–29 Oct 1925, *I.M. Johnston 4809* (S). Prov. Copiapó, Sector quebrada El León, 26.976625°S 70.773903°W, 70 m, 8 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3290* (BONN, ULS); Caldera on small point just north of town, 27°3'S, 22 Nov 1925, *I.M. Johnston 5067* (S); Copiapó, 400 m, 13 Jul 1938, *Ch.H. Andreas 885* (U [U1145297]); Tierra Amarilla, 700 m, Oct 1924, *E. Werdermann 456* (U [U1145305]); Estancia Castilla, road from Totoral to Panamericana, 27.919994°S 70.84531°W, 240 m, 28 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3869* (BONN, ULS). Prov. Huasco, Road Carrizal Bajo to Canto de Agua, c. 2 km from Carrizal Bajo, 28.112004°S 71.116139°W, 45 m, 7 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3279* (BONN, ULS); Quebrada Baratillo, 28°21'57'S 71°7'21'W, 150 m, 14 Sep 2003, *F. Luebert & L. Kritzner 1805* (EIF); Quebrada Baratillo, 28.363325°S 71.096321°W, 110 m, 7 Oct 2016, *F. Luebert, A. Stoll & T. Böhnert 3269* (BONN, ULS); hills E of Tres Playitas, 28.400532°S 71.16755°W, 160 m, 27 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3854* (BONN, ULS). Region IV Coquimbo: Prov. Elqui, Guayacán, s.a., *unknown s.n.* (S); Puente Juan Soldado, 29.656542°S 71.301174°W, 200 m, 27 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3831* (BONN, ULS); La Serena, 16 Sep 1947, *B. Sparre 2595* (S); Coquimbo, Jul–Aug 1958, *W.H. Harvey s.n.* (S); Coquimbo, 100 m, Nov 1923, *E. Werdermann 124* (U [U1145298]); Coquimbo, rocks behind the Fort, 29.93372°S 71.33691°W, 25 m, 24 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3809* (BONN, ULS); Coquimbo, rocks behind the Fort, 29.93372°S 71.33691°W, 25 m, 24 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3809A* (BONN, ULS); Herradura, 9 Aug 1917, *C. & I. Skottsberg 704* (S). Prov. Limarí, Ovalle, Río Limarí, 11 Oct 1947, *B. Sparre 2994* (S); N of Caleta El Toro, 30.737239°S 71.699907°W, 25 m, 23 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3794* (BONN, ULS); along road from Los Loros to Caleta

El Toro, 30.741021°S 71.65348°W, 50 m, 23 Sep 2017, *F. Luebert, T. Böhnert & F.F. Merklinger 3786* (BONN, ULS).

6.3.2 Species excluded from the Chilean flora

Ophryosporus floribundus (DC.) R.M.King & H.Rob., Phytologia 25: 66. 1972.
Basionym: *Piqueria floribunda* DC., Prodr. 5: 105. 1836.
Type. PERU. "Perou cordilliere, 1834, *T.P.X. Haenke s.n.* (holotype: G-DC [G00130596, photo!]; isotype: P [P00742191, photo!]).

This taxon is cited for Chile in Muñoz-Schick *et al.* (2001), Pinto & Luebert (2009), Zuloaga *et al.* (2008) and Rodriguez *et al.* (2018) based on four specimens. Three of them were collected by R. Pinto in the late 1990s at three coastal localities in northern Chile, Alto Chipana, Punta Lobos and Punta Gruesa (*Pinto s.n.*, SGO 142948, SGO 142949, and SGO 142950). Recent field work at the coastal localities has not resulted in any collections that match the type of *O. floribundus*. Rather we found plants that we identified as *O. anomalus*, another species that has been reported for this area (Johnston, 1932) and has been only sporadically collected since. The type of *O. floribundus* has opposite, solitary leaves and long internodes of about 3–4 cm in length. The type of *O. anomalus* has crowded leaves that are borne in fascicles and with very short internodes. The leaves of the Chilean coastal specimens assigned to *O. floribundus* vary in size and shape, some corresponding well to the type of *O. anomalus* being narrowly oblanceolate and with entire margins and an obtuse apex, others becoming more broadly lanceolate to triangular with dentate margins and an acute apex thus remotely resembling *O. floribundus* but actually more similar to those of *O. triangularis*. On younger shoots the leaves appear more or less opposite but generally they are borne in fascicles. The cypselae of the plants on both sheets of the type specimen of *O. anomalus* (*Cuming 953*, K) bear quite visible, slightly spreading squamellate pappus setae, while no pappus is recorded for *O. floribundus*. We therefore conclude that *O. floribundus* is restricted to Peru from the area of Lima, while the coastal plants in northern Chile should be referred to *O. anomalus*.

Specimens examined. PERU. Dept. Amazonas: Purruchuca [sic], s.a., *Mathews, A. 1015* (G). **Dept. Lima**: Prov. Canta, road from Canta to Lima below San José turnoff towards Lima, 11.49383°S 76.65187°W, 2322 m, 1 Mar 2018, *M. Weigend & K.A. Peña Ramos 9719* (BONN); road from Canta to Lima, road down from Canta to turn off San José, 11.49383°S 76.65187°W, 2322 m, 1 Mar 2018, *M. Weigend & K.A.*

Peña Ramos 9722 (BONN). Prov. Huarochirí, Matucana, c. 2400 m, 25 May 1940, *E. Asplund 11072* (S); Matucana, c. 2600 m, 25 May 1940, *E. Asplund 11079* (S).

Ophryosporus hoppii (B.L.Rob.) R.M.King & H.Rob., Phytologia 23: 399. 1972.
Basionym: *Trychinolepis hoppii* B.L.Rob., Contr. Gray Herb. 80: 6. 1928.
Type. PERU. Dept. Arequipa: Jul 1925, *W. Hopp 28* (holotype: B [probably destroyed], F neg. 14723!; lectotype, designated here: GH [GH00013302]). Epitype (designated here): PERU. Dept. Arequipa. Lomas of Mollendo, c. 4 km N of Islay, 230 m, 20 Nov 1983, *M.O. Dillon & D. Dillon 3926* (USM [74666]; isoepitypes: BONN!, F!, US [3026292, photo!]).

This taxon was originally described as a new genus and species, *Trychinolepis hoppii* (Robinson, 1928: 6), because of its irregularly lobed, squamellate pappus, which resembled that of the West Indian genus *Phania*, even though a habitual resemblance to the genus *Ophryosporus* was stated by the author (Robinson, 1928). Subsequent analyses led to this monotypic genus to be sunk into *Ophryosporus*, because the pappus remained the only difference to other species of *Ophryosporus* (King & Robinson, 1972b). Specimens assigned to *O. hoppii* in Peru have a squamellate pappus, where the setae are fused at the base and are rather conspicuous. Cypselae with this type of pappus are visible on the type specimen and can even be seen on online images. However, due to the loss of the holotype in B and the very fragmentary remnants of the isotype at GH, we have decided to epitypify the specimen collected by M.O. Dillon and D. Dillon in 1983, as it corresponds to the protologue of *T. hoppii* and has been collected in the same region. This specimen has all necessary characters suitable for identification and is a good reference for potential future work. Specimens which we could positively identify as *Ophryosporus hoppii* were all collected in Peru. Records of *O. hoppii* for Chile appear to be miss-identifications and belong to *O. pinifolius*, e.g. *Schlegel 4879* & *5092* (CONC, F). The pappus of *O. pinifolius* on the contrary, although squamellate, are much more inconspicuous (Fig. 2). Our own observations and extensive sampling in northern Chile failed to positively identify a plant that clearly fits into the species concept of *O. hoppii*. For example, a specimen from Chusmiza, N of Iquique (*O. Zöllner 2997*, U) is cited twice in Plos (2012), once for *O. hoppii* and a second time for *O. heptanthus*. The locality at Chusmiza was visited by the authors, and two species, *O. pinifolius* and *O. heptanthus* were confirmed. These two taxa grow in local sympatry here, yet are distinguished from one another by their pappus, which in *O. pinifolius* consists of minute squamellae and which in *O. heptanthus* is formed by setae, up to 4 mm long. It is possible that the two taxa were collected by Zöllner as one species, only for later herbarium identification to recognize the two different taxa in the two herbarium vouchers. A further herbarium record from Alto Patache in Chile (*H. Larraín 98200*, CONC) could not be found in CONC.

Specimens examined. PERU. Dept. Arequipa: Prov. Islay, Quebrada Guerreros, 456 m, 6 Apr 1998, *FLSP 2411* (HUSA, US); Ocoña, 5 Feb 1969, *J. Soukup 6426/6216* (US, USM). Prov. Castilla, Chuquibamba towards Aplao 15°51'52.3"S 72°36'56.4"W, 2231 m, 24 Mar 2019, *M. Weigend 9862/19–52* (BONN, USM).

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6.4 References

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Chapter 7

Final considerations

7 Final considerations

This thesis contains three chapters (2, 3 & 4) which deal with microevolutionary processes and biogeographic patterns of Atacama lineages, and these chapters form the core of this work. Two further chapters (5 & 6), taxonomic revisions of two genera in Chile and southern Peru, are also included. In the following, a conclusive summary of this thesis is provided and an outline of how the individual studies have contributed toward our understanding of plant evolution in the Atacama Desert is given. Potential avenues for further research are also discussed.

7.1 Microevolutionary processes and biogeographic patterns

The first study (Chapter 2) is a population genomic analysis of *Tillandsia landbeckii* Phil., a member of subgenus *Diaphoranthema* (Till, 1992; Smith & Till, 1998), which has invaded the hyper arid Peruvian and Atacama Desert systems approximately 5 Ma (Givnish *et al.*, 2011). *T. landbeckii* forms near-monospecific communities in areas receiving fog moisture. This moisture is absorbed by highly specialised leaf trichomes unique to this family (Benzing, 2000). Fog appears to be the dominant factor that determines the distribution of *T. landbeckii*, and its impact onto the coastal cordillera varies due to the geomorphological properties of the latter (Cereceda *et al.*, 2008), resulting in a highly patchy distribution of *Tillandsia* lomas and thus their geographical isolation from each other (Rundel *et al.*, 1997).

Although much work has been done on the process of physical establishment of these *Tillandsia* lomas (e.g. Rundel *et al.*, 1997; Pinto *et al.*, 2006; Latorre *et al.*, 2011; Borthagaray *et al.*, 2010; Koch *et al.*, 2019), and varying types of reproductive strategies within the subgenus *Diaphoranthema* and other allied taxa in subfamily Tillandsioideae have been described (Gilmartin & Brown, 1985; Masuzawa, 1986; Till, 1992), a population genomic analysis on a large scale for this species in the Atacama was so far not available.

At the outset of this study we were interested to know, whether the populations form as the result of dispersal, for which the species is well equipped through the production of wind-dispersed seeds, or if the populations are remnants of a once widespread metapopulation that became increasingly fragmented as a consequence of varying degrees of aridity in the Atacama Desert over geological time. For the Pleistocene in particular, climatic oscillations for the Atacama have been inferred from previous studies using methods of radiocarbon dating (Latorre *et al.*, 2002, 2003) or cosmogenic nuclide exposure dating (Ritter *et al.*, 2018, 2019). In our study, a number of research questions was addressed, with the aim to understand the current distribution of *Tillandsia* lomas in Chile, to characterise gene flow within and between populations and correlate this gene flow to possible geographic barriers, or to dispersability of the species.

Our results suggest that there are three genetically and geographically distinct population clusters of *Tillandsia* in Chile, with limited gene flow between these clusters. A heterozygosity analysis, as well as measures of population differentiation (F_{ST}) and the inbreeding coefficient (F_{IS}) of these three geographical clusters revealed, that the central cluster is genetically the most diverse (*i.e.* most sexually reproducing lineages), while the two outlying clusters to the North and South contain several populations with a high degree of clonality (vegetative reproduction). These results further suggest, that in terms of genetic connectivity between populations, abiotic factors function as barriers to gene flow between populations and between clusters of populations, but that the organism may overcome these barriers to a certain extent (and over time) through its ability to disperse effectively. We inferred founder effects resulting from repeated dispersal events via a stepping stone process as the likely origin of the southern and northern clusters. Populations may persist in isolation over time, and employ a mixed mating system that involves sexual as well as asexual reproduction.

A precise analysis of the climatic variables that determine presence or absence of *Tillandsia* in the Atacama Desert is still required. This could be achieved through the modeling of paleo -and current climate along the coast of northern Chile, bearing in mind, however, that such models would assume that the ecological requirements of the species remained constant over time. Although clearly fog is the most important source in delivering humidity, its impact on the coast has varied over geological time through the intensity of the Humbold Current (Garreaud *et al.*, 2010; Sáez *et al.*, 2012) and the position of the subtropical anticyclone, which lastly defines the temperature inversion and as such the position of the stratocumulus cloud deck (Garreaud *et al.*, 2008; Lobos Roco *et al.*, 2018). Little is known also about the intensity of fog required by these plants, about slope aspect favouring the establishment of *Tillandsia* lomas, or of the preferred temperature regimes. A study on the fine scale population structure following an elevational gradient has been provided by Koch *et al.* (2019) for a single large loma. This study provides evidence for the first time, that outbreeding is linked to a moth pollinator capable of covering the distances between lomas. However, perhaps the most intriguing question arising from our study remains unanswered: why do some populations reproduce predominantly sexually, while others reproduce predominantly clonally? A precise survey of flowering times, flowering behaviour and pollination syndrome based on multiple lomas should be envisaged as an immediate next step to fill the gaps in our knowledge of the reproductive system employed by *Tillandsia* *landbeckii.* In our study, we termed the northern and southern cluster as peripheral, yet this species is distributed much farther north into Peru and possibly Ecuador (Smith & Downs, 1977; Till, 1992). An inclusion of populations from the entire range of the species may shed additional light into the actual genetic processes responsible for shaping *Tillandsa* lomas.

Despite these remaining gaps in our knowledge, our study has made an important advance in understanding the genetic processes of a species in the hyper arid environments of the Atacama. A characterization of the *Tillandsia* lomas of Peru, a more explicit temporal time frame of the origin of populations, in combination with a more detailed knowledge of the past and present ecological requirements through attempts of climate modeling may render additional interesting results in the future.

The second study of this thesis (Chapter 3) is also a population genomic study based on a paleo-endemic lineage of the Atacama, *Huidobria fruticosa* Phil. (Loasaceae). While several ecological, taxonomic and biogeographic studies in the Loasaceae family have been published (e.g. Weigend *et al.*, 2004; Ackermann & Weigend, 2006; Acuña Castillo & Weigend, 2017; Acuña Castillo *et al.*, 2017, 2019), this present study is the first analysis of population structure for the genus and including an extensive sampling of over 350 individuals in 21 populations.

Huidobria currently contains two species, *H. fruticosa* and *H. chilensis*, both of which are endemic to the Atacama Desert (Grau, 1997). According to Acuña Castillo *et al.* (2019), these two species diverged between 40 and 60 Ma, and in combination with very clear morphological differences (Grau, 1997). This may warrant a reclassification into two distinct genera.

H. fruticosa has an Andean as well as coastal distribution. Like *Tillandsia*, the seeds of *Huidobria* are dispersed by wind (Weigend *et al.*, 2004) and probably build up a seed bank reservoir. Unlike *Tillandsia*, this species appears where water accumulates following precipitation and is often found in river canyons, with preliminary data suggesting that *Huidobria* taps ground water employing a strongly developed tap root (Acuña Castillo & Weigend, 2017; Jaeschke *et al.*, 2019). Following precipitation events, mass occurrences of this species have been observed, where a few months before there was barren land (pers. obs.). These ecological peculiarities, combined with the estimated divergence age of this lineage raised the question, whether we could detect in its genetic signature signs of the long-lasting aridity proposed for the Atacama by Dunai *et al.* (2005) or Hartley *et al.* (2005). If this was the case, we would expect high values of genetic differentiation (F_{ST}) as compared to e.g. neo-endemic lineages such as *Tillandsia.* A colonization of the coastal Atacama from the Andean populations through dispersal along the desert canyons that cross the inland desert pampa seemed feasible.

Our results are surprising, because they suggest a recent population structure of this ancient endemic lineage. Although *Huidobria* has likely witnessed the evolution of hyper aridity of the core Atacama, the genetic structure of populations is, according to our data, of Pleistocene origin, ca. 2 Ma and younger. Similar to the previous study of *Tillandsia*, we detected a split of population clusters into several more or less distinct genetic clusters that were geographically isolated from each other. Interestingly, apart from a geographically northern cluster, there was only very limited gene flow between coastal and Andean populations and the genetically most differentiated cluster consisted of the southernmost populations on the coast. This does not support our initial hypothesis of Andean origin of *Huidobria* with a subsequent colonization of the coast via river canyons, but points to a more complex population history along the coastal cordillera, possibly involving refugial areas where the species persisted during unfavourable conditions, as has been suggested for other Atacama lineages with a predominantly coastal distribution (Ossa *et al.*, 2013, 2017). While the precise origins of *Huidobria* remain elusive, the age of the splitting of population clusters which we retrieved in our study correlates remarkably well with phases of prolonged aridity dated to the same period by cosmogenic nuclide exposure dating of ancient shoreline terraces (Ritter *et al.*, 2018). This finding is also in line with other results obtained for Atacama lineages, which have shown a clear association of Pliocene-Pleistocene climatic oscillations to affect plant evolution (e.g. Baranzelli *et al.*, 2014; Hershkovitz *et al.*, 2006; Ossa *et al.*, 2013, 2017; Viruel *et al.*, 2012). Some aspects of future study of *Huidobria* should include the analysis of environmental parameters which determine the distribution of current *Huidobria* populations. Further, the soil seed bank deserves more attention, as this is an important parameter of the general population genetic diversity, which may even surpass the genetic diversity of extant populations.

The third chapter is an analysis of the origin and diversity of the genus *Eulychnia* Phil. (Cactaceae; Chapter 4). *Eulychnia* is a mostly Chilean genus, with a single species disjunctly distributed in southern Peru (Cullmann, 1958). Based on phylogenies obtained from two different molecular approaches, Sanger sequencing and genotyping by sequencing, we estimated the divergence of *Eulychnia* from its sister genus *Austrocactus* through molecular clock dating, and provided a biogeographical analysis of the diversification within *Eulychnia*. Through a complete taxon sampling, which was gathered during the several field work campaigns, we were able to obtain better resolution in our phylogenies than was achieved in previous studies by Hernández-Hernández *et al.* (2014) or Larridon *et al.* (2018). It is well known that increasing the number of species tends to increase the age of the clades (Linder & Rieseberg, 2004), so we anticipated a more precise age estimate, which was also achieved.

According to our results, *Eulychnia* originated at the Miocene-Pliocene boundary, approximately 7 Ma and subsequently diversified into the Atacama arid environments. The main finding of this study was, that the divergence time estimates between major clades of *Eulychnia* correspond to prolonged phases of aridity during the Pleistocene as demonstrated by Ritter *et al.* (2018), paralleling our previous results obtained for *Huidobria*. We suggest that the current allopatric distribution of *Eulychnia* is the consequence of habitat fragmentation during Pleistocene phases of aridity, which in turn caused the disjunct distribution of the *E. ritteri* into Peru as relic populations.

A further interesting finding was an incongruence between the phylogenies obtained from genotyping by sequencing and the phylogeny obtained from three plastid markers. The disjunct taxon *E. ritteri* from Peru clustered as sister to all other taxa in the GBS phylogeny, while in the cp-cladogram, it was retrieved as sister to the *E. breviflora* clade. This incongruence may be due to several reasons, including different phylogenetic signals from the two data sets, or ancestral interspecific hybridization/introgression. A further possibility may be chloroplast capture stemming from introgression events. This phenomenon is common in plants (Rieseberg & Soltis, 1991; Acosta & Premoli, 2010), and considering that introgression in *Eulychnia* is likely to occur (Ritter, 1980; Eggli & Leuenberger, 1998), this seems a logical explanation for the incongruence of our phylogenies. Further population genomic work may elucidate the evolutionary history of this genus further and particularly resolve remaining questions about the possible hybrid origin of current species.

7.2 Taxonomic investigations

Floristic accounts in the traditional sense such as produced for the Atacama Desert by Philippi (1860); Johnston (1929, 1932); Dillon & Luebert (2014) or Dillon *et al.* (2011) are nowadays often associated with much painstaking work for little scientific impact. Yet producing basic floristic inventories, taxonomic accounts and nomenclatural revisions are much needed efforts toward taxonomic and nomenclatural coherence, as the foundation not only of any subsequent investigations, but also to communicate such investigations to a broad scientific community in a standardised fashion.

The genus *Ophryosporus* with currently over 40 accepted species is disjunct between the Andes from Colombia to Chile and the Atlantic Forest in southern Brazil and northern Argentina (King & Robinson, 1972a). The here presented taxonomic revision (Chapter 6) forms the basis for any future work on the genus in Chile, because it clarifies the number of species present in the country, as well as their taxonomic and nomenclatural status, since no recent published revision of the genus is available (with the exception of an unpublished thesis by Plos (2012)). A taxonomic treatment of *Ophryosporus* throughout its entire distribution range is still much needed. For Chile, a population genomic analysis of the six species herein recognised, and based on ca. 800 individuals is currently in preparation.

Eulychnia Phil., Cactaceae, is a dominant feature of the Atacama Desert (Merklinger, 2018). No systematic revision since Ritter (1980, 1981) exists, and the *Eulychnia* names described by Philippi remained untypified until the study of Leuenberger & Eggli (2000). Names have been added sporadically (Hoxey & Klaassen, 2011; Guerrero & Walter, 2019) using numerous morphological characters or phylogenetic position, or names have been accepted provisionally based on no obvious foundation at all (Hunt, 2006). The revision presented here (Chapter 5) could be regarded as the logical consequence of our previous study of *Eulychnia* (Chapter 4), in which we show that the genus is of recent origin and likely in the process of speciation. Based on these results we devised a taxonomic concept that reflects the findings from our molecular phylogenetic analyses, and which includes an analysis of the morphological diversity within *Eulychnia*, while resolving taxonomic and nomenclatural problems traditionally associated with the genus (Leuenberger & Eggli, 2000; Hunt, 2002).

Several biotic and abiotic processes work in synergy to explain the current distribution and dynamics of plant populations in the Atacama Desert. The variation in climate, in particular phases of prolonged aridity are a key element in shaping the microevolutionary processes that ultimately cause the divergence and diversification of lineages. Plant evolution on a deep geological timescale is directly impacted by such geological and climatological processes (Axelrod, 1967; Gillespie & Roderick, 2014). The Atacama is an extreme environment, where evolutionary processes have been affected by long-term aridity. According to Stebbins (1952) or Gaston & Spicer (2004), extinction rates and spatio-temporal species turnover and associated genetic processes are more severe under arid conditions than in more stable environments. Climatic oscillations favour the contraction and subsequent expansion of habitats, and cause individuals and populations to become isolated, and to undergo genetic drift or acquire new adaptive traits, sometimes reconnect with other species, and ultimately speciate. This is probably the key aspect shared by all studies presented here. On the contrary, the key aspect in which these studies differ from each other, must be sought in their individual evolutionary history, with the time scales of these processes as the key difference hindering generalising statements of plant evolutionary processes in arid environments.

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Summary

Microevolutionary patterns and taxonomic investigations of the Atacama Desert flora

Plant evolutionary processes in the hyper arid Atacama Desert of northern Chile are here investigated using our species/species complexes as case studies based on the following research questions:

(1) Are populations of *Tillandsia* genetically isolated from each other or do they maintain gene flow across potential barriers such as patches of hyper arid landscape or surface rivers?
(2) Have populations of *Huidobria* undergone long-term isolation in parallel to the long-lasting aridity of the Atacama Desert?
(3) When did *Eulychnia* originate, and when and how did it diversify into the Atacama Desert?
(4) Can the considerations regarding of question (3) contribute to produce a taxonomic concept for *Eulychnia*?
(5) How many species of *Ophryosporus* are there in Chile?

These research questions are addressed employing a combination of modern population genetic methods (genotyping by sequencing, GBS), molecular phylogenetic methods of Sanger sequencing, as well as traditional alpha taxonomy based on morphological analyses and literature/herbarium review and research.

The dissertation is presented in a cumulative style. It consists of papers that have already been published or accepted for publication, as well as chapters currently in working progress and soon to be submitted to the relevant journals. Each of the five chapters contains its respective introduction and conclusive considerations.

Chapter 1 is a general introduction, which provides an overview of the Atacama Desert, and in which the central research questions of this dissertation are formulated; an overview of the dissertation is provided.

Chapter 2 is a population genomic study of *Tillandsia landbeckii* (Bromeliaceae). Genetic diversity and gene flow within and between populations was analysed, including a heterozygosity analysis, F-statistics (F_{ST} and F_{IS}), genetic structure, isolation by distance and an interpretation of the data to explain the current distribution of the sampled lomas in the light of landscape evolution in the Atacama. Our results show that founder effects through stepping-stone dispersal are likely responsible for the formation of *Tillandsia* lomas, and that individuals of different lomas reproduce sexually and/or asexually (clonally). Gene flow between lomas belonging to three geo-

graphically and genetically distinct clusters is limited and appears to be impacted by barriers, possibly surface rivers and distance across hyper arid landscape.

Chapter 3 is a population genomic study of *Huidobria fruticosa* (Loasaceae), an ancient endemic lineage that diverged from its closest known relative between 40–60 Mya. The aim of this chapter was to correlate the evolution of this species to the evolution of aridity in the Atacama Desert. It was expected to find a high genetic differentiation mirroring long-term isolation of populations in aridity. However, our analysis of SNP data employing population genomic methods such as measures of genetic diversity $\theta\pi$, F-statistics (F_{ST}), genetic structure and measures of time units based on an assumption of mutation rate showed, that the populations of this species are probably of recent origin since the Pleistocene. As in *Tillandsia*, the populations fell into genetically and geographically distinct clusters, with an estimated divergence age from each other matching geological data which suggests phases of prolonged aridity as the cause of these population cluster-splits. Our results further suggest that effective dispersal and a soil seed bank impact the genetic diversity in *Huidobria* and probably mitigate extinction risk of sub-populations.

Chapter 4 provides an estimation of the divergence time between two genera of the Cactaceae, *Eulychnia* and its sister genus *Austrocactus*. The aim of this study to elucidate the origins of *Eulychnia* and to reconstruct the diversification of this genus into the Atacama Desert. Phylogenies were produced from two differing data sets based on a complete taxon sampling of the genus *Eulychnia*. The first phylogeny was based on three plastid markers which were merged into a previously published family-wide phylogeny of the Cactaceae. A second phylogeny was calculated using SNP data obtained from GBS. Both phylogenies were molecular clock dated and a biogeographical analysis was produced. Our results provide an estimated divergence age of *Eulychnia* from *Austrocactus* approximately 7 Mya toward the Miocene-Pliocene boundary. Our biogeographic analysis revealed, that *Eulychnia* has evolved as a coastal taxon, and diversified in response to prolonged phases of aridity during the Pleistocene. Diversification age estimates correlate, as in the previous study on *Huidobria*, with geological results obtained from cosmogenic nuclide exposure dating and we interpret the current allopatric distribution of *Eulychnia* taxa as the result of habitat fragmentation through prolonged arid phases. The very recent diversification within this genus in combination with distinct hybrid zones at range limits, points to the preliminary assumption that speciation processes are still underway, with taxa not yet having reached full reproductive isolation. This idea has led to the following chapter.

Chapter 5 provides a taxonomic revision of the genus *Eulychnia* in the light of the results obtained from the previous chapter and the idea that speciation in *Eulychnia* has not yet reached reproductive isolation between taxa. The genus has a

checkered taxonomic history and posed several issues regarding the type specimens of names provided by Friedrich Ritter. The original names published by Philippi remained untypified until twenty years ago. Several recently published species needed a new taxonomic circumscription. In this synopsis we accept three species and nine subspecies of *Eulychnia* in line with morphological variation, geographic distribution and phylogenetic results.

Chapter 6 provides a taxonomic revision of the genus *Ophryosporus* for Chile. There is no recent revision of the genus and confusion existed over names applied to Chilean plants. Some Chilean members of *Ophryosporus* have only been collected sporadically and a morphological analysis was required to ascertain their taxonomic relationship. Nomenclatural issues were resolved, including some necessary lectotypifications and an epitypification of names, and a key to the Chilean species of *Ophryosporus* was provided. This work recognises six species of *Ophryosporus* in Chile and forms the basis for any further studies on the genus.

List of publications

Atacama Desert

Merklinger FF and Luebert F (2020) Notes on *Ophryosporus* in Chile. *Phytokeys* (in press).

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Abbreviations

alt.	altitude
bp	basepair
cf.	*confer/conferatur* (compare)
coll. no.	collection number
comb.	combination
CRC	Collaborative Research Centre
DEC	Dispersal Extinction Cladogenesis
Dept.	Department
DNA	deoxyribonucleic acid
e.g.	*exempli gratia* (for example)
elev.	elevation
et al.	*et alia* (and others)
Fig.	figure
GBS	genotyping by sequencing
HPD	highest posterior density
i.e	*id est* (in other words)
in prep.	in preparation
in rev.	in review
incl.	*inclusus*
K	thousand
m	metres
Ma	*mega annum* (million years)
ML	Maximum Likelihood
mya	million years ago
ng	nanogram
nov.	*novel* (new)
PP	posterior probability
Prov.	Province
s.a.	*sine anno* (without year)
s.n.	*sine numerus* (without number)
SEM	Scanning Electron Microscopy
SFB	Sonderforschungsbereich
sp.	species
spp.	species (plural)
stat.	status
subm.	submitted
subsp.	subspecies
var.	variety
µl	microlitre

Appendix A

Supplementary data to Chapter 2

Table S1: Summary of F_{ST} and number of sites in a pairwise comparison among all populations.

Pop 1	**Pop 2**	**Sum F_{ST}**	**Sites**	**Mean F_{ST}**
Pop 18	Pop 5	285.31311321772	1956	0.14586559980456
Pop 18	Pop 4	277.314990488705	1547	0.179259851641051
Pop 18	Pop 6	369.528837258273	1702	0.217114475474896
Pop 18	Pop 7	287.18778990629	1500	0.191458526604193
Pop 18	Pop 11	293.446989089316	1845	0.159049858584995
Pop 18	Pop 12	269.48244085741	1883	0.143113351490924
Pop 18	Pop 1	480.925768337026	1672	0.287635028909705
Pop 18	Pop 20	340.696731911899	1717	0.19842558643675
Pop 18	Pop 3	275.383316891033	1348	0.20429029442955
Pop 18	Pop 2	290.489179533626	1280	0.226944671510646
Pop 18	Pop 8	271.394782214798	2148	0.126347663973369
Pop 18	Pop 9	294.526919577473	2082	0.141463458010313
Pop 18	Pop 10	290.929804330722	2537	0.114674735644746
Pop 18	Pop 15	274.691454854623	2203	0.124689720769234
Pop 18	Pop 14	267.034976247175	1963	0.136034119331215
Pop 18	Pop 13	258.387755901136	1830	0.141195495027943
Pop 18	Pop 17	268.069859799225	1799	0.149010483490397
Pop 18	Pop 21	309.49545374034	1900	0.162892344073863
Pop 18	Pop 19	296.951520761084	2151	0.138052775807106
Pop 18	Pop 16	197.738760774509	1649	0.119914348559435
Pop 5	Pop 4	87.221779419933	1815	0.04805607681539
Pop 5	Pop 6	184.046495385797	2053	0.089647586646759
Pop 5	Pop 7	174.98346381796	2054	0.085191559794528
Pop 5	Pop 11	154.680717800332	2260	0.068442795486872
Pop 5	Pop 12	142.367095969211	2288	0.062223381105424
Pop 5	Pop 1	338.213309395748	1917	0.176428434739566
Pop 5	Pop 20	356.935548675936	2331	0.15312550350748
Pop 5	Pop 3	138.707855450845	1940	0.071498894562291
Pop 5	Pop 2	177.811501438735	1886	0.094279693233688
Pop 5	Pop 8	126.218752591356	2610	0.048359675322359
Pop 5	Pop 9	159.848214284625	2559	0.062465109138189
Pop 5	Pop 10	121.145573734803	2740	0.044213713041899
Pop 5	Pop 15	155.597379649868	2640	0.058938401382526
Pop 5	Pop 14	127.538583331837	2398	0.053185397552893
Pop 5	Pop 13	122.86919648913	2322	0.052915243966034
Pop 5	Pop 17	341.574413064626	2562	0.13332334623912
Pop 5	Pop 21	337.646738593317	2486	0.135819283424504
Pop 5	Pop 19	319.813404304495	2732	0.117062007432099
Pop 5	Pop 16	164.929637170344	2289	0.072053139873457
Pop 4	Pop 6	267.927832497579	1889	0.141835803333816
Pop 4	Pop 7	158.243574586284	1678	0.094304871624723

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Table S1: Continued from previous page

Pop 1	**Pop 2**	**Sum F_{ST}**	**Sites**	**Mean F_{ST}**
Pop 4	Pop 11	145.446403366579	1900	0.076550738613989
Pop 4	Pop 12	133.816549864187	1947	0.068729609586126
Pop 4	Pop 1	423.068833301805	1752	0.241477644578656
Pop 4	Pop 20	378.081880895449	1975	0.191433863744531
Pop 4	Pop 3	110.573942819412	1550	0.071338027625427
Pop 4	Pop 2	153.343034056068	1478	0.103750361336988
Pop 4	Pop 8	111.033245584495	2291	0.048464969700783
Pop 4	Pop 9	157.463374147435	2235	0.070453411251649
Pop 4	Pop 10	145.017396097798	2642	0.054889249090764
Pop 4	Pop 15	150.150431620936	2316	0.064831792582442
Pop 4	Pop 14	112.146913077241	2051	0.0546791385067
Pop 4	Pop 13	103.334734667452	1957	0.052802623744227
Pop 4	Pop 17	360.40975698822	2208	0.163229056606984
Pop 4	Pop 21	346.296644836073	2138	0.161972237996293
Pop 4	Pop 19	336.774182890726	2386	0.141145927447916
Pop 4	Pop 16	155.023954336387	1927	0.08044834163798
Pop 6	Pop 7	297.975580808503	1825	0.163274290853974
Pop 6	Pop 11	261.470375056867	2160	0.121051099563365
Pop 6	Pop 12	241.540117977198	2175	0.111052927805608
Pop 6	Pop 1	315.424212311482	1868	0.168856644706361
Pop 6	Pop 20	362.251713311186	2097	0.172747598145534
Pop 6	Pop 3	294.137698963258	1729	0.170120126641561
Pop 6	Pop 2	308.787786694675	1653	0.186804468659816
Pop 6	Pop 8	253.404091276876	2407	0.105277977265009
Pop 6	Pop 9	254.650399930594	2347	0.108500383438685
Pop 6	Pop 10	195.779552196641	2552	0.076716125468903
Pop 6	Pop 15	251.475328465755	2459	0.102267315358176
Pop 6	Pop 14	252.750949887853	2247	0.112483733817469
Pop 6	Pop 13	254.159206205949	2103	0.120855542656181
Pop 6	Pop 17	344.611102123971	2354	0.146393841174159
Pop 6	Pop 21	354.773670015517	2316	0.153183795343487
Pop 6	Pop 19	325.549312904601	2542	0.128068179742172
Pop 6	Pop 16	254.254221869653	2078	0.122355255952672
Pop 7	Pop 11	182.386223878495	1823	0.100047297794018
Pop 7	Pop 12	145.767171380932	1860	0.078369446978996
Pop 7	Pop 1	471.246895774431	1885	0.249998353195984
Pop 7	Pop 20	366.959484725045	1929	0.190233014372755
Pop 7	Pop 3	155.790579877112	1456	0.106999024640874
Pop 7	Pop 2	181.165997201547	1355	0.133701842953171
Pop 7	Pop 8	125.263067819679	2106	0.059479139515517
Pop 7	Pop 9	178.420258850671	2103	0.084840826842925
Pop 7	Pop 10	166.647072665028	2518	0.066182316388017
Pop 7	Pop 15	176.954234106647	2254	0.078506758698601

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Table S1: Continued from previous page

Pop 1	**Pop 2**	**Sum F_{ST}**	**Sites**	**Mean F_{ST}**
Pop 7	Pop 14	132.529179357686	1948	0.068033459629202
Pop 7	Pop 13	118.501405090739	1798	0.065907344321879
Pop 7	Pop 17	349.052258203434	2186	0.159676238885377
Pop 7	Pop 21	352.317438932035	2158	0.163261093110303
Pop 7	Pop 19	330.172896225187	2369	0.139372265185812
Pop 7	Pop 16	159.112379757652	1806	0.088102092889065
Pop 11	Pop 12	118.958246235954	2047	0.058113456881267
Pop 11	Pop 1	373.776236981075	2108	0.177313205398992
Pop 11	Pop 20	359.937032183908	2263	0.159053041177158
Pop 11	Pop 3	183.977964394191	1851	0.099393821930951
Pop 11	Pop 2	209.003560734073	1758	0.118887122146799
Pop 11	Pop 8	146.275731217733	2396	0.061049971292877
Pop 11	Pop 9	165.760258642461	2345	0.070686677459472
Pop 11	Pop 10	158.070348312079	2769	0.057085716255717
Pop 11	Pop 15	158.322318182587	2464	0.064254187574102
Pop 11	Pop 14	127.73240113984	2152	0.059355204990632
Pop 11	Pop 13	140.29405473967	2084	0.067319604001761
Pop 11	Pop 17	331.885683151984	2426	0.1368036616455
Pop 11	Pop 21	271.598328189239	2338	0.116166949610453
Pop 11	Pop 19	285.999495936476	2560	0.111718553100186
Pop 11	Pop 16	176.661169747924	2125	0.08313466811667
Pop 12	Pop 1	385.780014164743	2154	0.179099356622443
Pop 12	Pop 20	337.6329119192	2285	0.14776057414407
Pop 12	Pop 3	165.741669197808	1910	0.086775743035501
Pop 12	Pop 2	190.164301065139	1821	0.104428501408643
Pop 12	Pop 8	123.248145596602	2434	0.050636049957519
Pop 12	Pop 9	157.258055424161	2406	0.065360787790591
Pop 12	Pop 10	143.37340801618	2798	0.051241389569757
Pop 12	Pop 15	125.388691054419	2453	0.051116465982233
Pop 12	Pop 14	101.569426209501	2118	0.047955347596554
Pop 12	Pop 13	105.797076975003	2058	0.051407714759477
Pop 12	Pop 17	308.827416572773	2439	0.126620506999907
Pop 12	Pop 21	279.070893071007	2393	0.116619679511495
Pop 12	Pop 19	270.951227406027	2588	0.104695219244987
Pop 12	Pop 16	150.906858970103	2151	0.070156605750861
Pop 1	Pop 20	463.053026070927	2077	0.222943199841563
Pop 1	Pop 3	486.105640960451	1793	0.271113017825126
Pop 1	Pop 2	487.111082433086	1694	0.287550816076202
Pop 1	Pop 8	452.667983702932	2499	0.181139649340909
Pop 1	Pop 9	448.952032916645	2415	0.185901462905443
Pop 1	Pop 10	406.251509768339	2657	0.152898573492036
Pop 1	Pop 15	413.458162251014	2488	0.166180933380632
Pop 1	Pop 14	429.938365435881	2284	0.188239214288914

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Table S1: Continued from previous page

Pop 1	**Pop 2**	**Sum F_{ST}**	**Sites**	**Mean F_{ST}**
Pop 1	Pop 13	455.359625507479	2209	0.206138354688764
Pop 1	Pop 17	443.758913274429	2299	0.193022580806624
Pop 1	Pop 21	375.203832367103	2125	0.176566509349225
Pop 1	Pop 19	399.601690544724	2453	0.162903257458102
Pop 1	Pop 16	432.737805339255	2136	0.202592605495906
Pop 20	Pop 3	350.369517236396	1773	0.197613940911673
Pop 20	Pop 2	345.233402447691	1699	0.203198000263503
Pop 20	Pop 8	329.801871035326	2277	0.144840523072168
Pop 20	Pop 9	303.611187804374	2213	0.137194391235596
Pop 20	Pop 10	290.700175885981	2650	0.109698179579615
Pop 20	Pop 15	301.425670209933	2335	0.12909022278798
Pop 20	Pop 14	344.609080722793	2350	0.146642162009699
Pop 20	Pop 13	346.384221660981	2218	0.156169622029297
Pop 20	Pop 17	54.4523728261447	1954	0.027867130412561
Pop 20	Pop 21	222.48568793375	1873	0.118785738352242
Pop 20	Pop 19	101.348989210831	2146	0.04722692880281
Pop 20	Pop 16	289.375958235438	2058	0.140610280969601
Pop 3	Pop 2	34.3451885032789	1049	0.03274088513182
Pop 3	Pop 8	124.476404433505	2139	0.058193737463069
Pop 3	Pop 9	170.961291442154	2077	0.082311647300026
Pop 3	Pop 10	159.629162593005	2499	0.063877215923572
Pop 3	Pop 15	169.536056618445	2219	0.076402008390467
Pop 3	Pop 14	123.840075459866	1955	0.063345307140596
Pop 3	Pop 13	106.001960884438	1795	0.059054017205815
Pop 3	Pop 17	338.162392006607	2037	0.16601001080344
Pop 3	Pop 21	343.917144230783	2017	0.170509243545257
Pop 3	Pop 19	323.637506249438	2254	0.143583631876414
Pop 3	Pop 16	137.724356229079	1698	0.081109750429375
Pop 2	Pop 8	157.160147445767	2038	0.077114890797727
Pop 2	Pop 9	203.46468893279	1999	0.101783236084437
Pop 2	Pop 10	189.303096085415	2441	0.077551452718319
Pop 2	Pop 15	197.057111233311	2142	0.091996783955794
Pop 2	Pop 14	152.994952049657	1877	0.08151036337222
Pop 2	Pop 13	138.701969628741	1721	0.08059382314279
Pop 2	Pop 17	332.579268127985	1964	0.169337712896123
Pop 2	Pop 21	335.890666236288	1937	0.173407674876762
Pop 2	Pop 19	315.082023166472	2157	0.146074187837956
Pop 2	Pop 16	153.759311361169	1619	0.094971779716596
Pop 8	Pop 9	95.310718672224	2172	0.043881546350011
Pop 8	Pop 10	89.4372192445428	2595	0.034465209728148
Pop 8	Pop 15	120.591457897239	2360	0.051098075380186
Pop 8	Pop 14	95.7819072161254	2476	0.038684130539631
Pop 8	Pop 13	81.3476034949179	2361	0.03445472405545

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Table S1: Continued from previous page

Pop 1	**Pop 2**	**Sum F_{ST}**	**Sites**	**Mean F_{ST}**
Pop 8	Pop 17	314.229042783989	2495	0.125943504121839
Pop 8	Pop 21	325.46405002278	2491	0.130655981542666
Pop 8	Pop 19	300.055704882611	2700	0.111131742549115
Pop 8	Pop 16	134.564692404667	2409	0.055859150022693
Pop 9	Pop 10	63.7561116610781	2559	0.024914463329847
Pop 9	Pop 15	122.755693887693	2300	0.053372040820736
Pop 9	Pop 14	138.677559960092	2443	0.056765272189968
Pop 9	Pop 13	131.841472801919	2337	0.056414836457817
Pop 9	Pop 17	288.486223802456	2418	0.119307784864539
Pop 9	Pop 21	318.61825067976	2397	0.132923759148836
Pop 9	Pop 19	280.624511906561	2611	0.107477790848932
Pop 9	Pop 16	170.699828778864	2358	0.072391784893496
Pop 10	Pop 15	118.440797261335	2730	0.043384907421735
Pop 10	Pop 14	128.447544102303	2865	0.044833348726807
Pop 10	Pop 13	118.617828503464	2729	0.043465675523439
Pop 10	Pop 17	276.064132524655	2856	0.096661110827961
Pop 10	Pop 21	304.731742523538	2824	0.107907840836947
Pop 10	Pop 19	266.777967536488	3047	0.087554305066127
Pop 10	Pop 16	156.472359061011	2773	0.056427103880639
Pop 15	Pop 14	100.344812173008	2397	0.041862666738844
Pop 15	Pop 13	120.321251336381	2381	0.050533914882983
Pop 15	Pop 17	278.46772677467	2506	0.111120401745678
Pop 15	Pop 21	282.426835079418	2483	0.113744194554739
Pop 15	Pop 19	253.060515147918	2664	0.094992685866336
Pop 15	Pop 16	165.552323044616	2449	0.067599968576813
Pop 14	Pop 13	72.4910218149225	2062	0.03515568468231
Pop 14	Pop 17	321.273832110834	2510	0.127997542673639
Pop 14	Pop 21	307.169884874745	2473	0.124209415638797
Pop 14	Pop 19	291.467765660633	2642	0.11032088026519
Pop 14	Pop 16	135.082638428388	2199	0.061429121613637
Pop 13	Pop 17	327.696989463905	2420	0.135411979117316
Pop 13	Pop 21	328.276476482895	2399	0.13683888140179
Pop 13	Pop 19	307.87621153365	2592	0.118779402597859
Pop 13	Pop 16	122.267271385403	2064	0.059238019082075
Pop 17	Pop 21	192.06081808178	2037	0.094286115896799
Pop 17	Pop 19	83.0158139042619	2265	0.036651573467665
Pop 17	Pop 16	258.175776044564	2223	0.116138450762287
Pop 21	Pop 19	145.567215989559	2163	0.06729875912601
Pop 21	Pop 16	272.846155786343	2272	0.120090737582017
Pop 19	Pop 16	244.722483438338	2443	0.100172936323511

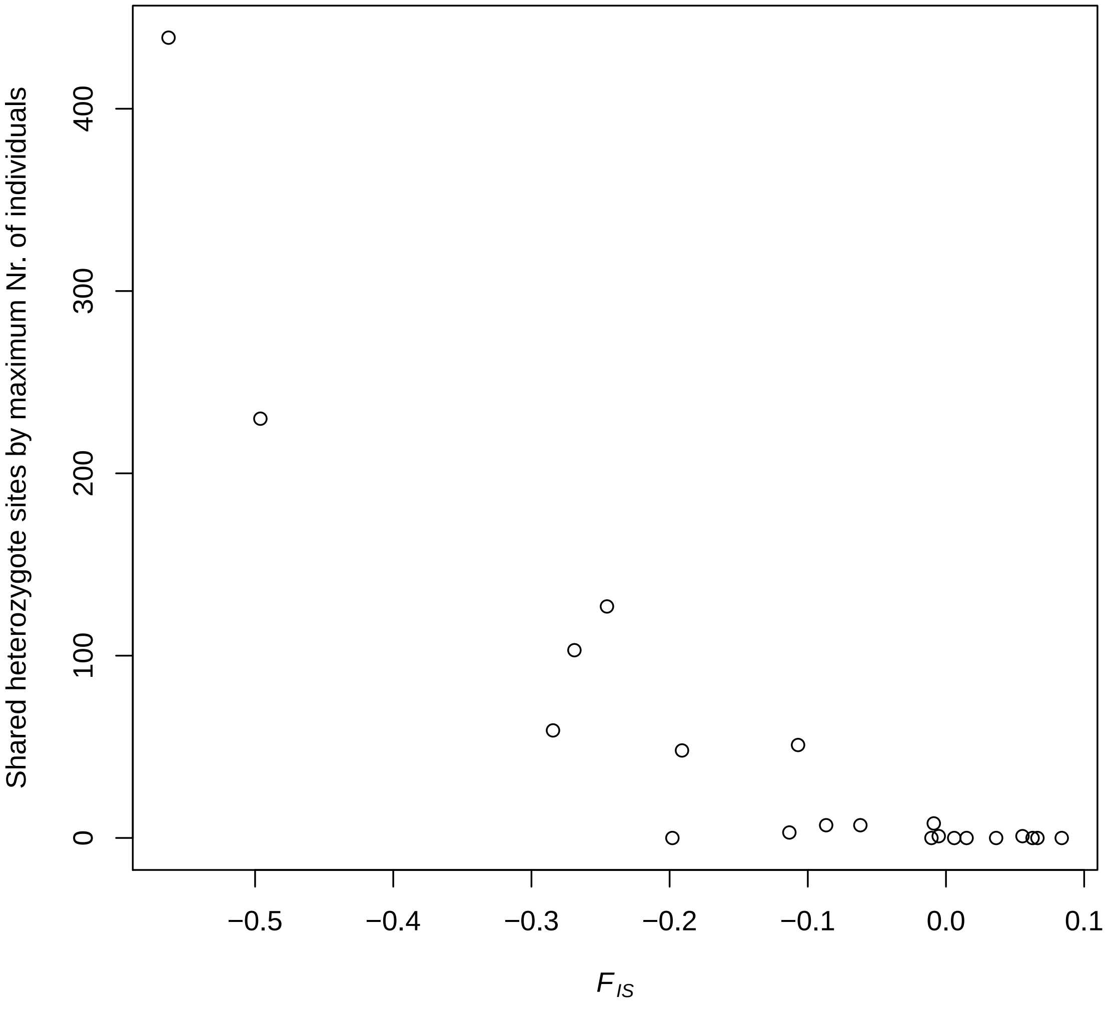

Figure S1: Plot of inbreeding coefficient F_{IS} in relation to the shared heterozygous sites by the maximum number of individuals.

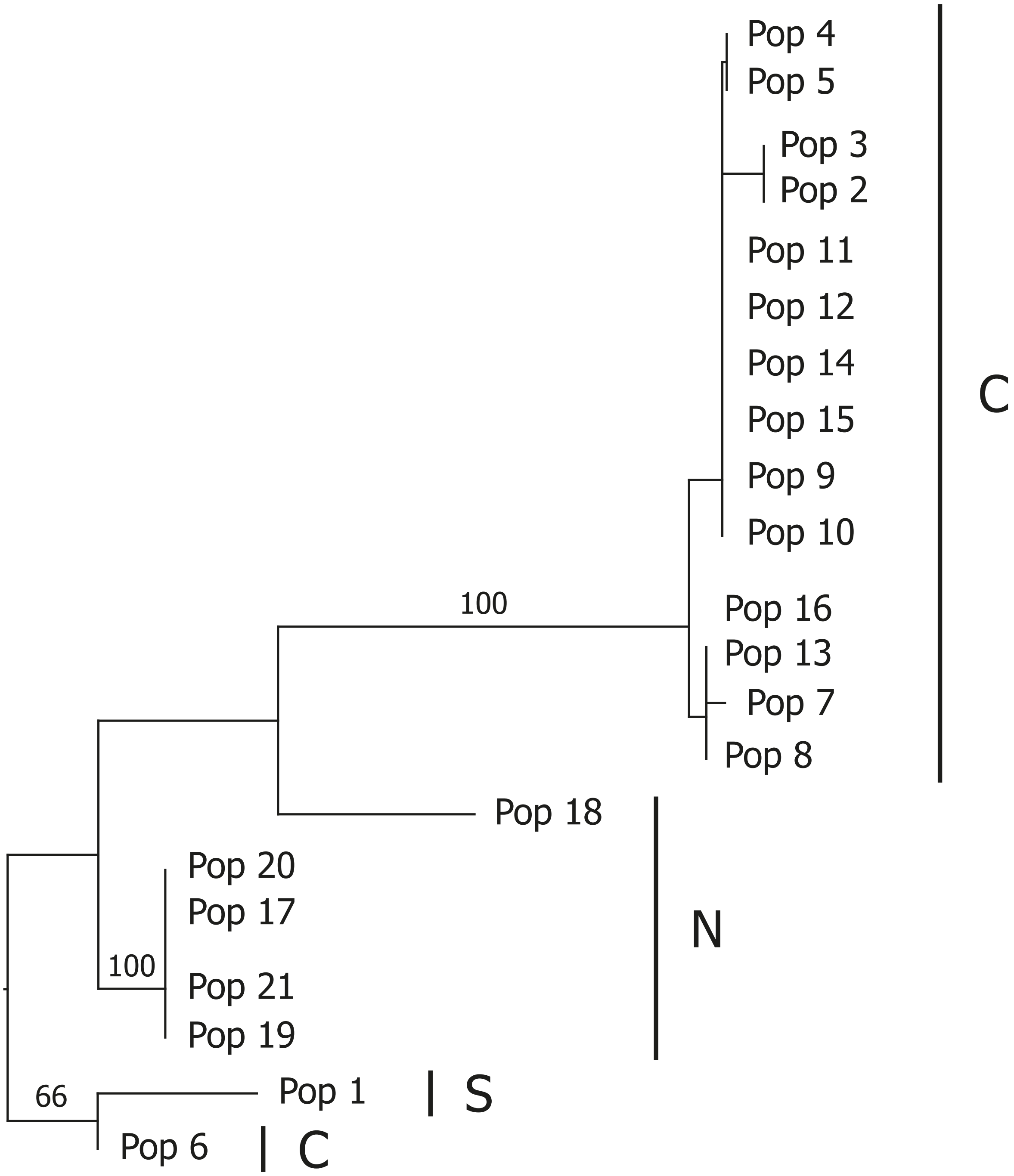

Figure S2: Maximum likelihood tree (RAxML) based on population consensus sequences using the GTRCATI model and with 100 bootstrap replicates. The tree reflects the grouping in three geographical clusters (S, C, N). The position of Pop 6 outside the central cluster could be due to the effect of missing data caused by the sampling of only eight individuals of that population.

Appendix B

Supplementary data to Chapter 3

Table S1: Summary table of all sampled *Huidobria fruticosa* populations.

Name	**Population**	**Sample**	**Department**	**Region**	**Locality**	**Latitude**	**Longitude**	**Cluster**
HF 110 1	17	ED1526	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 2	17	ED1337	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 3	17	ED1527	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 4	17	ED1528	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 5	17	ED1529	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 6	17	ED1338	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 7	17	ED1530	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 8	17	ED1339	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 9	17	ED1531	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 110 10	17	ED1532	Region I	Tarapacá	Tarapacá	-19.907274	-69.489514	NC
HF 118 1	3	ED3755	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 2	3	ED3756	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 3	3	ED3757	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 4	3	ED3758	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 5	3	ED3759	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 6	3	ED3760	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 7	3	ED3761	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 8	3	ED3762	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 9	3	ED3763	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	SC
HF 118 10	3	ED3764	Region II	Antofagasta	Cerro Paranal	-24.75786	-70.489705	AC
HF 184 1	13	ED2400	Region II	Antofagasta	NW of Chuquicamata	-22.303184	-69.076781	AC
HF 184 2	13	ED2401	Region II	Antofagasta	NW of Chuquicamata	-22.303598	-69.076633	AC
HF 184 3	13	ED2402	Region II	Antofagasta	NW of Chuquicamata	-22.304188	-69.076332	AC
HF 184 4	13	ED2403	Region II	Antofagasta	NW of Chuquicamata	-22.305883	-69.076033	AC
HF 184 5	13	ED2404	Region II	Antofagasta	NW of Chuquicamata	-22.306532	-69.075795	AC
HF 184 6	13	ED2405	Region II	Antofagasta	NW of Chuquicamata	-22.306949	-69.075614	AC
HF 184 7	13	ED2406	Region II	Antofagasta	NW of Chuquicamata	-22.307308	-69.075568	AC
HF 184 8	13	ED2407	Region II	Antofagasta	NW of Chuquicamata	-22.308041	-69.075065	AC
HF 184 9	13	ED2408	Region II	Antofagasta	NW of Chuquicamata	-22.308498	-69.075494	AC
HF 184 10	13	ED2409	Region II	Antofagasta	NW of Chuquicamata	-22.308507	-69.075207	AC
HF 184 11	13	ED2410	Region II	Antofagasta	NW of Chuquicamata	-22.30895	-69.074946	AC
HF 184 12	13	ED2411	Region II	Antofagasta	NW of Chuquicamata	-22.309388	-69.074815	AC
HF 184 13	13	ED2412	Region II	Antofagasta	NW of Chuquicamata	-22.309445	-69.074621	AC
HF 184 14	13	ED2413	Region II	Antofagasta	NW of Chuquicamata	-22.309507	-69.074481	AC
HF 184 15	13	ED2414	Region II	Antofagasta	NW of Chuquicamata	-22.309875	-69.074672	AC
HF 184 16	13	ED2415	Region II	Antofagasta	NW of Chuquicamata	-22.310261	-69.074889	AC
HF 184 17	13	ED2416	Region II	Antofagasta	NW of Chuquicamata	-22.310566	-69.07484	AC
HF 184 18	13	ED2417	Region II	Antofagasta	NW of Chuquicamata	-22.310943	-69.074819	AC
HF 184 19	13	ED2418	Region II	Antofagasta	NW of Chuquicamata	-22.311368	-69.075008	AC
HF 184 20	13	ED2419	Region II	Antofagasta	NW of Chuquicamata	-22.311569	-69.075051	AC

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Table S1: Continued from previous page

Name	**Population**	**Sample**	**Department**	**Region**	**Locality**	**Latitude**	**Longitude**	**Cluster**
HF 184 21	13	ED2420	Region II	Antofagasta	NW of Chuquicamata	-22.293359	-69.095077	AC
HF 184 22	13	ED2421	Region II	Antofagasta	NW of Chuquicamata	-22.293468	-69.095877	AC
HF 184 23	13	ED2422	Region II	Antofagasta	NW of Chuquicamata	-22.293297	-69.096618	AC
HF 185 1	14	ED2423	Region II	Antofagasta	W of Chuquicamata	-22.288609	-69.176007	AC
HF 185 2	14	ED2424	Region II	Antofagasta	W of Chuquicamata	-22.288605	-69.176565	AC
HF 185 3	14	ED2425	Region II	Antofagasta	W of Chuquicamata	-22.289063	-69.17776	AC
HF 185 4	14	ED2426	Region II	Antofagasta	W of Chuquicamata	-22.2893	-69.178468	AC
HF 185 5	14	ED2427	Region II	Antofagasta	W of Chuquicamata	-22.289135	-69.178771	AC
HF 185 6	14	ED2428	Region II	Antofagasta	W of Chuquicamata	-22.28945	-69.179197	AC
HF 185 7	14	ED2429	Region II	Antofagasta	W of Chuquicamata	-22.289899	-69.185081	AC
HF 185 8	14	ED2430	Region II	Antofagasta	W of Chuquicamata	-22.289702	-69.185293	AC
HF 185 9	14	ED2431	Region II	Antofagasta	W of Chuquicamata	-22.289681	-69.185362	AC
HF 185 10	14	ED2432	Region II	Antofagasta	W of Chuquicamata	-22.289496	-69.185684	AC
HF 185 11	14	ED2433	Region II	Antofagasta	W of Chuquicamata	-22.289595	-69.185979	AC
HF 185 12	14	ED2434	Region II	Antofagasta	W of Chuquicamata	-22.289574	-69.186272	AC
HF 185 13	14	ED2435	Region II	Antofagasta	W of Chuquicamata	-22.28946	-69.186266	AC
HF 185 14	14	ED2436	Region II	Antofagasta	W of Chuquicamata	-22.289162	-69.186707	AC
HF 185 15	14	ED2437	Region II	Antofagasta	W of Chuquicamata	-22.289078	-69.186919	AC
HF 185 16	14	ED2438	Region II	Antofagasta	W of Chuquicamata	-22.288469	-69.190986	AC
HF 185 17	14	ED2439	Region II	Antofagasta	W of Chuquicamata	-22.288282	-69.193044	AC
HF 185 18	14	ED2440	Region II	Antofagasta	W of Chuquicamata	-22.288411	-69.193249	AC
HF 185 19	14	ED2441	Region II	Antofagasta	W of Chuquicamata	-22.287983	-69.194229	AC
HF 185 20	14	ED2442	Region II	Antofagasta	W of Chuquicamata	-22.288094	-69.196525	AC
HF 2019 1	1	ED5162	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 2	1	ED5163	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 3	1	ED5164	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 4	1	ED5165	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 5	1	ED5166	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 6	1	ED5167	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 7	1	ED5168	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 8	1	ED5169	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 9	1	ED5170	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 10	1	ED5171	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 11	1	ED5172	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 12	1	ED5173	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 13	1	ED5174	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 2019 14	1	ED5175	Region II	Antofagasta	Quebrada San Ramón	-25.38642	-70.4441	SC
HF 303 1	12	ED1830	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786979	-68.164579	AC
HF 303 2	12	ED1831	Region II	Antofagasta	Tilomonte to Tilopozo	-23.787264	-68.165472	AC
HF 303 3	12	ED1832	Region II	Antofagasta	Tilomonte to Tilopozo	-23.787264	-68.165472	AC
HF 303 4	12	ED1833	Region II	Antofagasta	Tilomonte to Tilopozo	-23.78712	-68.165255	AC

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Table S1: Continued from previous page

Name	**Population**	**Sample**	**Department**	**Region**	**Locality**	**Latitude**	**Longitude**	**Cluster**
HF 303 5	12	ED1834	Region II	Antofagasta	Tilomonte to Tilopozo	-23.787248	-68.165474	AC
HF 303 6	12	ED1835	Region II	Antofagasta	Tilomonte to Tilopozo	-23.787248	-68.165474	AC
HF 303 7	12	ED1836	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786487	-68.166327	AC
HF 303 8	12	ED1837	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786487	-68.166327	AC
HF 303 9	12	ED1838	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786279	-68.166236	AC
HF 303 10	12	ED1839	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786279	-68.166236	AC
HF 303 11	12	ED1840	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786279	-68.166236	AC
HF 303 12	12	ED1841	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786279	-68.166236	AC
HF 303 13	12	ED1842	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786086	-68.165966	AC
HF 303 14	12	ED1843	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786086	-68.165966	AC
HF 303 15	12	ED1844	Region II	Antofagasta	Tilomonte to Tilopozo	-23.786086	-68.165966	AC
HF 303 16	12	ED1845	Region II	Antofagasta	Tilomonte to Tilopozo	-23.78604	-68.165855	AC
HF 303 17	12	ED1846	Region II	Antofagasta	Tilomonte to Tilopozo	-23.785886	-68.165731	AC
HF 303 18	12	ED1847	Region II	Antofagasta	Tilomonte to Tilopozo	-23.785886	-68.165731	AC
HF 303 19	12	ED1848	Region II	Antofagasta	Tilomonte to Tilopozo	-23.785886	-68.165731	AC
HF 303 20	12	ED1849	Region II	Antofagasta	Tilomonte to Tilopozo	-23.785727	-68.165499	AC
HF 303 21 182 1	12	ED2397	Region II	Antofagasta	Tilomonte to Tilopozo	-23.782144	-68.163171	AC
HF 303 22 182 2	12	ED2398	Region II	Antofagasta	Tilomonte to Tilopozo	-23.781869	-68.162475	AC
HF 303 23 182 3	12	ED2399	Region II	Antofagasta	Tilomonte to Tilopozo	-23.781869	-68.162475	AC
HF 423 1	6	ED1533	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 2	6	ED1534	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 3	6	ED1535	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 4	6	ED1536	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 5	6	ED1537	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 6	6	ED1538	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 7	6	ED1539	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 8	6	ED1540	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 9	6	ED1541	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 10	6	ED1316	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 11	6	ED1317	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 12	6	ED1318	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 13	6	ED1319	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 14	6	ED1320	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 423 15	6	ED1542	Region II	Antofagasta	Panamericana km 95	-24.287122	-70.295257	SC
HF 430 1	2	ED1543	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 2	2	ED1544	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 3	2	ED1545	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 4	2	ED1546	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 5	2	ED1321	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 6	2	ED1322	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 7	2	ED1547	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC

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Name	**Population**	**Sample**	**Department**	**Region**	**Locality**	**Latitude**	**Longitude**	**Cluster**
HF 430 8	2	ED1548	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 9	2	ED1549	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 10	2	ED1550	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 11	2	ED1551	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 12	2	ED1552	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 13	2	ED1553	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 14	2	ED1554	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 15	2	ED1555	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 16	2	ED1556	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 17	2	ED1557	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 18	2	ED1558	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 19	2	ED1323	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 430 20	2	ED1559	Region II	Antofagasta	Lala Kama (Paposo)	-25.019794	-70.380574	SC
HF 451 1	4	ED1560	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 2	4	ED1561	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 3	4	ED1562	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 4	4	ED1324	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 5	4	ED1563	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 6	4	ED1325	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 7	4	ED1326	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 8	4	ED1564	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 9	4	ED1327	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 10	4	ED1565	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 11	4	ED1566	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 12	4	ED1328	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 13	4	ED1567	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 14	4	ED1568	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 15	4	ED1569	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 16	4	ED1570	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 17	4	ED1571	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 18	4	ED1572	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 19	4	ED1573	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 451 20	4	ED1574	Region II	Antofagasta	Quebrada Caleta Botija	-24.510737	-70.553191	SC
HF 452 1	8	ED1329	Region II	Antofagasta	El Cobre to Antofagasta	-24.27407	-70.519198	SC
HF 452 2	8	ED1330	Region II	Antofagasta	El Cobre to Antofagasta	-24.27407	-70.519198	SC
HF 452 3	8	ED1331	Region II	Antofagasta	El Cobre to Antofagasta	-24.27407	-70.519198	SC
HF 452 4	8	ED1575	Region II	Antofagasta	El Cobre to Antofagasta	-24.27407	-70.519198	SC
HF 452 5	8	ED1332	Region II	Antofagasta	El Cobre to Antofagasta	-24.27407	-70.519198	SC
HF 452 6	8	ED1333	Region II	Antofagasta	El Cobre to Antofagasta	-24.27407	-70.519198	SC
HF 453 1	5	ED1576	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 2	5	ED1334	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC

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Name	**Population**	**Sample**	**Department**	**Region**	**Locality**	**Latitude**	**Longitude**	**Cluster**
HF 453 3	5	ED1577	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 4	5	ED1578	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 5	5	ED1579	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 6	5	ED1335	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 7	5	ED1580	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 8	5	ED1581	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 9	5	ED1582	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 10	5	ED1583	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 11	5	ED1584	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 12	5	ED1585	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 13	5	ED1336	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 14	5	ED1586	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 15	5	ED1587	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 16	5	ED1588	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 17	5	ED1589	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 18	5	ED1590	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 19	5	ED1591	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 453 20	5	ED1592	Region II	Antofagasta	El Cobre to Antofagasta	-24.29918333	-70.44526667	SC
HF 530 1	7	ED3128	Region II	Antofagasta	Quebrada El Cobre	-24.284374	-70.510859	SC
HF 530 2	7	ED3129	Region II	Antofagasta	Quebrada El Cobre	-24.284278	-70.511048	SC
HF 530 3	7	ED3130	Region II	Antofagasta	Quebrada El Cobre	-24.284176	-70.511193	SC
HF 530 4	7	ED3131	Region II	Antofagasta	Quebrada El Cobre	-24.283833	-70.511156	SC
HF 530 5	7	ED3132	Region II	Antofagasta	Quebrada El Cobre	-24.283057	-70.512062	SC
HF 532 1	9	ED3133	Region II	Antofagasta	El Cobre Mirador	-24.255206	-70.426387	SC
HF 532 2	9	ED3134	Region II	Antofagasta	El Cobre Mirador	-24.255104	-70.426357	SC
HF 532 3	9	ED3135	Region II	Antofagasta	El Cobre Mirador	-24.252137	-70.425451	SC
HF 532 4	9	ED3136	Region II	Antofagasta	El Cobre Mirador	-24.251894	-70.425731	SC
HF 532 5	9	ED3137	Region II	Antofagasta	El Cobre Mirador	-24.250302	-70.425435	SC
HF 532 6	9	ED3138	Region II	Antofagasta	El Cobre Mirador	-24.250269	-70.425575	SC
HF 532 7	9	ED3139	Region II	Antofagasta	El Cobre Mirador	-24.249009	-70.425195	SC
HF 532 8	9	ED3140	Region II	Antofagasta	El Cobre Mirador	-24.249016	-70.425108	SC
HF 532 9	9	ED3141	Region II	Antofagasta	El Cobre Mirador	-24.249008	-70.42496	SC
HF 532 10	9	ED3142	Region II	Antofagasta	El Cobre Mirador	-24.249078	-70.424774	SC
HF 532 11	9	ED3143	Region II	Antofagasta	El Cobre Mirador	-24.249071	-70.424459	SC
HF 532 12	9	ED3144	Region II	Antofagasta	El Cobre Mirador	-24.24871	-70.424592	SC
HF 532 13	9	ED3145	Region II	Antofagasta	El Cobre Mirador	-24.248605	-70.424872	SC
HF 532 14	9	ED3146	Region II	Antofagasta	El Cobre Mirador	-24.248483	-70.425067	SC
HF 532 15	9	ED3147	Region II	Antofagasta	El Cobre Mirador	-24.24777	-70.42494	SC
HF 532 16	9	ED3148	Region II	Antofagasta	El Cobre Mirador	-24.247538	-70.424901	SC
HF 532 17	9	ED3149	Region II	Antofagasta	El Cobre Mirador	-24.247302	-70.424912	SC
HF 532 18	9	ED3150	Region II	Antofagasta	El Cobre Mirador	-24.247118	-70.424982	SC

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Name	Population	Sample	Department	Region	Locality	Latitude	Longitude	Cluster
HF 532 19	9	ED3151	Region II	Antofagasta	El Cobre Mirador	-24.247211	-70.425349	SC
HF 532 20	9	ED3152	Region II	Antofagasta	El Cobre Mirador	-24.24772	-70.425573	SC
HF 533 1	10	ED3153	Region II	Antofagasta	El Cobre to Antofagasta	-24.232285	-70.366932	SC
HF 533 2	10	ED3154	Region II	Antofagasta	El Cobre to Antofagasta	-24.232451	-70.366945	SC
HF 533 3	10	ED3155	Region II	Antofagasta	El Cobre to Antofagasta	-24.232299	-70.366724	SC
HF 533 4	10	ED3156	Region II	Antofagasta	El Cobre to Antofagasta	-24.23214	-70.36658	SC
HF 533 5	10	ED3157	Region II	Antofagasta	El Cobre to Antofagasta	-24.232141	-70.366299	SC
HF 533 6	10	ED3158	Region II	Antofagasta	El Cobre to Antofagasta	-24.23201	-70.366219	SC
HF 533 7	10	ED3159	Region II	Antofagasta	El Cobre to Antofagasta	-24.232136	-70.366054	SC
HF 533 8	10	ED3160	Region II	Antofagasta	El Cobre to Antofagasta	-24.231987	-70.366014	SC
HF 533 9	10	ED3161	Region II	Antofagasta	El Cobre to Antofagasta	-24.232049	-70.3659	SC
HF 533 10	10	ED3162	Region II	Antofagasta	El Cobre to Antofagasta	-24.231873	-70.365755	SC
HF 533 11	10	ED3163	Region II	Antofagasta	El Cobre to Antofagasta	-24.231857	-70.365576	SC
HF 533 12	10	ED3164	Region II	Antofagasta	El Cobre to Antofagasta	-24.231915	-70.365017	SC
HF 533 13	10	ED3165	Region II	Antofagasta	El Cobre to Antofagasta	-24.231687	-70.365008	SC
HF 533 14	10	ED3166	Region II	Antofagasta	El Cobre to Antofagasta	-24.231752	-70.364772	SC
HF 533 15	10	ED3167	Region II	Antofagasta	El Cobre to Antofagasta	-24.231709	-70.364639	SC
HF 533 16	10	ED3168	Region II	Antofagasta	El Cobre to Antofagasta	-24.231532	-70.36434	SC
HF 533 17	10	ED3169	Region II	Antofagasta	El Cobre to Antofagasta	-24.23153	-70.364	SC
HF 533 18	10	ED3170	Region II	Antofagasta	El Cobre to Antofagasta	-24.231385	-70.363631	SC
HF 533 19	10	ED3171	Region II	Antofagasta	El Cobre to Antofagasta	-24.231207	-70.363084	SC
HF 533 20	10	ED3172	Region II	Antofagasta	El Cobre to Antofagasta	-24.23093	-70.361886	SC
HF 537 1	15	ED3173	Region II	Antofagasta	Quebrada Tocopilla	-22.065987	-70.161023	TC
HF 537 2	15	ED3174	Region II	Antofagasta	Quebrada Tocopilla	-22.068399	-70.1618	TC
HF 537 3	15	ED3175	Region II	Antofagasta	Quebrada Tocopilla	-22.068999	-70.162298	TC
HF 537 4	15	ED3176	Region II	Antofagasta	Quebrada Tocopilla	-22.069346	-70.162412	TC
HF 537 5	15	ED3177	Region II	Antofagasta	Quebrada Tocopilla	-22.069627	-70.162516	TC
HF 537 6	15	ED3178	Region II	Antofagasta	Quebrada Tocopilla	-22.069882	-70.162862	TC
HF 537 7	15	ED3179	Region II	Antofagasta	Quebrada Tocopilla	-22.070313	-70.162956	TC
HF 537 8	15	ED3180	Region II	Antofagasta	Quebrada Tocopilla	-22.070528	-70.162983	TC
HF 537 9	15	ED3181	Region II	Antofagasta	Quebrada Tocopilla	-22.070841	-70.163312	TC
HF 537 10	15	ED3182	Region II	Antofagasta	Quebrada Tocopilla	-22.071248	-70.163135	TC
HF 537 11	15	ED3183	Region II	Antofagasta	Quebrada Tocopilla	-22.071395	-70.163391	TC
HF 537 12	15	ED3184	Region II	Antofagasta	Quebrada Tocopilla	-22.07197	-70.163537	TC
HF 537 13	15	ED3185	Region II	Antofagasta	Quebrada Tocopilla	-22.072431	-70.163642	TC
HF 537 14	15	ED3186	Region II	Antofagasta	Quebrada Tocopilla	-22.072617	-70.163818	TC
HF 537 15	15	ED3187	Region II	Antofagasta	Quebrada Tocopilla	-22.072566	-70.164033	TC
HF 537 16	15	ED3188	Region II	Antofagasta	Quebrada Tocopilla	-22.072624	-70.164406	TC
HF 537 17	15	ED3189	Region II	Antofagasta	Quebrada Tocopilla	-22.072517	-70.164684	TC
HF 537 18	15	ED3190	Region II	Antofagasta	Quebrada Tocopilla	-22.072686	-70.164988	TC
HF 537 19	15	ED3191	Region II	Antofagasta	Quebrada Tocopilla	-22.072612	-70.165399	TC

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Name	**Population**	**Sample**	**Department**	**Region**	**Locality**	**Latitude**	**Longitude**	**Cluster**
HF 537 20	15	ED3192	Region II	Antofagasta	Quebrada Tocopilla	-22.072874	-70.165827	TC
HF 540 1	16	ED3193	Region I	Tarapacá	Quebrada Huatacondo	-20.931268	-69.105833	SC
HF 540 2	16	ED3194	Region I	Tarapacá	Quebrada Huatacondo	-20.931268	-69.105833	SC
HF 540 3	16	ED3195	Region I	Tarapacá	Quebrada Huatacondo	-20.931268	-69.105833	SC
HF 540 4	16	ED3196	Region I	Tarapacá	Quebrada Huatacondo	-20.931268	-69.105833	SC
HF 540 5	16	ED3197	Region I	Tarapacá	Quebrada Huatacondo	-20.931268	-69.105833	SC
HF 540 6	16	ED3198	Region I	Tarapacá	Quebrada Huatacondo	-20.931268	-69.105833	SC
HF 540 7	16	ED3199	Region I	Tarapacá	Quebrada Huatacondo	-20.931268	-69.105833	SC
HF 540 8	16	ED3200	Region I	Tarapacá	Quebrada Huatacondo	-20.946778	-69.153977	SC
HF 615 1	11	ED4506	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 2	11	ED4507	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 3	11	ED4508	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 4	11	ED4509	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 5	11	ED4510	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 6	11	ED4511	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 7	11	ED4512	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 8	11	ED4513	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 9	11	ED4514	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 10	11	ED4515	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 11	11	ED4516	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 12	11	ED4517	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 13	11	ED4518	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 14	11	ED4519	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 615 15	11	ED4520	Region II	Antofagasta	SE Antofagasta	-24.22561	-70.10584	SC
HF 661 1	18	ED2443	Region I	Tarapacá	Pachica	-19.850789	-69.438952	NC
HF 661 2	18	ED2444	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 3	18	ED2445	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 4	18	ED2446	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 5	18	ED2447	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 6	18	ED2448	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 7	18	ED2449	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 8	18	ED2450	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 9	18	ED2451	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 10	18	ED2452	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 11	18	ED2453	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 12	18	ED2454	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 13	18	ED2455	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 14	18	ED2456	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 15	18	ED2457	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 16	18	ED2458	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 17	18	ED2459	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC

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Table S1: Continued from previous page

Name	**Population**	**Sample**	**Department**	**Region**	**Locality**	**Latitude**	**Longitude**	**Cluster**
HF 661 18	18	ED2460	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 19	18	ED2461	Region I	Tarapacá	Pachica	-19.858381	-69.437255	NC
HF 661 20	18	ED2462	Region I	Tarapacá	Pachica	-19.875419	-69.454873	NC
HF 662 1	20	ED2463	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 2	20	ED2464	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 3	20	ED2465	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 4	20	ED2466	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 5	20	ED2467	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 6	20	ED2468	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 7	20	ED2469	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 8	20	ED2470	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 9	20	ED2471	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 10	20	ED2472	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 11	20	ED2473	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 12	20	ED2474	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 13	20	ED2475	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 14	20	ED2476	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 15	20	ED2477	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 16	20	ED2478	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 17	20	ED2479	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 18	20	ED2480	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 19	20	ED2481	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 662 20	20	ED2482	Region I	Tarapacá	Pisagua Viejo	-19.552119	-70.194231	NC
HF 663 1	19	ED2483	Region I	Tarapacá	Quebrada Aroma	-19.573032	-69.35055	NC
HF 663 2	19	ED2484	Region I	Tarapacá	Quebrada Aroma	-19.572731	-69.350672	NC
HF 663 3	19	ED2485	Region I	Tarapacá	Quebrada Aroma	-19.57211	-69.351117	NC
HF 663 4	19	ED2486	Region I	Tarapacá	Quebrada Aroma	-19.571581	-69.35168	NC
HF 663 5	19	ED2487	Region I	Tarapacá	Quebrada Aroma	-19.571086	-69.351944	NC
HF 663 6	19	ED2488	Region I	Tarapacá	Quebrada Aroma	-19.570007	-69.351957	NC
HF 663 7	19	ED2489	Region I	Tarapacá	Quebrada Aroma	-19.574828	-69.354078	NC
HF 663 8	19	ED2490	Region I	Tarapacá	Quebrada Aroma	-19.57825	-69.361455	NC
HF 663 9	19	ED2491	Region I	Tarapacá	Quebrada Aroma	-19.578536	-69.361456	NC
HF 663 10	19	ED2492	Region I	Tarapacá	Quebrada Aroma	-19.578783	-69.361165	NC
HF 663 11	19	ED2493	Region I	Tarapacá	Quebrada Aroma	-19.578771	-69.366172	NC
HF 663 12	19	ED2494	Region I	Tarapacá	Quebrada Aroma	-19.579112	-69.366194	NC
HF 663 13	19	ED2495	Region I	Tarapacá	Quebrada Aroma	-19.580143	-69.365637	NC
HF 663 14	19	ED2496	Region I	Tarapacá	Quebrada Aroma	-19.581752	-69.364957	NC
HF 663 15	19	ED2497	Region I	Tarapacá	Quebrada Aroma	-19.584862	-69.381192	NC
HF 663 16	19	ED2498	Region I	Tarapacá	Quebrada Aroma	-19.585743	-69.380873	NC
HF 663 17	19	ED2499	Region I	Tarapacá	Quebrada Aroma	-19.586611	-69.380587	NC
HF 663 18	19	ED2500	Region I	Tarapacá	Quebrada Aroma	-19.583766	-69.387666	NC

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Table S1: Continued from previous page

Name	**Population**	**Sample**	**Department**	**Region**	**Locality**	**Latitude**	**Longitude**	**Cluster**
HF 663 19	19	ED2501	Region I	Tarapacá	Quebrada Aroma	-19.591192	-69.434506	NC
HF 663 20	19	ED2502	Region I	Tarapacá	Quebrada Aroma	-19.591821	-69.435212	NC
HF 663 21	19	ED2503	Region I	Tarapacá	Quebrada Aroma	-19.591213	-69.435108	NC
HF 663 22	19	ED2504	Region I	Tarapacá	Quebrada Aroma	-19.592229	-69.436678	NC
HF 663 23	19	ED2505	Region I	Tarapacá	Quebrada Aroma	-19.592314	-69.436856	NC
HF 663 24	19	ED2506	Region I	Tarapacá	Quebrada Aroma	-19.612113	-69.47057	NC
HF 663 25	19	ED2507	Region I	Tarapacá	Quebrada Aroma	-19.635062	-69.556525	NC
HF 678 1	21	ED2508	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 2	21	ED2509	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 3	21	ED2510	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 4	21	ED2511	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 5	21	ED2512	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 6	21	ED2513	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 7	21	ED2514	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 8	21	ED2515	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 9	21	ED2516	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 10	21	ED2517	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 11	21	ED2518	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 12	21	ED2519	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 13	21	ED2520	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 14	21	ED2521	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 15	21	ED2522	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 16	21	ED2523	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 17	21	ED2524	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 18	21	ED2525	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 19	21	ED2526	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC
HF 678 20	21	ED2527	Region I	Tarapacá	Quebrada Camina	-19.32942	-69.50792	NC

Table S2: Estimation of sequence time divergence between population clusters using a “triple comparison method”.

Seq 0	Seq 1	Seq 2	Ti 01	Tv 01	Ti 02	Tv 02	Ti 12	Tv 12	Sites	JC 01	JC 02	JC 12	K2P 01	K2P 02	K2P 12	JC frac	K2P frac
Hc	HF 110 9	HF 540 6	2700	1772.5	2695	1761	98.5	83.5	74621	0.0625	0.0622	0.0024	0.0627	0.0625	0.0024	0.0392	0.0390
Hc	HF 110 9	HF 184 17	2681	1757.5	2678.5	1738.5	98.5	80	72740	0.0636	0.0633	0.0025	0.0639	0.0636	0.0025	0.0387	0.0386
Hc	HF 110 9	HF 303 10	2663.5	1738.5	2657.5	1726	97.5	87.5	72077	0.0637	0.0634	0.0026	0.0639	0.0637	0.0026	0.0404	0.0403
Hc	HF 110 9	HF 185 9	2672.5	1758.5	2670	1746	98.5	81.5	72477	0.0638	0.0635	0.0025	0.0640	0.0638	0.0025	0.0391	0.0389
Hc	HF 678 10	HF 540 6	2709.5	1774.5	2699	1764	104	87.5	74751	0.0625	0.0622	0.0026	0.0628	0.0624	0.0026	0.0411	0.0410
Hc	HF 678 10	HF 184 17	2686.5	1755.5	2678.5	1737.5	104	84	72726	0.0637	0.0633	0.0026	0.0640	0.0636	0.0026	0.0408	0.0406
Hc	HF 678 10	HF 303 10	2667.5	1736.5	2657.5	1725	101.5	93.5	72111	0.0637	0.0634	0.0027	0.0639	0.0636	0.0027	0.0426	0.0425
Hc	HF 678 10	HF 185 9	2677	1756.5	2670	1745	101	86.5	72474	0.0638	0.0635	0.0026	0.0641	0.0638	0.0026	0.0407	0.0406
Hc	HF 663 10	HF 540 6	2699.5	1774.5	2689.5	1759	99	90.5	74978	0.0622	0.0618	0.0025	0.0624	0.0620	0.0025	0.0408	0.0407
Hc	HF 663 10	HF 184 17	2681	1762.5	2672.5	1739.5	103	87	72826	0.0636	0.0632	0.0026	0.0639	0.0634	0.0026	0.0412	0.0411
Hc	HF 663 10	HF 303 10	2664.5	1746.5	2653.5	1729	98	95.5	72350	0.0636	0.0632	0.0027	0.0638	0.0634	0.0027	0.0423	0.0421
Hc	HF 663 10	HF 185 9	2671.5	1763.5	2662	1747	99	88.5	72414	0.0639	0.0635	0.0026	0.0641	0.0637	0.0026	0.0407	0.0406
Hc	HF 662 16	HF 540 6	2700	1774	2690.5	1761	100	88	74852	0.0623	0.0620	0.0025	0.0625	0.0622	0.0025	0.0405	0.0404
Hc	HF 662 16	HF 184 17	2673	1755	2665.5	1734.5	101.5	84.5	72466	0.0637	0.0633	0.0026	0.0640	0.0636	0.0026	0.0405	0.0403
Hc	HF 662 16	HF 303 10	2657	1738	2646.5	1724	100	94	71892	0.0638	0.0634	0.0027	0.0640	0.0636	0.0027	0.0425	0.0424
Hc	HF 662 16	HF 185 9	2663	1756	2655	1742	99	87	71967	0.0641	0.0637	0.0026	0.0643	0.0640	0.0026	0.0405	0.0404
Hc	HF 110 9	HF 533 5	2729.5	1797.5	2725.5	1784	95.5	76.5	75086	0.0629	0.0626	0.0023	0.0631	0.0628	0.0023	0.0366	0.0364
Hc	HF 110 9	HF 423 13	2707.5	1802.5	2703	1792	96.5	85.5	75076	0.0626	0.0624	0.0024	0.0628	0.0626	0.0024	0.0388	0.0387
Hc	HF 110 9	HF 453 5	2713	1783	2706.5	1768.5	96	94.5	74323	0.0631	0.0628	0.0026	0.0633	0.0630	0.0026	0.0408	0.0407
Hc	HF 110 9	HF 615 5	2701	1778	2694.5	1757.5	94	78.5	73065	0.0640	0.0635	0.0024	0.0642	0.0638	0.0024	0.0371	0.0370
Hc	HF 678 10	HF 533 5	2733.5	1795.5	2725.5	1783	99	80.5	75228	0.0628	0.0625	0.0024	0.0630	0.0627	0.0024	0.0382	0.0380
Hc	HF 678 10	HF 423 13	2716	1802.5	2707	1793	100.5	88.5	75425	0.0624	0.0622	0.0025	0.0627	0.0624	0.0025	0.0403	0.0401
Hc	HF 678 10	HF 453 5	2720	1783	2708.5	1770	99.5	99	74562	0.0630	0.0626	0.0027	0.0632	0.0628	0.0027	0.0425	0.0423
Hc	HF 678 10	HF 615 5	2706	1776	2694.5	1756.5	97.5	81.5	73028	0.0640	0.0636	0.0025	0.0643	0.0638	0.0025	0.0385	0.0383
Hc	HF 663 10	HF 533 5	2729.5	1802.5	2720.5	1784	100.5	83.5	75264	0.0628	0.0624	0.0024	0.0630	0.0626	0.0024	0.0391	0.0390
Hc	HF 663 10	HF 423 13	2703	1805.5	2694	1790	98	90.5	75282	0.0624	0.0621	0.0025	0.0626	0.0623	0.0025	0.0403	0.0402
Hc	HF 663 10	HF 453 5	2710	1786	2700	1767	97.5	102	74684	0.0628	0.0623	0.0027	0.0630	0.0626	0.0027	0.0428	0.0426
Hc	HF 663 10	HF 615 5	2697	1781	2685.5	1755.5	96	85.5	72901	0.0641	0.0635	0.0025	0.0643	0.0638	0.0025	0.0391	0.0389
Hc	HF 662 16	HF 533 5	2727	1799	2718.5	1784	98.5	82	75038	0.0629	0.0625	0.0024	0.0631	0.0628	0.0024	0.0384	0.0383
Hc	HF 662 16	HF 423 13	2703.5	1802	2694	1790	97	91	75062	0.0626	0.0623	0.0025	0.0628	0.0625	0.0025	0.0402	0.0401
Hc	HF 662 16	HF 453 5	2710	1782.5	2700	1766.5	99	100	74465	0.0629	0.0625	0.0027	0.0631	0.0627	0.0027	0.0427	0.0425
Hc	HF 662 16	HF 615 5	2693	1775.5	2682.5	1753	95.5	83.5	72688	0.0641	0.0636	0.0025	0.0644	0.0639	0.0025	0.0386	0.0385
Hc	HF 540 6	HF 533 5	2726	1781	2728	1777	68.5	53	75220	0.0624	0.0624	0.0016	0.0627	0.0627	0.0016	0.0259	0.0258
Hc	HF 540 6	HF 423 13	2692	1776	2695.5	1774.5	74	55.5	74923	0.0621	0.0622	0.0017	0.0624	0.0624	0.0017	0.0278	0.0277
Hc	HF 540 6	HF 453 5	2696.5	1754.5	2698.5	1753.5	68.5	62	74371	0.0624	0.0624	0.0018	0.0626	0.0626	0.0018	0.0282	0.0281
Hc	HF 540 6	HF 615 5	2699	1758.5	2699.5	1747.5	64	56	72985	0.0637	0.0635	0.0016	0.0639	0.0638	0.0016	0.0259	0.0258
Hc	HF 184 17	HF 533 5	2707	1763.5	2707.5	1766	60.5	47.5	73319	0.0636	0.0636	0.0015	0.0638	0.0639	0.0015	0.0232	0.0231
Hc	HF 184 17	HF 423 13	2679.5	1756.5	2681.5	1761	71	49.5	73346	0.0631	0.0632	0.0016	0.0633	0.0634	0.0016	0.0261	0.0260
Hc	HF 184 17	HF 453 5	2694	1753.5	2693.5	1756	64.5	49.5	73498	0.0631	0.0631	0.0016	0.0633	0.0634	0.0016	0.0246	0.0245
Hc	HF 184 17	HF 615 5	2700.5	1761	2698.5	1756.5	60	46.5	73161	0.0636	0.0635	0.0015	0.0638	0.0637	0.0015	0.0229	0.0228
Hc	HF 303 10	HF 533 5	2691	1755	2694	1752	61.5	51	72907	0.0636	0.0636	0.0015	0.0638	0.0638	0.0015	0.0243	0.0242
Hc	HF 303 10	HF 423 13	2660	1745	2665	1743	69.5	51	72714	0.0632	0.0632	0.0017	0.0634	0.0634	0.0017	0.0263	0.0262
Hc	HF 303 10	HF 453 5	2669.5	1737	2673.5	1735.5	63.5	56.5	72771	0.0631	0.0632	0.0017	0.0634	0.0634	0.0017	0.0261	0.0260
Hc	HF 303 10	HF 615 5	2680	1751	2681.5	1741.5	64	50.5	72498	0.0638	0.0636	0.0016	0.0640	0.0639	0.0016	0.0248	0.0247
Hc	HF 185 9	HF 533 5	2696.5	1767	2698	1763	59	47	73055	0.0637	0.0637	0.0015	0.0640	0.0639	0.0015	0.0228	0.0227
Hc	HF 185 9	HF 423 13	2677	1767	2679	1766	69	51	73076	0.0634	0.0634	0.0016	0.0637	0.0637	0.0016	0.0259	0.0258
Hc	HF 185 9	HF 453 5	2678.5	1753	2679	1750	64	50	72889	0.0634	0.0634	0.0016	0.0636	0.0636	0.0016	0.0247	0.0246
Hc	HF 185 9	HF 615 5	2687.5	1765	2685.5	1754	57.5	48	72741	0.0639	0.0637	0.0015	0.0641	0.0639	0.0015	0.0228	0.0227
Hc	HF 110 9	HF 537 15	2708.5	1791.5	2705.5	1779	94	78.5	74965	0.0626	0.0623	0.0023	0.0628	0.0626	0.0023	0.0369	0.0368
Hc	HF 110 9	HF 537 2	2692.5	1781.5	2688	1768	94	84.5	74528	0.0626	0.0623	0.0024	0.0628	0.0625	0.0024	0.0384	0.0383
Hc	HF 110 9	HF 537 18	2699.5	1785	2690.5	1770.5	89.5	81.5	74940	0.0624	0.0620	0.0023	0.0626	0.0622	0.0023	0.0367	0.0366
Hc	HF 110 9	HF 537 9	2694.5	1782.5	2690	1770	93	81.5	74157	0.0629	0.0627	0.0024	0.0632	0.0629	0.0024	0.0375	0.0374
Hc	HF 678 10	HF 537 15	2714	1789.5	2706.5	1778	97.5	82.5	74991	0.0626	0.0623	0.0024	0.0628	0.0625	0.0024	0.0385	0.0384
Hc	HF 678 10	HF 537 2	2693	1775.5	2684	1763	99.5	85.5	74411	0.0626	0.0623	0.0025	0.0628	0.0625	0.0025	0.0399	0.0397
Hc	HF 678 10	HF 537 18	2703	1783.5	2689.5	1769.5	95	83	74878	0.0624	0.0620	0.0024	0.0627	0.0623	0.0024	0.0383	0.0381
Hc	HF 678 10	HF 537 9	2699	1780.5	2690	1769	98.5	82.5	74120	0.0630	0.0627	0.0024	0.0632	0.0629	0.0024	0.0389	0.0388

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Table S2: Continued from previous page

Seq 0	**Seq 1**	**Seq 2**	**Ti 01**	**Tv 01**	**Ti 02**	**Tv 02**	**Ti 12**	**Tv 12**	**Sites**	**JC 01**	**JC 02**	**JC 12**	**K2P 01**	**K2P 02**	**K2P 12**	**JC frac**	**K2P frac**
Hc	HF 663 10	HF 537 15	2708	1794.5	2699.5	1777	99	84.5	75292	0.0623	0.0619	0.0024	0.0625	0.0622	0.0024	0.0393	0.0392
Hc	HF 663 10	HF 537 2	2686.5	1780.5	2678	1762	97.5	91.5	74431	0.0626	0.0622	0.0025	0.0628	0.0624	0.0025	0.0408	0.0406
Hc	HF 663 10	HF 537 18	2697	1787.5	2683	1768.5	94	85	75178	0.0622	0.0617	0.0024	0.0624	0.0619	0.0024	0.0385	0.0384
Hc	HF 663 10	HF 537 9	2688.5	1784.5	2680	1767	96.5	87.5	73898	0.0631	0.0627	0.0025	0.0633	0.0630	0.0025	0.0396	0.0395
Hc	HF 662 16	HF 537 15	2705.5	1791	2697.5	1777	98	82	75207	0.0623	0.0620	0.0024	0.0625	0.0622	0.0024	0.0386	0.0384
Hc	HF 662 16	HF 537 2	2681.5	1775	2673	1760	96	88	74160	0.0626	0.0623	0.0025	0.0629	0.0625	0.0025	0.0398	0.0396
Hc	HF 662 16	HF 537 18	2693.5	1783	2680	1766.5	93	84.5	75045	0.0622	0.0617	0.0024	0.0624	0.0619	0.0024	0.0382	0.0381
Hc	HF 662 16	HF 537 9	2685.5	1780	2677	1766	95	85	73696	0.0632	0.0628	0.0024	0.0634	0.0631	0.0024	0.0388	0.0387
Hc	HF 540 6	HF 537 15	2720	1780	2719.5	1778	46	49	75537	0.0621	0.0620	0.0013	0.0623	0.0623	0.0013	0.0203	0.0202
Hc	HF 540 6	HF 537 2	2699	1766	2698.5	1764	55.5	64	74825	0.0622	0.0621	0.0016	0.0624	0.0624	0.0016	0.0257	0.0256
Hc	HF 540 6	HF 537 18	2706.5	1769	2701.5	1765.5	52	60.5	75428	0.0618	0.0617	0.0015	0.0620	0.0619	0.0015	0.0242	0.0241
Hc	HF 540 6	HF 537 9	2706	1773	2705.5	1772	51.5	61	74306	0.0628	0.0628	0.0015	0.0631	0.0630	0.0015	0.0241	0.0240
Hc	HF 184 17	HF 537 15	2694.5	1750.5	2692.5	1756	48	53.5	73142	0.0634	0.0634	0.0014	0.0636	0.0637	0.0014	0.0219	0.0218
Hc	HF 184 17	HF 537 2	2674	1737.5	2671.5	1742	59	64.5	72673	0.0633	0.0633	0.0017	0.0635	0.0636	0.0017	0.0269	0.0268
Hc	HF 184 17	HF 537 18	2683	1741	2676.5	1744.5	53	59.5	73110	0.0631	0.0630	0.0015	0.0633	0.0633	0.0015	0.0244	0.0243
Hc	HF 184 17	HF 537 9	2685.5	1746.5	2682.5	1752	52.5	64.5	72963	0.0633	0.0634	0.0016	0.0636	0.0636	0.0016	0.0253	0.0252
Hc	HF 303 10	HF 537 15	2677.5	1744	2679	1741	50	56	72634	0.0635	0.0635	0.0015	0.0637	0.0637	0.0015	0.0230	0.0229
Hc	HF 303 10	HF 537 2	2664	1735	2666	1731	58	56	72442	0.0633	0.0633	0.0016	0.0636	0.0635	0.0016	0.0249	0.0248
Hc	HF 303 10	HF 537 18	2670.5	1736	2666.5	1731.5	55	57.5	72663	0.0632	0.0631	0.0015	0.0635	0.0633	0.0015	0.0245	0.0244
Hc	HF 303 10	HF 537 9	2672.5	1742	2674	1739	52.5	55	72473	0.0635	0.0635	0.0015	0.0638	0.0637	0.0015	0.0234	0.0233
Hc	HF 185 9	HF 537 15	2681.5	1757.5	2679	1757	47	53.5	72537	0.0638	0.0638	0.0014	0.0641	0.0640	0.0014	0.0217	0.0216
Hc	HF 185 9	HF 537 2	2665	1745.5	2662	1744	59	64.5	72385	0.0635	0.0635	0.0017	0.0638	0.0637	0.0017	0.0269	0.0268
Hc	HF 185 9	HF 537 18	2670.5	1747	2663	1744.5	53.5	60.5	72508	0.0635	0.0634	0.0016	0.0638	0.0636	0.0016	0.0248	0.0247
Hc	HF 185 9	HF 537 9	2676.5	1755	2673	1754	56.5	65	72581	0.0637	0.0636	0.0017	0.0639	0.0639	0.0017	0.0263	0.0262
Hc	HF 533 5	HF 537 15	2728	1789	2727	1791	64	60	75255	0.0626	0.0626	0.0016	0.0628	0.0628	0.0016	0.0264	0.0263
Hc	HF 533 5	HF 537 2	2706.5	1774	2704.5	1774	65.5	65	74682	0.0625	0.0625	0.0017	0.0628	0.0627	0.0017	0.0280	0.0279
Hc	HF 533 5	HF 537 18	2714	1775	2707.5	1775.5	58	60.5	75039	0.0623	0.0623	0.0016	0.0626	0.0625	0.0016	0.0254	0.0253
Hc	HF 533 5	HF 537 9	2714.5	1782	2712.5	1784	64.5	63	74431	0.0630	0.0630	0.0017	0.0632	0.0632	0.0017	0.0272	0.0271
Hc	HF 423 13	HF 537 15	2704	1793.5	2704	1793.5	76	59	75242	0.0623	0.0623	0.0018	0.0625	0.0625	0.0018	0.0288	0.0287
Hc	HF 423 13	HF 537 2	2683	1779.5	2681	1778.5	72.5	64	74676	0.0623	0.0622	0.0018	0.0625	0.0625	0.0018	0.0294	0.0293
Hc	HF 423 13	HF 537 18	2689.5	1781.5	2684	1779.5	66	62	75081	0.0620	0.0619	0.0017	0.0623	0.0622	0.0017	0.0275	0.0274
Hc	HF 423 13	HF 537 9	2690.5	1785.5 2688	1785	73	62.5	74448	0.0627	0.0626	0.0018	0.0629	0.0629	0.0018	0.0291	0.0290	
Hc	HF 453 5	HF 537 15	2705.5	1765.5	2707	1765.5	67.5	65	74504	0.0625	0.0626	0.0018	0.0628	0.0628	0.0018	0.0285	0.0284
Hc	HF 453 5	HF 537 2	2683	1753.5	2683	1751.5	71.5	72	73836	0.0626	0.0626	0.0019	0.0629	0.0628	0.0019	0.0311	0.0310
Hc	HF 453 5	HF 537 18	2696.5	1760.5	2692.5	1758	63.5	69.5	74570	0.0623	0.0622	0.0018	0.0625	0.0624	0.0018	0.0287	0.0286
Hc	HF 453 5	HF 537 9	2689	1759	2689	1759.5	70.5	71.5	73611	0.0630	0.0630	0.0019	0.0632	0.0632	0.0019	0.0307	0.0305
Hc	HF 615 5	HF 537 15	2703.5	1766	2705.5	1775	62	62	73268	0.0636	0.0638	0.0017	0.0639	0.0640	0.0017	0.0266	0.0265
Hc	HF 615 5	HF 537 2	2681.5	1754	2681.5	1760.5	64.5	64.5	72858	0.0635	0.0636	0.0018	0.0637	0.0638	0.0018	0.0279	0.0278
Hc	HF 615 5	HF 537 18	2691	1755.5	2687.5	1762	59	60.5	73135	0.0634	0.0634	0.0016	0.0636	0.0637	0.0016	0.0258	0.0257
Hc	HF 615 5	HF 537 9	2692.5	1762.5	2692	1770.5	59	66	73133	0.0635	0.0636	0.0017	0.0638	0.0639	0.0017	0.0269	0.0268
Hc	North	Andean	2679.1875	1757.5	2671.3125	1742.4375	100.1875	87.4375	72982.625	0.0634	0.0631	0.0026	0.0636	0.0633	0.0026	0.0407	0.0406
Hc	North	Coastal	2712.46875	1790.75	2703.75	1774.65625	97.53125	87.65625	74448.5625	0.0631	0.0627	0.0025	0.0633	0.0629	0.0025	0.0396	0.0395
Hc	Andean	Coastal	2689.65625	1759.03125	2691.125	1756.0625	64.9375	51.53125	73342.125	0.0633	0.0632	0.0016	0.0635	0.0635	0.0016	0.0251	0.0250
Hc	North	Mid-coastal	2696.875	1784.09375	2688.25	1769.4375	95.625	84.21875	74618.5625	0.0626	0.0623	0.0024	0.0628	0.0625	0.0024	0.0387	0.0385
Hc	Andean	Mid-coastal	2684.15625	1751.59375	2681.90625	1751	53.0625	59.03125	73262.9375	0.0631	0.0631	0.0015	0.0634	0.0633	0.0015	0.0243	0.0242
Hc	Coastal	Mid-coastal	2698.28125	1771.03125	2696.65625	1772.71875	66.0625	64.1875	74235.5625	0.0628	0.0628	0.0018	0.0630	0.0630	0.0018	0.0280	0.0279

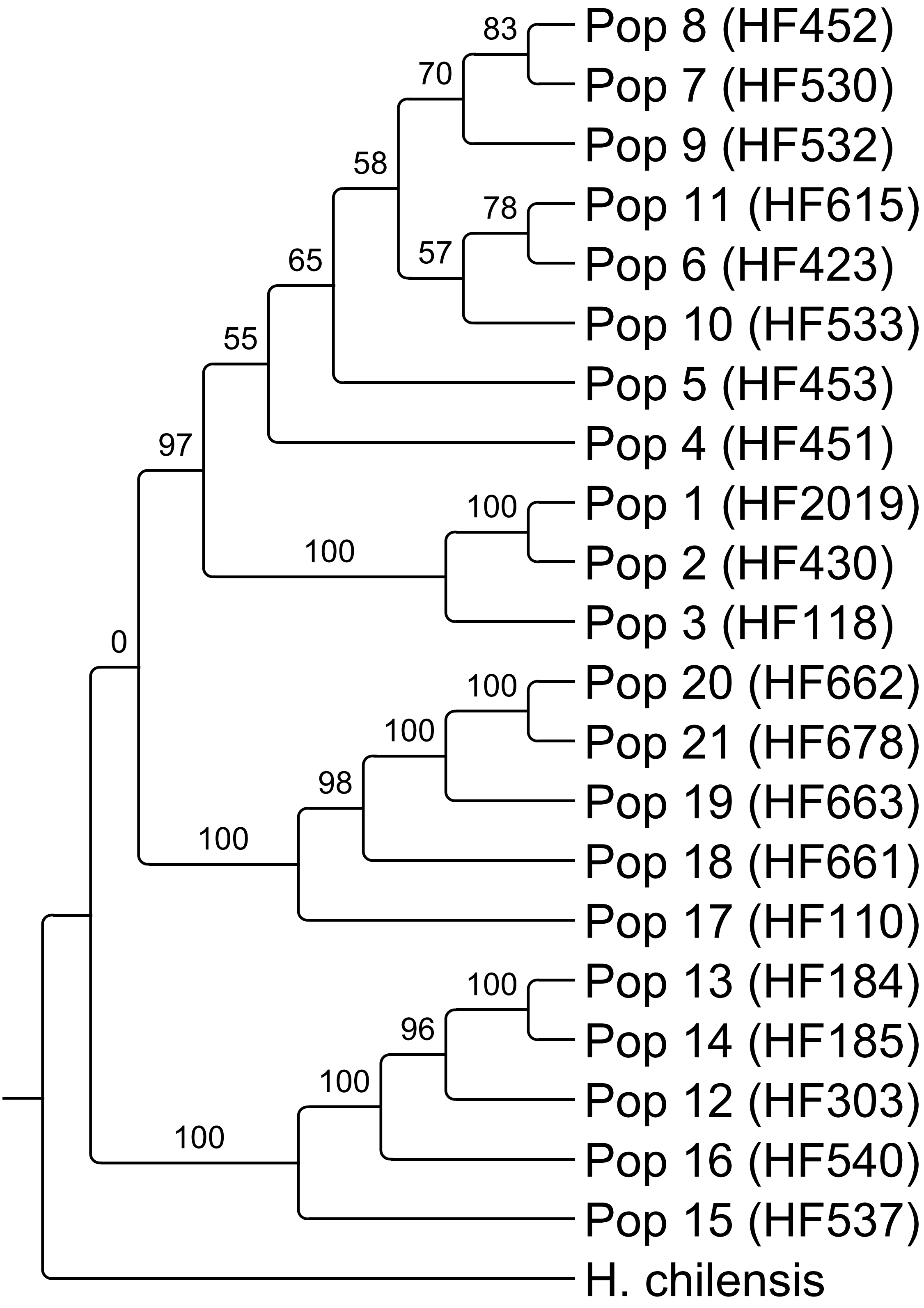

Figure S1: Maximum likelihood phylogeny of the *H. fruticosa* populations using *H. chilensis* as an outgroup. Numbers represent bootstrap values.

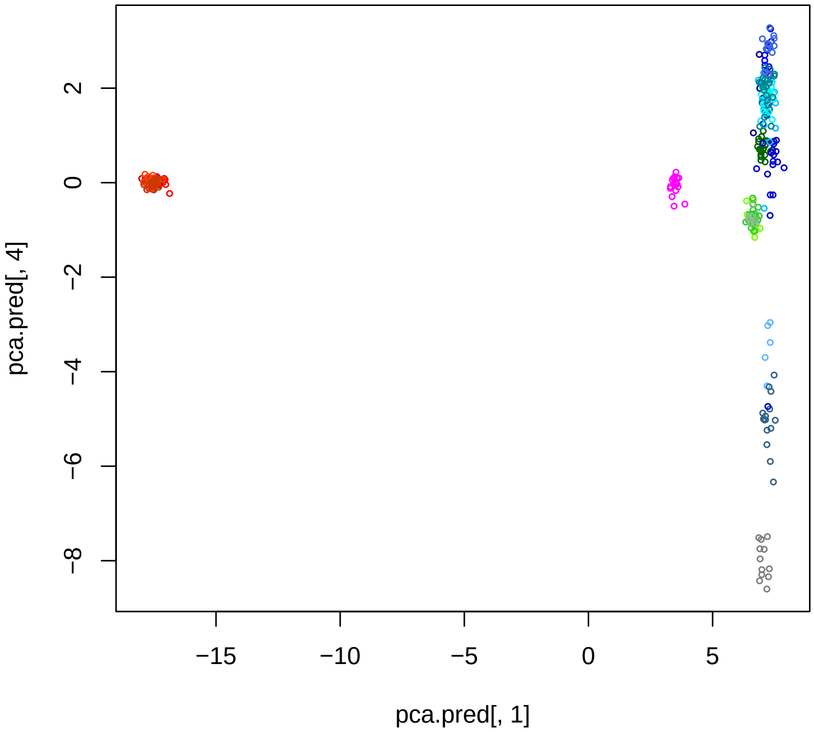

Figure S2: Principal Component Analysis (PCA) axes 1–4.

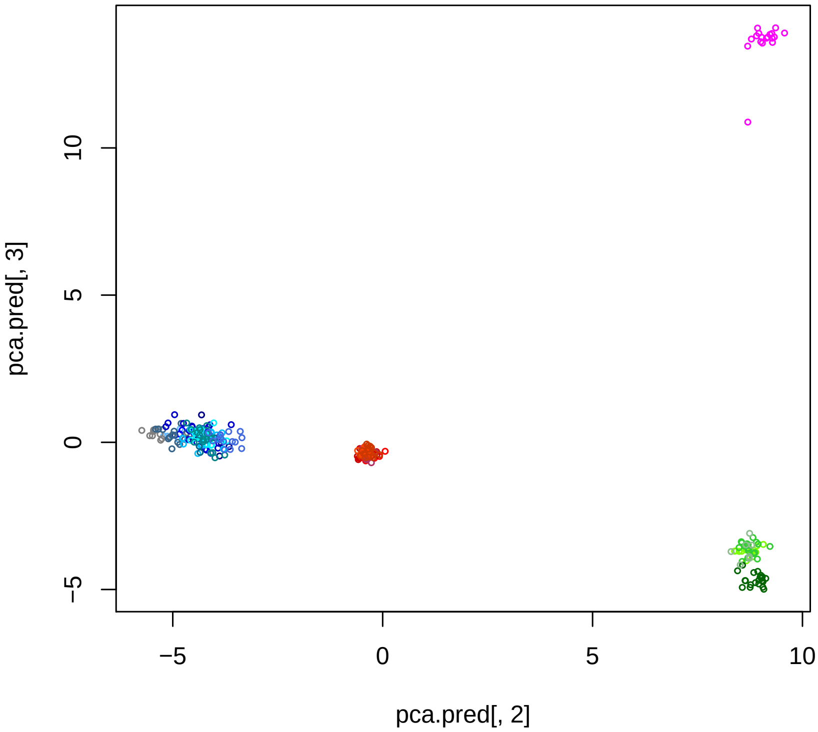

Figure S3: Principal Component Analysis (PCA) axes 2–3.

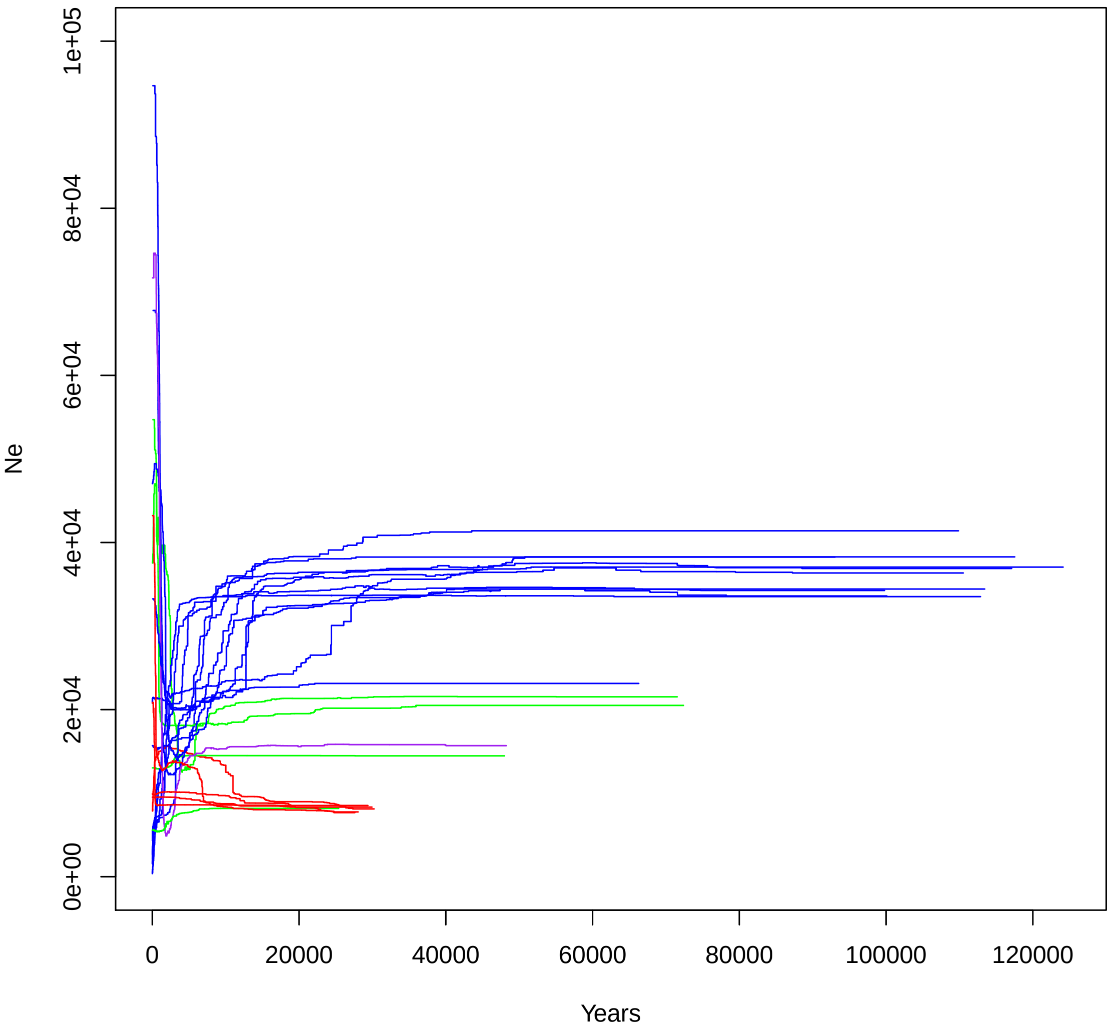

Figure S4: Estimation of past population sizes from allele frequency spectra.

Appendix C

Supplementary data to Chapter 4

Table S1: Summary of all taxa used in this study including voucher information. For locotype collections the oldest available name at the species or variety level was used, indicated by an asterisk in the Lab number column. The usage of currently not accepted names does not reflect our taxonomic opinion.

Taxon	**Country**	**Locality**	**Sample**	**Voucher**	**Herbarium**	**Latitude**	**Longitude**
Austrocactus bertinii	Argentina	Sierra Grande	ED3490	cult. Sarnes 9121/3	BONN	-41.6000	-65.4200
A. coxii	Argentina	Nahuel Huapi	ED3492	cult. Sarnes 546/2	BONN	-40.0333	-71.1800
A. sp.	Argentina	Río Senguer	ED3491	cult. Sarnes 4613	BONN	-45.4700	-69.8300
A. spiniflorus	Chile	Farellones	ED3494	cult. Sarnes 3401/2	BONN	-33.3670	-70.3000
A. subandinus	Argentina	Los Molles	ED3493	cult. Sarnes 6842/1	BONN	-35.2600	-69.9800
Corryocactus brevistylus	Chile	Chusmiza	ED5158	F.F. Merklinger 2019-22	BONN	-19.7231	-69.2206
C. erectus	Peru	Huancarpay	ED4540	F.F. Merklinger 2018-127	BONN	-13.6129	-71.7239
Eulychnia acida	Chile	Tongoy	ED5135*	F.F. Merklinger 2019-2	BONN	-30.3092	-71.5918
E. acida	Chile	Illapel	ED3110	F. Luebert 3767	BONN	-31.6041	-71.1260
E. acida	Chile	Quebrada Los Choros	ED5138*	F.F. Merklinger 2019-6	BONN	-29.3161	-71.2506
E. acida	Chile	Quebrada Agua Grande	n/a	F. Kattermann 1242	SGO	-28.5559	-71.1938
E. acida	Chile	99 km S of Vallenar	n/a	Eggli & Leuenberger 3034	ZSS	-29.2990	-71.0327
E. acida	Chile	13 km N of Tilama	n/a	Eggli & Leuenberger 2550	ZSS	-31.9818	-71.1410
E. acida	Chile	El Palqui	n/a	F.F. Merklinger 2018-97	BONN	-30.7254	-70.9487
E. acida	Chile	Forest Reserve El Tambo	n/a	F.F. Merklinger 2018-90	BONN	-31.7954	-71.0058
E. acida	Chile	S of Combarbalá	n/a	F.F. Merklinger 2018-93	BONN	-31.2925	-71.1954
E. acida	Chile	La Ligua	n/a	Eggli & Leuenberger 2564	ZSS	-31.0542	-71.0282
E. acida	Chile	Los Loros to Caleta El Toro	n/a	F. Luebert 3782	BONN	-30.7602	-71.6444
E. acida	Chile	Road to the Fray Jorge NP	n/a	Eggli & Leuenberger 1852	ZSS	-30.6148	-71.5557
E. acida	Chile	Ovalle	n/a	Eggli & Leuenberger 2566	ZSS	-30.4822	-71.0712
E. acida	Chile	Ovalle, Pichasca	n/a	A.Troncoso A. s.n.	SGO	-30.3918	-70.8581
E. acida	Chile	Road to Los Choros	n/a	Eggli & Leuenberger 2960	ZSS	-29.3095	-71.2687
E. acida	Chile	3 km SW of Los Choros	n/a	Eggli & Leuenberger 2968	ZSS	-29.3035	-71.3293
E. acida	Chile	La Higuera	n/a	Eggli & Leuenberger 2672	ZSS	-29.3393	-71.1618
E. acida	Chile	Road to Los Choros	n/a	F. Luebert 3834	BONN	-29.3393	-71.2041
E. acida	Chile	Road to Los Choros	n/a	F. Luebert 3834A	BONN	-29.3393	-71.2041
E. acida	Chile	2 km SE of Chungungo	n/a	Eggli & Leuenberger 3039	ZSS	-29.4520	-71.2865
E. acida	Chile	Observatory Tololo	n/a	Eggli & Leuenberger 3041	ZSS	-30.0465	-70.8195
E. acida	Chile	37 km E of La Serena	n/a	F. Ritter 232	SGO	-29.9842	-70.8745
E. acida	Chile	Vicuña	n/a	Eggli & Leuenberger 3053	ZSS	-29.9070	-70.5270
E. acida	Chile	Marquesa to Viñita Baja	n/a	F. Luebert 3826	BONN	-29.8738	-70.8608
E. acida	Chile	D-485 towards Paiguano	n/a	F.F. Merklinger 2018-84	BONN	-29.9849	-70.5417
E. acida	Chile	Coquimbo	n/a	Philippi s.n.	SGO	-29.9500	-71.3333
E. acida	Chile	2 km W of El Molle	n/a	F. Kattermann 1013	SGO	-29.9667	-70.9500
E. acida	Chile	La Serena, 1 km E of El Peñon	n/a	Eggli & Leuenberger 2577	ZSS	-30.1447	-71.2155
E. acida	Chile	Cuesta Las Cardas	n/a	F.F. Merklinger 2018-79A	BONN	-30.3013	-71.2573
E. acida	Chile	Quebrada Santa Gracia	n/a	Eggli & Leuenberger 3081	ZSS	-29.8092	-71.0842

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Table S1: Continued from previous page

Taxon	**Country**	**Locality**	**Sample**	**Voucher**	**Herbarium**	**Latitude**	**Longitude**
E. acida	Chile	Punta Teatinos	n/a	F.F. Merklinger 2018-86	BONN	-29.7904	-71.2965
E. acida	Chile	Tongoy	n/a	Eggli & Leuenberger 3085	ZSS	-30.3085	-71.5920
E. acida	Chile	104 km S of Vallenar	n/a	Eggli & Leuenberger 1840	ZSS	-29.3441	-71.0400
E. acida (hybrid)	Chile	Cuesta Buenos Aires	ED5137*	F.F. Merklinger 2019-5	BONN	-29.5785	-71.2483
E. acida var. *elata*	Chile	Hzda Castilla	ED3114	F. Luebert 3866	BONN	-27.9200	-70.8453
E. acida var. *elata*	Chile	Canto del Agua	ED5143*	F.F. Merklinger 2019-11	BONN	-28.2240	-70.8268
E. acida var. *elata*	Chile	Caleta Pajonales	ED5152*	F.F. Merklinger 2018-71	BONN	-27.6877	-70.9140
E. acida var. *elata*	Chile	Nantoco	ED5153*	F. Luebert 3881	BONN	-27.6281	-70.3832
E. acida var. *elata*	Chile	Chorillos to El Chapote	n/a	F. Ritter 651	SGO	-28.1509	-70.9000
E. acida var. *elata*	Chile	16 km SE of Canto del Agua	n/a	Eggli & Leuenberger 3015A	ZSS	-28.2367	-70.8222
E. acida var. *elata*	Chile	Toward Canto del Agua	n/a	F.F. Merklinger 2018-74	BONN	-28.1534	-71.0308
E. acida var. *elata*	Chile	W of Castillo	n/a	F. Ritter s.n.	L	-27.8094	-70.6919
E. acida var. *elata*	Chile	Between Castillo and Totoral	n/a	F. Ritter 651 (loc.6)	SGO	-27.9346	-70.8224
E. acida var. *elata*	Chile	11 km S of Copiapó	n/a	Eggli & Leuenberger 1812	ZSS	-27.4660	-70.3768
E. acida var. *elata*	Chile	Copiapó	n/a	F. Ritter 651	L	-27.3667	-70.3314
E. acida var. *elata*	Chile	NE of Copiapó	n/a	F. Ritter 651 (loc.5)	SGO	-27.3659	-70.2918
E. acida var. *elata*	Chile	Paipote-Diego de Almagro	n/a	M. Muñoz 1988	SGO	-27.3169	-70.1636
E. acida var. *procumbens*	Chile	Freirina	ED3113	F. Luebert 3848	BONN	-28.5150	-71.0798
E. acida var. *procumbens*	Chile	Llano Choros	ED5139*	F.F. Merklinger 2019-7	BONN	-29.1037	-71.4508
E. acida var. *procumbens*	Chile	Los Choros	ED4234	F.F. Merklinger 2018-80	BONN	-29.1758	-71.4857
E. acida var. *procumbens*	Chile	Freirina	n/a	F. Ritter 650	L	-28.5092	-71.0811
E. acida var. *procumbens*	Chile	Freirina	n/a	F. Ritter s.n.	L	-28.5092	-71.0811
E. acida var. *procumbens*	Chile	Hills above Freirina	n/a	F.F. Merklinger 2018-72	BONN	-28.5185	-71.0562
E. acida var. *procumbens*	Chile	45 km W of Domeyko	n/a	Eggli & Leuenberger 2583	ZSS	-28.9647	-71.3397
E. acida var. *procumbens*	Chile	c. 17 km W of Vallenar	n/a	Eggli & Leuenberger 2994	ZSS	-28.5298	-70.9275
E. acida var. *procumbens*	Chile	Quebr. Cuesta La Arena	n/a	Eggli & Leuenberger 3016	ZSS	-28.6465	-71.1373
E. acida var. *procumbens*	Chile	Alto del Carmen to San Félix	n/a	Eggli & Leuenberger 2993	ZSS	-28.8742	-70.4435
E. acida var. *procumbens*	Chile	8 km E of Alto del Carmen	n/a	Eggli & Leuenberger 3032	ZSS	-28.7773	-70.4173
E. acida var. *procumbens*	Chile	34 km N of Domeyko	n/a	Eggli & Leuenberger 2970	ZSS	-28.7768	-70.7807
E. acida var. *procumbens*	Chile	22 km E of Vallenar	n/a	Eggli & Leuenberger 3033	ZSS	-28.8262	-70.6392
E. acida var. *procumbens*	Chile	20 km W of Vallenar	n/a	F. Ritter 650 (loc.3)	SGO	-28.5444	-70.9534
E. acida var. *procumbens*	Chile	Below Domeyko	n/a	F. Ritter 232	SGO	-28.9547	-70.8908
E. acida var. *procumbens*	Chile	Chañar de Aceituna	n/a	M. Muñoz S. 1160	SGO	-28.9521	-71.3331
E. acida var. *procumbens*	Chile	Road Trapiche to Choros	n/a	F. Kattermann 1232	SGO	-29.3412	-71.1620
E. acida var. *procumbens*	Chile	E of Punta Choros	n/a	F. Luebert 3841	BONN	-29.2402	-71.4489
E. acida var. *procumbens*	Chile	Domeyko-Freirina	ED5140*	F.F. Merklinger 2019-8	BONN	-28.7536	-71.1373
E. aricensis	Chile	Arica	ED4541	cult. Schneider s.n.	n/a	-18.6531	-70.3000
E. aricensis	Chile	Cerro Camaraca	n/a	F. Ritter 197	ZSS	-18.6531	-70.3000
E. aricensis	Chile	Alto Camaraca	n/a	W. Krahn 945	ZSS	-18.6531	-70.3000
E. barquitensis	Chile	Barquito	ED3117	F. Luebert 3898	BONN	-26.3642	-70.6349
E. barquitensis	Chile	Barquito	n/a	F. Ritter 215	L	-26.3606	-70.6425

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Table S1: Continued from previous page

Taxon	Country	Locality	Sample	Voucher	Herbarium	Latitude	Longitude
E. barquitensis	Chile	c. 5 km S of Chañaral	n/a	Eggli 2906	ZSS	-26.3767	-70.6483
E. breviflora	Chile	Coquimbo	ED3111	F. Luebert 3808	BONN	-29.9337	-71.3369
E. breviflora	Chile	Quebrada Copiapó	ED5144*	F.F. Merklinger 2019-12	BONN	-27.3373	-70.9068
E. breviflora	Chile	Chungungo	ED5150*	F.F. Merklinger 2019-27	BONN	-29.4597	-71.2885
E. breviflora	Chile	Tres Playitas	ED5141*	F.F. Merklinger 2019-9	BONN	-28.4003	-71.1675
E. breviflora	Chile	Carrizal Bajo-Canto del Agua	ED5142*	F.F. Merklinger 2019-10	BONN	-28.1061	-71.1372
E. breviflora	Chile	Huasco Bajo	n/a	M.Muñoz S. 1934	SGO	-28.4608	-71.1693
E. breviflora	Chile	Tres Playitas	n/a	F. Kattermann 1026	SGO	-28.4333	-71.1833
E. breviflora	Chile	Tres Playitas	n/a	F. Luebert 3852	BONN	-28.4005	-71.1676
E. breviflora	Chile	Huasco Bajo to Carrizal Bajo	n/a	Eggli & Leuenberger 1833	ZSS	-28.4021	-71.1741
E. breviflora	Chile	Huasco Bajo to Carrizal Bajo	n/a	Eggli & Leuenberger 3000	ZSS	-28.3897	-71.1863
E. breviflora	Chile	8 km E of Carrizal Bajo	n/a	Eggli & Leuenberger 2600	ZSS	-28.1150	-71.0990
E. breviflora	Chile	Below Totoral	n/a	F. Ritter 214A	SGO	-27.8877	-70.9699
E. breviflora	Chile	Carrizal Bajo	n/a	W. Maechler s.n.	ZSS	-28.0817	-71.1469
E. breviflora	Chile	Toward Canto del Agua	n/a	F.F. Merklinger 2018-73	BONN	-28.1063	-71.1372
E. breviflora	Chile	Toward Canto del Agua	n/a	F.F. Merklinger 2018-75	BONN	-28.1534	-71.0308
E. breviflora	Chile	N of access road to Tongoy	n/a	Eggli & Leuenberger 3082A	ZSS	-30.0398	-71.3812
E. breviflora	Chile	15 km S of Coquimbo	n/a	Eggli & Leuenberger 2676	ZSS	-30.0713	-71.3632
E. breviflora	Chile	Coquimbo	n/a	Philippi s.n.	HAL	-29.9500	-71.3333
E. breviflora	Chile	Coquimbo	n/a	unknown s.n. (poss. half of type)	SGO	-29.9500	-71.3333
E. breviflora	Chile	Coquimbo	n/a	Philippi s.n.	K	-29.9500	-71.3333
E. breviflora	Chile	Coquimbo	n/a	Philippi s.n.	MO	-29.9500	-71.3333
E. breviflora	Chile	Playa Chica de Totoralillo	n/a	F.F. Merklinger 2018-87	BONN	-30.0712	-71.3745
E. breviflora	Chile	La Serena, E of El Peñon	n/a	Eggli & Leuenberger 2575	ZSS	-30.1447	-71.2155
E. breviflora	Chile	N of Punta Teatinos	n/a	Eggli & Leuenberger 1844	ZSS	-29.8203	-71.2808
E. breviflora	Chile	Cuesta Los Porotitos	n/a	F.F. Merklinger 2018-85	BONN	-29.7908	-71.3002
E. breviflora	Chile	6 km de Punta de Teatinos	n/a	C. Muñoz P. 4304	SGO	-29.7669	-71.2631
E. breviflora	Chile	14 km SW of Chungungo	n/a	Eggli & Leuenberger 3040A	ZSS	-29.5315	-71.3167
E. breviflora	Chile	Road to El Tofo	n/a	Eggli & Leuenberger 3036	ZSS	-29.4485	-71.2543
E. breviflora var. *tenuis*	Chile	Caldera	ED3116	F. Luebert 3894	BONN	-26.9633	-70.7401
E. breviflora var. *tenuis*	Chile	Cerro Copiapó (El Morro)	ED5145*	F.F. Merklinger 2019-13	BONN	-27.1447	-70.9197
E. breviflora var. *tenuis*	Chile	c. 5 km N of Puerto Viejo	n/a	F.F. Merklinger 2018-70	BONN	-27.3899	-70.8339
E. breviflora var. *tenuis*	Chile	c. 10 km S of Bahia Inglesa	n/a	Eggli & Leuenberger 1795	ZSS	-27.1391	-70.9154
E. breviflora var. *tenuis*	Chile	S of Caldera, El Morro	n/a	F.F. Merklinger 2018-69	BONN	-27.1354	-70.9199
E. breviflora var. *tenuis*	Chile	Caldera	n/a	F. Ritter 215A	L	-27.0681	-70.8192
E. breviflora var. *tenuis*	Chile	46 km S of Chañaral	n/a	Eggli & Leuenberger 2868	ZSS	-26.7167	-70.7358
E. breviflora var. *tenuis*	Chile	17 km S of Chañaral	n/a	Eggli & Leuenberger 1790	ZSS	-26.4282	-70.6903
E. breviflora var. *tenuis*	Chile	12 km S of Chañaral	n/a	Eggli & Leuenberger 2630C	ZSS	-26.4282	-70.6903
E. castanea	Chile	Los Molles	ED3109	F. Luebert 3757	BONN	-32.2425	-71.5157
E. castanea	Chile	Totoralillo	ED5134*	F.F. Merklinger 2019-1	BONN	-31.9903	-71.5102
E. castanea	Chile	Caleta El Toro	ED4667*	F. Luebert 3791	BONN	-30.7372	-71.6999

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Table S1: Continued from previous page

Taxon	**Country**	**Locality**	**Sample**	**Voucher**	**Herbarium**	**Latitude**	**Longitude**
E. castanea	Chile	Tongoy	ED5136*	F.F. Merklinger 2019-3	BONN	-30.3092	-71.5918
E. castanea	Chile	Pichidangui	n/a	F. Ritter 241 (loc.2)	SGO	-32.1400	-71.5294
E. castanea	Chile	Panamericana N of Los Vilos	n/a	Eggli & Leuenberger 1669	ZSS	-31.8553	-71.5134
E. castanea	Chile	Los Vilos	n/a	F. Ritter 241	SGO	-31.9128	-71.5003
E. castanea	Chile	Pichidangui	n/a	Eggli & Leuenberger 2933	ZSS	-32.1250	-71.5333
E. castanea	Chile	Los Vilos to Totoralillo	n/a	F.F. Merklinger 2018-89	BONN	-32.0145	-71.5099
E. castanea	Chile	3 km W of Puerto Oscuro	n/a	Eggli & Leuenberger 3088	ZSS	-31.4212	-71.4280
E. castanea	Chile	2 km N of Caleta Oscuro	n/a	F.F. Merklinger 2018-95	BONN	-31.4032	-71.6030
E. castanea	Chile	Parque Eolico de Arrayan	n/a	F.F. Merklinger 2018-88	BONN	-30.5449	-71.6930
E. castanea	Chile	Parque Nacional Fray Jorge	n/a	Kraus s.n.	ZSS	-30.6631	-71.6697
E. castanea	Chile	Parque Nacional Fray Jorge	n/a	Eggli & Leuenberger 3086D	ZSS	-30.6323	-71.7077
E. castanea	Chile	Ovalle, Quebrada San Pedro	n/a	Eggli & Leuenberger 2945	ZSS	-30.9867	-71.6387
E. castanea	Chile	Tongoy	n/a	Eggli & Leuenberger 3083	ZSS	-30.3085	-71.5920
E. castanea	Chile	La Ballena	n/a	F. Luebert 4116	BONN	-32.2910	-71.4744
E. castanea	Chile	N of Puente El Chivato	n/a	Eggli & Leuenberger 2678	ZSS	-32.2380	-71.4970
E. castanea	Chile	Los Molles	n/a	Landbeck s.n.	SGO	-32.2067	-71.4464
E. castanea	Chile	Los Molles	n/a	Eggli & Leuenberger 2932A	ZSS	-32.2430	-71.5148
E. castanea	Chile	21 km N of Papudo	n/a	Eggli & Leuenberger 1656	ZSS	-32.2384	-71.4972
E. castanea (hybrid)	Chile	Tres Playitas	n/a	F. Luebert 3852A	BONN	-28.4005	-71.1676
E. hybrid	Chile	2 km SE of Chungungo	n/a	Eggli & Leuenberger 3039A	ZSS	-29.4520	-71.2865
E. hybrid	Chile	1 km E of El Peñon	n/a	Eggli & Leuenberger 2576	ZSS	-30.1450	-71.2155
E. hybrid	Chile	1 km E of El Peñon	n/a	Eggli & Leuenberger 2576A	ZSS	-30.1450	-71.2155
E. hybrid	Chile	Punta Teatinos	n/a	Eggli & Leuenberger 1846	ZSS	-29.8222	-71.3120
E. iquiquensis	Chile	Punta Gruesa	ED3122	F. Luebert 4016	BONN	-20.3777	-70.1398
E. iquiquensis	Chile	Alto Chipana	ED5149*	F.F. Merklinger 2019-19	BONN	-21.2937	-70.0414
E. iquiquensis	Chile	Iquique	n/a	F. Ritter 202	SGO	-20.2131	-70.1503
E. iquiquensis	Chile	Above Playa Lobito	n/a	Eggli & Leuenberger 2835	ZSS	-20.3995	-70.1598
E. iquiquensis	Chile	Punta Gruesa	n/a	R. Pinto s.n.	SGO	-20.3667	-70.1500
E. iquiquensis	Chile	Alto Chipana	n/a	R. Pinto s.n.	SGO	-21.2667	-70.0500
E. iquiquensis	Chile	Alto Chipana	n/a	M.A. Trivelli s.n.	SGO	-21.2937	-70.0414
E. iquiquensis	Chile	Quebrada Caracoles	n/a	Eggli & Leuenberger 2701	ZSS	-23.6187	-70.3328
E. iquiquensis	Chile	Quebrada La Chimba	n/a	R. Rodríguez 3092	SGO	-23.5467	-70.3644
E. iquiquensis	Chile	Quebrada La Chimba	n/a	F. Kattermann 1076	SGO	-23.5333	-70.3500
E. iquiquensis	Chile	La Chimba, quebr. Guanaco	n/a	F.F. Merklinger 2018-56	BONN	-23.5492	-70.3581
E. iquiquensis	Chile	c. 38 km S of Tocopilla	n/a	Eggli & Leuenberger 2843	ZSS	-22.3712	-70.2280
E. iquiquensis var. *pullilana*	Chile	El Cobre	ED3120	F. Luebert 3967	BONN	-24.2710	-70.5176
E. iquiquensis var. *pullilana*	Chile	Near El Cobre	n/a	Eggli & Leuenberger 2850	ZSS	-24.2975	-70.4940
E. iquiquensis var. *pullilana*	Chile	El Cobre	n/a	F. Ritter 479B	L	-30.3833	-71.2000
E. iquiquensis var. *pullilana*	Chile	Hills above El Cobre	n/a	F.F. Merklinger 2018-59	BONN	-24.3017	-70.5135
E. iquiquensis var. *pullilana*	Chile	Hills S of Caleta Coloso	n/a	F. Luebert 3976	BONN	-23.8263	-70.4868
E. iquiquensis var. *pullilana*	Chile	Hills S of Caleta Coloso	n/a	F.F. Merklinger 2018-57	BONN	-23.8131	-70.4934

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Table S1: Continued from previous page

Taxon	**Country**	**Locality**	**Sample**	**Voucher**	**Herbarium**	**Latitude**	**Longitude**
E. morromorenoensis	Chile	Morro Moreno	ED3121	F. Luebert 3983	BONN	-23.4911	-70.5918
E. morromorenoensis	Chile	Cerro Moreno	n/a	F. Ritter 202A	ZSS	-23.4656	-70.5644
E. morromorenoensis	Chile	Cerro Moreno	n/a	F. Ritter 479	ZSS	-23.4656	-70.5644
E. morromorenoensis	Chile	Morro Moreno	n/a	Eggli & Leuenberger 2694	ZSS	-23.4880	-70.5997
E. ritteri	Peru	Chala	ED3489	cult. Gibraltar BG	n/a	-15.8536	-74.2361
E. ritteri	Peru	Lomas de Atiquipa	ED4430*	F.F. Merklinger 2018-151	BONN	-15.7215	-74.3918
E. ritteri	Peru	Above Chala	n/a	E. Markus s.n.	ZSS	-15.7694	-74.1897
E. saint-pieana	Chile	Chañaral	ED3118	F. Luebert3910	BONN	-26.2967	-70.6313
E. saint-pieana	Chile	El Caleuche	ED5146*	F.F. Merklinger 2019-14	BONN	-26.4130	-70.6493
E. saint-pieana	Chile	La Madera	ED5147*	F.F. Merklinger 2019-15	BONN	-25.7727	-70.6835
E. saint-pieana	Chile	Las Tórtolas	ED5148*	F.F. Merklinger 2019-16	BONN	-25.5573	-70.6164
E. saint-pieana	Chile	Sierra Esmeralda	n/a	P.C. Hutchinson 421	ZSS	-25.8863	-70.6496
E. saint-pieana	Chile	Quebrada Cachina	n/a	F. Kattermann 1274	SGO	-25.9101	-70.6267
E. saint-pieana	Chile	Pan de Azúcar	n/a	F. Kattermann 1054	ZSS	-26.0000	-70.6000
E. saint-pieana	Chile	Road to Caleta Esmeralda	n/a	F. Luebert 3914	BONN	-25.8938	-70.6482
E. saint-pieana	Chile	Falda Verde	n/a	Eggli 2887	ZSS	-26.2902	-70.6378
E. saint-pieana	Chile	Chañaral	n/a	F. Ritter 479A	SGO	-26.3736	-70.3381
E. spec. 1	Chile	Piedra Colgada	ED3115	F. Luebert 3884	BONN	-27.3059	-70.4954
E. spec. 1	Chile	Piedra Colgada	n/a	F. Ritter 215A (loc.2)	SGO	-27.3111	-70.4956
E. spec. 1	Chile	Piedra Colgada	n/a	F.F. Merklinger 2018-68	BONN	-27.3059	-70.4954
E. spec. 1	Chile	Piedra Colgada	n/a	F.F. Merklinger 2019-25	BONN	-27.3059	-70.4954
E. spec. 2	Chile	La Serena	ED3112	F. Luebert 3814	BONN	-29.8531	-71.2547
E. spec. 2	Chile	La Serena	n/a	F.F. Merklinger 2019-4	BONN	-29.8531	-71.2547
E. taltalensis	Chile	Taltal	ED3119	F. Luebert 3921	BONN	-25.4279	-70.4356
E. taltalensis	Chile	Paposo	ED5151*	F. Luebert 3933	BONN	-25.0066	-70.4500
E. taltalensis	Chile	Taltal	n/a	E. Werdermann 869	BM	-25.4069	-70.4853
E. taltalensis	Chile	Taltal	n/a	F. Ritter 214	L	-25.4069	-70.4853
E. taltalensis	Chile	Quebrada Taltal	n/a	Eggli & Leuenberger 2639A	ZSS	-25.4858	-70.4285
E. taltalensis	Chile	Quebrada San Ramón	n/a	Eggli & Leuenberger 2661	ZSS	-25.3823	-70.3875
E. taltalensis	Chile	Cerro Perales	n/a	Eggli 2915A	ZSS	-25.4250	-70.4210
E. taltalensis	Chile	17 km N of Taltal	n/a	Eggli & Leuenberger 1755	ZSS	-25.2684	-70.4393
E. taltalensis	Chile	20 km N of Taltal	n/a	F.F. Merklinger 2018-62	BONN	-25.2395	-70.4287
E. taltalensis	Chile	17 km N of Paposo	n/a	F.F. Merklinger 2018-61	BONN	-24.8938	-70.5227
E. taltalensis	Chile	32 km N of Paposo	n/a	F.F. Merklinger 2018-60	BONN	-24.7603	-70.5501
E. taltalensis	Chile	Miguel Díaz	n/a	F. Luebert 3954	BONN	-24.5488	-70.5651
E. vallenarensis	Chile	Vallenar	ED4802	F. Luebert 4041	BONN	-28.7186	-70.7641
E. vallenarensis	Chile	Panamericana km 645	n/a	P. Guerrero 1258	CONC	-28.7134	-70.7610

Table S2: List of primers for the three plastid DNA regions used in this study. Location = region in the chloroplast genome; Primer = lab name of the Primer; Direct. = forward (F) or reverse (R) reading direction of the primer.

Location	**Primer**	**Sequence**	**Direction**	**Reference**
*trn*K	trnK-Fbryo1	GGGTTGCTAACTCAATGGTAGAG	F	Wicke and Quandt 2009
*psb*A	psbA-R	CGCGTCTCTCTAAAATTGCAGTCAT	R	K. Steele in Johnson and Soltis 1995
*mat*K	matK710F	GTATCGCACTATGTWTCATTTGA	F	Johnson and Soltis 1995
*mat*K	matkEu2193R	GAAGAATCTCTTGGTAAGATC	R	this study
*mat*K	matkEu2084R	GTTTACGAGCCAAAGTTCTAGC	R	this study
*trn*L$_{\mathrm{UGU}}$	trnL15	A(AT)TGGTAGACGCTRCGGACT	F	Noben et al. 2017
*trn*F$_{\mathrm{GAA}}$	trnF39	TTTGAACTGGTGACACRAGGA	R	Noben et al. 2017
*trn*L$_{\mathrm{UAA}}$	trnL-C	CGAAATCGGTAGACGCTACG	F	Taberlet et al. 1991
*trn*L$_{\mathrm{UAA}}$	trnTF-D	GGGGATAGAGGGACTTGAAC	R	Taberlet et al. 1991
*trn*L	trnL-460F	GAGAATAAAGATAGAGTCC	F	Worberg et al. 2007
*trn*F$_{\mathrm{GAA}}$	trnL-F	ATTTGAACTGGTGACACGAG	R	Taberlet et al. 1991
*rpl*16 Ex1	F71	GGCTATGCTTAGTGTGTGACTCGTT	F	Jordan et al. 1996
*rpl*16 Ex2	rpl16R	GTAATCCAAGCTGGTTCAAGTGC	R	Olsson et al. 2009
*rpl*16 Ex1	rpl16F	CTATGCTTAGTGTGTGACTC	F	Campagna and Downie 1998
*rpl*16 Ex2	rpl16R	TCTTCCTCTATGTTGTTTACG	R	Campagna and Downie 1998

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Table S3: PCR program *trn*K-*mat*K

	Temperature [°C]	**Time [s]**	
1	95	120	
2	50	60	
3	72	90	
4	95	30	
5	50	60	39x
6	72	90	
7	8	break	

Table S4: PCR program *PCR programm trn*L-*trn*F

	Temperature [°C]	**Time [s]**	
1	94	120	
2	94	30	
3	52	60	35x
4	72	60	
5	72	600	
6	8	break	

Table S5: PCR program *rpl*16

	Temperature [°C]	**Time [s]**	
1	95	120	
2	95	30	
3	56	60	35x
4	68	90	
5	68	240	
6	8	break	

Table S6: Geographical distribution of the taxa used in the BioGeoBEARS analyses.

Taxon	**A**	**B**	**C**	**D**	**E**	**F**
Eulychnia iquiquensis					X	
E. taltalensis					X	
E. saint-pieana					X	
E. breviflora var. *tenuis*					X	
E. spec. 1				X		
E. breviflora			X			
E. acida var. *elata*		X				
E. acida var. *procumbens*		X	X			
E. acida		X	X			
E. castanea			X			
E. spec. 2			X			
E. ritteri						X
Austrocactus	X					

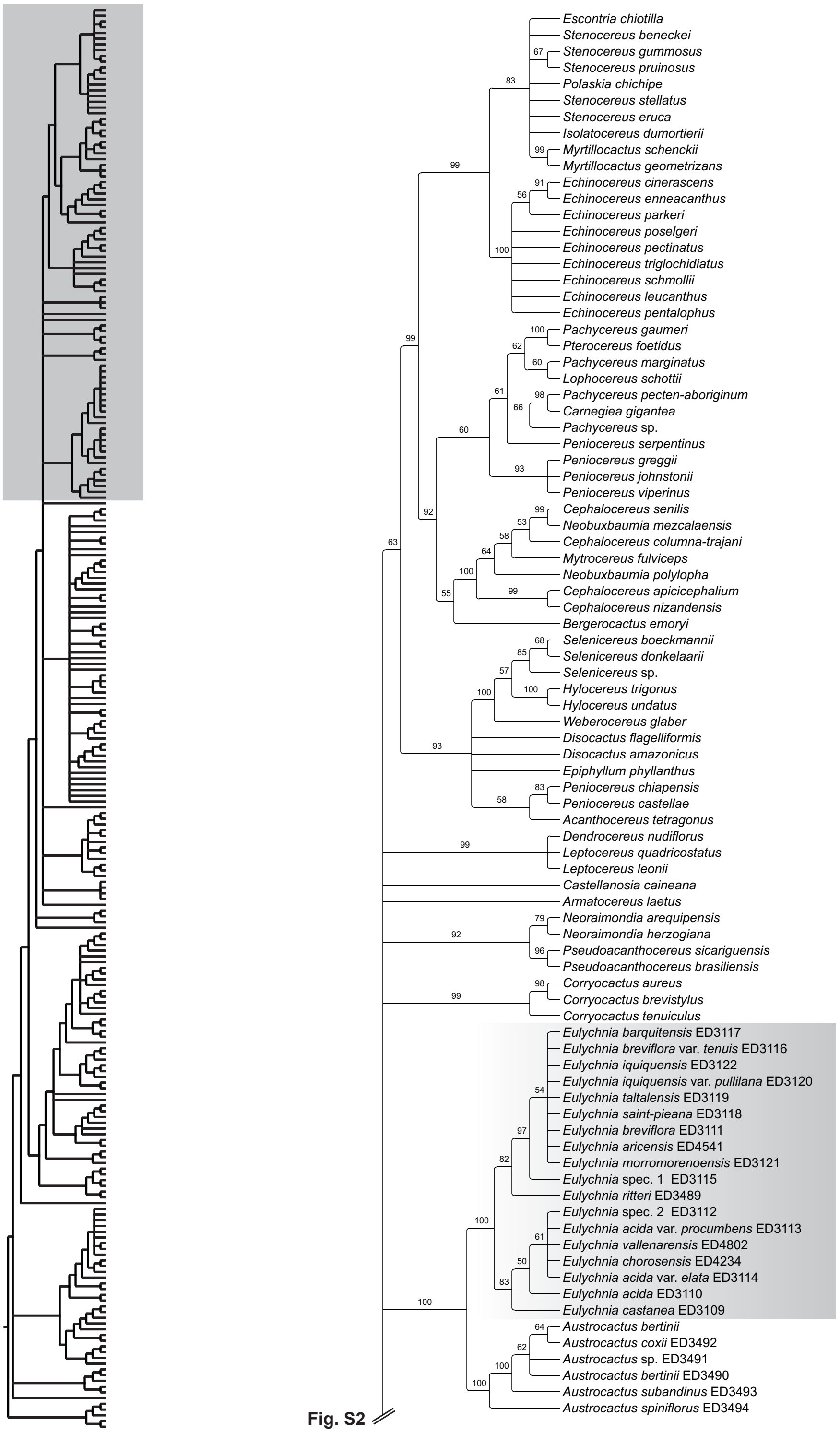

Figure S1: Maximum likelihood phylogeny of the Cactaceae family (part one of three), based on the dataset taken from Hernández-Hernández (2014) and including our own increased sampling of *Eulychnia* (shaded in grey) and *Austrocactus*. Numbers at nodes represent bootstrap values >50%.

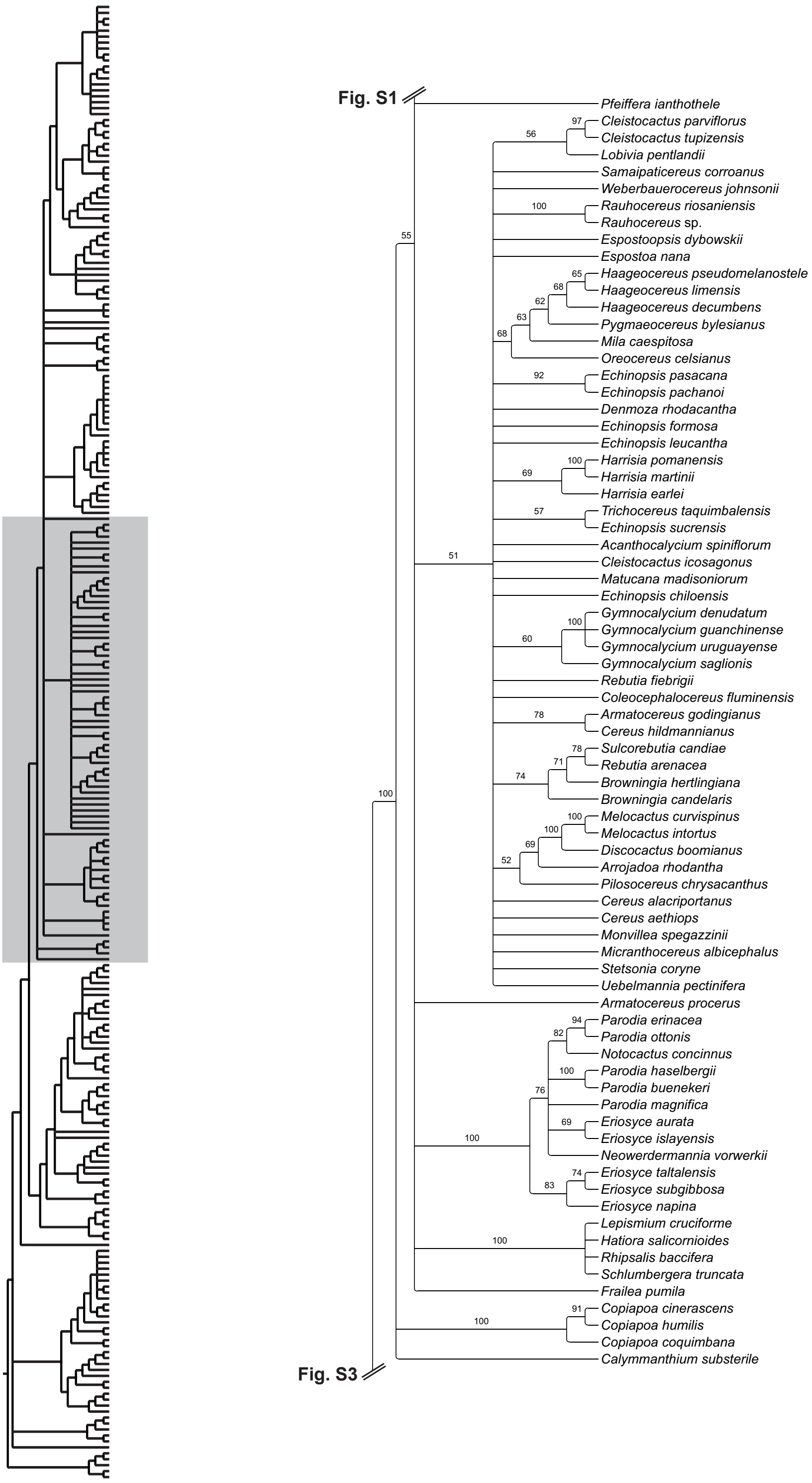

Figure S2: Maximum likelihood phylogeny of the Cactaceae family (part two of three), based on the dataset taken from Hernández-Hernández (2014).

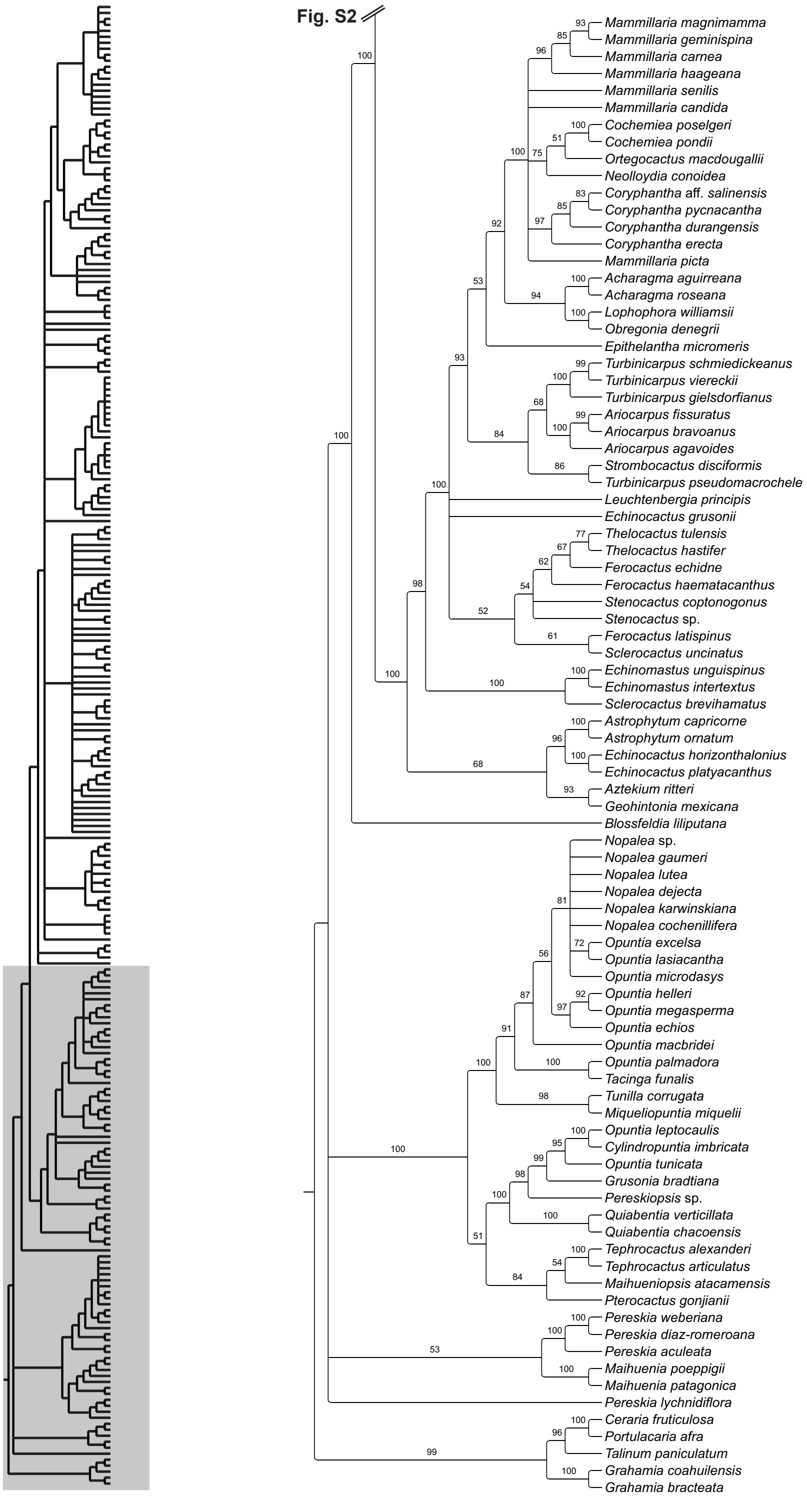

Figure S3: Maximum likelihood phylogeny of the Cactaceae family (three one of three), based on the dataset taken from Hernández-Hernández (2014).

Eulychnia Assembly of GBS sequences

August 24, 2020

1 Overall parameter setting and assembly start

[1]:
```
## imports
import ipyrad as ip
import ipyrad.analysis as ipa
import ipyparallel as ipp
import toytree
import toyplot
print("ipyrad v. {}".format(ip.__version__))
print("toytree v. {}".format(toytree.__version__))
```

```
/home/nees/anaconda3/envs/ipyrad_09/lib/python3.7/site-
packages/sklearn/utils/deprecation.py:144: FutureWarning: The
sklearn.neighbors.nearest_centroid module is  deprecated in version 0.22 and
will be removed in version 0.24. The corresponding classes / functions should
instead be imported from sklearn.neighbors. Anything that cannot be imported
from sklearn.neighbors is now part of the private API.
  warnings.warn(message, FutureWarning)
```

```
ipyrad v. 0.9.17
toytree v. 1.0.8
```

1.1 Open a separate terminal to start clusters

[ ]:
```
## ipcluster start --n=8
```

[2]:
```
## connect to cluster
ipyclient = ipp.Client()
ipyclient.ids
print(ip.cluster_info(ipyclient))
```

```
Parallel connection | GenomicsAGQ: 8 cores
None
```

[3]:
```
## assembly name
data = ip.Assembly ("Eulychnia")
```

```
New Assembly: Eulychnia
```

[6]:
```
## set parameters
data.set_params("project_dir", "/home/nees/Ipyrad_Eul/Eul_Assembly/")
data.set_params("sorted_fastq_path", "/home/nees/Ipyrad_Eul/Eul_Seqfiles/*.fastq.
 ↪gz")
data.set_params("datatype", "ddrad")
data.set_params("restriction_overhang",("TGCAG", "GGCC"))
data.set_params("filter_adapters", "2")
data.set_params("min_samples_locus", "150")
data.set_params("output_formats", "*")
data.set_params("mindepth_statistical", 10)
data.set_params("clust_threshold", 0.9)
data.set_params("max_Hs_consens", (0.05))
##print all parameters
data.get_params()
```

[6]:
```
0   assembly_name             Eulychnia
1   project_dir               ~/Ipyrad_Eul/Eul_Assembly
2   raw_fastq_path
3   barcodes_path
4   sorted_fastq_path         ~/Ipyrad_Eul/Eul_Seqfiles/*.fastq.gz
5   assembly_method           denovo
6   reference_sequence
7   datatype                  ddrad
8   restriction_overhang      ('TGCAG', 'GGCC')
9   max_low_qual_bases        5
10  phred_Qscore_offset       33
11  mindepth_statistical      10
12  mindepth_majrule          6
13  maxdepth                  10000
14  clust_threshold           0.9
15  max_barcode_mismatch      0
16  filter_adapters           2
17  filter_min_trim_len       35
18  max_alleles_consens       2
19  max_Ns_consens            0.05
20  max_Hs_consens            0.05
21  min_samples_locus         150
22  max_SNPs_locus            0.2
23  max_Indels_locus          8
24  max_shared_Hs_locus       0.5
25  trim_reads                (0, 0, 0, 0)
26  trim_loci                 (0, 0, 0, 0)
27  output_formats            ['l', 'p', 's', 'n', 'k', 'a', 'g', 'G', 'u',
'v', 't', 'm']
28  pop_assign_file
29  reference_as_filter
```

[7]:
```
## run step 1 & 2 of the assembly
data.run("12", force =True)
```

```
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:26 | loading reads        | s1 |
[####################] 100% 0:03:47 | processing reads     | s2 |
```

[8]:
```
## run step 3-6 of the assembly
data.run("3456", force = True)
```

```
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:32 | dereplicating        | s3 |
[####################] 100% 0:12:24 | clustering/mapping   | s3 |
[####################] 100% 0:00:01 | building clusters    | s3 |
[####################] 100% 0:00:00 | chunking clusters    | s3 |
[####################] 100% 0:21:49 | aligning clusters    | s3 |
[####################] 100% 0:00:32 | concat clusters      | s3 |
[####################] 100% 0:00:04 | calc cluster stats   | s3 |
[####################] 100% 0:00:57 | inferring [H, E]     | s4 |
[####################] 100% 0:00:04 | calculating depths   | s5 |
[####################] 100% 0:00:07 | chunking clusters    | s5 |
[####################] 100% 0:06:14 | consens calling      | s5 |
[####################] 100% 0:00:08 | indexing alleles     | s5 |
[####################] 100% 0:00:02 | concatenating inputs | s6 |
[####################] 100% 0:00:40 | clustering tier 1    | s6 |
[####################] 100% 0:00:00 | concatenating inputs | s6 |
[####################] 100% 0:00:32 | clustering across    | s6 |
[####################] 100% 0:00:03 | building clusters    | s6 |
[####################] 100% 0:01:03 | aligning clusters    | s6 |
```

[4]:
```
## load assembly object
data = ip.load_json("./Eulychnia.json")
```

```
loading Assembly: Eulychnia
from saved path: ~/Ipyrad_Eul/Eul_Assembly/Eulychnia.json
```

[5]:
```
## exclude samples from assembly with low read number
keep_list = [i for i in data.samples.keys() if i not in ["Aus_sp_ED3491"]]
data1 = data.branch("data1", subsamples = keep_list)
```

[13]:
```
## assembly with 8 or more samples shared across all loci = 20 % | 80 % missing␣
 ↪data
pops8 = data1.branch("pops8")
pops8.populations = {
    "ingroup": (8, [i for i in pops8.samples if "Eul_" in i]),
    "outgroup": (0, [i for i in pops8.samples if "Aus_" in i]),
    }
```

```
pops8.run("7", force = True)

## assembly with 12 or more samples shared across all loci = 30 % | 70 % missing␣
 ↪data
pops12 = data1.branch("pops12")
pops12.populations = {
    "ingroup": (12, [i for i in pops12.samples if "Eul_" in i]),
    "outgroup": (0, [i for i in pops12.samples if "Aus_" in i]),
    }
pops12.run("7", force = True)

## assembly with 16 or more samples shared across all loci = 40 % | 60 % missing␣
 ↪data
pops16 = data1.branch("pops16")
pops16.populations = {
    "ingroup": (16, [i for i in pops16.samples if "Eul_" in i]),
    "outgroup": (0, [i for i in pops16.samples if "Aus_" in i]),
    }
pops16.run("7", force = True)

## assembly with 20 or more samples shared across all loci = 50 % | 50 % missing␣
 ↪data
pops20 = data1.branch("pops20")
pops20.populations = {
    "ingroup": (20, [i for i in pops20.samples if "Eul_" in i]),
    "outgroup": (0, [i for i in pops20.samples if "Aus_" in i]),
    }
pops20.run("7", force = True)
```

```
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:03 | applying filters     | s7 |
[####################] 100% 0:00:13 | building arrays      | s7 |
[####################] 100% 0:00:07 | writing conversions  | s7 |
[####################] 100% 0:00:42 | indexing vcf depths  | s7 |
[####################] 100% 0:00:39 | writing vcf output   | s7 |
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:04 | applying filters     | s7 |
[####################] 100% 0:00:12 | building arrays      | s7 |
[####################] 100% 0:00:06 | writing conversions  | s7 |
[####################] 100% 0:00:35 | indexing vcf depths  | s7 |
[####################] 100% 0:00:36 | writing vcf output   | s7 |
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:03 | applying filters     | s7 |
[####################] 100% 0:00:12 | building arrays      | s7 |
[####################] 100% 0:00:05 | writing conversions  | s7 |
[####################] 100% 0:00:30 | indexing vcf depths  | s7 |
[####################] 100% 0:00:33 | writing vcf output   | s7 |
```

```
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:03 | applying filters     | s7 |
[####################] 100% 0:00:11 | building arrays      | s7 |
[####################] 100% 0:00:05 | writing conversions  | s7 |
[####################] 100% 0:00:24 | indexing vcf depths  | s7 |
[####################] 100% 0:00:29 | writing vcf output   | s7 |
```

[4]:
```
## load assembly object
data2 = ip.load_json("./data1.json")
```

```
loading Assembly: data1
from saved path: ~/Ipyrad_Eul/Eul_Assembly/data1.json
```

[29]:
```
## create a subset with only Eulychnia samples for STRUCTURE analysis
keep_list1 = [i for i in data.samples.keys() if i not in "Aus_ber_ED3490"
"Aus_cox_ED3492" "Aus_sp_ED3491" "Aus_spin_ED3494" "Aus_sub_ED3493"
"Corr_Aus_Out_ED4540" "Corr_Aus_Out_ED5158"]
Eul_str = data2.branch("Eul_str", subsamples = keep_list1)
sorted(keep_list1)
```

[29]:
```
['Eul_aci_ED3110',
 'Eul_aci_ED5135',
 'Eul_aci_ED5137',
 'Eul_aci_ED5138',
 'Eul_aci_elata_ED3114',
 'Eul_aci_elata_ED5143',
 'Eul_aci_elata_ED5152',
 'Eul_aci_elata_ED5153',
 'Eul_aci_proc_Chorros_ED4234',
 'Eul_aci_proc_Chorros_ED5139',
 'Eul_aci_proc_Freirina_ED3113',
 'Eul_barq_ED3117',
 'Eul_brev_ED3111',
 'Eul_brev_ED5141',
 'Eul_brev_ED5142',
 'Eul_brev_ED5144',
 'Eul_brev_ED5150',
 'Eul_brev_ten_ED3116',
 'Eul_brev_ten_ED5145',
 'Eul_cast_ED3109',
 'Eul_cast_ED4667',
 'Eul_cast_ED5134',
 'Eul_cast_ED5136',
 'Eul_iqui_ED3122',
 'Eul_iqui_ED5149',
 'Eul_iqui_aricensis_ED4541',
 'Eul_iqui_morro_ED3121',
```

```
 'Eul_iqui_pull_ED3120',
 'Eul_rit_ED3489',
 'Eul_rit_ED4430',
 'Eul_saint-pieana_ED3118',
 'Eul_saint_pieana_ED5146',
 'Eul_saint_pieana_ED5147',
 'Eul_saint_pieana_ED5148',
 'Eul_spec1_PieCol_ED3115',
 'Eul_spec2_LaSerena_ED3112',
 'Eul_tal_ED3119',
 'Eul_tal_ED5151',
 'Eul_vall_ED4802',
 'Eul_vall_ED5140']
```

[30]:

```
## branch to create several data sets with different parameters and excluding␣
 ↪Corr & Aus
Eul_str_min8 = Eul_str.branch("Eul_str_min8")
Eul_str_min8.set_params("min_samples_locus", 8)
Eul_str_min8.run("7", force = True)

##create a branch for outputs with min_samples=12
Eul_str_min12 = Eul_str.branch("Eul_str_min12")
Eul_str_min12.set_params("min_samples_locus", 12)
Eul_str_min12.run("7", force = True)

##create a branch for outputs with min_samples=16
Eul_str_min16 = Eul_str.branch("Eul_str_min16")
Eul_str_min16.set_params("min_samples_locus", 16)
Eul_str_min16.run("7", force = True)

##create a branch for outputs with min_samples=20
Eul_str_min20 = Eul_str.branch("Eul_str_min20")
Eul_str_min20.set_params("min_samples_locus", 20)
Eul_str_min20.run("7", force = True)
```

```
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:06 | applying filters     | s7 |
[####################] 100% 0:00:09 | building arrays      | s7 |
[####################] 100% 0:00:06 | writing conversions  | s7 |
[####################] 100% 0:00:23 | indexing vcf depths  | s7 |
[####################] 100% 0:00:21 | writing vcf output   | s7 |
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:03 | applying filters     | s7 |
[####################] 100% 0:00:09 | building arrays      | s7 |
[####################] 100% 0:00:05 | writing conversions  | s7 |
[####################] 100% 0:00:19 | indexing vcf depths  | s7 |
[####################] 100% 0:00:19 | writing vcf output   | s7 |
```

```
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:03 | applying filters     | s7 |
[####################] 100% 0:00:08 | building arrays      | s7 |
[####################] 100% 0:00:05 | writing conversions  | s7 |
[####################] 100% 0:00:16 | indexing vcf depths  | s7 |
[####################] 100% 0:00:18 | writing vcf output   | s7 |
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 0:00:03 | applying filters     | s7 |
[####################] 100% 0:00:07 | building arrays      | s7 |
[####################] 100% 0:00:04 | writing conversions  | s7 |
[####################] 100% 0:00:13 | indexing vcf depths  | s7 |
[####################] 100% 0:00:16 | writing vcf output   | s7 |
```

1.2 Phylogenetic downstream analyses

```
[ ]: # conda install raxml -c bioconda
     # conda install toytree -c eaton-lab
     # conda install tetrad -c eaton-lab -c conda-forge
```

```
[3]: ## Load assemblies from their JSON file
     pops8 = ip.load_json("./pops8.json")
     pops12 = ip.load_json("./pops12.json")
     pops16 = ip.load_json("./pops16.json")
     pops20 = ip.load_json("./pops20.json")
```

```
loading Assembly: pops8
from saved path: ~/Ipyrad_Eul/Eul_Assembly/pops8.json
loading Assembly: pops12
from saved path: ~/Ipyrad_Eul/Eul_Assembly/pops12.json
loading Assembly: pops16
from saved path: ~/Ipyrad_Eul/Eul_Assembly/pops16.json
loading Assembly: pops20
from saved path: ~/Ipyrad_Eul/Eul_Assembly/pops20.json
```

1.3 RAxML — ML concatenation tree inference

```
[11]: ## loop to run RAxML on all four assemblies (pops8 -pops20)
      for dset in [pops8, pops12, pops16, pops20]:
          rax = ipa.raxml(
              workdir = "./Euly_RAxML_FINAL",
              name = dset.name,
              data = dset.outfiles.phy,
              N = 1000,
              T = 8,
              o = ["Corr_Aus_Out_SFB635_ED4540", "Corr_Aus_Out_ED5158"])
          #rax.run(force = True)
```

```
## Print the raxml command
print(rax.command)
```

```
/home/nees/anaconda3/envs/ipyrad_09/bin/raxmlHPC-PTHREADS-AVX2 -f a -T 8 -m
GTRGAMMA -n pops20 -w /home/nees/Ipyrad_Eul/Eul_Assembly/Euly_RAxML_FINAL -s
/home/nees/Ipyrad_Eul/Eul_Assembly/pops20_outfiles/pops20.phy -p 54321 -N 1000
-x 12345
```

[12]:

```
## access the resulting tree files
rax.trees
```

[12]:

```
bestTree
~/Ipyrad_Eul/Eul_Assembly/Euly_RAxML_FINAL/RAxML_bestTree.pops20
bipartitions
~/Ipyrad_Eul/Eul_Assembly/Euly_RAxML_FINAL/RAxML_bipartitions.pops20
bipartitionsBranchLabels
~/Ipyrad_Eul/Eul_Assembly/Euly_RAxML_FINAL/RAxML_bipartitionsBranchLabels.pops20
bootstrap
~/Ipyrad_Eul/Eul_Assembly/Euly_RAxML_FINAL/RAxML_bootstrap.pops20
info
~/Ipyrad_Eul/Eul_Assembly/Euly_RAxML_FINAL/RAxML_info.pops20
```

[27]:

```
## plot the resulting tree in the notebook with toytree
#tre = toytree.tree(rax.trees.bipartitions)
tre = toytree.tree("./Euly_RAxML_FINAL/RAxML_bipartitions.pops12")
rtre = tre.root(wildcard = "Corr")
# use canvas and axes function in order use export function
canvas, axes = rtre.ladderize(1).draw(
    width = 2000,
    height = 1200,
    tip_labels_align = True,
    node_labels = rtre.get_node_values("support"),
    node_sizes=0,
    node_labels_style={"font-size": "12px",
                       "baseline-shift": "5px",
                       "-toyplot-anchor-shift": "-15px"},
    )
```

[28]:

```
## exporting figures in SVG, PDF ...
import toyplot.svg
import toyplot.pdf
toyplot.svg.render(canvas, "Euly_RAxML_FINAL/RAxML_pops12.svg")
toyplot.pdf.render(canvas, "Euly_RAxML_FINAL/RAxML_pops12.pdf")
```

[9]:

```
## plot the resulting tree in the notebook with toytree
#tre = toytree.tree(rax.trees.bipartitions)
#tre = toytree.tree("./Euly_RAxML_FINAL/RAxML_bipartitions.pops12")
```

```
rtre = tre.root(wildcard = "Corr")
# use canvas and axes function in order use export function
canvas, axes = rtre.ladderize(1).draw(
    width = 1500,
    height = 900,
    tip_labels_align = True,
    node_labels = rtre.get_node_values("support"),
    node_sizes=0,
    node_labels_style={"font-size": "12px",
                       "baseline-shift": "7px",
                       "-toyplot-anchor-shift": "-13px"},
    )
```

```
        ---------------------------------------------------------------------------

        NameError                                 Traceback (most recent call last)

        <ipython-input-9-878a252523a0> in <module>
          1 ## plot the resulting tree in the notebook with toytree
    ----> 2 tre = toytree.tree(rax.trees.bipartitions)
          3 #tre = toytree.tree("./Euly_RAxML_FINAL/RAxML_bipartitions.pops12")
          4 rtre = tre.root(wildcard = "Corr")
          5 # use canvas and axes function in order use export function


        NameError: name 'rax' is not defined
```

2 tetrad - quartet tree inference

[5]:

```
tet = ipa.tetrad(
    name = 'Eul_Tet',
    workdir ="./Eul_Tetrad",
    data = "./pops12_outfiles/pops12.snps.hdf5",
    mapfile = "./pops12_outfiles/pops12.snpsmap",
    nboots = 1000
    )

#tet.run(ipyclient=ipyclient, force = True)
```

```
loading snps array [46 taxa x 129119 snps]
max unlinked SNPs per quartet [nloci]: 16758
quartet sampler [random, nsamples**2.8]: 45260 / 163185
```

[6]:
```
## access the resulting tree files
tet.trees
```

[6]:
```
('tree', '/home/nees/Ipyrad_Eul/Eul_Assembly/Eul_Tetrad/Eul_Tet.tree')
('cons', '/home/nees/Ipyrad_Eul/Eul_Assembly/Eul_Tetrad/Eul_Tet.tree.cons')
('boots', '/home/nees/Ipyrad_Eul/Eul_Assembly/Eul_Tetrad/Eul_Tet.tree.boots')
('nhx', '/home/nees/Ipyrad_Eul/Eul_Assembly/Eul_Tetrad/Eul_Tet.tree.nhx')
```

[32]:
```
tre = toytree.tree("./Eul_Tetrad/Eul_Tet.tree.cons")
rtre = tre.root(wildcard = "Corr_Aus_")
#rtre.draw(tip_labels_align=True, node_labels="support")

# use canvas and axes function in order use export function
canvas, axes = rtre.ladderize(1).draw(
    width = 2000,
    height = 1200,
    #use_edge_length = False,
    tip_labels_align = True,
    node_labels = rtre.get_node_values("support"),
    node_sizes=0,
    node_labels_style={"font-size": "16px",
                       "baseline-shift": "7px",
                       "-toyplot-anchor-shift": "-13px"},
    )
```

[33]:
```
## exporting figures in SVG, PDF ...
import toyplot.svg
import toyplot.pdf
toyplot.svg.render(canvas, "Eul_Tetrad/Eul_Tetrad_pops12.svg")
toyplot.pdf.render(canvas, "Eul_Tetrad/Eul_Tetrad_pops12.pdf")
```

[21]:
```
mtre = toytree.mtree(tet.trees.boots)
mtre.treelist = [i.root(["Corr_Aus_Out_ED5158", "Corr_Aus_Out_ED4540"]) for i in␣
 ↪mtre.treelist]
canvas, axes = mtre.draw_cloud_tree(
   height=600,
   width=400,
   use_edge_lengths=False,
   html=True,
);
```

[22]:
```
## exporting figures in SVG, PDF ...
import toyplot.svg
import toyplot.pdf
toyplot.svg.render(canvas, "Eul_Tetrad/Eul_cloudtree_pops12.svg")
toyplot.pdf.render(canvas, "Eul_Tetrad/Eul_cloudtree_pops12.pdf")
```

2.1 Plotting RAxML & Tetrad trees besides one another

[23]:

```
print(mtre)
```

```
<toytree.Multitree.MultiTree object at 0x7ff9c1a8dfd0>
```

[45]:

```
## Load trees
Eul_ML_pops12 = toytree.tree("./Euly_RAxML_FINAL/RAxML_bipartitions.pops12")
Eul_Tet_pops12 = toytree.tree("./Eul_Tetrad/Eul_Tet.tree.cons")
#Eul_Tetcloud_pops12 = toytree.mtree("./Eul_Tetrad/Eul_Tet.trees.boots")
#mtre = toytree.mtree(tet.trees.boots)

## root the trees
rEul_ML_pops12 = Eul_ML_pops12.root(wildcard = "Corr_Aus_")
rEul_Tet_pops12 = Eul_Tet_pops12.root(wildcard = "Corr_Aus_")
#rEul_Tetcloud_pops12 = Eul_Tetcloud_pops12.root(wildcard = "Corr_Aus_")
#mtre.treelist = [i.root(["Corr_Aus_Out_ED5158", "Corr_Aus_Out_ED4540"]) for i␣
 ↪in mtre.treelist]

# set dimensions of the canvas
canvas = toyplot.Canvas(width=3000, height=1200)

# dissect canvas into multiple cartesian areas (x1, x2, y1, y2)
ax0 = canvas.cartesian(bounds=('2%', '40%', '5%', '95%'))
ax1 = canvas.cartesian(bounds=('35%', '70%', '5%', '95%'))
#ax2 = canvas.cartesian(bounds=('65%', '95%', '5%', '95%'))

# call draw with the 'axes' argument to pass it to a specific cartesian area
style = {
    "tip_labels_align": True,
    "tip_labels_style": {"font-size": "9px"},
    "node_labels_style": {"font-size": "12px",
                          "baseline-shift": "7px",
                          "-toyplot-anchor-shift": "-13px"},
}
rEul_ML_pops12.ladderize(1).draw(
    axes=ax0, **style, node_sizes=0,
    node_labels = rEul_ML_pops12.get_node_values("support"));
rEul_Tet_pops12.ladderize(1).draw(
    axes=ax1, **style, node_sizes=0,
    node_labels = rEul_Tet_pops12.get_node_values("support"));
#Eul_Tetcloud_pops12.draw_cloud_tree(
#    axes=ax2, **style, node_sizes=0,
#    node_labels = True);
#mtre.draw_cloud_tree(
 #  axes=ax2,use_edge_lengths=False,
  # html=True,);
```

```

# hide the axes (e.g, ticks and splines)
ax0.show=False; ax1.show=False; #ax2.show=False
```

[41]:
```
## exporting figures in SVG, PDF ...
import toyplot.svg
import toyplot.pdf
toyplot.svg.render(canvas, "./Eul_Tetrad/Eul_ML_Tetrad_pops12.svg")
toyplot.pdf.render(canvas, "./Eul_Tetrad/Eul_ML_Tetrad_pops12.pdf")
```

2.2 Structure analyses

[4]:
```
## imports
import ipyrad as ip
import ipyrad.analysis as ipa
import ipyparallel as ipp
import toytree
print("ipyrad v. {}".format(ip.__version__))
print("toytree v. {}".format(toytree.__version__))
```

```
ipyrad v. 0.9.17
toytree v. 1.0.8
```

[5]:
```
## ipcluster start --n=8
## connect to cluster
ipyclient = ipp.Client()
print("{} engines found".format(len(ipyclient)))
```

```
8 engines found
```

[6]:
```
## Structure
import toyplot
str_data = "/home/nees/Ipyrad_Eul/Eul_Assembly/Eul_str_min12_outfiles/
 ↪Eul_str_min12.snps.hdf5"
```

[146]:
```
#group individuals into populations
imap = {
    "Erit": ["Eul_rit_ED4430", "Eul_rit_ED3489"],
    "Eahybrid": ["Eul_aci_ED5137", "Eul_spec2_LaSerena_ED3112"],
    "Ecast": ["Eul_cast_ED3109", "Eul_cast_ED5134", "Eul_cast_ED4667"],
    "Eaci": ["Eul_aci_ED5135", "Eul_aci_ED3110", "Eul_aci_ED5138"],
    "Ech": ["Eul_aci_proc_Chorros_ED5139", "Eul_aci_proc_Chorros_ED4234",␣
 ↪"Eul_vall_ED5140", "Eul_aci_proc_Freirina_ED3113", "Eul_vall_ED4802"],
    "Ela": ["Eul_aci_elata_ED5143", "Eul_aci_elata_ED5152",␣
 ↪"Eul_aci_elata_ED5153", "Eul_aci_elata_ED3114"],
```

```
    "Ebrev": ["Eul_brev_ED5150", "Eul_cast_ED5136", "Eul_brev_ED3111",␣
 ↪"Eul_brev_ED5144", "Eul_spec1_PieCol_ED3115", "Eul_brev_ED5141",␣
 ↪"Eul_brev_ED5142"],
    "Eten":["Eul_brev_ten_ED3116", "Eul_brev_ten_ED5145"],
    "Esp": ["Eul_saint-pieana_ED3118", "Eul_saint_pieana_ED5146",␣
 ↪"Eul_barq_ED3117"],
    "Etal": ["Eul_saint_pieana_ED5147", "Eul_tal_ED3119",␣
 ↪"Eul_saint_pieana_ED5148", "Eul_tal_ED5151", "Eul_iqui_pull_ED3120"],
    "Eiqu": ["Eul_iqui_morro_ED3121", "Eul_iqui_ED5149",␣
 ↪"Eul_iqui_aricensis_ED4541", "Eul_iqui_ED3122"]
}

#require that 50% of samples have data in each group
minmap = {i: 0.5 for i in imap}
```

[10]:

```
## create a STRUCTURE object
struct = ipa.structure(
    name = 'Eul_min12_Str',
    data = str_data,
    imap=imap,
    minmap=minmap,
    mincov=0.9,
)
```

```
15 previous results loaded for run [Eul_min12_Str]
Samples: 40
Sites before filtering: 82524
Filtered (indels): 12209
Filtered (bi-allel): 3185
Filtered (mincov): 48380
Filtered (minmap): 45907
Filtered (combined): 52424
Sites after filtering: 30100
Sites containing missing values: 15493 (51.47%)
Missing values in SNP matrix: 30395 (2.52%)
```

[22]:

```
#run structure and plot results

struct.mainparams.burnin = 100000
struct.mainparams.numreps = 500000
```

[23]:

```
struct.run(nreps=20, kpop=[2, 3, 4, 5, 6], auto=True, force=True)
```

```
Parallel connection | GenomicsAGQ: 8 cores
[####################] 100% 2 days, 14:00:10 | running 100 structure jobs
```

[24]:

```
#analyze results, choosing K
etable = struct.get_evanno_table([2, 3, 4, 5, 6])
etable
```

[24]:

```
   Nreps       lnPK      lnPPK     deltaK  estLnProbMean  estLnProbStdev
2     20      0.000      0.000   0.000000     -84779.145      728.000425
3     20   -978.215   6705.510   2.926155     -85757.360     2291.577462
4     20   5727.295  53401.730  31.900927     -80030.065     1673.986754
5     20 -47674.435  81153.895   0.616143    -127704.500   131712.794003
6     20  33479.460      0.000   0.000000     -94225.040    50834.866814
```

[25]:

```
# get canvas object and set size
canvas = toyplot.Canvas(width=600, height=300)

# plot the mean log probability of the models in red
axes = canvas.cartesian(ylabel="estLnProbMean")
axes.plot(etable.estLnProbMean * -1, color="darkred", marker="o")
axes.y.spine.style = {"stroke": "darkred"}

# plot delta K with its own scale bar of left side and in blue
axes = axes.share("x", ylabel="deltaK", ymax=etable.deltaK.max() + etable.deltaK.
 ↪max() * .25)
axes.plot(etable.deltaK, color="steelblue", marker="o");
axes.y.spine.style = {"stroke": "steelblue"}

# set x labels
axes.x.ticks.locator = toyplot.locator.Explicit(range(len(etable.index)), etable.
 ↪index)
axes.x.label.text = "K (N ancestral populations)"
```

[171]:

```
#Barplots
k = 6
table = struct.get_clumpp_table(k)
```

```
[K6] 20/20 results permuted across replicates (max_var=0).
```

[172]:

```
# sort list by columns
table.sort_values(by=list(range(k)), inplace=True)

# or, sort by a list of names (here taken from imap)
import itertools
onames = list(itertools.chain(*imap.values()))
table = table.loc[onames]
```

[173]:

```
# build barplot
canvas = toyplot.Canvas(width=1500, height=500)
axes = canvas.cartesian(bounds=("20%", "80%", "20%", "60%"))
```

```
axes.bars(table)

# add labels to x-axis
ticklabels = [i for i in table.index.tolist()]
axes.x.ticks.locator = toyplot.locator.Explicit(labels=ticklabels)
axes.x.ticks.labels.angle = -90
axes.x.ticks.show = True
axes.x.ticks.labels.offset = 10
axes.x.ticks.labels.style = {"font-size": "12px"}
```

[174]:

```
##exporting figures in svg, pdf
import toyplot.svg
import toyplot.pdf
toyplot.svg.render(canvas, "Eul_struct_K6.svg")
toyplot.pdf.render(canvas, "Eul_struct_K6.pdf")
```

Appendix D

Supplementary data to Chapter 5

Table S1: Complete geo-referenced specimen data of all vouchers considered in this study.

Taxon	Country	Region	Province	Locality	Latitude	Longitude	Elevation (m)	Date	Collector	coll. no.	Herbarium
E. acida subsp. *acida*	Chile	III, Atacama	prov. Huasco	Quebrada Agua Grande, road Huasco to Caleta Tongoy	-28.55589	-71.19381		1 Nov 2007	F.Kattermann	1242	(SGO160944, as *E. breviflora*)
E. acida subsp. *acida*	Chile	III, Atacama	prov. Huasco	Vallenar, 99 km S of Vallenar along the Panamericana (8 km S of Incahuasi)	-29.29900	-71.03267	560 m	25 Oct 1997	Eggli & Leuenberger	3034	(ZSS18451, SGO145973)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	17 km W of the Panamericana at Trapiche following the road to Los Choros	-29.30950	-71.26867	180 m	19 Oct 1997	Eggli & Leuenberger	2960	(ZSS18422)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	3 km SW of Los Choros	-29.30350	-71.32933	40 m	19 Oct 1997	Eggli & Leuenberger	2968	(ZSS18440)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	0.5–1 km N of Punta Teatinos	-29.82028	-71.28083		2 Dec 1991	Eggli & Leuenberger	1846	(ZSS12916, SGO145688)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	Quebrada Los Choros along road to Punta Choros	-29.31613	-71.25063	210 m	17 Sep 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-6	(EIF, BONN, K)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	La Higuera, 4 km NW of Punta Alta (El Trapiche) at the Panamericana towards Choros Bajos, Quebrada de los Choros	-29.33933	-71.16183	200 – 300 m	19 Dec 1994	Eggli & Leuenberger	2672	(ZSS17459, SGO144143)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	Along road from Panamericana to Los Choros	-29.339337	-71.204058	250 m	27 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3834	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	Along road from Panamericana to Los Choros	-29.339337	-71.204058	250 m	27 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3834A	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	104 km S of Vallenar along Panamericana towards La Serena	-29.344140	-71.040017	430 m	1 Dec 1991	Eggli & Leuenberger	1840	(ZSS12920, SGO145683).
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	La Higuera, 2 km SE of Chungungo / Cruz Grande towards the Panamericana	-29.45200	-71.28650	120 m	25 Oct 1997	Eggli & Leuenberger	3039	(ZSS18288)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	N of La Serena, Cuesta Buenos Aires along Panamericana	-29.57849	-71.24825	300 m	17 Sep 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-5	(EIF, BONN, K)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	N of La Serena, Punta Teatinos	-29.79037	-71.29653	200 m	9 Sep 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-86	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	La Serena, Quebrada de Santa Gracia, 19 km N of El Islón towards Almirante Latorre	-29.80917	-71.08417	250 m	30 Oct 1997	Eggli & Leuenberger	3081	(ZSS18496, SGO144234)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	Road from Marquesa to Viñita Baja	-29.873778	-70.860812	700 m	26 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3826	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	Vicuña, 18 km W of Guanta on road CH41 towards Vicuña	-29.90700	-70.52700	950 m	27 Oct 1997	Eggli & Leuenberger	3053	(ZSS18374, SGO144112)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	Coquimbo	-29.95000	-71.33333		Sep 1885	Philippi	s.n.	(SGO052702, as type of *E. breviflora* but see comment by Eggli & Leuenberger)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	2 km W of El Molle	-29.96667	-70.95000	300 m	1 Feb 1996	F.Kattermann	1013	(SGO, photo)

Continued on next page

Table S1 – *Continued from previous page*

Taxon	**Country**	**Region**	**Province**	**Locality**	**Latitude**	**Longitude**	**Elevation (m)**	**Date**	**Collector**	**coll. no.**	**Herbarium**
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	D-485 towards Paiguano	-29.98494	-70.54169	921 m	8 Sep 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-84	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	37 km E of La Serena	-29.984236	-70.874471		s.a.	F.Ritter	232	(SGO125184, loc.2)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	2 km S of main road La Serena - Vicuña towards Observatory Tololo at entrance gate	-30.04650	-70.81950	620 m	26 Oct 1997	Eggli & Leuenberger	3041	(ZSS18373, SGO144111)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	La Serena, 1 km E of El Peñon	-30.14467	-71.21550	160 – 250 m	4 Dec 1994	Eggli & Leuenberger	2577	(ZSS17511, SGO144131)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	N of La Serena, Cuesta Las Cardas	-30.30133	-71.25728	510 m	7 Sep 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-79A	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	Coquimbo, 22 km W of Tongoy towards Puerto Aldea	-30.30850	-71.59200	20 – 50 m	31 Oct 1997	Eggli & Leuenberger	3085	(ZSS18466, SGO144249)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Elqui	Puerto Aldea, W of Tongoy	-30.30923	-71.59180	24 m	16 Sep 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-2	(EIF, BONN, K)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Limarí	Ovalle, Pichasca	-30.391775	-70.858125	750 m	2 May 1980	A.TroncosoA.	s.n.	(SGO128139)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Limarí	Ovalle, 13 km from asphalt road Ovalle-La Serena	-30.48217	-71.07117	380 m	3 Dec 1994	Eggli & Leuenberger	2566	(ZSS17442, SGO144123)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Limarí	5 km W of the Panamericana following the road to the Fray Jorge NP	-30.614771	-71.555678	190 – 220 m	3 Dec 1991	Eggli & Leuenberger	1852	(ZSS12915)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Limarí	Road from Los Loros to Caleta El Toro	-30.760181	-71.644371	250 m	23 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3782	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Limarí	Between Monte Patria and El Palqui above a gravel producing plant along D-55	-30.72539	-70.94874	800 m	14 Sep 2018	F.F.Merklinger & A.Kozok	2018-97	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Limarí	10 km N of Cogotí beyond La Isla towards La Ligua, c. 1 km S of La Ligua	-31.05417	-71.02817	720 m	2 Dec 1994	Eggli & Leuenberger	2564	(ZSS17443, SGO145768)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Choapa	S of Combarbalá near Reserva Nacional Las Chinchillas	-31.2925	-71.19541	640 m	13 Sep 2018	F.F.Merklinger & A.Kozok	2018-93	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Choapa	Road from Illapel to Combarbalá, near Illapel	-31.604095	-71.125953	450 m	22 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3767	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Choapa	Forest Reserve El Tambo near town of Salamanca	-31.79541	-71.00582	510 m	12 Sep 2018	F.F.Merklinger & A.Kozok	2018-90	(ULS, BONN)
E. acida subsp. *acida*	Chile	IV, Coquimbo	prov. Choapa	Los Vilos, between Tilama and Caimanes, 13 km N of Tilama	-31.98183	-71.14100	700 m	30 Nov 1994	Eggli & Leuenberger	2550	(ZSS17514, SGO145789)
E. acida subsp. *acidita*	Chile	IV, Coquimbo	prov. Elqui	N of La Serena along Panamericana, Fundo Juan Soldado	-29.8531	-71.2547	19 m	25 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3814	(ULS, BONN)
E. acida subsp. *acidita*	Chile	IV, Coquimbo	prov. Elqui	N of La Serena, Fundo Juan Soldado along Panamericana	-29.8531	-71.2547	20 m	17 Sep 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-4	(EIF, BONN, K).
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Elqui	Coquimbo, 22 km W of Tongoy towards Puerto Aldea	-30.30850	-71.59200	20 – 50 m	31 Oct 1997	Eggli & Leuenberger	3083	(ZSS18342, SGO144236)

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Table S1 – *Continued from previous page*

Taxon	**Country**	**Region**	**Province**	**Locality**	**Latitude**	**Longitude**	**Elevation (m)**	**Date**	**Collector**	**coll. no.**	**Herbarium**
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Elqui	Puerto Aldea, W of Tongoy	-30.30923	-71.59180	24 m	16 Sep 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-3	(EIF, BONN, K)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Limarí	Parque Eolico de Arrayan	-30.54494	-71.69301	24 m	10 Sep 2018	F.F.Merklinger, A.Kozok	2018-88	(ULS, BONN)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Limarí	Ovalle, Parque Nacional Fray Jorge	-30.63233	-71.70767	20 – 200 m	1 Nov 1997	Eggli & Leuenberger	3086d	(ZSS18340)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Limarí	Ovalle, Parque Nacional Fray Jorge	-30.66306	-71.66972		18 Nov 1991	Kraus	s.n.	(ZSS17978, photos)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Limarí	N of Caleta El Toro	-30.737239	-71.699907	25 m	23 Sep 2017	F.Luebert,T.Böhnert,F.F.Merklinger	3791	(ULS, BONN)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Limarí	Ovalle, first hill N of the mouth of the Quebrada San Pedro	-30.98667	-71.63867	80 – 140 m	18 Oct 1997	Eggli & Leuenberger	2945	(ZSS18447, SGO144113)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Choapa	2 km N of Caleta Oscuro	-31.40321	-71.603	10 m	13 Sep 2018	F.F.Merklinger, A.Kozok	2018-95	(ULS, BONN)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Choapa	Mincha, Caleta Oscuro, 3 km W of Puerto Oscuro	-31.42117	-71.59467	20 – 100 m	2 Nov 1997	Eggli & Leuenberger	3088	(ZSS18358, SGO144241)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Choapa	7.3 km on Panamericana N of turnoff from Panamericana to Los Vilos	-31.855318	-71.513728	40 m	10 Nov 1991	Eggli & Leuenberger	1669	(ZSS08010, SGO144223)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Choapa	Los Vilos	-31.91278	-71.50028		May 1954	F.Ritter	241	(SGO125187)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Choapa	N of Totoralillo below Panamericana and Trattoria del Mar ”L'Encuentro”	-31.99028	-71.51018	20 m	16 Sep 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-1	(EIF, BONN, K)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Choapa	Between Los Vilos and Totoralillo along Panamericana	-32.01445	-71.5099	32 m	11 Sep 2018	F.F.Merklinger, A.Kozok	2018-89	(ULS, BONN)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Choapa	Los Vilos, Pichidangui	-32.12500	-71.53333	10 – 50 m	17 Oct 1997	Eggli & Leuenberger	2933	(ZSS18483, SGO144100)
E. acida subsp. *castanea*	Chile	IV, Coquimbo	prov. Choapa	Pichidangui	-32.14000	-71.52944		s.a.	F.Ritter	241	(SGO125186, loc.2)
E. acida subsp. *castanea*	Chile	V, Valparaíso	prov. Petorca	La Ligua, 2 km S of turnoff of access road to Los Molles, just N of Puente El Chivato	-32.23800	-71.49700	20 – 50 m	21 Dec 1994	Eggli & Leuenberger	2678	(ZSS17494, SGO145769)
E. acida subsp. *castanea*	Chile	V, Valparaíso	prov. Petorca	21 km N along Panamericana from turnoff to Papudo towards Los Vilos	-32.238389	-71.497166	0 – 20 m	10 Nov 1991	Eggli & Leuenberger	1656	(ZSS07989, SGO117503).
E. acida subsp. *castanea*	Chile	V, Valparaíso	prov. Petorca	Los Molles	-32.242464	-71.515703		Nov 1862	Landbeck	s.n.	(SGO052701, LT)
E. acida subsp. *castanea*	Chile	V, Valparaíso	prov. Petorca	Los Molles	-32.242464	-71.515703	20 m	21 Sep 2017	F.Luebert,T.Böhnert,F.F.Merklinger	3757	(ULS, BONN)
E. acida subsp. *castanea*	Chile	V, Valparaíso	prov. Petorca	La Ligua, Los Molles, extreme SW tip of peninsula at Virgin statuette	-32.24300	-71.51483	10 m	17 Oct 1997	Eggli & Leuenberger	2932a	(ZSS18250, photos)
E. acida subsp. *castanea*	Chile	V, Valparaíso	prov. Petorca	La Ballena	-32.29095	-71.47437	15 m	10 Oct 2019	F.Luebert	4116	(EIF, BONN, K)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	A 400 m cruce Paipote-Diego de Almagro	-27.316950	-70.163592		25 Oct 1984	M.Muñoz S.	1988	(SGO108213)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	mountain NE of Copiapó	-27.365882	-70.291834		s.a.	F.Ritter	651	(SGO125196, loc.5).
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	Copiapó	-27.36667	-70.33139		16 May 1905	F.Ritter	651	(L [U0007883/U160674B, Holo])
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	Along Panamericana S of Copiapó	-27.45849	-70.37648	835 m	3 Oct 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-26	(EIF, BONN, K)

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Table S1 – *Continued from previous page*

Taxon	**Country**	**Region**	**Province**	**Locality**	**Latitude**	**Longitude**	**Elevation (m)**	**Date**	**Collector**	**coll. no.**	**Herbarium**
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	11 km S of Copiapó following the Panamericana towards Santiago	-27.465991	-70.376828	820 – 850 m	29 Nov 1991	Eggli & Leuenberger	1812	(ZSS12908, as *E. acida*, SGO145705)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	Road from Panamericana to Nantoco, c. 5 km past Panamericana	-27.62808	-70.38315	1000 m	29 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3881	(ULS, BONN)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	N of Caleta Pajonales along road	-27.68774	-70.914	72 m	3 Sep 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-71	(ULS, BONN)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	W of Castillo	-27.809369	-70.691954		11 May 1905	F.Ritter	s.n.	(L [U1601972], loc.3) Holo
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	Estancia Castilla, road from Totoral to Panamericana	-27.919994	-70.84531	240 m	28 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3866	(ULS, BONN)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Copiapó	Between Castillo and Totoral	-27.934554	-70.822443		s.a.	F.Ritter	651	(SGO125197, loc.6)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Huasco	Chorillos towards El Chapote	-28.150942	-70.900010		s.a.	F.Ritter	651	(SGO125195, loc.4)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Huasco	Road from Carrizal Bajo to Canto de Agua	-28.1534	-71.03077	161 m	4 Sep 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-74	(ULS, BONN)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Huasco	Road from Carrizal Bajo to Canto de Agua a few km before the Panamericana	-28.22400	-70.82675	321 m	18 Sep 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-11	(EIF, BONN, K)
E. acida subsp. *elata*	Chile	III, Atacama	prov. Huasco	16 km SE of Canto del Agua towards Panamericana	-28.23667	-70.82217	350 m	22 Oct 1997	Eggli & Leuenberger	3015a	(ZSS18326)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	Freirina	-28.50917	-71.08111		16 May 1905	F.Ritter	650	(L [U160433B] Holo)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	Freirina	-28.50917	-71.08111		11 May 1905	F.Ritter	s.n.	(L [U1601973]) ISO
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	Hills above Freirina	-28.514953	-71.079746	160 m	27 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3848	(ULS, BONN)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	Above Freirina	-28.51852	-71.05616		s.a.	F.Ritter	650	(SGO125194, loc.4)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	Hills above Freirina	-28.51852	-71.05616	178 m	4 Sep 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-72	(ULS, BONN)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	north of Nicolasa	-28.52361	-71.00861		s.a.	F.Ritter	650	(ZSS SR13520 [loc. 5, sem., paratype])
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	Freirina, c. 17 km W of Vallenar towards Huasco	-28.52983	-70.92750	300 m	22 Oct 1997	Eggli & Leuenberger	2994	(ZSS18299, SGO146016)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	20 km W of Vallenar	-28.544427	-70.953428		s.a.	F.Ritter	650	(SGO125193, loc.3)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	Freirina, 24 km S of Freirina, on gravel road towards Torres de Labrar, Quebrada Cuesta La Arena	-28.64650	-71.13733	550 m	23 Oct 1997	Eggli & Leuenberger	3016	(ZSS18333, SGO146145)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	20 km S of Vallenar, Panamericana road km 645	-28.71338	-70.76097	741 m	9 Aug 2018	P.Guerrero	1258	(CONC)
E. acida subsp. *procumbens*	Chile	III, Atacama	prov. Huasco	20 km S of Vallenar, Panamericana road km 645	-28.71857	-70.76411	740 m	10 May 2019	F.Luebert & A.Sandoval	4041	(BONN, EIF)

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Appendix E

Supplementary data to Chapter 6

Table S1: Complete geo-referenced specimen data of all vouchers considered in this study.

Taxon	**Country**	**Region**	**Province**	**Locality**	**Latitude**	**Longitude**	**Elev. (m)**	**Date**	**Collector**	**coll. No.**	**Herbarium**
O. anomalus	Chile	I, Tarapacá	Iquique	Alto Chipana	-21.26667	-70.05000		15 Oct 1997	W.Sielfeld	7	SGO143038
O. anomalus	Chile	I, Tarapacá	Iquique	Alto Chipana	-21.26667	-70.05000		21 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3427A	BONN, ULS
O. anomalus	Chile	I, Tarapacá	Iquique	Alto Chipana	-21.26667	-70.05000		1 Oct 2019	F.Luebert, F.F.Merklinger & J.Ruhm	4102	EIF, BONN, K
O. anomalus	Chile	I, Tarapacá	Iquique	Alto Punta Gruesa	-20.36667	-70.15000		14 Dec 1997	R.Pinto	s.n.	SGO142948
O. anomalus	Chile	I, Tarapacá	Iquique	Alto Punta Lobos	-21.03333	-70.15000	800 m	14 Jan 1998	R.Pinto	s.n.	SGO142950
O. anomalus	Chile	I, Tarapacá	Iquique	Alto Punta Patache	-20.81667	-70.15000	800 m	6 Dec 1997	R.Pinto	s.n.	SGO142949
O. anomalus	Chile	I, Tarapacá	Iquique	Alto Punta Patache	-20.81667	-70.15000		22 Jan 2000	R.Pinto	s.n.	SGO [photo]
O. anomalus	Chile	II, Antofagasta	Tocopilla	Cobija	-22.55500	-70.25972		s.a.	C.Gaudichaud	s.n.	F1012247
O. anomalus	Chile	II, Antofagasta	Tocopilla	Cobija, quebrada Aguada Cañas	-22.55500	-70.25972	500–800 m	4 Apr 1949	W.Biese	3088	SGO096693
O. anomalus	Chile	II, Antofagasta	Tocopilla	Cerro Rosario	-20.30000	-69.73333		1 Nov 1941	M.R.Espinosa	s.n.	SGO143254
O. anomalus	Chile	II, Antofagasta	Tocopilla	Tocopilla	-22.09194	-70.19778		27 Oct 1930	F.Jaffuel	1026	GH
O. floribundus	Peru	Lima, Lima	Canta	road from Canta to Lima below San José turnoff towards Lima	-11.49383	-76.65187	2322 m	1 Mar 2018	M.Weigend & K.A.Peña Ramos	9719	BONN
O. floribundus	Peru	Lima, Lima	Canta	road from Canta to Lima, road down from Canta to turn off San José	-11.49383	-76.65187	2322 m	1 Mar 2018	M.Weigend & K.A.Peña Ramos	9722	BONN
O. floribundus	Peru	Lima, Lima	Huarochirí	Matucana	-11.85000	-76.40000	c. 2400 m	25 May 1940	E.Asplund	11072	S
O. floribundus	Peru	Lima, Lima	Huarochirí	Matucana	-11.85000	-76.40000	c. 2600 m	25 May 1940	E.Asplund	11079	S
O. heptanthus	Chile	I, Tarapacá	Tamarugal	Chusmiza	-19.68444	-69.18889	3200 m	10 Jan 1969	O.Zöllner	2997	L125727
O. heptanthus	Chile	I, Tarapacá	Tamarugal	quebrada de Chusmiza	-19.68469	-69.18386	3350 m	18 Feb 2003	M.F.Gardner & S.G.Knees	6534	SGO, E
O. heptanthus	Chile	I, Tarapacá	Tamarugal	Chusmiza	-19.68006	-69.18042	3380 m	27 Apr 2008	M.Muñoz & A.Moreira	4940	SGO157269
O. heptanthus	Chile	I, Tarapacá	Tamarugal	at the entrance of the Andean village Chusmiza	-19.6788	-69.17956	3392 m	2 Oct 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-20	BONN, EIF, K
O. heptanthus	Peru	Moquegua, Moquegua	Sanchez Cerro	Puquina, outside Puquina towards Arequipa	-16.61094	-70.19189	3174 m	29 Mar 2019	M.Weigend	9994/19-182	BONN
O. heptanthus	Peru	Puno, Puno	Puno	Huerta N of Puno	-15.67250	-70.19472	3840 m	22 Mar 1957	H.Ellenberg	238A	U [U1145284]
O. heptanthus	Peru	Puno, Puno	Puno	Huerta N of Puno	-15.67250	-70.19472	4100 m	22 Mar 1957	H.Ellenberg	292	U [U1145286]
O. heptanthus	Peru	Puno, Puno	Puno	Huerta N of Puno	-15.67250	-70.19472	3840 m	22 Mar 1957	H.Ellenberg	238	U [U1145285]
O. heptanthus	Peru	Puno, Puno	Azángaro	Checayani, NE of Azángaro	-14.90833	-70.19611	3980 m	28 Mar 1957	H.Ellenberg	461	U [U1145288]
O. heptanthus	Peru	Puno, Puno	Lampa	Pucará	-15.03944	-70.36750	3900 m	22 Aug 1957	H.Ellenberg	2753A	U [U1145287]
O. heptanthus	Peru	Ayacucho, Ayacucho	Lucanas	a few km from Puente Toro Muerte	-14.71531	-74.54575	3589 m	21 Mar 2019	M.Weigend	9841/19-32	BONN
O. hoppii	Peru	Arequipa, Arequipa	Islay	quebrada Guerreros	-17.01222	-72.04611	456 m	6 Apr 1998	FLSP	2411	HUSA, US
O. hoppii	Peru	Arequipa, Arequipa	Islay	lomas of Mollendo, c. 4 km N of Islay	-16.87611	-72.10222	230 m	20 Nov 1983	M.O.Dillon & D.Dillon	3926	BONN, F

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Table S1: Continued from previous page

Taxon	**Country**	**Region**	**Province**	**Locality**	**Latitude**	**Longitude**	**Elev. (m)**	**Date**	**Collector**	**coll. No.**	**Herbarium**
O. hoppii	Peru	Arequipa, Arequipa	Islay	Ocoña	-16.27778	-73.01083		5 Feb 1969	J.Soukup	6426/6216	US, USM
O. hoppii	Peru	Arequipa, Arequipa	Castilla	Chuquibamba towards Aplao	-15.86453	-72.61567	2231 m	24 Mar 2019	M.Weigend	9862/19-52	BONN, USM
O. johnstonii	Chile	II, Antofagasta	Antofagasta	vicinity of Miguel Díaz, directly N of quebrada Iscuña, c. 55 km N of Paposo	-24.55000	-70.55000	100–300 m	15 Dec 1987	M.O.Dillon & S.Teillier	5292	BONN
O. johnstonii	Chile	II, Antofagasta	Antofagasta	vicinity of Aguada de Miguel Díaz	-24.55000	-70.55000		1–4 Dec 1925	I.M.Johnston	5310	SGO059042
O. johnstonii	Chile	II, Antofagasta	Antofagasta	vicinity of Aguada Cardón	-24.55000	-70.55000		30 Nov 1925	I.M.Johnston	5259	S
O. johnstonii	Chile	II, Antofagasta	Antofagasta	rocky slopes of Aguada Cardón	-24.74173	-70.54385	210 m	15 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3384	BONN, ULS
O. johnstonii	Chile	II, Antofagasta	Antofagasta	Aguada Cardón	-24.741717	-70.542687	210 m	5 Oct 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3950	BONN, ULS
O. paradoxus	Chile	III, Atacama	Copiapó	Jorquera-valley	-28.04528	-69.96222		12 Jan 1970	O.Zöllner	4682	L3661656
O. paradoxus	Chile	III, Atacama	Huasco	below El Chivato	-28.90000	-70.06667	1800 m	4 Jan 1926	I.M.Johnston	5870	S
O. paradoxus	Chile	III, Atacama	Huasco	Resguardo	-28.96667	-70.16667	1530 m	4 Jan 1926	I.M.Johnston	5863	S
O. paradoxus	Chile	III, Atacama	Huasco	valley San Félix	-28.92389	-70.40306	1180 m	16 Dec 1941	E.Pisano V.& R.Bravo F.	1089	SGO
O. paradoxus	Chile	IV, Coquimbo	Elqui	valley of Río Turbio between Rivadavia and Guanta	-29.98250	-70.56417	900 m	18–19 Jan 1926	I.M.Johnston	6271	S
O. paradoxus	Chile	IV, Coquimbo	Elqui	near Guanaqueros	-30.19722	-71.42167		24 Jul 1973	O.Zöllner	6827	L3661655
O. paradoxus	Chile	IV, Coquimbo	Elqui	road from Marquesa to Viñita Baja	-29.954529	-70.964978	340 m	26 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3822	BONN, ULS
O. paradoxus	Chile	IV, Coquimbo	Elqui	road from Marquesa to Viñita Baja	-29.873778	-70.860812	700 m	26 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3825	BONN, ULS
O. paradoxus	Chile	IV, Coquimbo	Elqui	road to Andacollo, c. 6 km before Andacollo	-30.201205	-71.092169	900 m	24 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3807	BONN, ULS
O. paradoxus	Chile	IV, Coquimbo	Limarí	Ovalle, Fray Jorge	-30.66306	-71.66972	215 m	13 Aug 1917	C.& I. Skottsberg	746	S
O. paradoxus	Chile	IV, Coquimbo	Limarí	Ovalle, Fray Jorge	-30.66306	-71.66972	200 m	Nov 1925	E.Werdermann	892	U [U1145306], S
O. paradoxus	Chile	IV, Coquimbo	Limarí	Ovalle, Fray Jorge	-30.66306	-71.66972		Oct 1947	B.Sparre	3061	S
O. paradoxus	Chile	IV, Coquimbo	Limarí	Villaseca near Huamalata	-30.568064	-71.150966	270 m	24 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3799	BONN, ULS
O. paradoxus	Chile	IV, Coquimbo	Limarí	road from Alcones to Los Loros	-30.78262	-71.587161	330 m	23 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3781	BONN, ULS
O. paradoxus	Chile	IV, Coquimbo	Limarí	hotel Termas de Socos	-30.732502	-71.493507	80 m	23 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3777	BONN, ULS

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Table S1: Continued from previous page

Taxon	Country	Region	Province	Locality	Latitude	Longitude	Elev. (m)	Date	Collector	coll. No.	Herbarium
O. paradoxus	Chile	IV, Coquimbo	Limarí	road from Los Loros to Caleta El Toro	-30.741021	-71.65348	50 m	23 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3790	BONN, ULS
O. paradoxus	Chile	IV, Coquimbo	Choapa	road from Combarbalá to Canela Baja, few km after Los Pozos	-31.363888	-71.260395	500 m	22 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3775	BONN, ULS
O. paradoxus	Chile	IV, Coquimbo	Choapa	road from Illapel to Combarbalá, near Illapel	-31.604095	-71.125953	450 m	22 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3772	BONN, ULS
O. paradoxus	Chile	V, Valparaíso	Petorca	La Ligua, 5 km from Petorca on road from Pedequa	-32.45222	-71.23083	550 m	27 Nov 1938	C.R.Worth & J.L.Morrison	16704	S
O. paradoxus	Chile	V, Valparaíso	San Felipe de Aconcagua	cuesta Las Chilcas	-32.851797	-70.875068	560 m	19 Jul 2003	F.Luebert & L.Kritzner	1757	EIF
O. paradoxus	Chile	V, Valparaíso	San Felipe de Aconcagua	cuesta Las Chilcas	-32.851797	-70.875068	380 m	20 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3741	BONN, ULS
O. paradoxus	Chile	V, Valparaíso	San Felipe de Aconcagua	cuesta Las Chilcas	-32.851797	-70.875068	380 m	20 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3741A	BONN, ULS
O. paradoxus	Chile	V, Valparaíso	San Antonio		-33.58333	-71.50000		Nov 1927	O.Buchtien	3450	L3661663
O. paradoxus	Chile	V, Valparaíso	Valparaíso		-33.03583	-71.62944		14 Jan 1947	E.Wall & B.Sparre	45	S
O. paradoxus	Chile	V, Valparaíso	Valparaíso	rocks near the sea	-33.03583	-71.62944		17 Nov 1895	O.Buchtien	s.n.	S
O. paradoxus	Chile	V, Valparaíso	Quillota	Parque Nacional La Campana, Cerro La Campana	-32.97962	-71.12783	480 m	30 Dec 2000	F.Luebert	1398	EIF
O. paradoxus	Chile	RM, Metropolitana	Melipilla	La Barriga	-33.50000	-70.98333		Oct 1964	O.Zöllner	1765	L3661658
O. paradoxus	Chile	RM, Metropolitana	Melipilla	Curacaví	-33.39750	-71.12694		Dec 1967	O.Zöllner	2245	L3661657
O. pinifolius	Chile	I, Tarapacá	Tamarugal	quebrada de Parca	-19.985261	-69.098117	3261 m	22 Mar 2017	F.F.Merklinger & A.Stoll	2017-12	BONN, ULS
O. pinifolius	Chile	I, Tarapacá	Tamarugal	Usmagama, road from Usmagama to Limacsina	-19.78771	-69.207368	2434 m	26 Mar 2017	F.F.Merklinger & A.Stoll	2017-22	BONN, ULS
O. pinifolius	Chile	I, Tarapacá	Tamarugal	road to Usmagama, turnoff ruta CH-15	-19.730253	-69.218684	2956 m	26 Mar 2017	F.F.Merklinger & A.Stoll	2017-26	BONN, ULS
O. pinifolius	Chile	I, Tarapacá	Tamarugal	road to Usmagama, turnoff ruta CH-15	-19.730253	-69.218684	2956 m	26 Mar 2017	F.F.Merklinger & A.Stoll	2017-19	BONN, ULS
O. pinifolius	Chile	I, Tarapacá	Tamarugal	cuesta Usmagama, km 3.9	-19.730154	-69.217046	3050 m	28 Oct 2016	F.Luebert & T.Böhnert	3452	BONN, ULS
O. pinifolius	Chile	I, Tarapacá	Tamarugal	Chusmiza, above town	-19.683025	-69.183376	3400 m	21 Mar 2017	F.Luebert & T.Böhnert	3642	BONN, ULS
O. pinifolius	Chile	I, Tarapacá	Tamarugal	at entrance of Andean village Chusmiza	-19.6783	-69.1793	3393 m	2 Oct 2019	F.F.Merklinger, F.Luebert & J.Ruhm	2019-21	BONN, EIF, K

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Table S1: Continued from previous page

Taxon	**Country**	**Region**	**Province**	**Locality**	**Latitude**	**Longitude**	**Elev. (m)**	**Date**	**Collector**	**coll. No.**	**Herbarium**
O. pinifolius	Chile	I, Tarapacá	Tamarugal	2 km above the village of Chusmiza in quebrada de Chusmiza at km 76 alongside the new road to Colchane	-19.68158	-69.18639	3406 m	18 Feb 2003	M.F.Gardner & S.G.Knees	6512	SGO150393, E
O. pinifolius	Chile	I, Tarapacá	Tamarugal	above Jaiña	-19.551255	-69.242848	2750 m	21 Mar 2017	F.Luebert & T.Böhnert	3631	BONN, ULS
O. pinifolius	Chile	I, Tarapacá	Tamarugal	quebrada de Soga	-19.514361	-69.381274	2400 m	16 Mar 2017	F.Luebert, T.Böhnert & F.F.Merklinger, A.Stoll & D.Quandt	3455	BONN, ULS
O. pinifolius	Chile	I, Tarapacá	Tamarugal	quebrada Aroma	-19.514361	-69.381274	2300 m	16 Mar 2017	F.F.Merklinger	2017-6	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	along road through Illapata	-18.94831	-69.50272	2300 m	28 Mar 2017	F.F.Merklinger & A.Stoll	2017-41	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	between Esquina and Pachica	-18.927444	-69.552944	2298 m	28 Mar 2017	F.F.Merklinger & A.Stoll	2017-43	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	quebrada Chokaya, from Codpa into Camarones valley toward Pachica	-18.88289	-69.664972	2373 m	28 Mar 2017	F.F.Merklinger & A.Stoll	2017-40	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	quebrada Chokaya, from Codpa into Camarones valley toward Pachica	-18.86473	-69.68034	2235 m	28 Mar 2017	F.F.Merklinger & A.Stoll	2017-30	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	quebrada de Vitor NW of Palca	-18.827041	-69.677724	2085 m	28 Mar 2017	F.F.Merklinger & A.Stoll	s.n.	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	Codpa, between Codpa and Timar	-18.762747	-69.69828	2393 m	29 Mar 2017	F.F.Merklinger & A.Stoll	2017-46	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	shortly after Timar	-18.747992	-69.699137	2307 m	29 Mar 2017	F.F.Merklinger & A.Stoll	2017-47	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	shortly after Timar	-18.736382	-69.706777	2447 m	29 Mar 2017	F.F.Merklinger & A.Stoll	2017-54	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	Timar, between Timar and Tignamar	-18.717336	-69.663483	2840 m	29 Mar 2017	F.F.Merklinger & A.Stoll	2017-51	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	Palca	-18.83333	-69.66667	2200 m	30 Oct 1964	F.Schlegel	5092	CONC, F [photo, as *Piqueria floribunda*]
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	between Timar and Tignamar, shortly before Tignamar	-18.663898	-69.560963	3354 m	29 Mar 2017	F.F.Merklinger & A.Stoll	2017-53	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	Tignamar	-18.61667	-69.46667	3100 m	11 Sep 1963	F.Schlegel	4879	CONC, F [photo as *Piqueria floribunda*]
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	W of Tignamar along road	-18.57918	-69.52785	3300 m	29 Mar 2017	F.F.Merklinger & A.Stoll	2017-55	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	Saxamar	-18.56667	-69.48333	c. 3000 m	20 Mar 2015	A.Moreira & F.Luebert	2456	BONN
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	NE of Saxamar	-18.55108	-69.50015	3500 m	29 Mar 2017	F.F.Merklinger & A.Stoll	2017-57	BONN, ULS

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Table S1: Continued from previous page

Taxon	**Country**	**Region**	**Province**	**Locality**	**Latitude**	**Longitude**	**Elev. (m)**	**Date**	**Collector**	**coll. No.**	**Herbarium**
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	along A-31 near Belen	-18.48545	-69.52782	3500 m	29 Mar 2017	F.F.Merklinger & A.Stoll	2017-56	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	along road Putre-Arica	-18.45314	-69.76415	3102 m	1 Apr 2017	F.F.Merklinger & A.Stoll	2017-62	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	along road Putre-Arica	-18.21033	-69.56082	3500 m	1 Apr 2017	F.F.Merklinger & A.Stoll	2017-65	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Arica	road A-135 from Panamericana to Puquios	-18.254661	-69.828852	3100 m	14 Oct 2017	F.Luebert, T.Böhnert & F.F.Merklinger	4008	BONN, ULS
O. pinifolius	Chile	XV, Arica y Parinacota	Parinacota	Paychama [Pachama]	-18.42972	-69.52917	3600 m	10 Mar 1927	C.Troll	3244	B, M
O. triangularis	Chile	II, Antofagasta	Tocopilla	quebrada 2–3 km N of Tocopilla above old Caleta Duendes	-22.08333	-70.18333	150–200 m	18 Oct 1988	M.O.Dillon & D.Dillon	5718	BONN
O. triangularis	Chile	II, Antofagasta	Tocopilla	Tercera quebrada Tocopilla	-22.0558	-70.17662	300 m	18 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3413	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Tocopilla	quebrada La Higuera, S Tocopilla	-22.30194	-70.21611		30 Sep 2005	F.Luebert, N.Garcia & N.Schulz	2569/963	EIF
O. triangularis	Chile	II, Antofagasta	Tocopilla	quebrada above Caleta Buena, S Mantos de la Luna	-22.43075	-70.22186	640 m	10 Oct 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3989	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	near Cobija	-22.55500	-70.25972		19 Dec 1971	O.Zöllner	4593	L153863
O. triangularis	Chile	II, Antofagasta	Antofagasta	Juan López	-23.51205	-70.53365	150 m	19 Jul 2003	M.Antonissen	7	EIF
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada La Chimba	-23.55000	-70.36667	300–550 m	17 Dec 1987	M.O.Dillon & J.T.S.Teillier	5321	BONN
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada La Chimba	-23.55000	-70.36667	380–480 m	11 Nov 1988	M.O.Dillon & D.Dillon	5881	BONN
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada La Chimba	-23.53567	-70.35887	460 m	17 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3408	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	c. 7 km N of Paposo	-24.95000	-70.48333	40 m	14 Dec 1987	M.O.Dillon & J.T.S.Teillier	5262	BONN
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada Panul	-24.777263	-70.531618	190 m	5 Oct 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3944	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada Panul	-24.777263	-70.531618	190 m	5 Oct 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3944A	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada just S Panul	-24.773468	-70.533915	180 m	4 Oct 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3939	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada Cardón	-24.74173	-70.54385	210 m	15 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3389	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada Cardón	-24.741717	-70.542687	210 m	5 Oct 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3949	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	El Rincon, al N de Paposo	-24.93333	-70.50000		17 Sep 1941	C.Muñoz P.& G.T.Johnson	2902	SGO118350
O. triangularis	Chile	II, Antofagasta	Antofagasta	Paposo, base Cerro Carneros	-24.94000	-70.47906	160 m	23 Oct 2009	A.Moreira & F.Luebert	1200	SGO158780

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Taxon	Country	Region	Province	Locality	Latitude	Longitude	Elev. (m)	Date	Collector	coll. No.	Herbarium
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada Portezuelo	-25.0124	-70.446467	550 m	13 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3357	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada Matancilla, c. 5 km S of Punta Grande	-25.11667	-70.45000	170–350 m	27 Oct 1988	M.O.Dillon, D.Dillon, V.Asencio & M.Villarroel O.	5750	BONN
O. triangularis	Chile	II, Antofagasta	Antofagasta	Cachinalcito	-25.18611	-70.43583		28 Nov 1925	I.M.Johnston	5173	S
O. triangularis	Chile	II, Antofagasta	Antofagasta	c. 20 km N of Taltal, quebrada Anchuña	-25.23543	-70.42594	183 m	31 Aug 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-63	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	c. 20 km N of Taltal, quebrada Anchuña	-25.23543	-70.42594	183 m	31 Aug 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-64	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	c. 20 km N of Taltal, quebrada Anchuña	-25.23543	-70.42594	183 m	31 Aug 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-65	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	c. 20 km N of Taltal, quebrada Anchuña	-25.23543	-70.42594	183 m	31 Aug 2018	F.F.Merklinger, A.Kozok & D.Quandt	2018-66	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada El Médano	-24.84167	-70.52500	300 m	8 Oct 1941	E.Pisano V.& R.Bravo F.	398	SGO
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada San Ramón	-25.38578	-70.43658	120 m	11 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3346	BONN, ULS
O. triangularis	Chile	II, Antofagasta	Antofagasta	quebrada San Ramón	-25.38578	-70.43658		17 Sep 1968	O.Zöllner	2851	U [U3661650]
O. triangularis	Chile	II, Antofagasta	Antofagasta	Cerro Perales, c. 5 km E of Taltal	-25.41667	-70.41667	550 m	21 Nov 1988	M.O.Dillon & D.Dillon	6002	BONN
O. triangularis	Chile	II, Antofagasta	Antofagasta	hills SE of Taltal	-25.48333	-70.48528		25 Nov 1925	I.M.Johnston	5080	S
O. triangularis	Chile	II, Antofagasta	Antofagasta	Posado Hidalgo	-25.75000	-70.58333		13 Dec 1925	I.M.Johnston	5661	S
O. triangularis	Chile	II, Antofagasta	Antofagasta	road Panamericana toward Caleta Esmeralda	-25.895921	-70.581052	500 m	9 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3308	BONN, ULS
O. triangularis	Chile	III, Atacama	Chañaral	21 km W of Panamericana on northern route to Pan de Azucar NP	-26.13333	-70.61667	85 m	30 Sep 1988	M.O.Dillon, D.Dillon & V.Pobleto	5609	BONN
O. triangularis	Chile	III, Atacama	Chañaral	Falda Verde, hills N of Chañaral	-26.296721	-70.631252	75–600 m	1 Oct 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3905	BONN
O. triangularis	Chile	III, Atacama	Chañaral	hills back of El Barquito	-26.36056	-70.64278		28–29 Oct 1925	I.M.Johnston	4809	S
O. triangularis	Chile	III, Atacama	Copiapó	sector quebrada El León	-26.976625	-70.773903	70 m	8 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3290	BONN, ULS
O. triangularis	Chile	III, Atacama	Copiapó	Caldera on small point just north of town	-27.06806	-70.81917		22 Nov 1925	I.M.Johnston	5067	S
O. triangularis	Chile	III, Atacama	Copiapó	Copiapó	-27.36667	-70.33139	400 m	13 Jul 1938	Ch.H.Andreas	885	U [U1145297]
O. triangularis	Chile	III, Atacama	Copiapó	Tierra Amarilla	-27.46611	-70.26250	700 m	Oct 1924	E.Werdermann	456	U [U1145305]
O. triangularis	Chile	III, Atacama	Copiapó	Estancia Castilla, road from Totoral to Panamericana	-27.919994	-70.84531	240 m	28 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3869	BONN, ULS

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Taxon	**Country**	**Region**	**Province**	**Locality**	**Latitude**	**Longitude**	**Elev. (m)**	**Date**	**Collector**	**coll. No.**	**Herbarium**
O. triangularis	Chile	III, Atacama	Huasco	road Carrizal Bajo to Canto de Agua, c. 2 km from Carrizal Bajo	-28.112004	-71.116139	45 m	7 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3279	BONN, ULS
O. triangularis	Chile	III, Atacama	Huasco	quebrada Baratillo	-28.36583	-71.12250	150 m	14 Sep 2003	F.Luebert & L.Kritzner	1805	EIF
O. triangularis	Chile	III, Atacama	Huasco	quebrada Baratillo	-28.363325	-71.096321	110 m	7 Oct 2016	F.Luebert, A.Stoll & T.Böhnert	3269	BONN, ULS
O. triangularis	Chile	III, Atacama	Huasco	Vallenar	-28.590017	-70.737164		1860	Volckmann	s.n.	GH[14290], SGO
O. triangularis	Chile	III, Atacama	Huasco	hills E of Tres Playitas	-28.400532	-71.16755	160 m	27 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3854	BONN, ULS
O. triangularis	Chile	IV, Coquimbo	Elqui	Herradura	-29.98889	-71.35000		9 Aug 1917	C.& I. Skottsberg	704	S
O. triangularis	Chile	IV, Coquimbo	Elqui	Guayacán	-29.96611	-71.34139		s.a.	unknown	s.n.	S
O. triangularis	Chile	IV, Coquimbo	Elqui	Puente Juan Soldado	-29.656542	-71.301174	200 m	27 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3831	BONN, ULS
O. triangularis	Chile	IV, Coquimbo	Elqui	La Serena	-29.90444	-71.24889		16 Sep 1947	B.Sparre	2595	S
O. triangularis	Chile	IV, Coquimbo	Elqui	Coquimbo	-29.95306	-71.33333		Jul–Aug 1958	W.H.Harvey	s.n.	S
O. triangularis	Chile	IV, Coquimbo	Elqui	Coquimbo	-29.95306	-71.33333	100 m	Nov 1923	E.Werdermann	124	U [U1145298]
O. triangularis	Chile	IV, Coquimbo	Elqui	Coquimbo, rocks behind the Fort	-29.93372	-71.33691	25 m	24 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3809	BONN, ULS
O. triangularis	Chile	IV, Coquimbo	Elqui	Coquimbo, rocks behind the Fort	-29.93372	-71.33691	25 m	24 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3809A	BONN, ULS
O. triangularis	Chile	IV, Coquimbo	Limarí	Ovalle, Río Límari	-30.67389	-71.40611		11 Oct 1947	B.Sparre	2994	S
O. triangularis	Chile	IV, Coquimbo	Limarí	N of Caleta El Toro	-30.737239	-71.699907	25 m	23 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3794	BONN, ULS
O. triangularis	Chile	IV, Coquimbo	Limarí	road from Los Loros to Caleta El Toro	-30.741021	-71.65348	50 m	23 Sep 2017	F.Luebert, T.Böhnert & F.F.Merklinger	3786	BONN, ULS