Historical Biogeography and Macroevolution of the Atacama Desert Flora

Dissertation

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for myself

Chasing Guanacos in the southern Atacama Desert near Punta del Chorro.

Preface and Acknowledgments

The extreme aridity of the South American Atacama Desert in northern Chile is one of the most challenging environments for human, animal and plant life. It is common sense in the scientific community that the hyperarid conditions in this desert are longlasting, probably since the Miocene or earlier. Recent studies revealed, however, a more heterogeneous climate history throughout the Pleistocene and early Holocene. While nowadays commercial aspects – as the mining of raw materials – are main reasons for the presence of humans in the Atacama Desert, the environmental conditions were much milder about 10K years ago. Archaeological findings show that the Atacama was already inhabited at that time. Increased rainfall in the Andes as well as a higher intensity of El Niño and La Niña events fed entire wetlands. But, while ancient cultures throughout the globe developed flourishing societies, climate change prevented that from happening in the Atacama Desert.

After Europeans reached the New World at the end of the 15th century, a new time of exploitation and colonization began, first along the Atlantic but soon also along the Pacific coast. Gold and silver were the most sought-after goods at that time. In the name of the Christian God, everyone tried to get hold of it as much as possible. The murder of the last Inca King Atahualpa 1533 in Cajamarca (Peru) by the Spaniards under the leadership of Francisco Pizarro or the rise and fall of Potosi in present-day Bolivia are just two examples of the atrocious and moral decline of the time, which Eduardo Galeano describes in his book ‘The Open Veins of Latin America’ with all its cruelty.

But with the Europeans also came the naturalists and besides the exploitation of the continent, the exploration of its immense biodiversity began. Naturally, the mysterious Amazon rainforest and the tropical Andes first aroused the interest of early explorers. The exploration of the Atacama Desert in northern Chile, on the other hand, had to wait until Thaddaeus Haenke (1761-1816), a naturalist from Bohemia, came to explore the geology as well as the flora and fauna of the Atacama Desert. In the early nineteenth century a young naturalist named Charles Darwin passed the Atacama Desert on his journey around the world onboard the H.M.S. Beagle. Not the journey itself but the knowledge he gained from it was to give the world one of the most fundamental works about the origin of life on earth. On his excursions into the interior of the Atacama Desert near Copiapó, Darwin discovered layers of shells of existing species embedded in calcareous rocks hundreds of meters above the sea level. The conclusion that this part of the continent must have been uplifted since thousands of years, later-on turned out to be a crucial puzzle piece in the formulation of his theory of biological evolution.

Twenty years later, the German emigrant Rudolph Amandus Philippi – in his new position as director of the Natural History Museum of Santiago de Chile – was commissioned to explore the area of the Atacama Desert until 24° S. The northern part of the Atacama became Chilean only in 1884, as a result of the Saltpeter War with Peru and Bolivia. He was the first to travel systematically through the desert and, in addition to geology, devoted himself above all to the animal and plant world. Even today a large number of plant species bear his scientific author’s abbreviation ‘Phil.’. His report, published in German and Spanish, was a useful and entertaining travel companion to the field trips undertaken for this work. In his time, only a few people dared to venture into the desert, but in the following years more and more researchers took on the still arduous journey. Another milestone in the botanical exploration of the desert is the work of Karl Friedrich Reiche, another German immigrant, who published the ‘Flora de Chile’ around the turn of the century. In the nineteen-twenties a young American set out to explore the southern coast of the Atacama Desert. His observations of the desert and above all his botanical expertise make the ‘Papers on the Flora of Northern Chile’ by Ivan Murray Johnston the absolute standard reading for every botanist who sets out to explore the Atacama Desert. His plant descriptions are so accurate and each of his taxonomic decisions are so clearly formulated that most of the species he described are still valid today. In addition, his extremely precise location information allowed me and my travel companions to collect some plant species that have not been found since Johnston himself first collected and described them. In the following years more came to seek out new species in the Atacama Desert (e.g., Michael O. Dillon) including a growing number of Chilean botanists like Carlos Muñoz Pizarro, Melica Muñoz-Schick or in more recent years Federico Luebert, to name just a few. Today, research in the Atacama Desert is constantly shifting from a purely descriptive to a more interdisciplinary approach, trying to understand landscape and biotic evolution and their interdependent processes.

This thesis, carried out at the Nees Institute for Biodiversity of Plants (University of Bonn), is embedded in such an interdisciplinary Collaborative Research Center: 'Earth – Evolution at the Dry Limit'. With its focus on the Atacama Desert the project is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation, Projektnummer 268236062 – SFB 1211).

The here presented work would not have been possible without a number of people who supported me during the last three years. First of all, my sincere thanks go to my first supervisor Prof. Dr. Maximilian Weigend and my second supervisor Prof. Dr. Dietmar Quandt for giving me the opportunity to prove myself and for bearing my peculiarities during the last years. I am very grateful for the substantial support and trust you both have given me.

During all this time, Dr. Federico Luebert has been a constant companion I could always rely on and who always found encouraging words when I needed them. I have benefited from his knowledge and experience in so many ways that a simple thank you seems insufficient. The experience to work in a team together with Federico and Felix Merklinger was one of the best things during the last years. I sincerely hope for many more field trips and evenings together with 'lomo y vino tinto' in the desert. Thank you, both of you.

Among the many people involved in this project Claudia Schütte was one of the most helpful and supportive colleagues. Her patience and helpfulness during the laboratory work have tremendously contributed to the success of this work, for which I am extremely grateful. Thomas Joßberger provided constant support in handling our herbarium specimens, thank you. Furthermore, I would like to thank Dr. Julio Schneider (Senckenberg, Frankfurt) for sharing his knowledge about the secrets of *Cristaria* taxonomy, and for checking my identifications. Dr. Alexander Stoll (CEAZA, La Serena, Chile) deserves a very special thank you for supporting the field work in Chile and managing the shipment of our samples. I also thank Dr. Dörte Harpke and Dr. Frank Blattner (IPK Gatersleben) as well as the staff of the IPK Genome Center for support regarding the sequencing of our samples. Without the outstanding contribution of Prof. Dr. Tibur Dunai and his research group (University of Cologne; namely: Christian Tiede, Dr. Benedikt Ritter, Dr. Damián López and Joel Mohren) this project would not have been possible. Thanks guys, for an amazing time in Chile and all the energy you put into making this project a success. Dr. Dirk Hoffmeister, I would like to thank for his help regarding the data handling at the CRC database. Further, I wish to thank all colleagues in the framework of the CRC1211.

Among my colleagues at the Nees Institute I would like to thank first Constantin Poretschkin for five amazing years as an office companion and friend, I will miss our discussions and the fun we had. Further, I would like to thank Dr. Jens Mutke for the countless support over the years and that he tolerated me as a room neighbor. Dr. Julius Jeiter, I want to thank you for

always having an open ear and the constant supply of coffee. To Elke Hedke I would like to express my gratitude for keeping everything together and being supportive in so many ways, and to Karola Maul for her occasional support in the lab but mainly for all our discussions. Prof. Dr. Annette Scheersoi as well as the members of her working group, namely: Dr. Lara Weiser, Dr. Jonathan Hense, Amélie Tessartz and Julian Kokot, I would like to thank for a great time during the last years. I have always appreciated the collegial and friendly contact and I look forward too many more lunches and coffee breaks in good company. Further I would like to thank Jonathan Ruhm, Dr. Stefan Abrahamczyk, Andreas Mues and Dr. Julia Bechteler. During the years I worked on this thesis valuable members of the Institute left: I want to thank Dr. Juliana Chacon (LMU Munich), Dr. Norbert Holstein (NHM London, UK), Dr. Rafael Acuña-Castillo (University of Costa Rica) as well as PD Dr. Marcus Lehnert (Universität Halle-Wittenberg).

My past before my scientific career was significantly influenced by the time and the colleagues in the Botanical Gardens Bonn. For support in many different ways as well as friendship I would like to thank Dr. Wolfram Lobin, Michael Neumann, Anne Schulz and Bernhard Reinken to just name a few on behalf of the entire staff. Prof. Dr. em. Wilhelm Barthlott had a great influence on my development as a botanist. His botanical lecture series, which I was fortunate enough to attend during my apprenticeship, have laid an important foundation for my current expertise, for which I am very grateful.

Friends outside the university are especially valuable as they help to free your mind from all the idiocy of everyday university life. I am very grateful for meeting Chris Halla as well as for the trust he showed me during the last years as a climbing partner and friend.

During the last years before and during my PhD thesis I did not give my family as much attention and presence as they deserve. Therefore, I would like to thank my mother, father and my two brothers for the patience and constant support.

I am infinitely grateful in so many ways to all of you who are listed here and to those who I inexcusably forgot to list. However, all this is dwarfed by the support that I have received from my partner in all those years we have been together. I will not even try to list or describe in what ways she supported or what she means to me, as I could never do justice to the magnitude of the influence she had on me. Thank you, Conny.

Contents

Chapter 1

General Introduction

Southern Atacama coastal cordillera between Pan de Azucar National Park and Taltal. Columnar Cactaceae (*Eulychnia* sp.) are dominating elements.

„While travelling through these deserts one feels like a prisoner shut up in a gloomy court, who longs to see something green and to smell a moist atmosphere.“

Charles R. Darwin
Journal of Researches into the Natural History and Geology of the
Countries Visited during the Voyage of HMS Beagle round the World,
under the Command of Capt. Fitz Roy, R.N. (1st publ. 1839)

1.1 Deserts – a brief overview

On a global perspective, deserts are not particularly known as hotspots of biodiversity (Barthlott *et al.* 2005), although some deserts have acquired a surprisingly high plant species richness, e.g., the deserts of Central Asia (Zhang *et al.* 2016), Australia (Byrne *et al.* 2008) or the Atacama Desert in South America (Rauh 1986; Dillon & Hoffmann 1997). The term desert is widely applied for many different ecosystems throughout the globe and a detailed consideration of this topic would go beyond the scope of this introductory chapter. Nevertheless, it can generally be said that deserts are barren landscapes where water is a limiting factor. They can be classified based on different aspects, e.g., temperature, age, the prevailing substrate or the geographical location. Polar or ice deserts for instance are characterized by the absence of liquid water due to the extreme climatic conditions driven by their geographical location. The Sahara Desert in turn is one of the largest deserts on earth, but with approx. 2 to 3 mya also one of the youngest (Zhang *et al.* 2014). Apart from the availability of water, different kind of substrates also have a limiting effect on plant life. Generally, apart from polar deserts, it can be differentiated between rock or stone as well as salt or sand deserts. Humus is barely present in any of those substrate types due to a general lack of organic matter and, consequently, also several associated properties such as water holding capacity or nutrient matter. Apart from polar (e.g., Antarctica) and temperate deserts as in Central Asia (e.g., the Thaar or Gobi), the majority of deserts are found in the tropics and subtropics, in Africa (e.g., the Kalahari) and in North America (e.g., the Sonora and Chihuahuan Deserts). While most of the listed deserts are usually found inland, only few are actually restricted to coastal regions like the Namib at the southwestern coast of Africa, the coastal margins of the Arabian desert or the Peruvian-Chilean deserts along the Pacific coast of southwest South America (Rauh 1986; Stuut & Lamy 2004).

In order to categorize the different types of deserts the concept of aridity is commonly applied (Houston 2006). The degree of dryness is indicated here by the so-called aridity index (A_i). This index is calculated by the ratio of mean annual precipitation and annual potential evapotranspiration (Houston 2006). Based to this index three categories can be differentiated: semiarid (0.2-0.5 A_i), arid (0.03-0.2 A_i) and hyperarid with an aridity index < 0.03 A_i. Adaptation strategies to survive in such harsh conditions are quite diverse. Apart from well-known adaptations like leaf or stem succulence (Hernández-Hernández *et al.* 2014), plants have for example altered their life cycle by shortening generation times (Holmgren *et al.* 2006), developed alternative photosynthetic pathways (Kadereit *et al.* 2010; Lauterbach *et al.* 2019) or epidermal traits (Gutiérrez-Ortega *et al.* 2018). It has been shown that the adaptation to dry environments led to diversification (e.g., Garcia-Porta *et al.* 2019; Zhu *et al.* 2019), a famous example is the genus *Atriplex* L. (Amaranthaceae) where the C_4 clade harbors most of the diversity of the group (Kadereit *et al.* 2010).

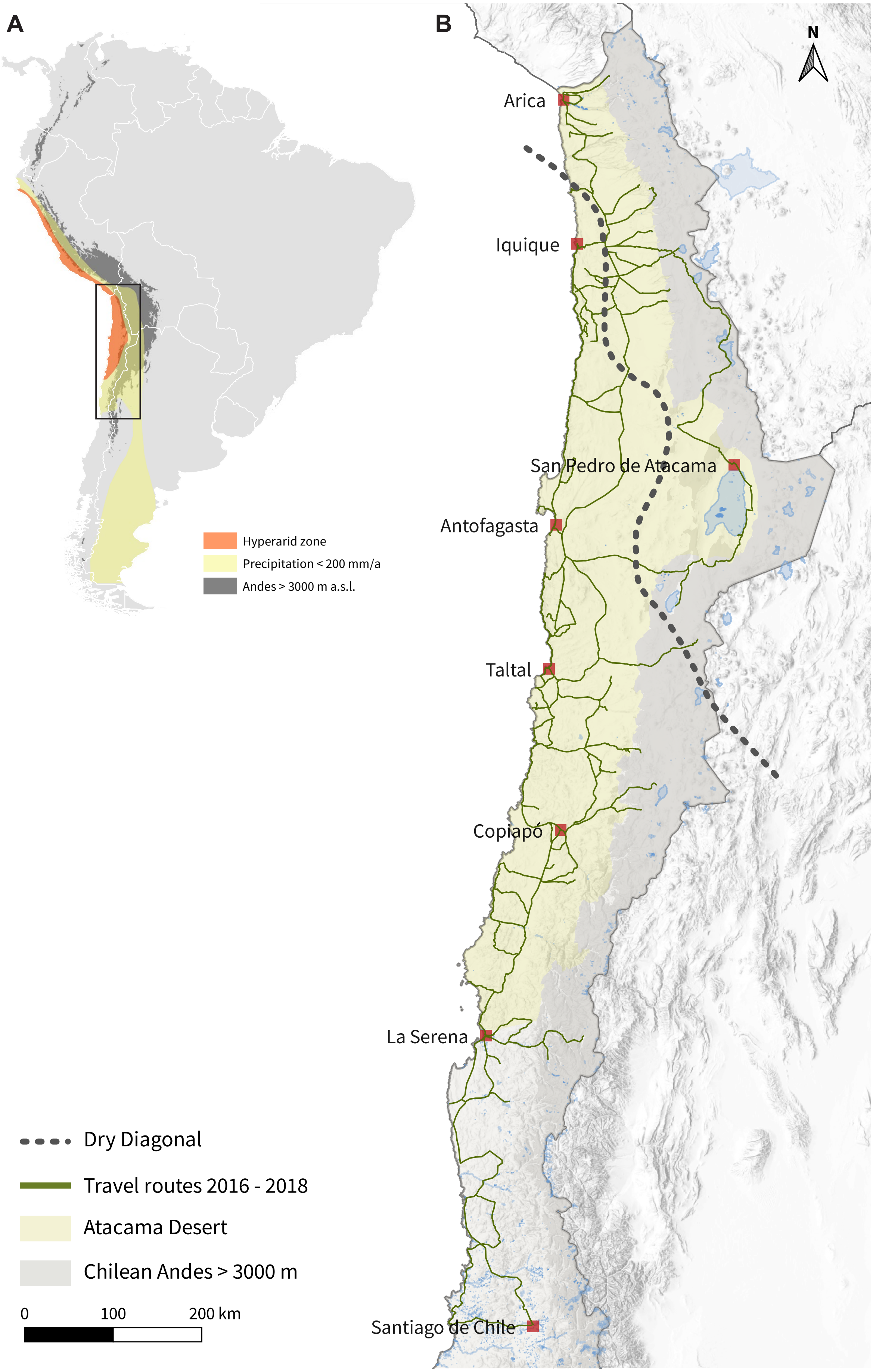
1.2 The Atacama Desert

The Peruvian-Chilean deserts are considered as the driest places on earth (Garreaud *et al.* 2010). As shown in Figure 1A, a hyperarid corridor extends from the northern coastal part of Peru to northern Chile, traverses the Andes and continues towards Patagonia east of the Andes (Houston & Hartley 2003). Precipitation is very sparse while moisture is usually available along the coast in the form of fog. However, while in terms of aridity the Peruvian-Chilean deserts share continuous conditions, both deserts differ in terms of topography, climate and, most importantly for this work, vegetation (Rauh 1986; Rundel *et al.* 1991; Dillon & Hoffmann 1997). Ranging from approximately 5° S in northern Peru to 30° S near La Serena (Chile) the Peruvian-Chilean deserts are limited by the Pacific Ocean in the west and the high Andes in the east (Figure 1A; Rauh 1986). Compared to their size and despite of their hyperaridity, the Peruvian desert and the Chilean Atacama Desert in particular harbor a surprisingly high number of plant species (Dillon & Hoffmann 1997; Dillon 2005).

1.2.1 Geography of the Atacama Desert

The Chilean Atacama Desert itself, as understood in this work, spans about 1,300 km from the Peruvian border north of the town of Arica in Chile (~18° S) to La Serena in the south (~30° S). It is flanked by the Pacific Ocean and a coastal mountain range in the west and the Andean foothills in the east followed by the high Andes (see Figure 1B). The Atacama's east-west extension is about 100 km, only in the area around San Pedro de Atacama the desert penetrates up to 200 km into the Andes. Between the coastal cordillera, which reaches elevations from 800 to over 1800 m, and the Andes, the inland of the Atacama Desert is characterized by a high plateau of approx. 1,000 m a.s.l. (Figure 3) which in the north is indented by very deep valleys, the so-called quebradas. Those quebradas, of which the biggest ones transport water from the high Andes towards the coast (e.g., Quebrada Camarones; Figure 2A), provide azonal vegetation while the rest of the plateau is cut-off from any water supply. On the other hand, some of the quebradas do not drain into the Pacific Ocean (Figure 2B), but rather into basins on the plateau which have no drainage through the coastal mountains. Instead, water evaporates there creating landscapes such as the Pampa Tamarugal inland of Iquique, which are often very salty and contain only a very specialized flora. Some of those basins have developed into salt pans like the Salar Grande south of Iquique or the Salar de Atacama. Further south between Copiapó and Vallenar, the coastal cordillera is lower in elevation, allowing for fog and the non-seasonal El Niño events to penetrate further inland so creating the famous flowering desert (Figure 2E).

Figure 1: Overview maps of the Atacama Desert. **A**) South America with the Andes above 3,000 m shown in dark gray; the hyperarid zone of the Peruvian-Chilean deserts are indicated in orange and arid environments with precipitation below 200 mm per year in yellow (after Houston & Hartley 2003). **B**) Map of northern Chile with the Atacama Desert as understood in this work given in yellow and the high Andes of Chile given in gray; major cities are indicated and the travel routes undertaken for this work are given in green lines; the Dry Diagonal after Houston (2006) is indicated by a dotted line. ▶

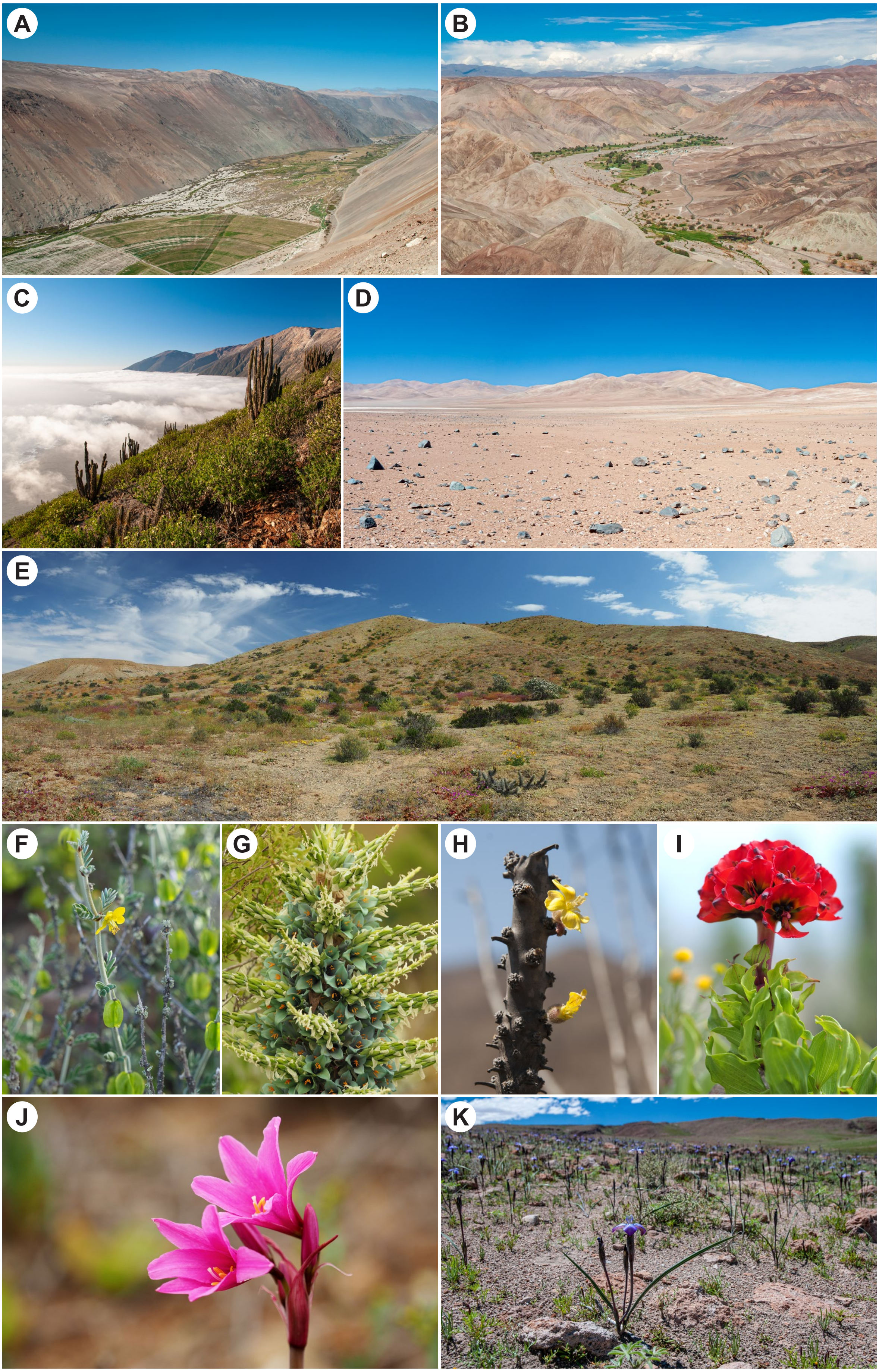

A
B
N
Hyperarid zone
Precipitation < 200 mm/a
Andes > 3000 m a.s.l.
Arica
Iquique
San Pedro de Atacama
Antofagasta
Taltal
Copiapó
La Serena
Santiago de Chile
Dry Diagonal
Travel routes 2016 - 2018
Atacama Desert
Chilean Andes > 3000 m
0
100
200 km

1.2.2 Climatic conditions

A combination of three main factors are responsible for the current but long-lasting arid conditions in the Atacama Desert. The position in the subtropical high-pressure belt which ensures the stability of arid climatic conditions probably since the Jurassic (Hartley *et al.* 2005). And while the cold Humboldt current restricts the uptake of moisture by onshore winds coming from the Pacific (Rundel *et al.* 1991), the Andes act as a significant rain-shadow blocking Atlantic air masses (Houston & Hartley 2003). This situation results in a seasonal and spatial separation of the Atacama Desert into two major climatic regimes: The easterlies, which prevail during the austral summer (December, January, February) bring precipitation from the east side of the Andes to the north-east of the Atacama Desert, while the westerlies during the austral winter (June, July, August) bring mainly fog but also occasional precipitation from the Pacific to the coast in the south-west (Garreaud 2009). Between those two climate regimes a corridor with hyperarid conditions is prevailing, forming the so called Dry Diagonal (see dotted line in Figure 2B; Houston 2006). Along this Dry Diagonal the Atacama is one of driest places on earth, where no precipitation has been recorded since decades.

The influence of the westerlies, which bring humid air from the Pacific due to a northward shift of the Pacific anticyclone as well as the Intertropical Convergence Zone, is gradually decreasing towards the north (Garreaud 2009). In the very south of the Atacama Desert, where the coastal cordillera is lower, fog and precipitation can occur further inland. In the coastal area around Taltal, thick stratus clouds below 1,000 m get caught on the western flanks of the coastal cordillera and a rich vegetation dominated by shrubs prevails (Figure 2C). Further north, this so called loma vegetation rapidly decreases towards Antofagasta and can only be observed around Iquique after freak events during El Niño years (Dillon & Hoffmann 1997). On the other side, in the north east, of the Atacama Desert the easterlies during the austral summer bring precipitation over the Andes. However, this annual precipitation rapidly decreases towards the dry core of the Atacama Desert from 300 mm in the high Andes at approx. 5,000 m a.s.l. to less than 20 mm at 2,300 m (Houston & Hartley 2003).

Figure 2: Landscapes and plants of the Atacama Desert. **A**) Quebrada Camarones near the coast, along the Pan-American Highway between Iquique and Arica. **B**) Quebrada Tamarugal with the village Pachica in the Andean foothills above Huara, northeast of Iquique. **C**) The fertile belt and loma vegetation above Paposo; the coastal fog gets caught on the slopes of the coastal cordillera and condensates on shrubs like *Euphorbia lactiflua* Phil. (Euphorbiaceae) or *Eulychnia taltalensis* (F.Ritter) Hoxey (Cacatacaeae). **D**) The absolute desert only few kilometers inland from Paposo. **E**) Coastal loma vegetation of the southern Atacama coast only few kilometers inland near Tatoral; the rich vegetation is the result of an El Niño event in 2017. **F**) *Bulnesia chilensis* Gay (Zygophyllaceae) found in the Atacama pampa between Copiapó and Vallenar; this species is sister to all remaining *Bulnesia* species found east of the Andes and in Peru (see Chapter 3). **G**) *Puya alpestris* (Poepp.) Gay (Bromeliaceae) a common element of the Mediterranean vegetation of Central Chile, although, the species is also found in the southern Atacama Desert coastal region. **H**) *Oxalis gigantea* Barnéoud (Oxalidaceae) a common element of the coastal vegetation between Huasco and Quebrada El Médano north of Paposo. **I**) *Bomarea ovallei* (Phil.) Ravenna (Alstromeriaceae) is locally endemic to the coastal area around Carrizal Bajo north of Huasco. **J**) *Rhodophiala laeta* Phil (Amaryllidaceae) a geophyte found in the vicinity of Paposo. **K**) *Mastigostyla cyrtophylla* I.M. Johnst. (Iridaceae) a geophytic herb found in the transition zone from the Andean Atacama vegetation towards the high Andean vegetation above 3000 m; picture was taken at 2,900 m along the Altos de Pica transect (see chapter 2). ▶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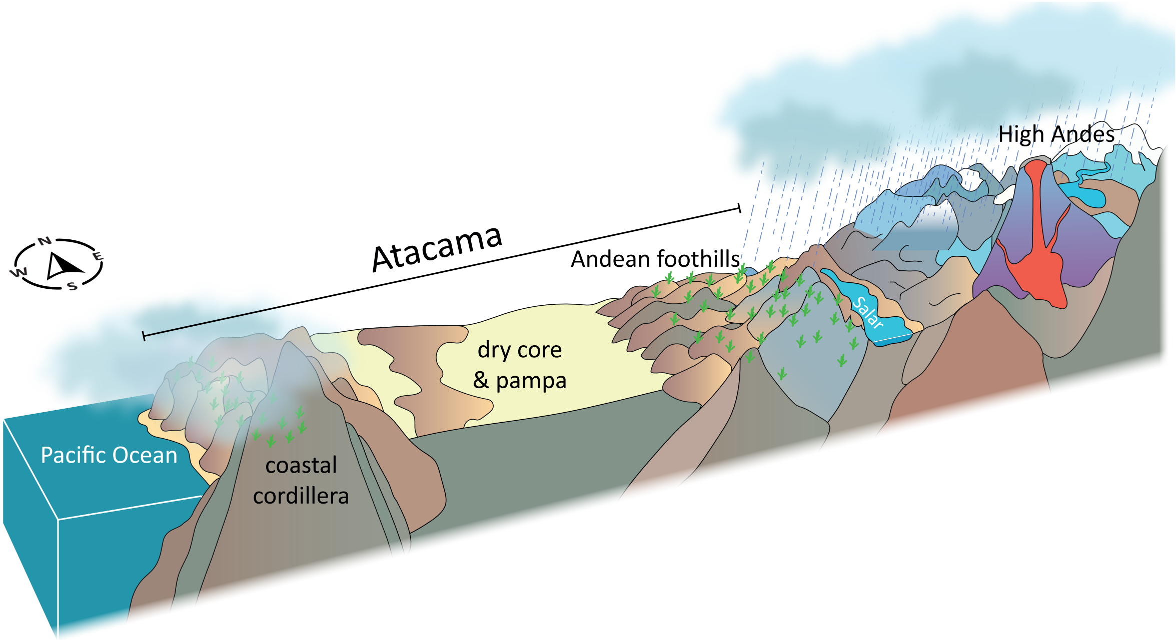

Figure 3: An idealized east-west profile of the Atacama Desert with the Pacific Ocean and the coastal cordilleras in the west, the hyperarid core in the center and the Andean foothills and high Andes in the east (figure modified after Pérez-Fodich *et al.* 2014).

Apart from the above described seasonality of climatic conditions in the Atacama Desert, the cyclic El Niño Southern Oscillation (ENSO) which occurs irregularly every few years during the austral winter, and the subsequent La Niña phenomenon in the following season have a major impact throughout the Pacific region and most particularly on arid and hyperarid areas (Holmgren *et al.* 2006). ENSO events have dramatic influence on the precipitation regime of the Atacama Desert and subsequently also on plant life (Dillon & Rundel 1990), for example by shaping germination rhythms of deserts plants (Gutiérrez *et al.* 2000). However, future climate change might result in increased intensity and frequency of ENSO events leading towards more associated freak events like floods in the Atacama Desert (Wang *et al.* 2019).

1.2.3 Geological and climatological history

The factors which are responsible for the arid to hyperarid conditions are long-lasting and probably exist since the Oligocene or earlier (Dunai *et al.* 2005; Hartley *et al.* 2005). Andean orogeny is one of the major geological factors responsible for the prevailing climatic conditions in the Atacama Desert due to its barrier function preventing moisture transport from the Amazon basin. Furthermore, it also has fundamental impact on landscape and biological evolution throughout South America (Hoorn *et al.* 2010; Luebert & Weigend 2014; Antonelli *et al.* 2018; Perrigo *et al.* 2019). However, the different stages of the Andean uplift are not yet fully understood. In general, the driving force for the rise of the Andes is the subduction of the Nazca Plate under the South American Plate which probably has started approx. 30 to 50 mya (Barnes & Ehlers 2009). For a more detailed introduction on the different processes of Andean orogeny and its influence on the Atacama Desert please see the introductory part of chapter 6.

Although it has been shown that arid and hyperarid conditions are long-lasting in the Atacama Desert, climate conditions were not homogeneous throughout time (Zachos *et al.* 2001; Gayó *et al.* 2005). Compared to the knowledge regarding the paleoclimate during the Neogene, climatic conditions throughout the Quaternary are better understood (Ritter *et al.* 2019). Recent evidence suggests the existence of several episodes in which lakes held large amounts of water in the dry core of the Atacama Desert (Ritter *et al.* 2018). Archeological findings from the dry core of the Atacama Desert even prove early settlements 13,000 to 11,000 years before present, which overlap in time with the presence of wetlands such as riparian woodlands (Latorre *et al.* 2013). Those fluctuating climatic conditions in the Quaternary had probably major impact on plant life in the Atacama Desert (Díaz *et al.* 2019).

1.3 Floristic Diversity in the Atacama Desert

Despite its surprisingly diverse flora, species richness is not evenly distributed among the genera native to the Atacama Desert (see Figure 2F–K for some impressions). Only ten genera comprise more than ten species and not all of them are the product of a single radiation process, but rather of multiple incursions with subsequent minor radiations like in *Oxalis* L. (Heibl & Renner 2012), *Heliotropium* L. sect. *Cochranea* (Miers) Kuntze (Luebert & Wen 2008) or *Cryptantha* Lehm. ex G.Don (Guilliams *et al.* 2017). The coastal lomas of the Atacama Desert alone harbor at least 550 species from 225 genera and up to 80 families (Dillon & Hoffmann 1997). According to Dillon & Hoffmann (1997) the most speciose families are Asteraceae (65 ssp.), Solanaceae (56 spp.), Fabaceae (35 spp.), Cactaceae (30 – 40 spp.) and Boraginaceae (26 spp.). However, as over 20 years have passed since this estimation actual numbers of plant species richness are probably higher. As mentioned above, ten genera in the Atacama Desert have ten or more species and are therefore considered as speciose in this work: *Nolana* L.f. (49 spp.; Solanaceae), *Adesmia* DC (~20 spp.; Fabaceae), *Cistanthe* Spach (~19 spp.; Montiaceae), *Copiapoa* Britton & Rose, (19 spp.; Cactaceae), *Heliotropium* L. (18 spp.; Heliotropiaceae), *Cristaria* Cav. (~19 spp.; Malvaceae), *Atriplex* L. (15 spp.; Amaranthaceae), *Oxalis* (14 spp.; Oxalidaceae), *Solanum* L. (12 spp.; Solanaceae) and *Spergularia* (Pers.) J.Presl & C.Presl (~10 spp.; Caryophyllaceae; Rosas 1989; Rundel *et al.* 1991; Muñoz-Schick 1995; Dillon & Hoffmann 1997; Dillon 2005; Luebert & Wen 2008; Zuloaga *et al.* 2008; Dillon *et al.* 2009; Heibl & Renner 2012; Larridon *et al.* 2015). *Cryptantha* also needs to be listed here but no exact numbers exist for the Atacama although the majority of South American taxa are native to Chile (Johnston 1927). Phylogenetic studies already exist for the majority of these genera, but were missing for *Adesmia*, *Cristaria*, *Atriplex* and *Spergularia*. Among those, taxonomic revisions exist for *Atriplex* and *Cristaria* (Rosas 1989; Muñoz-Schick 1995).

As mentioned above the flora is mainly restricted to the coastal cordillera and the Andean foothills (Gutiérrez *et al.* 1998). The coastal flora has received the most attention throughout the years (e.g., Johnston 1929; Rundel *et al.* 1991; Dillon & Hoffmann 1997; Schulz *et al.* 2011).

In turn, less is known about the flora of the Andean foothills, and several authors postulate that this flora is significantly different compared to the coastal flora as well as towards Peru (Rauh 1986; Rundel *et al.* 1991; Luebert & Pliscoff 2017). An in-depth overview of the floristic composition is given in the introduction of chapter 2.

The biogeographic history of the Atacama Desert flora has been a study subject for a long time. Early studies already provided first insights into the floristic connection towards other dry plant formations of South America (Sarmiento 1975). Based on a comparison of genera between different dry plant formations Sarmiento (1975) showed a floristic connection due to similarities of Atacama plant groups towards the Central Andes as well as Inter Andean dry valleys. Luebert (2011) in turn identified four individual so-called floristic elements based on phylogenetic and biogeographic information for 53 plant linages present in the Atacama Desert.

1.4 Hypotheses and research questions

The study of floras in arid environments is particularly interesting because the lower ecological complexity makes it easier to identify evolutionary processes responsible for the origin of new species. Stebbins (1952) already formulated a concept in which "aridity is a stimulus for plant evolution". The present study takes up this concept and addresses the following hypotheses:

Highest species richness in the Atacama Desert is found along the coastal cordillera and the Andean foothills separated by a barrier, the hyperarid core. Peru harbors a rich but different coastal desert flora compared to northern Chile. If so, (H1) **the coastal and the Andean part of the Atacama Desert as well as the Peruvian desert are three floristically separated units**. The following questions will be addressed in order to test this hypothesis: (Q1) What is the floristic composition and spatial variability of the vegetation near the dry core of the Atacama Desert? (Q2) Does a floristic connection exist between the Atacama Desert and the Peruvian desert? And (Q3) does a genetic connection exist between the Atacama Desert and the Peruvian coastal desert?

As shown by previous studies, the colonization events of certain plant lineages took place during the Miocene. This implies that, (H2) **the origin of endemic plant lineages coincides with climatic and geological factors responsible for (hyper-) aridity in the Atacama Desert during the middle to late Miocene**. In order to test this assumption, I address the following three questions: (Q4) What are the ages of the endemic lineages in the Atacama Desert? (Q5) Are Atacama lineages the product of single colonization events or multiple incursions into the Atacama Desert? And (Q6) is the timing of the colonization of plant lineages in the Atacama Desert correlated to climatic and geological events?

The global cooling trend since the middle Miocene followed by oscillating climatic conditions during the Quaternary is characterized by several cycles of glaciation and interglacial periods and subsequent variations in sea levels. In the Atacama Desert those conditions resulted in increased fluctuations of dry and more humid phases. This implies that (H3) **the diversification of speciose Atacama clades correlates with increasing aridity during the Miocene and Pliocene as well as the Quaternary climate oscillation.** The three following questions can provide answers that allow this hypothesis to be tested: (Q7) How did the spatiotemporal diversification of speciose plant lineages take place? (Q8) What is the influence of the Quaternary climate oscillation on diversification processes of speciose Atacama plant clades? And, (Q9) how is the status of current speciation processes and is it adequately reflected in current taxonomic concepts for speciose Atacama clades?

1.5 Overview of the Dissertation

The dissertation presented here is a cumulative work consisting of articles that have either been published, submitted or included as manuscripts that will be submitted later for publication in peer-reviewed journals. Supplementary materials are provided as appendices at the end of this document for the chapter 3, 5, 6 and 7. In the following, general information is given for each chapter including its status of publication:

Chapter 2: In this chapter the floristic composition and spatiotemporal variability of the flora along the coastal range and the Andean foothills, as well as its connection towards Peru is analyzed based on presence-abundance data. For this chapter, vegetation assessments along four altitudinal transects along the Andean foothills were conducted, compared and analyzed together with published data. The manuscript is based on the Master thesis of Jonathan Ruhm but has been reviewed by all co-authors and submitted for publication to *PLoS One*. Supplementary material including different databases is available online from the CRC database (https://doi.org/10.5880/CRC1211DB.29; Ruhm *et al.* 2019) and is, due to its extent, not included in the Appendix.

Chapter 3: Here, the historical biogeography of the Zygophyllaceae was investigated in the framework of a family-wide dated phylogeny. Zygophyllaceae are found frequently in arid environments of the tropics and subtropics. The Atacama Desert harbors five species of this family but all belong to different genera. The here presented manuscript is currently under review at *Frontiers of Biogeography*. Supplementary material including additional figures and results as well as tables including a species list and GenBank accession numbers can be found in Appendix A. Aligned sequence information as well as an R script written for the ancestral area reconstruction are available online from the CRC database (https://doi.org/10.5880/CRC1211DB.32; Böhnert *et al.* 2019c) or from the authors GitHub repository (https://github.com/TimBoeh/HistBiogeoZygo).

Chapter 4: Prior to the phylogenetic study on the genus *Atriplex* (Amaranthaceae) in chapter 5, a nomenclatural correction had to be formalized. In this chapter the priority rule following Art. 11 of the International Code of Nomenclature (Turland *et al.* 2017) was applied. It is discussed why *Atriplex retusa* Gay, published in 1849, takes priority over the previously accepted name *Atriplex deserticola* Phil., published in 1860. This chapter was published in *Phytotaxa* (Böhnert *et al.* 2018).

Chapter 5: *Atriplex*, with 15 species of shrubs, is one of the most speciose genera in the Atacama Desert. In this chapter, two different phylogenetic approaches using plastid sequence information were combined in order to date the age of the Atacama species. Next generation sequencing information and an increased sampling was used to gain deeper insights into the biogeographic and evolutionary history of the group. The manuscript presented has not been published and must be seen as the fundament for a publication in the near future. Supplementary material including taxon sampling, GenBank accession numbers as well as a Python script used to assemble and to analyze the next generation sequencing data is provided in Appendix B.

Chapter 6: The genus *Cristaria* (Malvaceae) is one of the most species rich genera and, apart from one species found in Peru, one on two Pacific islands, the genus is virtually endemic to the Atacama Desert. In this chapter, the most comprehensive sampling of the genus is presented and analyzed in a phylogenetic context using three plastid DNA regions and a Bayesian Molecular clock dating approach. This chapter has been published 2019 in a special issue about the Atacama Desert in *Global and Planetary Change* (Böhnert *et al.* 2019b). Supplementary material is available in Appendix C and encompasses additional figures as well as a species list including GenBank accessions. Aligned sequence information and tree files are available from the CRC database (https://doi.org/10.5880/CRC1211DB.26; Böhnert *et al.* 2019a).

Chapter 7: Here, a genotyping-by-sequencing (GBS) approach similar to the one in chapter 5 was used to gain a deeper understanding of the biogeographic history and the underlying macro-evolutionary processes responsible for the *in-situ* radiation of the genus *Cristaria*. This chapter represents an advanced state of a manuscript which is planned to be submitted in a slightly modified form in the near future. In Appendix D supplementary materials are provided, including additional figures, taxon sampling, assembly statistics, georeferenced voucher information as well as two Python scripts documenting the DNA assembly and phylogenetic reconstruction using the GBS data.

Chapter 8: In this final chapter all results are discussed with regard to the earlier formulated hypotheses and research questions. A final conclusion and an outlook on future research questions in relation to the individual chapters presented in this dissertation will put the results into a broader context.

1.6 References

Antonelli A, Kissling WD, Flantua SGA, Bermúdez MA, Mulch A, Muellner-Riehl AN, Kreft H, Linder HP, Badgley C, Fjeldså J, Fritz SA, Rahbek C, Herman F, Hooghiemstra H & Hoorn C (2018) Geological and climatic influences on mountain biodiversity. *Nature Geoscience* 11 (10): 718–725. DOI: https://doi.org/10.1038/s41561-018-0236-z.

Barnes JB & Ehlers TA (2009) End member models for Andean Plateau uplift. *Earth-Science Reviews* 97 1-4: 105–132. DOI: https://doi.org/10.1016/j.earscirev.2009.08.003.

Barthlott W, Mutke J, Rafiqpoor D, Kier G & Kreft H (2005) Global centers of vascular plant diversity. *Nova Acta Leopoldina* 92 (342): 61–83.

Böhnert T, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider J, Quandt D & Weigend M (2019a) cpDNA alignments and phylogenetic trees of *Cristaria* (Malvaceae) from the Atacama Desert. *CRC1211 Database*. DOI: https://doi.org/10.5880/CRC1211DB.26.

Böhnert T, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider JV, Quandt D & Weigend M (2019b) Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert. *Global and Planetary Change* 181: 102992. DOI: https://doi.org/10.1016/j.gloplacha.2019.102992.

Böhnert T, Luebert F & Weigend M (2018) *Atriplex retusa*, the correct name for *A. deserticola* (Chenopodiaceae; Amaranthaceae sensu APG). *Phytotaxa* 373 (1): 95–98. DOI: https://doi.org/10.11646/phytotaxa.373.1.6.

Böhnert T, Weigend M, Merklinger FF, Quandt D & Luebert F (2019c) cpDNA alignments and R script for Historical Biogeography of Zygophyllaceae in the Atacama Desert. *CRC1211 Database*. DOI: https://doi.org/10.5880/CRC1211DB.32.

Byrne M, Yeates DK, Joseph L, Kearney M, Bowler J, Williams MAJ, Cooper S, Donnellan SC, Keogh JS, Leys R, Melville J, Murphy DJ, Porch N & Wyrwoll K-H (2008) Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology* 17 (20): 4398–4417. DOI: https://doi.org/10.1111/j.1365-294X.2008.03899.x.

Díaz FP, Latorre C, Carrasco-Puga G, Wood JR, Wilmshurst JM, Soto DC, Cole TL & Gutiérrez RA (2019) Multiscale climate change impacts on plant diversity in the Atacama Desert. *Global Change Biology* 25 (5): 1733–1745. DOI: https://doi.org/10.1111/gcb.14583.

Dillon MO (2005) The Solanaceae of the lomas formations of coastal Peru and Chile. *Monographs in Systematic Botany from the Missouri Botanical Garden* 104: 131–155.

Dillon MO & Hoffmann AE (1997) Lomas formations of the Atacama Desert northern Chile. In: Davis SD, Heywood VH, Herrera-MacBryde O, Villa-Lobos J & Hamilton AC, eds. *Centres of Plant Diversity, a Guide and Strategy for Their Conservation. Volume 3: The Americas*. United Kingdom: The World Wildlife Fund For Nature and IUCN - The World Conservation Union, *pp* 528–535.

Dillon MO & Rundel PW (1990) The Botanical Response of the Atacama and Peruvian Desert Floras to the 1982-83 El Niño Event. In: Glynn PW, ed. *Global Ecological Consequences of the 1982–83 El Nino—SouthernOscillation*: Elsevier, *pp* 487–504.

Dillon MO, Tu T, Xie L, Quipuscoa Silvestre V & Wen J (2009) Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution* 47 (5): 457–476. DOI: https://doi.org/10.1111/j.1759-6831.2009.00040.x.

Dunai TJ, González López GA & Juez-Larré J (2005) Oligocene–Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. *Geology* 33 (4): 321–324. DOI: https://doi.org/10.1130/G21184.1.

Garcia-Porta J, Irisarri I, Kirchner M, Rodríguez A, Kirchhof S, Brown JL, MacLeod A, Turner AP, Ahmadzadeh F, Albaladejo G, Crnobrnja-Isailovic J, La Riva I de, Fawzi A, Galán P, Göçmen B, Harris DJ, Jiménez-Robles O, Joger U, Jovanović Glavaš O, Karış M, Koziel G, Künzel S, Lyra M, Miles D, Nogales M, Oğuz MA, Pafilis P, Rancilhac L, Rodríguez N, Rodríguez Concepción B, Sanchez E, Salvi D, Slimani T, S'khifa A, Qashqaei AT, Žagar A, Lemmon A, Moriarty Lemmon E, Carretero MA, Carranza S, Philippe H, Sinervo B, Müller J, Vences M & Wollenberg Valero KC (2019) Environmental temperatures shape thermal physiology as well as diversification and genome-wide substitution rates in lizards. *Nature communications* 10 (1): 4077. DOI: https://doi.org/10.1038/s41467-019-11943-x.

Garreaud RD (2009) The Andes climate and weather. *Advances in Geosciences* 22: 3–11. DOI: https://doi.org/10.5194/adgeo-22-3-2009.

Garreaud RD, Molina A & Farias M (2010) Andean uplift, ocean cooling and Atacama hyperaridity: A climate modeling perspective. *Earth and Planetary Science Letters* 292 (1-2): 39–50. DOI: https://doi.org/10.1016/j.epsl.2010.01.017.

Gayó E, Hinojosa LF & Villagrán C (2005) On the persistence of Tropical Paleofloras in central Chile during the Early Eocene. *Review of Palaeobotany and Palynology* 137 (1-2): 41–50. DOI: https://doi.org/10.1016/j.revpalbo.2005.09.001.

Guilliams CM, Hasenstab-Lehman KE, Mabry ME & Simpson MG (2017) Memoirs of a frequent flier: Phylogenomics reveals 18 long-distance dispersals between North America and South America in the popcorn flowers (Amsinckiinae). *American Journal of Botany* 104 (11): 1717–1728. DOI: https://doi.org/10.3732/ajb.1700369.

Gutiérrez JR, Arancio G & Jaksic FM (2000) Variation in vegetation and seed bank in a Chilean semi-arid community affected by ENSO 1997. *Journal of Vegetation Science* 11 (5): 641–648. DOI: https://doi.org/10.2307/3236571.

Gutiérrez JR, López-Cortes F & Marquet PA (1998) Vegetation in an altitudinal gradient along the Río Loa in the Atacama Desert of northern Chile. *Journal of Arid Environments* 40 (4): 383–399. DOI: https://doi.org/10.1006/jare.1998.0462.

Gutiérrez-Ortega JS, Yamamoto T, Vovides AP, Angel Pérez-Farrera M, Martínez JF, Molina-Freaner F, Watano Y & Kajita T (2018) Aridification as a driver of biodiversity: a case study for the cycad genus *Dioon* (Zamiaceae). *Annals of Botany* 121 (1): 47–60. DOI: https://doi.org/10.1093/aob/mcx123.

Hartley AJ, Chong G, Houston J & Mather AE (2005) 150 million years of climatic stability: Evidence from the Atacama Desert, northern Chile. *Journal of the Geological Society* 162 (3): 421–424. DOI: https://doi.org/10.1144/0016-764904-071.

Heibl C & Renner SS (2012) Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. *Systematic Biology* 61 (5): 823–834. DOI: https://doi.org/10.1093/sysbio/sys034.

Hernández-Hernández T, Brown JW, Schlumpberger BO, Eguiarte LE & Magallón S (2014) Beyond aridification: Multiple explanations for the elevated diversification of cacti in the New World Succulent Biome. *New Phytologist* 202 (4): 1382–1397. DOI: https://doi.org/10.1111/nph.12752.

Holmgren M, Stapp P, Dickman CR, Gracia C, Graham S, Gutiérrez JR, Hice C, Jaksic F, Kelt DA, Letnic M, Lima M, López BC, Meserve PL, Milstead WB, Polis GA, Previtali MA, Richter M, Sabaté S & Squeo FA (2006) Extreme climatic events shape arid and semiarid ecosystems. *Frontiers in Ecology and the Environment* 4 (2): 87–95. DOI: https://doi.org/10.1890/1540-9295(2006)004[0087:ECESAA]2.0.CO;2.

Hoorn C, Wesselingh FP, ter Steege H, Bermúdez MA, Mora A, Sevink J, Sanmartín I, Sanchez-Meseguer A, Anderson CL, Figueiredo JP, Jaramillo C, Riff D, Negri FR, Hooghiemstra H, Lundberg J, Stadler T, Särkinen T & Antonelli A (2010) Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330 (6006): 927–931. DOI: https://doi.org/10.1126/science.1194585.

Houston J (2006) Variability of precipitation in the Atacama Desert: Its causes and hydrological impact. *International Journal of Climatology* 26 (15): 2181–2198. DOI: https://doi.org/10.1002/joc.1359.

Houston J & Hartley AJ (2003) The central Andean west-slope rainshadow and its potential contribution to the origin of hyper-aridity in the Atacama Desert. *International Journal of Climatology* 23 (12): 1453–1464. DOI: https://doi.org/10.1002/joc.938.

Johnston IM (1927) Studies in the Boraginaceae VI: A revision of the South American Boraginoideae. *Contributions from the Gray Herbarium of Harvard University* 78: 1–118.

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

Kadereit G, Mavrodiev EV, Zacharias EH & Sukhorukov AP (2010) Molecular phylogeny of Atripliceae (Chenopodioideae, Chenopodiaceae): Implications for systematics, biogeography, flower and fruit evolution, and the origin of C4 photosynthesis. *American Journal of Botany* 97 (10): 1664–1687. DOI: https://doi.org/10.3732/ajb.1000169.

Larridon I, Walter HE, Guerrero PC, Duarte M, Cisternas MA, Hernández CP, Bauters K, Asselman P, Goetghebeur P & Samain M-S (2015) An integrative approach to understanding the evolution and diversity of *Copiapoa* (Cactaceae), a threatened endemic Chilean genus from the Atacama Desert. *American Journal of Botany* 102 (9): 1506–1520. DOI: https://doi.org/10.3732/ajb.1500168.

Latorre C, Santoro CM, Ugalde PC, Gayo EM, Osorio D, Salas-Egaña C, Pol-Holz R de, Joly D & Rech JA (2013) Late Pleistocene human occupation of the hyperarid core in the Atacama Desert, northern Chile. *Quaternary Science Reviews* 77: 19–30. DOI: https://doi.org/10.1016/j.quascirev.2013.06.008.

Lauterbach M, Zimmer R, Alexa AC, Adachi S, Sage R, Sage T, MacFarlane T, Ludwig M & Kadereit G (2019) Variation in leaf anatomical traits relates to the evolution of C_4 photosynthesis in Tribuloideae (Zygophyllaceae). *Perspectives in Plant Ecology, Evolution and Systematics* 39: 125463. DOI: https://doi.org/10.1016/j.ppees.2019.125463.

Luebert F (2011) Hacia una fitogeografía histórica del Desierto de Atacama. *Revista de Geografía Norte Grande* (50): 105–133. DOI: https://doi.org/10.4067/S0718-34022011000300007.

Luebert F & Pliscoff P (2017) Sinopsis bioclimática y vegetacional de Chile. Santiago de Chile: *Editorial Universitaria*. 381 pp.

Luebert F & Weigend M (2014) Phylogenetic insights into Andean plant diversification. *Frontiers in Ecology and Evolution* 2 (192): 85. DOI: https://doi.org/10.3389/fevo.2014.00027.

Luebert F & Wen J (2008) Phylogenetic Analysis and Evolutionary Diversification of *Heliotropium* Sect. *Cochranea* (Heliotropiaceae) in the Atacama Desert. *Systematic Botany* 33 (2): 390–402. DOI: https://doi.org/10.1600/036364408784571635.

Muñoz-Schick M (1995) Revision del género *Cristaria* (Malvaceae) en Chile. *Boletín del Museo Nacional de Historia Natural Chile* 45: 45–110.

Pérez-Fodich A, Reich M, Álvarez F, Snyder GT, Schoenberg R, Vargas G, Muramatsu Y & Fehn U (2014) Climate change and tectonic uplift triggered the formation of the Atacama Desert's giant nitrate deposits. *Geology* 42 (3): 251–254. DOI: https://doi.org/10.1130/G34969.1.

Perrigo A, Hoorn C & Antonelli A (2019) Why mountains matter for biodiversity. *Journal of Biogeography* 524 (10): 300. DOI: https://doi.org/10.1111/jbi.13731.

Rauh W (1986) The Peruvian-Chilean Deserts. Chapter 7. In: Evenari M, Noy-Meir I & Goodall DW, eds. *Hot deserts and arid shrublands*. Amsterdam: Elsevier, *pp* 239–267.

Ritter B, Binnie SA, Stuart FM, Wennrich V & Dunai TJ (2018) Evidence for multiple Plio-Pleistocene lake episodes in the hyperarid Atacama Desert. *Quaternary Geochronology* 44: 1–12. DOI: https://doi.org/10.1016/j.quageo.2017.11.002.

Ritter B, Wennrich V, Medialdea A, Brill D, King G, Schneiderwind S, Niemann K, Fernández-Galego E, Diederich J, Rolf C, Bao R, Melles M & Dunai TJ (2019) "Climatic fluctuations in the hyperarid core of the Atacama Desert during the past 215 ka". *Scientific Reports* 9 (1): 5270. DOI: https://doi.org/10.1038/s41598-019-41743-8.

Rosas MR (1989) El genero *Atriplex* (Chenopodiaceae) en Chile. *Gayana Botánica* 46 1-2: 3–82.

Ruhm J, Böhnert T, Weigend M, Stoll A, Merklinger FF, Quandt D & Luebert F (2019) Spatial floristic data from the Atacama and Peruvian Desert. *CRC1211 Database*. DOI: https://doi.org/10.5880/CRC1211DB.29.

Rundel PW, Dillon MO, Palma B, Mooney HA, Gulmon SL & Ehrlinger JR (1991) The Phytogeography and Ecology of the Coastal Atacama and Peruvian Desert. *Aliso: A Journal of Systematic and Evolutionary Botany* 13 (1): 1–49.

Sarmiento G (1975) The Dry Plant Formations of South America and Their Floristic Connections. *Journal of Biogeography* 2 (4): 233. DOI: https://doi.org/10.2307/3037998.

Schulz N, Aceituno P & Richter M (2011) Phytogeographic divisions, climate change and plant dieback along the coastal desert of northern Chile. *Erdkunde* 65 (2): 169–187. DOI: https://doi.org/10.3112/erdkunde.2011.02.05.

Stebbins GL (1952) Aridity as a Stimulus to Plant Evolution. *The American Naturalist* 86 (826): 33–44. DOI: https://doi.org/10.1086/281699.

Stuut J-BW & Lamy F (2004) Climate variability at the southern boundaries of the Namib (southwestern Africa) and Atacama (northern Chile) coastal deserts during the last 120,000 yr. *Quaternary Research* 62 (3): 301–309. DOI: https://doi.org/10.1016/j.yqres.2004.08.001.

Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ & Smith GF (2017) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Glashütten: *Koeltz Botanical Books.*

Wang B, Luo X, Yang Y-M, Sun W, Cane MA, Cai W, Yeh S-W & Liu J (2019) Historical change of El Niño properties sheds light on future changes of extreme El Niño. *Proceedings of the National Academy of Sciences of the United States of America* 116 (45): 22512–22517. DOI: https://doi.org/10.1073/pnas.1911130116.

Zachos J, Pagani M, Sloan L, Thomas E & Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292 (5517): 686–693. DOI: https://doi.org/10.1126/science.1059412.

Zhang R, Liu T, Zhang J-L & Sun Q-M (2016) Spatial and environmental determinants of plant species diversity in a temperate desert. *Journal of Plant Ecology* 9 (2): 124–131. DOI: https://doi.org/10.1093/jpe/rtv053.

Zhang Z, Ramstein G, Schuster M, Li C, Contoux C & Yan Q (2014) Aridification of the Sahara desert caused by Tethys Sea shrinkage during the Late Miocene. *Nature* 513 (7518): 401–404. DOI: https://doi.org/10.1038/nature13705.

Zhu Y, Shan D, Wang B, Shi Z, Yang X & Liu Y (2019) Floristic features and vegetation classification of the Hulun Buir Steppe in North China: Geography and climate-driven steppe diversification. *Global Ecology and Conservation* 20: e00741. DOI: https://doi.org/10.1016/j.gecco.2019.e00741.

Zuloaga FO, Morrone O & Belgrano MJB, eds. (2008) Catálogo de las Plantas Vasculares del Cono Sur. Argentina, Sur de Brasil, Chile, Paraguay y Uruguay. Saint Louis Mo.: *Missouri Botanical Garden Press.*

Chapter 2

Spatial patterns of floristic diversity in the Atacama Desert

Vegetation of the Andean foothills at 2,900 m at the Tambillo transect. The dominating element: *Mirabilis trollii* Heimerl. (Nyctaginaceae).

This chapter is included here as a manuscript but has since been published as:

Ruhm J, **Böhnert T**, Weigend M, Merklinger FF, Stoll A, Quandt D & Luebert F (2020) Plant life at the dry limit-Spatial patterns of floristic diversity and composition around the hyperarid core of the Atacama Desert. *PLos ONE* 15(5), e0233729. DOI: https://doi.org/10.1371/journal.pone.0233729.

Own contribution: Conceptualization, data curation, field work, methodology, validation, review & editing, supervision.

Data availability: Supplementary materials are available online via the CRC database (DOI: https://doi.org/10.5880/CRC1211DB.29).

Abstract

Extreme arid conditions in the Atacama Desert in northern Chile have created a unique vegetation almost entirely restricted to the desert margins along the coast of the Pacific Ocean and the Andean range. In this study we provide data on the desert vegetation along elevational gradients at four localities from the western Andean slopes, between 19° and 21° S. Additionally, zonation of floristic data was explored. Three altitudinal zones could be classified and described in detail for each locality. Conspicuously divergent floras in the Atacama Desert have been recorded, on the one hand the coastal ‘lomas formations’ and on the other hand the Andean desert vegetation, separated by a rather narrow strip of absolute desert. In this study we investigate the floristic isolation of both regions by implementing similarity analyses for 21 localities from the coastal and Andean deserts in northern Chile. Our results show a drastic east-west divergence in pairwise floristic similarity, which is in stark contrast to a weaker north-south divergence. A biotic barrier, preventing plant exchange from east to west and vice versa, imposed by the harsh conditions of the desert pampa, is one possible explanation for this finding. Moreover, the coastal and Andean desert likely constitute divergent habitats, e.g., in seasonality. Essential differences in factors determining plant life between both regions have probably contributed to a divergent evolution of the floras. Both explanations – ecological divergence and isolation – are not mutually exclusive, but likely complementary. Additionally, we combined floristic data from northern Chile with data from southern Peru. Similarity analyses of the combined regions provide first evidence for the existence of a biotic north-south corridor along the western slope of the Andes. Sub-Andean distributions of several species are discussed in the light of floristic connectivity between Peruvian and Chilean Andean floristic clusters.

Keywords: Altitudinal zonation — Andes — Chile — hyperaridity — Peru — phytogeographic corridor — South America

2.1 Introduction

Although the Atacama Desert of northern Chile is one of the driest places on earth (Garreaud *et al.* 2010) it harbors a unique and diverse flora, well adapted to arid environments (Luebert & Pliscoff 2017). With few exceptions (e.g., riverbeds or canyons), vegetation is restricted to the desert margins along the Andean range to the east and at the Pacific coastal range to the west (Gutiérrez *et al.* 1998).

The climate of the Atacama Desert is characterized by a summer rainfall zone to the north-east and a winter rainfall zone to the south-west, and a hyperarid zone separating them. In the Andean range north of 24° S, precipitation occurs mostly in summer (Zhou & Lau 1998). During austral summer, moisture laden air masses from the Amazonian Basin and the Grand Chaco region pass the Andean range and bring rainfall to the western Andean slopes. Here, annual precipitation declines rapidly with decreasing altitude from over 300 mm at

5,000 m to less than 20 mm at 2,300 m (Houston & Hartley 2003). Along the coastal range, rainfall occurs mainly in winter. During the austral winter, humid air from the Pacific is transported towards the north Chilean coast enabled by a northward shift of the Intertropical Convergence Zone and the Pacific anticyclone (Vuille & Ammann 1997; Garreaud *et al.* 2009). However, humid air, originating above the Pacific, is blocked from entering the desert by the topographic barrier of the coastal range and a strong temperature inversion along the west coast of South America (Houston 2006). Below the temperature inversion along the coast, moisture is received as fog or drizzle, with thick stratus clouds accumulating and pressed against the slopes of the coastal range, forming a fog zone (Rundel & Mahu 1976; Cereceda *et al.* 2008). The influence of this coastal fog reaches a minimum at the transition between winter and summer-rainfall zones. For the entire length of the Atacama an area of extreme hyperaridity is present in the desert center between 19° S and 25° S (Houston 2006). Due to these conditions, the Atacama Desert is characterized by a strong west to east moisture gradient and the desert pampa or absolute desert bears virtually no plant life (Arroyo *et al.* 1988).

Vegetation along the Andean range shows strong altitudinal zonation due to decreasing temperature and aridity. Desert vegetation along the Andes, designated as ‘pre-Puna’ in some studies, lies below the high Andean ‘tolares’ or Puna vegetation at elevations of typically 3,150 to 3,850 meters a.s.l. (Villagrán *et al.* 1981). Andean desert vegetation has been poorly investigated so far, whereas high Andean vegetation is quite well documented (Villagrán *et al.* 1981; 1982; Luebert & Gajardo 2000; Teillier 2004; Luebert & Gajardo 2005). Most plants in the Andean desert only emerge in those rare years where summer rainfall reaches lower elevations. At the coastal range, however, vegetation has been studied extensively (Schulz 2009). The coastal flora is largely endemic to the coastal ‘lomas formations’ and arguably distinct from that of the neighboring Andes (Rundel *et al.* 1991). This however has not been evaluated with floristic data. The level of endemism along the Andes is poorly investigated.

Several studies postulated the existence of a phytogeographical barrier separating the Peruvian from the Chilean coastal desert on the basis of floristic inventories and similarity analyses (Rundel *et al.* 1991; Galán De Mera *et al.* 1997; Dillon 2005; Pinto & Luebert 2009). Floristic connections between Andean deserts of Chile and Peru were first suggested by Schwarzer *et al.* (2010). Furthermore, based on floristic and faunistic data, Moreno *et al.* (1994) postulated a north-south corridor facilitating biotic exchanges along the Andes. Phylogenetic studies of several plant groups (Gengler-Nowak 2002; Moore & Jansen 2006; Luebert & Wen 2008) tend to support this idea. An isolated evolution of the coastal and the Andean deserts floras, respectively, has already been proposed by Rundel *et al.* (1991). However, we are not aware of any study evaluating either the isolation of the coastal versus Andean floras or the floristic connections between the Chilean and Peruvian Andean deserts. In the present study we provide novel floristic data sets for the Andean desert vegetation and altitudinal transects in northern Chile between 19° S and 21° S.

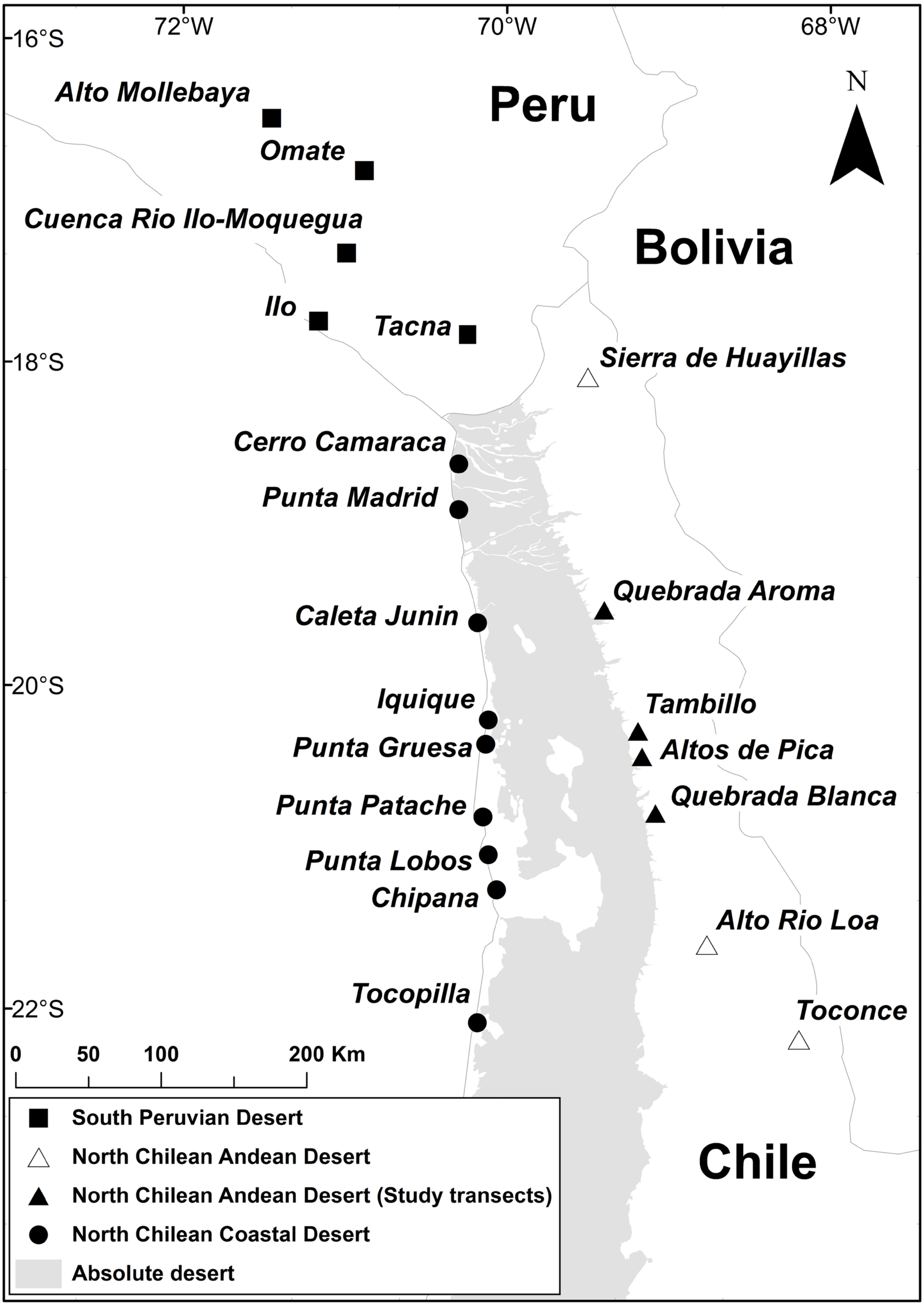

Figure 1: Overview of localities in southern Peru and northern Chile. Area in gray indicates the absolute desert according to Luebert & Pliscoff (2017).

Our aims are to **a**) give the first description of the desert vegetation along four altitudinal transects and **b**) explore the phytogeographic relationships between the coastal and Andean deserts of northern Chile and southern Peru using cluster analyses based on floristic data. We base our study on original data here presented, and previously published floristic data from Andean sites in northern Chile and southern Peru as well as from a range of localities along the southern Peruvian and northern Chilean coast.

2.2 Material and Methods

2.2.1 Study areas

In March 2017, floristic surveys were carried out at four localities along the Andean desert of the Tarapacá region in northern Chile (Figure 1). Climate data (Table 1) indicate remarkably 'humid' weather conditions for this area in 2017 in comparison to other years. Data on precipitation were provided by the Dirección General de Aguas, Chile. The following transects were studied:

Quebrada Aroma (19.52875° S, 69.37502° W to 19.57025° S, 69.46659° W): Vegetation of the Andean slopes along Quebrada Aroma was assessed between 2,600 and 2,200 meters with five plots. Due to accessibility, this transect did not reach the transition towards the Andean vegetation. Below 2,400 meters, vegetation is restricted to the cooler and wetter climate of the quebradas. The vegetation around Quebrada Aroma is dominated by shrubby species such as *Aphyllocladus denticulatus* (J. Remy) Cabrera (Asteraceae) and *Atriplex glaucescens* Phil. (Amaranthaceae) as well as columnar cacti such as *Browningia candelaris* (Meyen) Britton & Rose and smaller ascending species such as *Cumulopuntia sphaerica* (C.F. Först.) E.F. Anderson (Cactaceae).

Altos de Pica (20.38928° S, 69.08904° W to 20.48502 ° S, 69.22031° W): This transect is located at the slopes above the town of Pica along the road from Salar de Huasco down to Pica.

Table 1: Annual precipitation (mm) at three localities around the study area recorded from 2010 to 2018.

Year	**Sibaya** (2830 m) (19.53722° S, 69.20500° W)	**Poroma** (2880 m) (19.87166° S, 69.18222° W)	**Guataconco** (2460 m) (20.92750° S, 69.05277° W)
2010	6.8	22	21.5
2011	35.5	157	71.3
2012	82.3	154	53.1
2013	49.9	56	3
2014	23.6	33	0
2015	49.3	80.5	20
2016	33.4	55	0
2017	154.4	110	36
2018	27.6	53	7.4
Ø	51.42	80.6	23.59

Data were obtained from the Dirección General de Aguas, Chile (http://dga.cl, accessed: 11/05/2019).

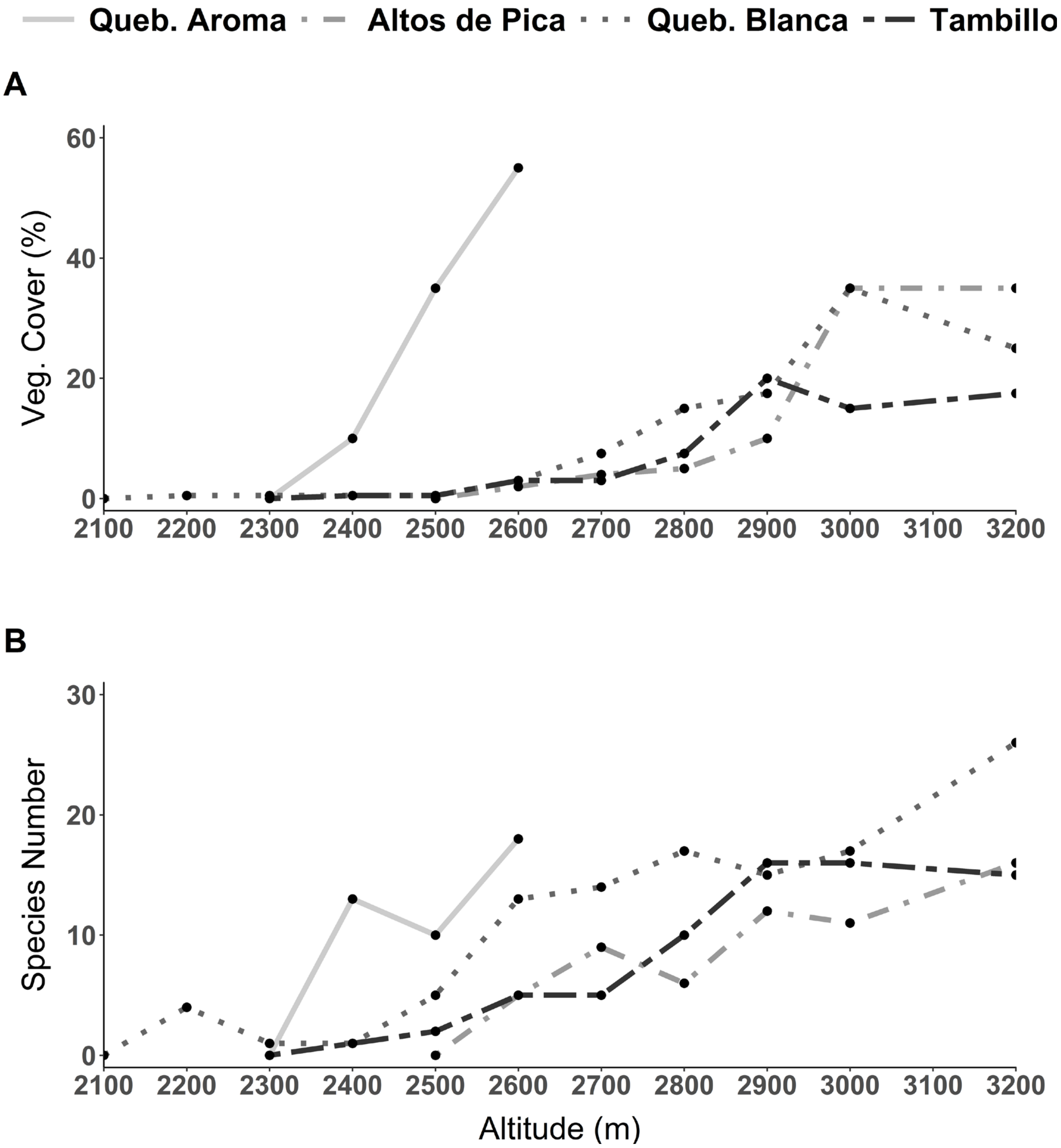

Figure 2: Vegetation changes across the elevation gradient from the four study transects. **A**) Vegetation cover in percentage. **B**) Species richness in total numbers.

Vegetation was assessed for ten plots between 3,200 and 2,200 meters. Vegetation was only found above 2,500 meters. Characteristic plants are shrubs and subshrubs such as *Atriplex glaucescens*, *Tiquilia paronychioides* (Phil.) A.T. Richardson (Ehretiaceae), but also forbs, for example the geophytic *Mastigostyla cyrtophylla* I.M. Johnst. (Iridaceae).

Quebrada Blanca (20.80247° S, 69.02584° W to 20.82217° S, 69.14174° W): Eleven plots were investigated between 3,200 to 2,100 meters along the road connecting the copper mine of Quebrada Blanca with the Pan-American Highway. The absolute desert starts below 2,100 meters. Besides the shrub *Atriplex glaucescens*, several herbaceous species were dominant, such as *Tetragonia microcarpa* Phil. (Aizoaceae), *Pectocarya anomala* I.M. Johnst. (Boraginaceae) and *Chorizanthe commissuralis* J. Remy (Polygonaceae).

Tambillo (20.30425° S, 69.11324° W to 20.26914° S, 69.32291° W): Starting at 3,200 meters, the altitudinal gradient follows the road between Salar de Huasco and the Pan-American Highway down to 2,000 meters. In this elevation range, vegetation was assessed for twelve plots. From 2,300 meters downwards, no plant species were recorded. Typical species in this area are the subshrub *Tiquilia grandiflora* (Phil.) A.T. Richardson (Ehretiaceae) along with *Munroa andina* Phil. (Poaceae) and *Mirabilis trollii* Heimerl (Nyctaginaceae).

2.2.2 Vegetation data and plant identification

The actual desert vegetation was segregated from the Andean vegetation by the absence of species typical for the high Andean 'tolares' (e.g., *Fabiana ramulosa* (Wedd.) Hunz. & Barboza; Luebert & Pliscoff 2017). Starting at the lower edge of the tolares flora, floristic inventories were done downhill for plots of 0.1 ha along altitudinal transects with one plot every 100 meters elevation towards the absolute desert. Sampling was stopped after two consecutive plots with no plants as evidence for having reached the absolute desert. Nearby species (outside the plots and indicated as such in the lists) were also recorded, to enrich the information of floristic diversity for each location following Mueller-Dombois & Ellenberg (1974, p. 61). In addition to the general plant cover of the plots, the cover-abundance of each species was estimated according to Braun-Blanquet (1932; 1964). For every species record a herbarium specimen was collected and a preliminarily identification was made in the field. Finally, specimens were deposited in the herbaria of the University of Bonn, Germany (BONN) and the University of La Serena, Chile (ULS; Table S1). Supplementary material tables from this study are available from the CRC database under Ruhm *et al.* (2019) and see also section 2.7 for supporting information.

Determination of herbarium specimens was carried out at Bonn University. Taxonomic studies on the genera and species within the focus region were used for specimen identification (Table S2). If these were not available, original descriptions of species were studied and whenever necessary, high-resolution scans of type specimens were used for comparison (https://plants.jstore.org). Scientific names were assigned and synonyms revised according to Zuloaga *et al.* (2008). Where species determination was not possible, specimens were assigned to morphospecies or labeled for pending confirmation. Information on lifeforms was taken from Zuloaga *et al.* (2008) and adjusted to the main groups of plant lifeforms according to Ellenberg and Mueller-Dombois (1967). Missing information was complemented either by consulting species descriptions or the inspection of conspecific herbarium specimens.

For a larger scale floristic comparison, additional data on the Andean desert flora of northern Chile was compiled from Villagrán *et al.* (1981; 1982) and Teillier (2004). Floristic information on localities from the coastal desert in northern Chile as well as the coastal and Andean

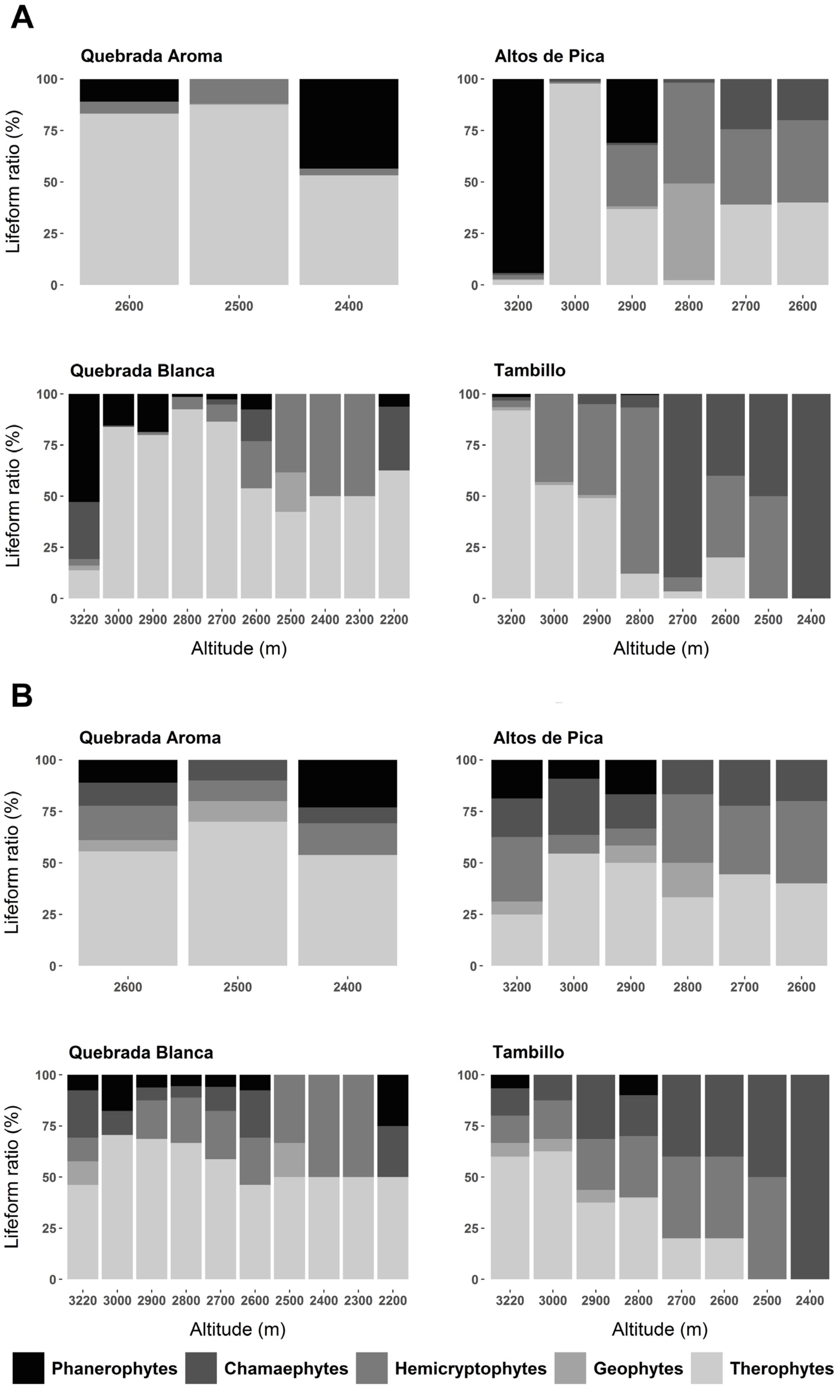

Figure 3: Lifeform spectra of the five dominant species for each elevational plot in the four study transects. **A**) Lifeform spectra based on vegetation cover. **B**) Lifeform spectra based on species number.

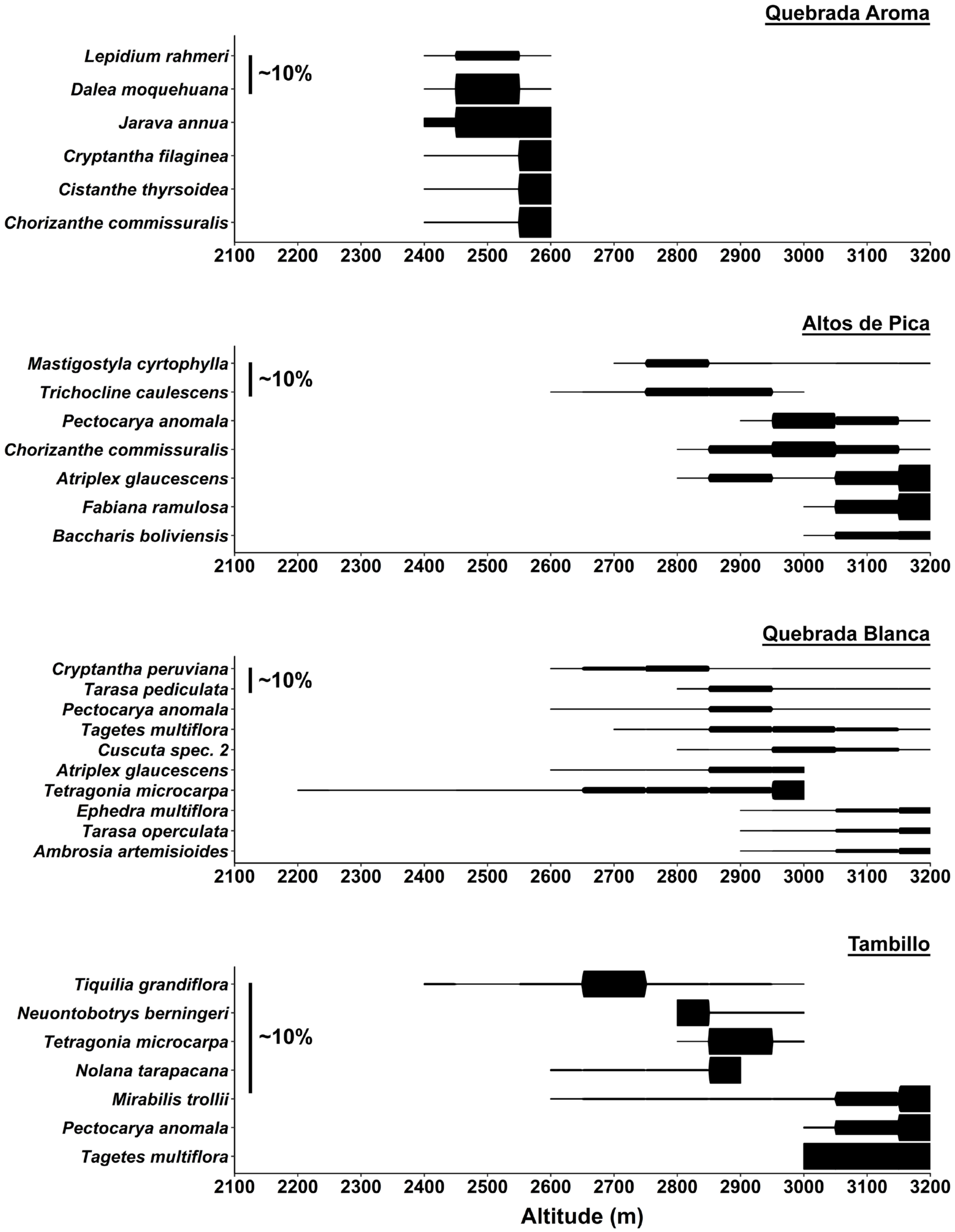

Figure 4: Abundance profiles of most dominant species along the four study transects. Abundance profiles are based on average cover values given in percentage (%) according to Tüxen & Ellenberg (1937). Most dominant species were selected by highest cover values. For each transect scalebars indicate average plant cover of about 10%.

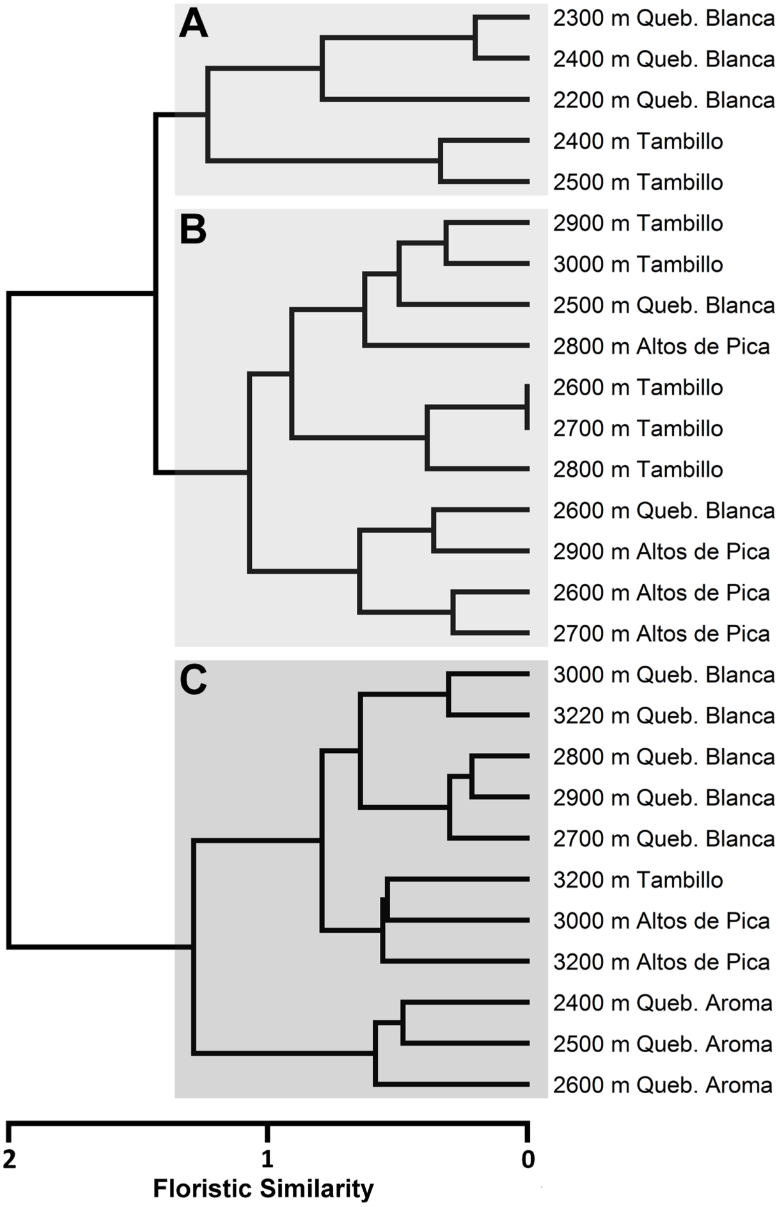

Figure 5: Classification of floristic distance between elevational plots from the four study transects. Clustering was conducted with the Ward's minimum variance method. Similarity analysis was performed using Sørensen index. **A**) Cluster of plots from lowest elevations. **B**) Cluster of plots from intermediate elevations. **C**) Cluster of plots from high elevations.

deserts in southern Peru were taken from Pinto and Luebert (2009), Schwarzer *et al.* (2010) and Arakaki *et al.* (2003). Scientific names of species were assigned and revised according to Brako and Zarucchi (1993) for Peru and Zuloaga *et al.* (2008) for Chile. A complete list of species across all these localities is given in Table S3. See Figure 1 for all localities included in the analyses.

2.2.3 Coverage and composition of the transects

For the vegetation analysis along the elevational gradients of the study transects (Quebrada Aroma, Altos de Pica, Quebrada Blanca and Tambillo), data were visualized and inspected in

R 3.4.2 (R Core Team 2017) and RStudio 1.1.383 (RStudio Team 2016). Estimates of cover-abundance (Braun-Blanquet 1932; 1964) for species from the study transects were transformed to average cover values (in %) according to Tüxen & Ellenberg (1937). Table S4 contains a comprehensive species list, average cover values of all species from the transects, their life form as well as general information about plots. Lifeform spectra based on vegetation cover on the one hand, and species numbers on the other hand were generated for the five main groups of self-supporting vascular plants for each elevational plot within the study transects (Ellenberg & Mueller-Dombois 1967). For lifeform spectra based on vegetation cover the percentage of each lifeform was calculated from the sum of all average cover values of species per plot. The four to five most dominating species were identified for each plot based on average cover values. Altitudinal abundance profiles were generated for all dominant species across the whole range of transects. If a species was not recorded for an individual plot, but found in plots below and above along a study transects, the missing data were imputed with the average of the upper and lower plot cover values of that species.

2.2.4 Similarity analyses

Similarity analyses and a classification of floristic data were performed using the R-package vegan 2.5-6 (Oksanen *et al.* 2019) to explore floristic zonation along altitudinal gradients. Pairwise floristic distances between plots were calculated using the Sørensen Similarity Index (Sørensen 1948). Similarity analyses were based on presence/absence data of species. Species recorded outside the actual study transects were excluded from these analyses. Hierarchical agglomerative clustering, using Ward's minimum variance criterion (Ward 1963), was employed for the classification of plots. Ward's method was selected on the basis of silhouette plots (Rousseeuw 1987) among four clustering methods (average linkage, complete linkage, single linkage, Ward's method), using the R-package fpc 2.2-3 (Henning 2019).

2.2.5 Floristic comparison

Floristic relationships between localities from coastal and Andean deserts of northern Chile and southern Peru were examined by conducting similarity analyses and classification of localities using identical parameters as described above. Regarding the floristic data, each transect was treated as a sample, pooling the species lists from all plots in the transect. In addition, the species records from outside the plots were included. Similarity analyses were then performed based on presence/absence data of species for each locality. A dendrogram and a heatmap were created with the R-packages gplots v3.0.1.1 (Warnes *et al.* 2019) and factoextra 1.0.5 (Kassambara & Mundt 2017) to display the results of the similarity analysis. These were followed by an ordination analysis based on floristic dissimilarities by Principal Coordinates Analysis (PCoA; Gower 1966) using the R-package ade4 1.7.13 (Chessel *et al.* 2004). Results of classification and ordination were subsequently combined and groups were visualized along the first two PCoA axes. Geographical distances were plotted against floristic dissimilarities. To calculate geographical distances between localities, the R-package geosphere 1.5.10 (Hijmans 2017) was employed.

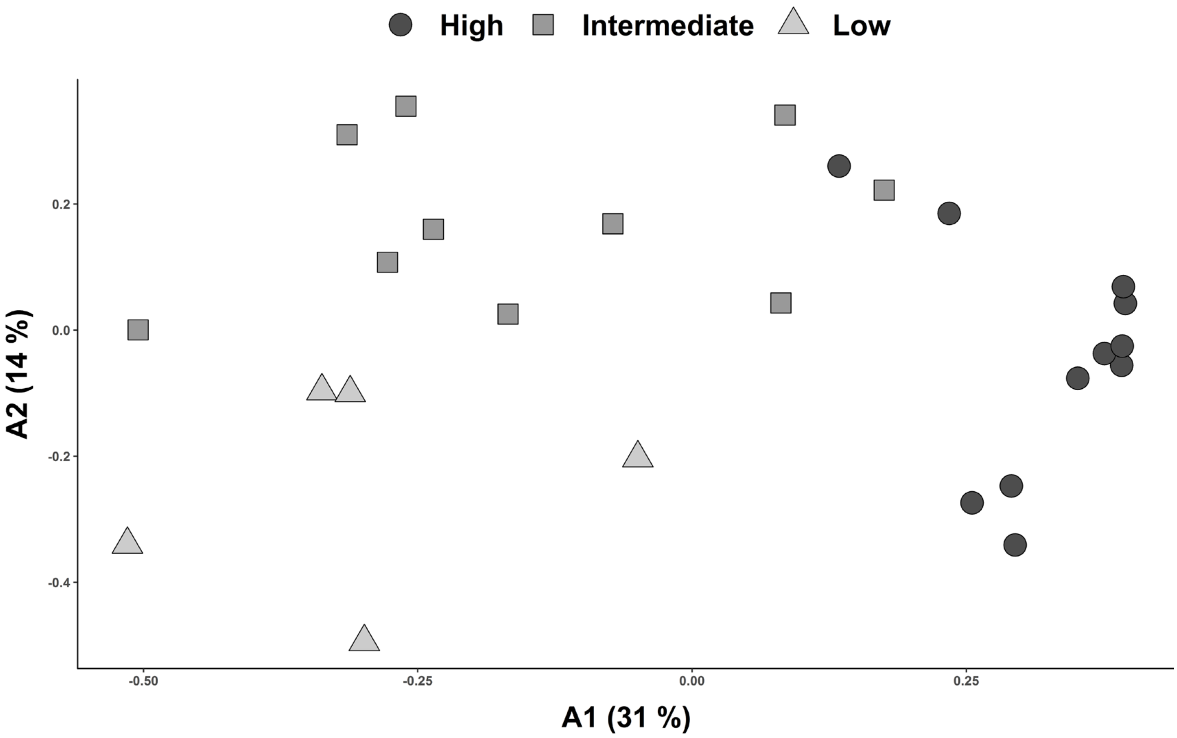

Figure 6: Ordination analysis of pairwise floristic similarity (Sørensen index) including plots from all four study transects. Plots are assigned to altitudinal vegetation zones (A, B, C) retrieved from cluster analysis (Figure 4). X-and y-axis correspond to the first two axes resulting from Principal Coordinates Analysis.

2.3 Results

2.3.1 Flora of the study transects

Examination of 153 herbarium specimens collected along the four study transects revealed 81 species and morphospecies in 62 genera and 26 families (Table S1). The most speciose families encountered are Asteraceae (11 spp.), Boraginaceae (8 spp.), Solanaceae (8 spp.) and Poaceae (7 spp.). Removing species recorded outside the plots reduces the species total to 68, including morphospecies.

2.3.2 Physiognomy and dominant species of the study transects

Quebrada Aroma: Species number as well as vegetation cover show a stronger decrease at lower altitudes compared to the other study transects (Figure 2). As indicated in Figure 2A vegetation cover in this transect is highest of the whole study area at each altitude. Across the whole range of the elevation gradient, hemicryptophytes and therophytes dominate the vegetation in both species number and abundance (Figure 3). Regarding vegetation cover woody species increase close to the desert center. (Figure 3A). *Jarava annua* (Mez) Peñailillo is consistently encountered across all elevations in high numbers (Figure 4). At 2,600 meters, besides *Jarava annua,* the most abundant species are *Chorizanthe commissuralis, Cistanthe thyrsoidea* (Reiche) Peralta & D.I. Ford, *Cryptantha filaginea* (Phil.) Reiche as well as *Atriplex glaucescens* (Figure 4). *Dalea moquehuana* J.F. Macbr. and *Lepidium rahmeri* dominate at 2,500 meters (Figure 4). At 2,400 meters, vegetation is dominated by *Jarava annua* and *Atriplex glaucescens*.

Altos de Pica: A sharp decline in vegetation cover by approximately two-thirds (Figure 2A) appears from 3,000 to 2,900 meters. Species number is reduced more gradually toward the lowest point close to the absolute desert (Figure 2B). At 3,200 meters, phanerophytes dominate in vegetation cover but become considerably less prominent at lower altitudes (Figure 3A). Additionally, an overall shift from predominantly woody to predominantly herbaceous species from higher to lower elevations is shown (Figure 3). At 3,200 meters the woody species *Baccharis boliviensis* (Wedd.) Cabrera, *Atriplex glaucescens* and *Fabiana ramulosa* are the most abundant species (Figure 4) marking the transition to the high Andean flora. At 3,000 meters the herbaceous species *Chorizanthe commissuralis* and *Pectocarya anomala* are common. At 2,900 and 2,800 meters, the most abundant species are *Pectocarya anomala*, *Atriplex glaucescens*, *Trichocline caulescens* Phil., *Mastigostyla cyrtophylla* and *Chorizanthe commissuralis*. Below 2,800 meters, dominant species cannot be distinguished.

Quebrada Blanca: Between 3,000 and 2,900 meters vegetation cover decreases by about half (Figure 2A). Regarding species number there are marked changes between 3,200 and 3,000 meters as well as between 2,600 and 2,400 meters. In each case species number decreases about a third (Figure 2B). Near the transition towards the Andean 'tolares' vegetation (3,200 m), phanerophytes and chamaephytes dominate the vegetation (e.g., *Ambrosia artemisioides* Meyen & Walp. ex Meyen, *Ephedra multiflora* Phil. ex Stapf, *Atriplex glaucescens*, *Tarasa operculata* (Cav.) Krapov.; Figures 3 & 4). At 3,000 meters, therophytes become the dominant lifeform. The most common species are *Pectocarya anomala*, *Tarasa pediculata*, *Cryptantha peruviana* I. M. Johnst., *Tetragonia microcarpa* and *Tagetes multiflora*. Intermediate and low altitudinal zones are not characterized by dominant species. From 2,400 to 2,200 meters, *Tetragonia microcarpa* and *Mirabilis trollii* are the only recorded species found in several plots. Towards the absolute desert, the woody species *Atriplex imbricata* (Moq.) D. Dietr. was found again along with the therophytes, albeit with low abundance (Table S4).

Tambillo: From 2,900 to 2,700 meters vegetation changes significantly regarding plant cover and species number, which are both reduced by more than half (Figure 2A, B). Vegetation cover is in general relatively low in comparison to the other transects (Figure 2A). At 3,200 meters, hemicryptophytes and therophytes represent the prevailing lifeforms (Figure 3). Even though woody species tend to increase downhill with increasing aridity, hemicryptophytes and therophytes continue to dominate the vegetation from 3,000 to 2,600 meters. At 2,400 meters there are only chamaephytes recognized. Between 3,200 and 3,000 meters dominate *Tagetes multiflora*, *Pectocarya anomala* and *Mirabilis trollii* (Figure 4). *Nolana tarapacana* (Phil.) I.M. Johnst., *Tetragonia microcarpa*, *Neuontobotrys berningeri* O.E. Schulz and *Tagetes multiflora* dominate at lower elevations between 2,900 and 2,800 meters. *Tiquilia grandiflora* becomes prevailing at 2,700 meters. At 2,500 meters and 2,400 meters only single individuals of *Tiquilia grandiflora* and *Mirabilis trollii* were found.

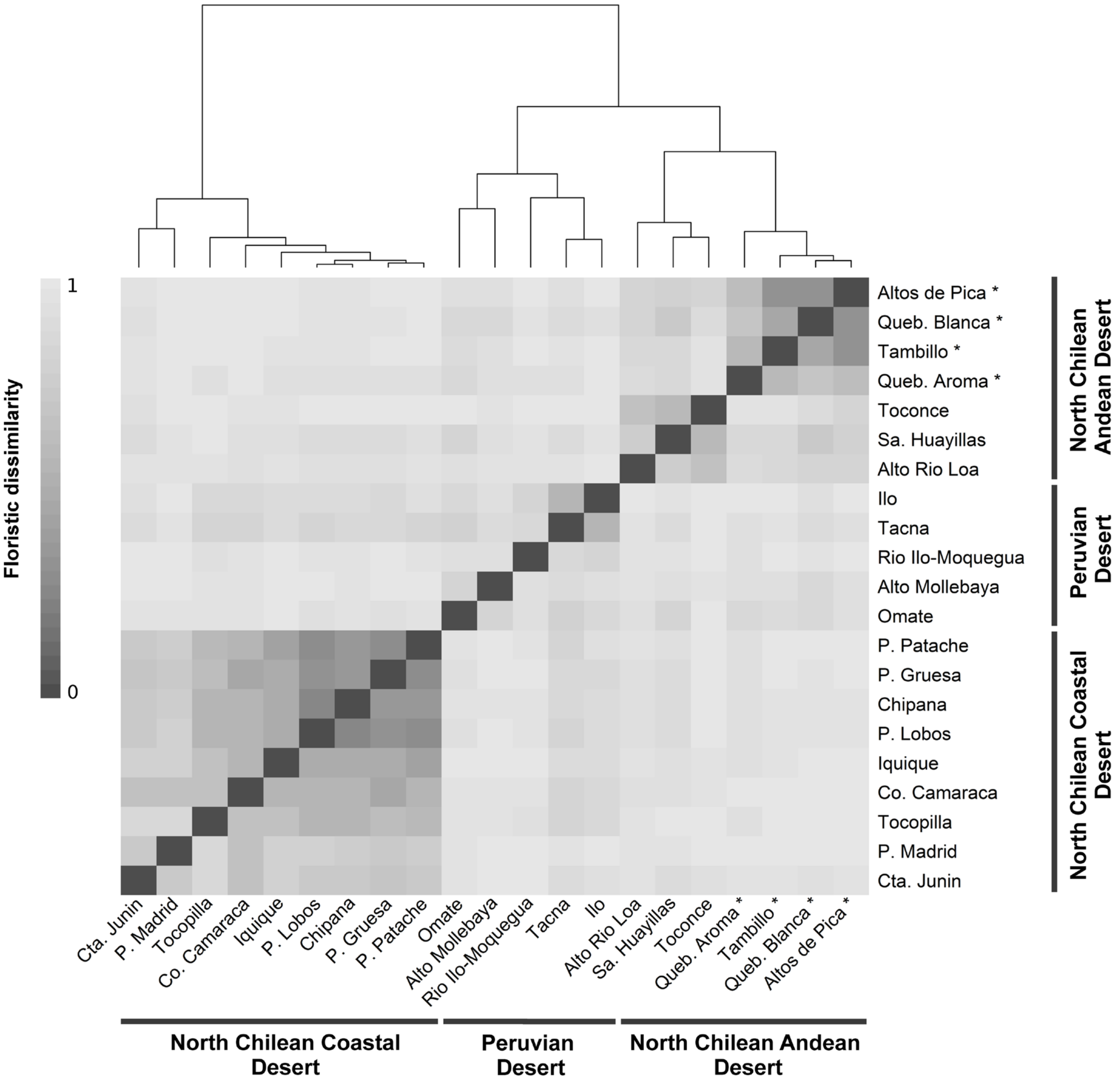

Figure 7: Heatmap of localities from the three floristic clusters derived from the dendrogram on the top (North Chilean Coastal Desert, North Chilean Andean Desert and Peruvian Desert). Classification was conditioned by Ward's minimum variance method. Similarity analysis was performed using Sørensen index. Localities of the study transects presented in this study are indicated by an asterisk (*).

2.3.3 Altitudinal vegetation zones

Classification of floristic similarity between the study plots reveals an elevation related clustering into three major altitudinal zones of desert vegetation along the Andes designated as Low (A), Intermediate (B) and High (C) (Figure 5; Table 2). Ordination analysis of the study plots assigned to the previously designated altitudinal zones, visualized along the first two axes (A1 and A2) of PCoA further on endorse the results of the classification analysis (Figure 6). Across all transects, vegetation zones exhibit constantly occurring species. Most constantly occurring species of the high vegetation zones (C) are *Lepidium rahmeri* Phil., *Tagetes multiflora* Kunth, *Mastigostyla cyrtophylla*, *Tetragonia microcarpa* and *Atriplex glaucescens* (Table 2; Table S4). In the intermediate vegetation zones (B), *Tetragonia microcarpa*, *Mirabilis trollii*,

Table 2: Altitudinal ranges (m) of vegetation zones for each study transect

	Low		Intermediate		High	
	min	max	min	max	min	max
Quebrada Aroma	-	-	-	-	2400	2600
Altos de Pica	-	-	2600	2900	3000	3200
Quebrada Blanca	2300	2400	2500	2600	2700	3220
Tambillo	2400	2500	2600	3000	-	3200

Mastigostyla cyrtophylla as well as *Munroa andina* are constantly encountered. In the Low vegetation zones (A), *Mirabilis trollii* is the only species found constantly (Table 2; Table S4). Elevational abundance profiles of dominant species for each transect are depicted in Figure 4.

2.3.4 Floristic relationships with other localities in Chile and Peru

Classification of floristic pairwise distances yields three distinct clusters: (1) North Chilean Coastal Desert (Cta. Junin, P. Madrid, Co. Camaraca, Iquique, P. Lobos, Chipana, P. Gruesa, P. Patache), (2) North Chilean Andean Desert (Alto Rio Loa, Sa. Huayillas, Toconce, Queb. Aroma, Queb. Blanca, Altos de Pica, Tambillo) and (3) Peruvian Desert (Omate, Alto Mollebaya, Rio Ilo-Moquegua, Tacna, Ilo) (Figures 1 & 7). The North Chilean Coastal Desert cluster is retrieved separately whereas the Peruvian Desert and North Chilean Andean Desert clusters are placed together. Two subgroups can be identified within the North Chilean Andean Desert cluster: One comprising the four localities from the Tarapacá region of this study, the other combining localities from regions further north (Arica y Parinacota) and south (Antofagasta), compiled from literature. Within the Peruvian Desert cluster, three localities (Rio Ilo-Moquegua, Tacna, Ilo) are separated from the Andean sites Omate and Alto Mollebaya. In the North Chilean Coastal Desert cluster, a core-group of very similar localities south of Iquique is retrieved together with increasingly dissimilar localities towards the north.

Coincident with the results of the classification, ordination places the three floristic clusters as clearly separated groups (Figure 8). Separation along the axis with the most explanatory power (A1: 27%) further underscores the predicted affinity between the Peruvian Desert cluster and North Chilean Andean Desert cluster as separated from the North Chilean Coastal Desert cluster. Pairwise floristic dissimilarities, compared to geographical distance between North Chilean Andean Desert and coastal localities, are nearly always higher than those within the individual clusters (Figure 9). There is a trend to increasing floristic dissimilarity within each floristic cluster with geographical distance along a north-south gradient. Floristic dissimilarity between the North Chilean Andean Desert and Peruvian Desert is high (> 0.8), regardless of geographical distance. The same is true for the comparisons between the North Chilean Coastal Desert and the Peruvian Desert. From a total of 281 species recorded in the North Chilean Coastal Desert and North Chilean Andean Desert cluster, only 14 species are common to both regions (Table S5), amounting to approximately 5 %. The Peruvian Desert and North Chilean Andean Desert cluster share about 7 % of the species (39 of 544; Table S6).

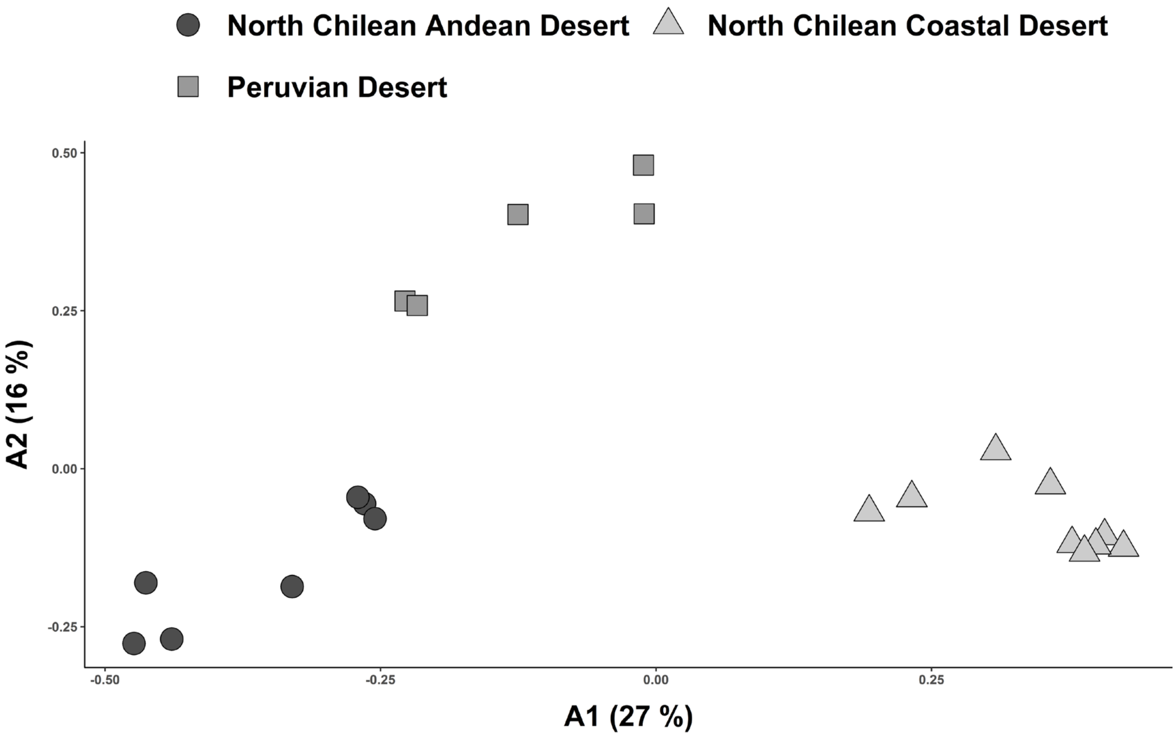

Figure 8: Ordination of values for pairwise floristic similarity (Sørensen index) including Chilean and Peruvian localities from the coast and Andes. Plots are assigned to altitudinal vegetation zones retrieved from cluster analysis (Figure 7). X-and y-axis correspond to the first two axes resulting from Principal Coordinates Analysis. The three floristic clusters are placed as clearly separated groups.

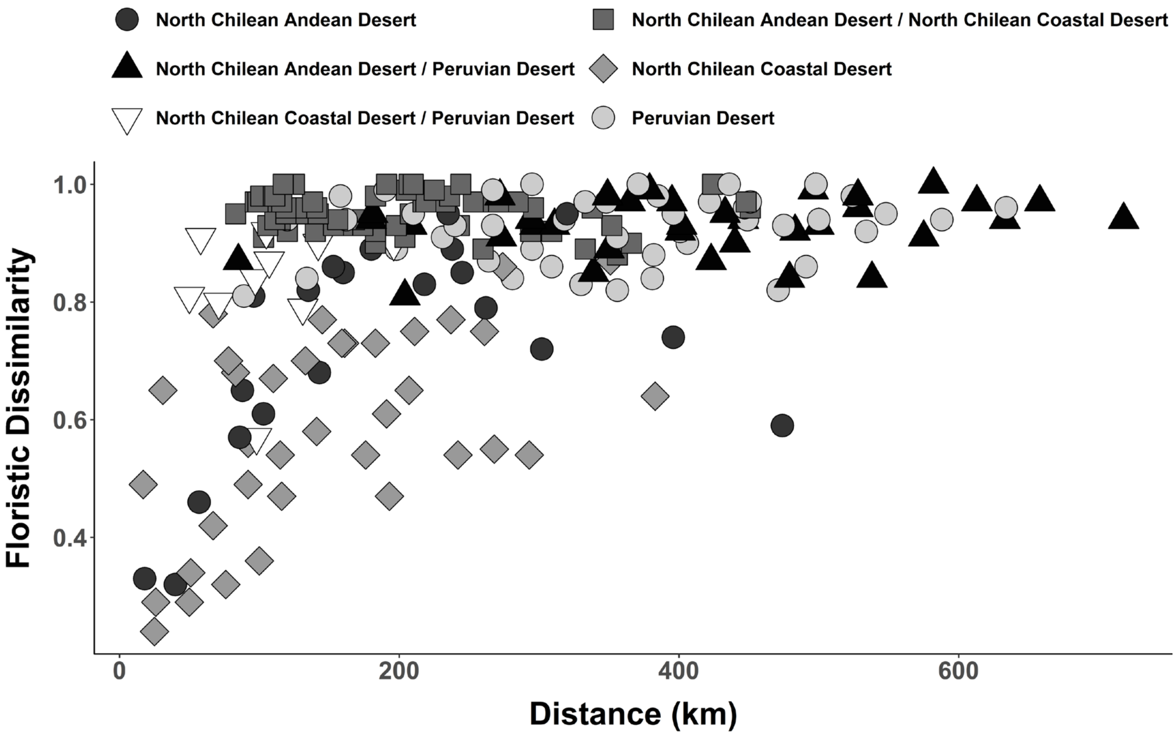

Figure 9: Floristic dissimilarity in relation to geographical distance shown for localities within each floristic cluster in comparison to localities from different floristic clusters.

2.4 Discussion

2.4.1 Altitudinal vegetation zonation of desert flora along study transects

This is the first study proposing an altitudinal vegetation zonation for the Andean Atacama Desert. Hierarchical agglomerative clustering as well as ordination (PCoA) show concordant results, underscoring that zonation is robust to change of method.

Our results are based on data gathered in one year of field work. Díaz *et al.* (2019) point out that it takes at least six years of observations to record over 90 % of all species present in a given area in the Atacama Desert and that precipitation is the main driver for interannual changes in productivity and plant species richness. We conducted floristic sampling along the study transects in a rather rainy year (Table 1), probably leading to enhanced plant proliferation. Hence, the vegetation and floristic data of our study also require completion with data collected across a longer period of time. However, floristic data for the Andean desert in northern Chile is still rather sparse and discontinuous and literature often only provides outdated floristic inventories with imprecise geographical context or sampling methods (e.g., Saiz *et al.* 2000). This hampers robust analyses of latitudinal changes in floristic composition along the Andean desert. In order to explore latitudinal changes in the zonation of the Andean desert vegetation in northern Chile, complementary and consistent floristic inventories of this region are required.

Villagrán *et al.* (1981) reported a reduction in the amplitude of desert or ‘pre-Puna’ vegetation from north to south along the Chilean Andes. This correlates with a gradual decrease of summer precipitation (Houston 2006). As a result, the absolute desert reaches higher elevations and approaches the Andean ‘tolares’ more closely at around 25° S (Arroyo *et al.* 1988; 1998; Luebert & Gajardo 2000). Arroyo *et al.* (1988) report that along the western slopes of the Andes, between 18° S and 19° S, woody species increase, whereas annuals decrease in number with increasing aridity towards the absolute desert. They suggest that drought-resistant woody perennials are better adapted to the extreme desert environment than annuals. Based on our data, this pattern can only be confirmed in one transect (‘Tambillo’). Although slightly less frequent, therophytes and/or hemicryptophytes are still highly represented even in plots at lower elevations (Figure 3). The conclusion of Arroyo *et al.* (1988) thus does no hold true across the entire region. This may be due to the fact that our sampling was conducted during a rainy year, thus capturing the emergence of these lifeforms. Unfortunately, Arroyo *et al.* (1988) provide no information on climatic conditions during their plant sampling. Díaz *et al.* (2019) suggest that seed banks of annual species can persist over decades in the Atacama Desert. Another explanation for these divergent results might be that the conclusions of Arroyo *et al.* (1988) are based on transects spanning a larger altitudinal range.

2.4.2 Floristic relationships and the barrier function of the absolute desert

Similarity analysis provides strong evidence for floristic isolation of the North Chilean Andean Desert and North Chilean Coastal Desert clusters (Figure 7). Comparison of floristic dissimilarity values to geographical distances supports these findings (Figure 9). High dissimilarity values between Andean and coastal localities in northern Chile, separated only by short distances (<200 km), contrast with lower dissimilarity values between equally distant localities within each cluster (Figures 1 & 9). Several authors (Villagrán *et al.* 1983; Rundel *et al.* 1991; Luebert 2010) have pointed out that floristic isolation appears to be the result of a permanent phytogeographical barrier. The hyperaridity of the Atacama Desert between 18° and 25° S hinders plant exchanges not only from north to south but especially from east to west. The divergent floristic composition may, however, also be due to the Andean desert and coastal desert in northern Chile constituting climatically divergent habitats: Coastal plants, for instance, depend on winter rainfall and moisture in form of fog or drizzle, whereas in the Andean desert the development of vegetation is mainly shaped by summer rainfall (Luebert & Pliscoff 2017). Other factors, such as differences in temperature (Luebert & Pliscoff 2017) or solar radiation (Molina *et al.* 2017) likely also play a role. The two explanations – isolation and ecological divergence – are likely not mutually exclusive and probably represent complementary causes for the floristic divergence of the coastal and Andean deserts in northern Chile. Dispersal across the desert core could take place as downslope dispersal of propagules from the Andes to the coast by landslides, alluvia or even streamflow (Nester *et al.* 2007) or from the coast toward the Andes by onshore winds (Ruttlant *et al.* 1998; Kalthoff *et al.* 2002) or via dispersal in either direction by animals crossing the desert (Beck & Vander Wall 2010; Fenner 2014). Conversely, the scattered, halophilic, extrazonal groundwater oases, e.g., in the Pampa de Tamarugal and on the Rio Loa, cannot have served as corridors or stepping stones since the respective species are not present in either the coastal or the Andean desert vegetation (Gutiérrez *et al.* 1998; Luebert & Pliscoff 2017). Only 14 species out of a total of 281 species (5 %) are found in both the coastal and Andean localities in northern Chile. This agrees with an earlier estimation (Rundel *et al.* 1991). Occurrence of species in both coastal and Andean deserts, despite the suggested phytogeographic east-west barrier, could be explained by migration routes through more humid zones connecting coast and Andes located north or south of the absolute desert. Indeed, inspection of geographical distributions of species shared between Andean and coastal localities in northern Chile reveals ranges beyond the latitudinal extension of the absolute desert for eleven species (Table S5). Based on current distribution data, only three of the 14 species present in coastal and Andean deserts, may have crossed the absolute desert: *Jarava annua*, *Solanum chilense* (Dunal) Reiche and *Polyachyrus sphaerocephalus* D. Don.

Climatic conditions in the Atacama Desert have oscillated at least since the last glacial maximum, with humid periods alternating with dryer periods (Betancourt *et al.* 2000) that have influenced the geographic extent of the absolute desert thus potentially contributing to the current distribution of plant species across the Atacama Desert. A detailed analysis of these aspects lies beyond the scope of this paper but is an interesting prospect for future studies.

Floristic affinities between the North Chilean Andean Desert and the Peruvian Desert cluster, separated from the North Chilean Coastal Desert (Figures 7 & 8), reinforce the idea of a floristic break along the coast between northern Chile and southern Peru (Rundel *et al.* 1991; Pinto & Luebert 2009). Our results also lend support to the idea of a north-south corridor for plant exchange along the Andes, bypassing the extreme environment of the absolute desert on both sides (Moreno *et al.* 1994). Luebert (2011) suggested, on the basis of climate studies (Houston & Hartley 2003; Garreaud *et al.* 2009), that generally wetter conditions along the western Andean slopes are likely to maintain such a corridor. About 7 % of the flora, accounting for 39 out of 544 species, are shared between localities from the Peruvian Desert and North Chilean Andean Desert. Most of these species exhibit broad distribution ranges, exceeding the Peruvian and North Chilean deserts subject of this study, suggesting that a biotic corridor along the Andean desert is not the only possibility to explain this pattern. Some species, however, are ranged to the Peruvian and northern Chilean deserts (e.g., *Ambrosia artemisioides, Exodeconus pusillus* (Bitter) Axelius) (Table S6), underscoring floristic connectivity between the Andean deserts of Chile and Peru (Moreno *et al.* 1994; Schwarzer *et al.* 2010). Unfortunately, only few phylogenetic and population genetic studies investigating this idea are available for Chilean-Peruvian desert plant groups (Gengler-Nowak 2002; Dillon *et al.* 2007; Luebert *et al.* 2009; Beddows *et al.* 2017; Böhnert *et al.* 2019).

The analyzed data were compiled from over a dozen different floristic inventories carried out between 1928 and 2017. Thus, a degree of inconsistency within the dataset, due to potential interannual variations in plant composition (Díaz *et al.* 2019), needs to be acknowledged. A possible further source for incongruence of floristic inventories is indicated by Jansen & Dengler (2010) who point towards the inconsistent use of plant names based on changing taxonomic concepts and the individual taxonomic understanding of the researcher. Moreover, limited vegetation data along the northern Chilean and Peruvian Andes is currently available, highlighting the need of further field floristic and vegetation studies. Only two Andean localities from southern Peru could be considered in the analyses. Still, our findings based on floristic data clearly indicate a phytogeographic connection between southern Peruvian and northern Chilean Andes for the first time.

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2.6 References

Arakaki M & Cano A (2003) Composición florística de la cuenca del río Ilo-Moquegua y Lomas de Ilo, Moquegua, Perú. *Revista Peruana de Biología* 10: 5–19.

Arroyo M, Castor C, Marticorena C, Muñoz M, Cavieres L, Matthei O, Squeo FA, Grosjean M & Rodriguez R (1998) The flora of Llullaillaco national park located in the transitional winter-summer rainfall area of the northern Chilean Andes. *Gayana Botánica* 55 (2): 93–110.

Arroyo M, Squeo FA, Armesto JJ & Villagrán C (1988) Effects of aridity on plant diversity in the northern Chilean Andes: Results of a natural experiment. *Annals of the Missouri Botanical Garden* 75 (1): 55–78.

Beck MJ & Vander Wall SB (2010) Seed dispersal by scatter-hoarding rodents in arid environments. *Journal of Ecology* 98 (6): 1300–1309. DOI: https://doi.org/10.1111/j.1365-2745.2010.01716.x.

Beddows I, Reddy A, Kloesges T & Rose LE (2017) Population genomics in wild tomatoes-the interplay of divergence and admixture. *Genome Biology and Evolution* 9 (11): 3023–3038. DOI: https://doi.org/10.1093/gbe/evx224.

Betancourt JL, Latorre C, Rech JA, Quade J & Rylander KA (2000) A 22,000-Year Record of Monsoonal Precipitation from Northern Chile's Atacama Desert. *Science* 289 (5484): 1542–1546. DOI: https://doi.org/10.1126/science.289.5484.1542.

Böhnert T, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider JV, Quandt D & Weigend M (2019) Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert. *Global and Planetary Change* 181: 102992. DOI: https://doi.org/10.1016/j.gloplacha.2019.102992.

Brako L & Zarucchi JL (1993) Catalogue of the flowering plants and gymnosperms of Peru. St. Louis, Mo: *Missouri Botanical Garden*. 1286 pp.

Braun-Blanquet J (1932) Plant sociology: The study of plant communities. New York: *McGraw-Hill.*

Braun-Blanquet J (1964) Pflanzensoziologie: Grundzüge der Vegetationskunde. Vienna: *Springer.*

Cereceda P, Larrain H, Osses P, Farías M & Egaña I (2008) The climate of the coast and fog zone in the Tarapacá Region, Atacama Desert, Chile. *Atmospheric Research* 87 (3-4): 301–311. DOI: https://doi.org/10.1016/j.atmosres.2007.11.011.

Chessel D, Drufour A & Thioulouse J. (2004) The ade4 Package – I: One-Table Methods. *R News* 7 (2): 47–52. https://cran.r-project.org/doc/Rnews/.

Díaz FP, Latorre C, Carrasco-Puga G, Wood JR, Wilmshurst JM, Soto DC, Cole TL & Gutiérrez RA (2019) Multiscale climate change impacts on plant diversity in the Atacama Desert. *Global Change Biology* 25 (5): 1733–1745. DOI: https://doi.org/10.1111/gcb.14583.

Dillon MO (2005) The Solanaceae of the lomas formations of coastal Peru and Chile. *Monographs in Systematic Botany from the Missouri Botanical Garden* 104: 131–155.

Dillon MO, Tu T, Soejima A, Yi T, Nie Z, Tye A & Wen J (2007) Phylogeny of *Nolana* (Nolaneae, Solanoideae, Solanaceae) as inferred from granule-bound starch synthase I (GBSSI) sequences. *Taxon* 56 (4): 1000–1011. DOI: https://doi.org/10.2307/25065900.

Ellenberg H & Mueller-Dombois D (1967) A key to Raunkiær plant life-forms with revised subdivisions. *Berichte des Geobotanischen Institutes ETH, Stiftung Rübel, Zürich* 37: 56–73.

Fenner M (2014) Seeds: The ecology of regeneration in plant communities. New York: *CABI Pub*. 307 pp.

Galán De Mera, A. J., Orellana V & J García JL (1997) Phytogeographical sectoring of the Peruvian coast. *Global Ecology and Biogeography Letters* 6 (5): 349-367. DOI: https://doi.org/10.2307/2997336.

Garreaud RD, Molina A & Farias M (2010) Andean uplift, ocean cooling and Atacama hyperaridity: A climate modeling perspective. *Earth and Planetary Science Letters* 292 (1-2): 39–50. DOI: https://doi.org/10.1016/j.epsl.2010.01.017.

Garreaud RD, Vuille M, Compagnucci R & Marengo J (2009) Present-day South American climate. *Palaeogeography, Palaeoclimatology, Palaeoecology* 281 (3-4): 180–195. DOI: https://doi.org/10.1016/j.palaeo.2007.10.032.

Gengler-Nowak K (2002) Reconstruction of the biogeographical history of Malesherbiaceae. *The Botanical Review* 68 (1): 171–188. DOI: https://doi.org/10.1663/0006-8101(2002)068[0171:ROTBHO]2.0.CO;2.

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53 (3-4): 325–338. DOI: https://doi.org/10.1093/biomet/53.3-4.325.

Gutiérrez JR, López-Cortes F & Marquet PA (1998) Vegetation in an altitudinal gradient along the Río Loa in the Atacama Desert of northern Chile. *Journal of Arid Environments* 40 (4): 383–399. DOI: https://doi.org/10.1006/jare.1998.0462.

Henning C (2019) fpc: Flexible procedures for clustering. https://CRAN.R-project.org/package=fpc.

Hijmans RJ (2017) geosphere: Spherical trigonometry. R package version 1.5-7. https://CRAN.R-project.org/package=geosphere.

Houston J (2006) Variability of precipitation in the Atacama Desert: its causes and hydrological impact. *International Journal of Climatology* 26 (15): 2181–2198. DOI: https://doi.org/10.1002/joc.1359.

Houston J & Hartley AJ (2003) The central Andean west-slope rainshadow and its potential contribution to the origin of hyper-aridity in the Atacama Desert. *International Journal of Climatology* 23 (12): 1453–1464. DOI: https://doi.org/10.1002/joc.938.

Jansen F & Dengler J (2010) Plant names in vegetation databases - a neglected source of bias. *Journal of Vegetation Science* 21 (6): 1179–1186. DOI: https://doi.org/10.1111/j.1654-1103.2010.01209.x.

Kalthoff N, Bischoff-Gauß I, Fiebig-Wittmaack M, Fiedler F, Thürauf J, Novoa E, Pizarro C, Castillo R, Gallardo L, Rondanelli R & Kohler M (2002) Mesoscale wind regimes in Chile at 30° S. *Journal of Applied Meteorology* 41 (9): 953–970. DOI: https://doi.org/10.1175/1520-0450(2002)041<0953:MWRICA>2.0.CO;2.

Kassambara A & Mundt F (2017) factoextra: Extract and visualize the results of multivariate data analyses. R package version 1.0.5. https://CRAN.R-project.org/package=factoextra.

Luebert F (2010). *Systematics, ecology and evolution of Heliotropium sect. Cochranea (Heliotropiaceae) and the biogeography of the Atacama Desert*. Dissertation, Freie Universität Berlin, Berlin.

Luebert F (2011) Hacia una fitogeografía histórica del Desierto de Atacama. *Revista de Geografía Norte Grande (*50): 105–133. DOI: https://doi.org/10.4067/S0718-34022011000300007.

Luebert F & Gajardo R (2000) Vegetación de los Andes áridos del norte de Chile. *Lazaroa* 21: 111–130.

Luebert F & Gajardo R (2005) Vegetación alto andina de Parinacota (norte de Chile) y una sinopsis de la vegetación de la Puna meridional. *Phytocoenologia* 35 (1): 79–128. DOI: https://doi.org/10.1127/0340-269X/2005/0035-0079.

Luebert F & Pliscoff P (2017) Sinopsis bioclimática y vegetacional de Chile. Santiago: *Editorial Universitaria*. 384 pp.

Luebert F & Wen J (2008) Phylogenetic analysis and evolutionary diversification of *Heliotropium* Sect. *Cochranea* (Heliotropiaceae) in the Atacama Desert. *Systematic Botany* 33 (2): 390–402. DOI: https://doi.org/10.1600/036364408784571635.

Luebert F, Wen J & Dillon MO (2009) Systematic placement and biogeographical relationships of the monotypic genera *Gypothamnium* and *Oxyphyllum* (Asteraceae: Mutisioideae) from the Atacama Desert. *Botanical Journal of the Linnean Society* 159 (1): 32–51. DOI: https://doi.org/10.1111/j.1095-8339.2008.00926.x.

Molina A, Falvey M & Rondanelli R (2017) A solar radiation database for Chile. *Scientific Reports* 7 (1): 14823. DOI: https://doi.org/10.1038/s41598-017-13761-x.

Moore MJ & Jansen RK (2006) Molecular evidence for the age, origin, and evolutionary history of the American desert plant genus *Tiquilia* (Boraginaceae). *Molecular Phylogenetics and Evolution* 39 (3): 668–687. DOI: https://doi.org/10.1016/j.ympev.2006.01.020.

Moreno P, Villagrán C, Marquet PA & Marshall LG (1994) Quarternary paleobiogeography of northern and central Chile. *Revista Chilena de Historia Natural* 67: 487–502.

Mueller-Dombois D & Ellenberg H (1974) Aims and methods of vegetation ecology. New York: *John Wiley and Sons*. 547 pp.

Nester PL, Gayó E, Latorre C, Jordan TE & Blanco N (2007) Perennial stream discharge in the hyperarid Atacama Desert of northern Chile during the latest Pleistocene. *Proceedings of the National Academy of Sciences of the United States of America* 104 (50): 19724–19729. DOI: https://doi.org/10.1073/pnas.0705373104.

Oksanen J, Blanchet FG, Kindt R, Legendre P, O'Hara RO, Simpson GL, Solymos MH, Stevens HH & Wagner H (2019) vegan: Community ecology package. R package version 2.5-4. https://CRAN.R-project.org/package=vegan.

Pinto R & Luebert F (2009) Datos sobre la flora vascular del desierto costero de Arica y Tarapacá, Chile, y sus relaciones fitogeográficas con el sur de Perú. *Gayana Botánica* 66 (1): 28–49. DOI: https://doi.org/10.4067/S0717-66432009000100004.

R Core Team (2017) R. A language and environment for statistical computing. Vienna, Austria: *R Foundation for Statistical Computing.*

Rousseeuw PJ (1987) Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. *Journal of Computational and Applied Mathematics* 20: 53–65. DOI: https://doi.org/10.1016/0377-0427(87)90125-7.

RStudio Team (2016) RStudio. Integrated development for R. Boston, MA: *RStudio, Inc.*

Ruhm J, Böhnert T, Weigend M, Stoll A, Merklinger FF, Quandt D & Luebert F (2019) Spatial floristic data from the Atacama and Peruvian Desert. *CRC1211 Database*. DOI: https://doi.org/10.5880/CRC1211DB.29.

Rundel PW, Dillon MO, Palma B, Mooney HA, Gulmon SL & Ehleringer JR (1991) The phytogeography and ecology of the coastal Atacama and Peruvian deserts. *A Journal of Systematic and Evolutionary Botany* 13 (1): 1–49. DOI: https://doi.org/10.5642/aliso.19911301.02.

Rundel PW & Mahu M (1976) Community structure and diversity in a coastal fog desert in northern Chile. *Flora* 165 (6): 493–505. DOI: https://doi.org/10.1016/S0367-2530(17)31888-1.

Ruttlant J, Fuenzalida H, Torres R & Figueroa D (1998) Interacción océano-atmósfera-tierra en la Región de Antofagasta (Chile, 23°S): Experimento DICLIMA. *Revista Chilena de Historia Natural* 71: 405–427.

Saiz F, Yates L, Nuñez C, Daza M, Varas ME & Vivar C (2000) Biodiversidad del complejo de artrópodos asociados al follaje de la vegetación del norte de Chile, II región. *Revista Chilena de Historia Natural* 73 (4): 671–692. DOI: https://doi.org/10.4067/S0716-078X2000000400011.

Schulz N (2009). *Loma-Formationen der Küsten-Atacama/Nordchile unter besonderer Berücksichtigung rezenter Vegetations- und Klimaveränderungen*. Dissertation, Naturwissenschaftlischen Fakultät der Universität Erlangen-Nürnberg, Erlangen.

Schwarzer C, Cáceres Huamaní F, Cano A, La Torre MI & Weigend M (2010) 400 years for long-distance dispersal and divergence in the northern Atacama Desert – Insights from the Huaynaputina pumice slopes of Moquegua, Peru. *Journal of Arid Environments* 74 (11): 1540–1551. DOI: https://doi.org/10.1016/j.jaridenv.2010.05.034.

Sørensen T (1948) A method of stabilizing groups of equivalent amplitude in plant sociology based on the similarity of species content and its application to analysis of the vegetation on Danish commons. *Biologiske Skrifter. K. Danske Videnskabernes Selskab* 5: 1–34.

Teillier S (2004) La vegetación de la cuenca media–alta del río Loa (3100-4150 msnm). Región de Antofagasta (II), Chile. http://www.chlorischile.cl/altoloa/altoloasinflora.htm.

Tüxen R & Ellenberg H (1937) Der systematische und ökologische Gruppenwert. Ein Beitrag zur Begriffsbildung und Methodik der Pflanzensoziologie. *Mitteilungen der Floristisch-soziologischen Arbeitsgemeinschaft* 3: 171–184.

Villagrán C, Armesto JJ & Arroyo M (1981) Vegetation in a high Andean transect between Turi and Cerro León in northern Chile. *Vegetatio* 48 (1): 3–16. DOI: https://doi.org/10.1007/BF00117356.

Villagrán C, Arroyo M & Armesto JJ (1982) La vegetacion de un transecto altitudinal en los Andes del norte de Chile (18–19°S). In: Veloso A & Bustos E, eds. *El ambiente natural y las poblaciones humanas de los Andes del norte de Chile (Arica, Lat. 18°28'S)*. Montevideo: Unesco, *pp* 13–70.

Villagrán C, Arroyo M & Marticorena C (1983) Effects of natural desertification on the distribution of the Andean flora in Chile. *Revista Chilena de Historia Natural* 56: 137–157. DOI: https://doi.org/http://bibliotecadigital.ciren.cl/.

Vuille M & Ammann C (1997) Regional snowfall patterns in the high, arid Andes. *Climatic Change* 36 (3/4): 413–423. DOI: https://doi.org/10.1023/A:1005330802974.

Ward JH (1963) Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association* 58 (301): 236–244. DOI: https://doi.org/10.2307/2282967.

Warnes GR, Bolker B, Bonebakker L, Gentleman R, Huber W, Liaw A, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M & Venables B (2019) gplots: Various R programming tools for plotting data. R package version 3.0.1.1. https://CRAN.R-project.org/package=gplots.

Zhou J & Lau KM (1998) Does a monsoon climate exist over South America? *Journal of Climate* 11 (5): 1020–1040. DOI: https://doi.org/10.1175/1520-0442(1998)011<1020:DAMCEO>2.0.CO;2.

Zuloaga FO, Morrone O & Belgrano MJB, eds. (2008) Catálogo de las plantas vasculares del Cono Sur. (Argentina, Sur de Brasil, Chile, Paraguay y Uruguay). St. Louis, Miss.: *Missouri Botanical Garden.*

2.7 Supporting information

Table S1: Altitudinal distribution range of species within the four study localities indicted for lowest (min) and highest (max) records along each gradient. For Quebrada Aroma elevations below 2,400 m refer to actual quebrada vegetation and data was not included in the analysis.

Table S2: A list of literature used for the identification of specimens recorded along the four study transects.

Table S3: Working table including presence/ absence data of 615 species for 21 localities in northern Chile and southern Peru.

Table S4: Working table including altitudinal vegetation and floristic data assessed along the four study transects. The data was used for the analysis of altitudinal zonation of desert vegetation in the Andean range. Values labelled with '-' indicate species recorded outside the actual study transects.

Table S5: Distribution ranges of species found in the North Chilean Andean Desert and North Chilean Coastal Desert cluster. From 281 species, 14 are found in both clusters accounting for 5 % of shared flora.

Table S6: Distribution ranges of Species found in the Peruvian Desert and in the North Chilean Andean Desert cluster. From 544 species, 39 are found in both clusters accounting for 7 % of shares flora.

Chapter 3

Historical assembly of Zygophyllaceae in the Atacama Desert

Pintoa chilensis Gay. This monotypic, prostrate subshrub is found in dry river beds in the southern Atacama Desert.

This chapter is included here as a manuscript but has since been published as:

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Own contribution: Conceptualization, data curation, field work, methodology, lab work, resources, validation, writing, review and editing. All authors contributed to revision.

Data availability: Supplementary materials are available online via the CRC database (DOI: https://doi.org/10.5880/CRC1211DB.32) and in **Appendix A**.

Abstract

The Atacama Desert harbors a unique arid-adapted flora with a high degree of endemism. However, the origin of the Atacama Desert flora is poorly understood. Zygophyllaceae is represented by five endemic species, one member of Zygophylloideae (*Fagonia chilensis*) and four members of Larreoideae. *Bulnesia chilensis* and *Porlieria chilensis* are the only representatives in the Atacama Desert of genera with disjunct distributions between Argentina, Peru and Chile, while *Metharme lanata* and *Pintoa chilensis* are monotypic endemic genera. Zygophyllaceae are thus a particularly suitable group for studying the historical assembly of the Atacama Desert flora as each these species may represent independent biogeographical events. We made use of published as well as original plastid DNA sequences (*rbc*L, *trn*L-*trn*F & *trn*S-*trn*G) to reevaluate the phylogenetic relationships of the Atacama Zygophyllaceae. Bayesian divergence time estimates as implemented in BEAST2 and ancestral area reconstruction with the Dispersal Extinction Cladogenesis approach using BioGeoBEARS were applied to infer ancestral ranges. We compiled the most complete data set of Larreoideae to date with 25 of 28 species. *Bulnesia rivas-martinezii* from Bolivia forms a clade with *Pintoa chilensis* from the Atacama Desert rendering the genus *Bulnesia* paraphyletic. Most representatives of Zygophyllaceae colonized the Atacama Desert during the Miocene, and only *Fagonia* L. dispersed more recently. The colonization history of the Atacama Desert in South America is reflected by three individual distribution patterns or floristic elements. The presence of *Bulnesia*, *Pintoa* and *Metharme* is best explained by Andean vicariance, while the southern Atacama Desert representative, *Porlieria chilensis*, has a continuous distribution into central Chile from where it probably dispersed further north. The only South American *Fagonia* species (*F. chilensis*) likely colonized the Chilean-Peruvian Coastal Desert via long distance dispersal from North America.

Keywords: Andes — arid environments — *Bulnesia* — Chile — historical biogeography — Larreoideae — *Metharme* — South America

3.1 Introduction

Located in northern Chile the Atacama Desert ranges from 18° S at the border region to Peru and 30° S around La Serena, while it is restricted in the east by the Andean mountain range and the Pacific Ocean to the west. Relative to its area the Atacama Desert is surprisingly species-rich harboring about 550 species with ca. 60 % are endemic to the Atacma Desert (Dillon & Hoffmann 1997). However, diversity is not equally distributed in the Atacama Desert. In its northern portion (approx. 18° to 26° S) most species can be found along the coastal range as well as along the Andean foothills, while only few species can survive the harsh conditions of the absolute desert at the inner core of the Atacama Desert. Further south the vegetation is more broadly distributed: whereas the conditions are still predominantly arid, absolute desert seperting the Andean and the coastal ranges (Villagrán *et al.* 1983; Rundel *et al.* 1991; Luebert & Pliscoff 2017).

The historical assembly of the Atacama Desert flora has been linked to the timing of the major factors controlling its aridity (Rundel *et al.* 1991). Recent evidence suggests that the age of aridity can be dated back to the Miocene or even Oligocene (Dunai *et al.* 2005). However, we still lack a detailed knowledge of the process, since an understanding of the timing of the onset of aridity remains elusive (Ritter *et al.* 2018), and only few studies have addressed the timing of diversifications of Atacama Desert plant groups (e.g., Luebert & Wen 2008; Dillon *et al.* 2009; Heibl & Renner 2012; Böhnert *et al.* 2019a).

Zygophyllaceae are key elements of world desert and semi-desert ecosystems, and their diversification is thought to be linked with increased aridity during the Oligocene-Miocene transition, especially in Zygophylloideae (Bellstedt *et al.* 2012; Wu *et al.* 2015; 2018). The family has a worldwide distribution, but is largely restricted to hot and dry regions. Five native genera of Zygophyllaceae are documented for the Atacama Desert, making it one of the Atacama groups with the highest phylogenetic diversity, although each genus is only represented by a single species. The genera *Bulnesia* Gay, *Metharme* Phil. ex Engler, *Pintoa* Gay and *Porlieria* Ruiz & Pav. belong to the New World endemic Larreoideae, only *Fagonia chilensis* Hook. & Arn. belongs to the sub-cosmopolitan Zygophylloideae. *Bulnesia* and *Porlieria* comprise four species each (Palacios & Hunziker 1984; Godoy-Bürki *et al.* 2018), while *Metharme* and *Pintoa* are monotypic (Beier *et al.* 2003).

Understanding the historical assembly of a flora requires evidence for the spatial and temporal origin of its component plant lineages. Based on a review of the distribution and phylogenetic relationships of 53 plant taxa in the Atacama Desert and their closely related species, Luebert (2011) recognized four distribution patterns or floristic elements: (1) tropical Andean, (2) central Chilean, (3) trans-Andean disjunct and (4) amphitropical disjunct. The two first elements represent species with closely related taxa distributed immediately north or south of the Atacama Desert, pointing to direct floristic exchanges between the Atacama Desert and its neighboring regions. Disjunct trans-Andean elements, with species distributed on both sides of the Andes, are likely the result of vicariance due to Andean uplift or of trans-Andean dispersal. American Amphitropical Disjunctions are explained as the result of long-distance dispersal (LDD) events between North and South America (Simpson *et al.* 2017).

All four floristic elements appear to be present among the five representatives of Zygophyllaceae in the Atacama Desert. *Bulnesia chilensis* Gay, *Pintoa chilensis* Gay and *Porlieria chilensis* I.M. Johnst. are found in the southern portion of the Atacama Desert, with *Porlieria chilensis* ranging into central Chile. *Metharme lanata* Phil. ex Engl. in turn is found at the dry limit in the northern part of the Atacama Desert. According to Lia *et al.* (2001) and Godoy-Bürki *et al.* (2018), *Bulnesia*, *Pintoa* and *Porlieria* have closely related species on the eastern side of the Andes in Argentina and Bolivia, but also in the tropical Andes and the Peruvian coastal desert respectively. For these genera, if a trans-Andean distribution due to vicariance driven by Andean uplift is to be supported, we expect that each Atacama Desert species is sister to a species distributed on the eastern side of the Andes and the split between those two sister species coincides with the timing of the Andean uplift. We also expect that floristic exchanges between the Atacama Desert, the tropical Andes and central Chile are more recent, as suggested in previous works for other taxa (Gengler–Nowak 2002; Schwarzer *et al.* 2010). South American *Fagonia* L. (*F. chilensis*) is widespread in the Chilean-Peruvian coastal Desert and has its sister species in SW USA and Mexico, where it is hypothesized to have originated (Porter 1974; Beier *et al.* 2004). A second American amphitropical disjunction, though not directly related to the Atacama Desert, is *Larrea* Cav., a single species of which is proposed to have colonized SW USA and Mexico from southern South America (Hunziker *et al.* 1972; Lia *et al.* 2001). Here we expect that *F. chilensis* is part of a clade of New World *Fagonia* and that the split from its closest relative can be dated to the Pliocene thus supporting the proposed hypothesis of Pliocene LDD colonization of the Atacama Desert.

This study takes previous phylogenetic studies on Zygophyllaceae as the starting point (Godoy-Bürki *et al.* 2018; Wu *et al.* 2018). We expand the sampling of South American taxa based on field studies and herbarium material to understand the historical assembly of Zygophyllaceae in the Atacama Desert. To this end, we (1) re-evaluate the phylogenetic relationships within Larreoideae with an expanded taxon sampling, (2) estimate divergence times of the Atacama taxa and (3) infer their ancestral geographical ranges.

3.2 Materials and Methods

3.2.1 Taxon sampling

We compiled a broad taxon sampling of Zygophyllaceae across all five subfamilies and 24 of the 25 accepted genera. Two representatives of the sister family Krameriaceae were used as the outgroup. Furthermore, we present a nearly comprehensive taxon sampling of Larreoideae, with only three accepted species missing, namely *Gonopterodendron carrapo* (Killip & Dugand) A.C. Godoy-Bürki a narrowly endemic and endangered species from Colombia, as well as *Porlieria arida* Rusby and *Izozogia nellii* G. Navarro (Navarro 1997) from Bolivia. For the genus *Bulnesia* we follow the new generic concept of Godoy-Bürki (2015) and Godoy-Bürki *et al.* (2018). The sampling of Larreoideae is based on the work of Godoy-Bürki *et al.* (2018), but complemented by original data from additional taxa from Chile, Peru and Bolivia. As a result, Larreoideae is represented by 25 out of 28 accepted species (89%), Zygophylloideae by 76 (42%) and Morkillioideae by three of the four accepted species. Subfamily Tribuloideae is represented by all six accepted genera, but only eight of the 62 species. A complete taxon list including GenBank accessions, vouchers and/or associated references are provided in the supplementary material (Appendix A; Table S1).

3.2.2 DNA extraction, amplification and sequencing

Genomic DNA was extracted from silica dried leaf tissue or herbarium specimens using the NucleoSpin Plant II kit (Machery-Nagel, Germany) following the manufacturer's protocol with an increased incubation time of 90 min. Sequences of three plastid DNA regions (*rbc*L, *trn*L-*trn*F, *trn*S-*trn*G) were amplified using the primer combinations and polymerase chain reaction (PCR) cycling conditions given in supplementary material Table S2 and Tables S3–S5, respectively. PCR products were purified through gel extraction using NucleoSpin Gel and PCR Clean-up kit (Machery-Nagel, Germany) following the manufacturer protocol. Sequencing was performed on a 3730XL DNA Analyzer (Applied Biosciences) by a sequencing service. Our sampling contains 132 samples, of which 47 are represented by one, 87 by two and 18 by three markers resulting in a total of 40% missing sequence information. The results of the Godoy-Bürki *et al.* (2018) show incongruence between plastid and nuclear datasets. Therefore, we decided not combine plastid and ITS sequence data in our analyses.

3.2.3 Alignment and phylogenetic analysis

Sequences were edited and manually aligned using PhyDe 0.9971 (Müller & Borsch 2005). One inversion site of two base pairs (bp) was detected in the *trn*S-*trn*G alignment and reverse-complemented and aligned for analysis following Quandt *et al.* (2003). In the *trn*L-*trn*F and trnS-*trn*G alignments, eight hotspot regions comprising a total of 111 bp were detected and excluded from the analysis. We defined hotspots as regions in the alignment in which poly-A/T stretches or overlapping microstructural mutations over many nucleotides made it difficult to assess homology accurately (Borsch *et al.* 2003; Löhne & Borsch 2005; Worberg *et al.* 2007). The position of these hotspots and the inversion in the alignments is reported in the supplementary material available from the CRC1211 public database (File S1 and S2; Böhnert *et al.* 2019b). Maximum Likelihood (ML) approach was employed to evaluate phylogenetic relationships prior to the dating and ancestral area reconstruction analysis of all Zygophyllaceae and Larreoideae. ML analysis was carried out with RAxML 8.2.10 (Stamatakis 2014) under the GTRCAT substitution model and 1,000 rapid bootstrap replicates, treating every gene region as a single partition. The final ML tree was plotted with the python package *toytree* (Eaton 2019).

3.2.4 Molecular clock dating

A Bayesian relaxed clock model as implemented in BEAST 2.5.1 (Bouckaert *et al.* 2014) was used to estimate divergence times within Zygophyllaceae. All species with more than one sample were found to be monophyletic, and we used a reduced dataset compared to the ML alignment (one sample per species except for *Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb., which is disjunct between Argentina and Peru) to set up an XML file in BEAUTI 2.5 (Bouckaert *et al.* 2014). The three partitions of the cpDNA regions were linked with respect to clock and tree models, but unlinked with respect to site model. We used bModelTest (Bouckaert & Drummond 2017) as implemented in BEAST2 instead of defining substitution

Table 1: Age constraints used for normal prior distribution in the BEAST analyses including mean and sigma values (Mean = node age, Min = minimum age, Max = maximum age).

Clade	Mean	Min	Max	Sigma	Source
Zygophyllaceae stem node	70	49	88	11	Bell *et al*. 2010; Wu *et al*. 2015
Zygophyllaceae crown node	60.9	34	90	16.3	Magallón *et al*. 2015
Larreoideae stem node	54.3	41.1	66.9	7.6	Wu *et al*. 2015
Zygophylloideae crown node	39.8	29.5	51.8	6.4	Wu *et al*. 2015

models for each partition. A relaxed lognormal clock with an estimated clock rate and a birth-death model as tree prior were specified (Drummond *et al.* 2006; Gernhard 2008). The fossil record within Zygophyllaceae is very sparse (reviewed by Bellstedt *et al.* 2012) and the few documented fossils cannot be confidently assigned to any member of extant genera. We therefore used a secondary calibration approach. In order to test the robustness of our approach we analyzed the data twice with two different calibration schemes. First, four clades were specified as monophyletic but only the stem node of Zygophyllaceae was used as secondary calibration point (calibration scheme 1) with normal prior distributions. Second, we applied age constraints on four nodes (calibration scheme 2) with normal prior distributions (Bell *et al.* 2010; Wu *et al.* 2015) (see Table 1, supplementary Figure S2 and Figure S3). The Markov Chain Monte Carlo (MCMC) was run for 100 million generations, sampling every 10,000 generations. The log file was checked using Tracer1.71 (Rambaut *et al.* 2018) and Maximum Clade Credibility Tree (MCCT) produced using TreeAnnotator, summarizing mean heights, excluding a burn-in of 10 % and a posterior probability limit of 0.95 was specified in order to obtain confidence intervals only for nodes with sufficient support. Finally, the R-packages ape 5.0 (Paradis & Schliep 2019), phylooch 1.5-5 (Heibl 2008 onwards), strap 1.4 (Bell & Lloyd 2015) and geoscale 2.0 (Bell 2015) were used in R 3.5.1 (R Core Team 2018) and RStudio 1.1.463 (RStudio Team 2016) to plot and annotate the dated tree. Phylogenetic and dating analysis were conducted on the CIPRES Gateway (Miller *et al.* 2010).

3.2.5 Ancestral area reconstruction

We employed the Dispersal Extinction Cladogenesis (DEC) approach described by Ree & Smith (2008) as implemented in the R package BioGeoBEARS 1.1.1 (Matzke 2013) to evaluate the colonization history and timing of the Atacama Desert by Zygophyllaceae in general and Larreoideae in particular. We conducted a two-step DEC analysis, one for the whole of Zygophyllaceae on a global scale and one for Larreoideae in the Americas. Due to conceptual and statistical problems (Ree & Sanmartín 2018) we did not use the DEC+j model. We used the time calibrated BEAST2 tree and removed the two outgroup taxa, ending up with 114 terminals from 113 species. We ran both analyses with two sets of assumptions (maximum number of areas sets to two and three) and without dispersal constraints over time, because they likely have little effects on the results (Chacón & Renner 2014). For the former, we categorized every sample in the phylogeny in five geographic groups corresponding to: (A) North and Central America, (B) South America, (C) Africa, the Mediterranean and the Arabian Peninsula, (D) Asia and (E) Australia. A subdivision of the Americas into two sub-regions was necessary as the New World *Fagonia* have an amphitropical disjunction. For the latter approach we extracted the Larreoideae from the same MCCT ending up with 26 terminals from 25 species. Two samples of *B. retama* (from Argentina and Peru, respectively) were included to assess the directionality of this trans-Andean disjunction. We categorized the Americas into five geographical units based on the distributional patterns of Larreoideae (Figure 2): North America (A) and Central America and the Caribbean (B) were separated into two distinct units taking into account the amphitropical disjunction of *Larrea*. We also included the northern part of South America (parts of Colombia and Venezuela) into B due to the occurrence of *Guaiacum* L. along the coast of northern South American and the disjunct distribution of *Gonopterodendron* (Griseb.) Godoy-Bürki between northern and southeastern South America (C). Further, we separated the Peruvian coastal desert (D) and the Atacama Desert (E) from the remaining parts of the continent in order to understand possible floristic exchanges between them and southeastern South America (C). The designation of areas for each taxon was based on geographical information derived from Zuloaga *et al.* (2008), Godoy-Bürki *et al.* (2018) and GBIF (http://www.gbif.org/). The geographical distribution of each taxon for the two analyses is documented in the supplementary material Tables S6 and S7 and in Godoy-Bürki *et al.* (2018). The R script used to analyze the ancestral area and to plot the results on the dated phylogeny is publicly available from the CRC1211 database (Böhnert *et al.* 2019b) and the GitHub repository (https://github.com/TimBoeh/HistBiogeoZygo).

3.3 Results

3.3.1 Phylogeny and divergence times

The final alignment including the three plastid DNA regions encompassed 132 samples corresponding to 115 taxa and had a length of 4,631 bp. The backbone topology in Zygophyllaceae was largely congruent between ML and the two BEAST2 analyses, receiving moderate to high bootstrap support (supplementary material Figure S1) and posterior probabilities (Figure 1 and supplementary material Figures S2 & S3). Both calibration schemes resulted in similar trees and divergence times estimations documented in supplementary material Table S8. Therefore, only the tree with four calibrated nodes is further described and discussed. New World *Fagonia* incl. *F. chilensis* forms a well-supported clade in both analyses with a crown node age of 4.71 Ma (95% HPD: 1.4–8.6). The stem node age of Larreoideae and Zygophylloideae was estimated at 52.5 Ma (95% HPD: 42.1–62.1), i.e. early Eocene. The crown node age of Larreoideae was dated to the late Oligocene at 28.6 Ma (95% HPD: 18.8–39.1). Within Larreoideae, the "*Guaiacum*-clade" (Figure 2) encompasses the genera *Porlieria, Gonopterodendron, Plectrocarpa* and *Guaiacum* with a crown node age of 23.6 Ma (95% HPD: 14.6–33.1).

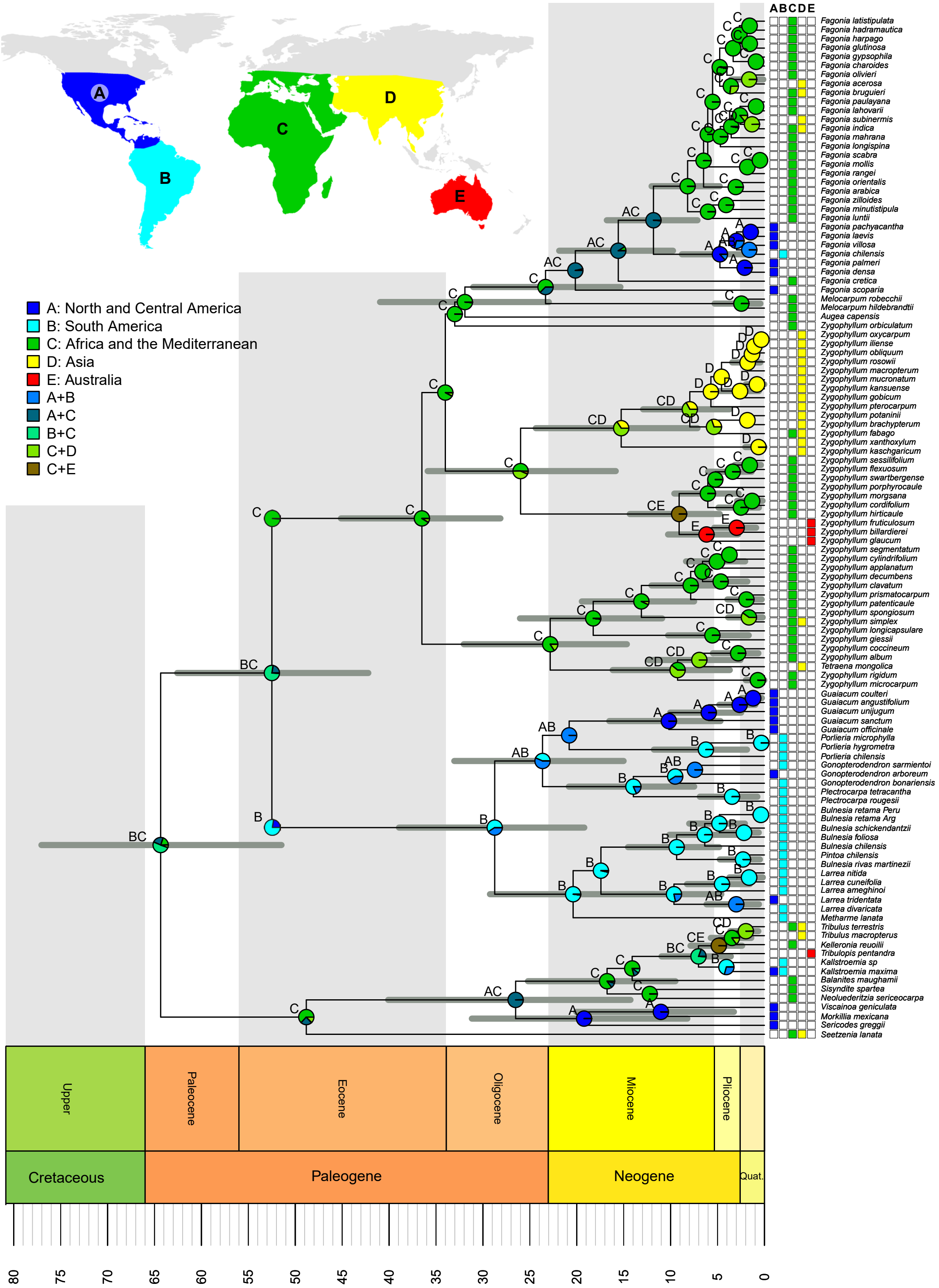

Figure 1: Global ancestral area reconstruction of Zygophyllaceae plotted on BEAST MCCT with outgroups removed and maximum area set to 2 (calibrations scheme 2). Only for nodes with posterior probability support ≥ 0.95 confidence intervals are shown. Squares between tips and tip labels indicate distributions assigned to each single species (A-E) with color codes corresponding to the areas indicated on the map and the legend. Pie charts depict relative probabilities of areas as estimated from the Dispersal Extinction Cladogenesis (DEC) analysis with BioGeoBEARS. Letters next to the pie charts indicate the areas with highest relative probabilities. Pie charts at corner nodes are only given when relevant for interpretation of biogeographic history.

Porlieria chilensis, the only species of this clade in the Atacama Desert, separated from its sister clade (*P. hygrometra* Peru + *P. microphylla* Argentina) in the late Miocene to early Pliocene (6.2 Ma, 95% HPD: 1.7–11.8). The "*Larrea*-clade" with a crown node age of 20.1 Ma (95% HPD: 11.9–29.2) comprises the genera *Bulnesia, Pintoa, Metharme* and *Larrea*. *Bulnesia chilensis* from the Atacama Desert diverged from its sister group (*B. retama* + *B. schickendantzii* + *B. foliosa*) from Argentina and Bolivia around the Miocene-Pliocene boundary (6.3 Ma, 95% HPD: 3–10.2). The monotypic genus *Pintoa* (*P. chilensis*) and *B. rivas-martinezii* from Bolivia form a clade with a crown node age of 2.2 Ma (95% HPD: 0.49–4.52). This clade is placed as sister to the genus *Bulnesia* with a divergence time estimated at 9.3 Ma (95% HPD: 4.8–14.6). The position of the genus *Metharme* within the "*Larrea*-clade" remains unresolved: in both analyses *Metharme* is sister to the remaining members of this clade, but with low support.

3.3.2 Ancestral area reconstruction

The two ancestral area reconstructions of the whole Zygophyllaceae using two different maximum areas settings are largely congruent, indicating a shared ancestry for the family in South America and Africa with a first split of the Tribuloideae, Morkillioideae and Seetzenioideae from Larreoideae and Zygophylloideae during the early Paleocene (Figure 1 & Figure S4). Larreoideae and Zygophylloideae separated from each other during the early Eocene from an ancestor distributed in South America and Africa. Ancestors of New World *Fagonia* colonized North America from the African-Mediterranean region during the early Miocene and diverged during the middle Miocene. The ancestor of *F. chilensis* subsequently dispersed from North America into South America during the late Pliocene and early Pleistocene. Both ancestral area reconstructions (Figure 2 & Figure S5) indicate that early Larreoideae were likely distributed in South America with at least three subsequent colonization events into northern South America as well as Central and North America. The analysis of Larreoideae suggests an ancestral area in southeastern South America (SE-SA) + the Atacama Desert during the middle Oligocene. The *Guaiacum* clade has a southeastern South American ancestor with multiple colonizations of Central and North America by *Guaiacum* throughout the Miocene and the arrival of *Gonopterodendron arboreum* in northern South America during the late Miocene. *Porlieria chilensis* and its sister group diverged in the late Miocene from a common ancestor distributed in eastern South America and the Atacama Desert, while the separation of *P. hygrometra* and *P. microphylla* took place < 0.5 Mya. In contrast, the second approach allowing a maximum number of areas of three points towards a more widespread ancestral range covering eastern South America, the Atacama Desert and Peru (Figure S5). The *Larrea* clade originated from a common ancestor in eastern South America and the Atacama Desert, with three independent vicariance events between these areas, one in *Metharme* (early Miocene), one in *Bulnesia* (late Miocene) and one in *Pintoa* (late Pliocene). A recent trans-Andean dispersal into the Peruvian Coastal Desert occurred in *B. retama* coming from east of the Andes.

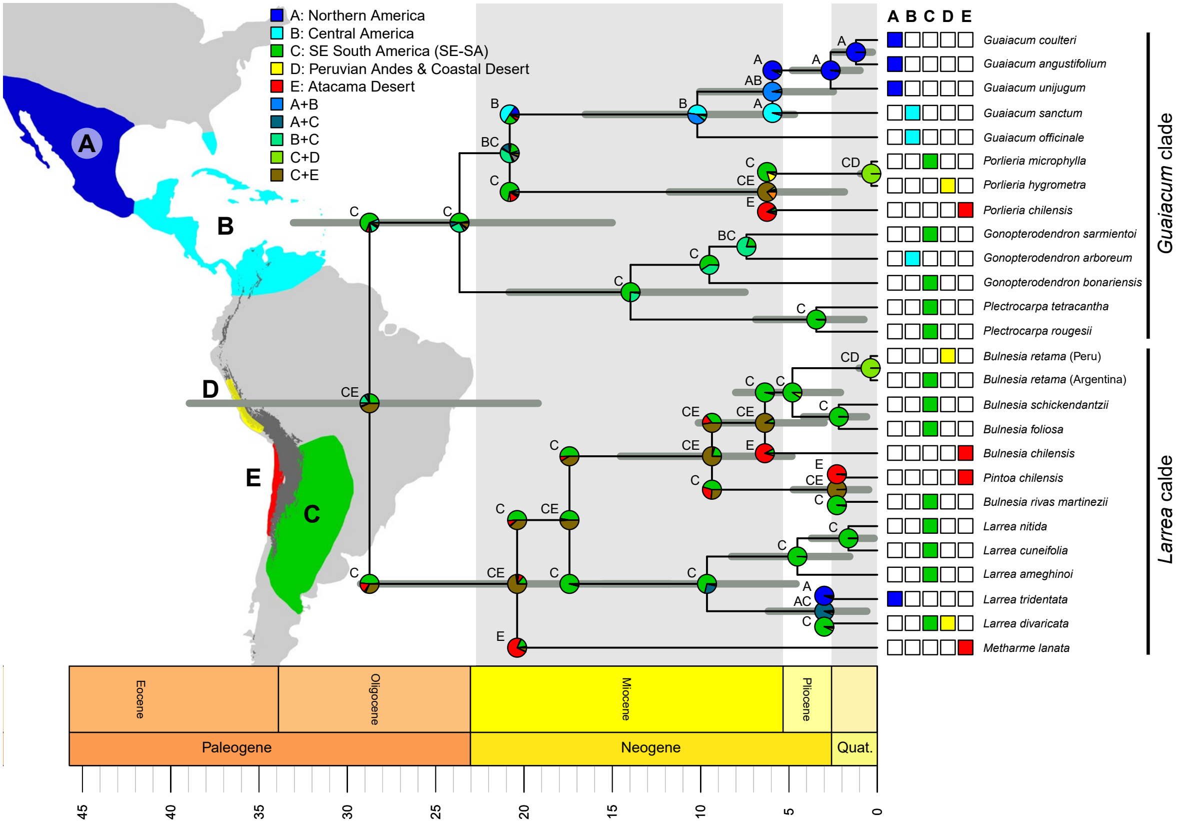

Figure 2: Ancestral area reconstruction of Larreoideae for the Americas plotted on the reduced BEAST tree from figure 1 with maximum area set to 2 (calibrations scheme 2). Only for nodes with posterior probability support ≥ 0.95 confidence intervals are shown. Squares between tips and tip labels indicate distributions assigned to each single species (A-E) with color codes corresponding to the areas indicated on the map and the legend. Pie charts at the nodes depict relative probabilities of areas as estimated from the Dispersal Extinction Cladogenesis (DEC) analysis with BioGeoBEARS. Letters next to the pie charts indicate the areas with highest relative probabilities. Pie charts at corner nodes are only given when relevant for interpretation of biogeographic history.

3.4 Discussion

3.4.1 Phylogeny and divergence times in Zygophyllaceae

The historical assembly of Zygophyllaceae in the New World, and the Atacama Desert in particular, is here analyzed for the first time, using a two-step ancestral area reconstruction approach based on plastid data. Dated phylogenies from plastid datasets as used in this study are known to produce conflicting results compared to gene trees from nuclear data (Middleton *et al.* 2014; Vargas *et al.* 2017). Nuclear phylogenies might provide further insights into the biogeographic history of Zygophyllaceae in the Atacama Desert.

Our results are largely congruent with previous phylogenetic analyses of the Zygophyllaceae. Only the position of the monotypic Seetzenioideae (*Seetzenia lanata* (Willd.) Bullock) differs between ML and Bayesian analyses as previously shown (Sheahan & Chase 2000; Wang *et al.* 2018; Wu *et al.* 2018). The time of origin of Zygophyllaceae is estimated to the Cretaceous–Paleogene boundary. At least three colonization events towards the Americas have been identified within Zygophyllaceae, one in each of the subfamilies Morkillioideae, Tribuloideae and Zygophylloideae. Ancestors of Morkillioideae colonized North America during the late Eocene and members of Tribuloideae (*Kallstroemia* Scop.) in the late Miocene. The last common ancestor of Larreoideae and Zygophylloideae is estimated to have been present in South America and Africa. Wu *et al.* (2018) proposed a shared ancestry between the New World and Africa, but without separating America into North and South. Approximately 50 Million years ago (early Eocene) the breakup of West Gondwana had already taken place and South America and Africa were no longer connected, though much closer to each other than today (Scotese *et al.* 1988). An ancient genetically connected population of early Zygophyllaceae with continuous gene flow via stepping stone dispersal distributed in Africa and South America seems possible (Morley 2003). However, an alternative scenario including one or multiple LDD events cannot be ruled out. Further, our expanded dataset supports, under both calibration schemes, that Larreoideae and Zygophylloideae started to diversify in the Miocene as previously suggested by Wu *et al.* (2018) based on a narrower sampling of Larreoideae. However, our analyses fail to fully resolve the position of monotypic *Metharme* and the relationships between *Porlieria* and *Guaiacum* remain tentative. The incorporation of *Bulnesia rivas-martinezii* revealed the paraphyly of *Bulnesia* since the species is sister to *Pintoa chilensis* in a clade sister to the remaining species of *Bulnesia*.

3.4.2 Historical assembly of the Atacama Desert flora

The majority of the ~35 species of *Fagonia* occur in the Old World and only 8 in the New World (Beier *et al.* 2004). Our data support the monophyly of New World *Fagonia* and a single LDD event to Central and North America from the Old World followed by a subsequent LDD to South America as previously hypothesized (Porter 1974). However, resolution in this part of the tree is quite limited and as in previous studies, the position of *F. scoparia* remains enigmatic and, according to Beier *et al.* (2004), a second colonization of North America from the Old World cannot be ruled out. The New World *Fagonia* clade has no internal resolution making further discussion on biogeographic events inappropriate. Nevertheless, a colonization of the Atacama Desert from north to south within the last 5 Ma seems evident (Simpson *et al.* 2017).

Among the four representatives of Larreoideae in the Atacama Desert, three have arisen in the desert region in northern Chile during the Miocene (*Bulnesia*, *Porlieria* and *Metharme*) and one during the transition from Pliocene to Pleistocene (*Pintoa*). The most recent common ancestor of *Porlieria* is reconstructed with a distribution in the Atacama Desert and southeastern South America in the late Miocene, at a time when the Altiplano-Puna Plateau had already reached elevations above 3000 m (Barnes & Ehlers 2009; Garzione *et al.* 2014) effectively isolating the eastern and the western lowlands, suggesting trans-Andean dispersal. However, since the stem lineage of *Porlieria* was distributed in eastern South America, the colonization of the Atacama Desert could have taken place since the earlier

Miocene. Therefore, Andean vicariance cannot be ruled out. Similar distribution patterns and divergence times estimations are documented for *Bulnesia* and the sister relationship of *B. chilensis* to the remaining species distributed in southeastern South America. These parallel results in *Porlieria* and *Bulnesia* suggest that the progressive Andean uplift during the Miocene led to the isolation of the lineages nowadays distributed on the western and eastern sides of the Andes.

In addition to trans-Andean dispersal and Andean vicariance of closely related taxa (Luebert 2011; Böhnert *et al.* 2019a), Palazzesi *et al.* (2012) suggested an alternative scenario for the genus *Viviana* (Vivianaceae). Based on pollen records from Patagonia they hypothesized an origin of the genus in southern South America during the Miocene and a subsequent northward displacement in parallel with increasing aridity during the late Miocene. The Southern and Central Andes would have then acted as a wedge, separating the eastern and western populations and inhibiting gene flow. The highly supported sister relationship between *Pintoa chilensis* and *B. rivas-martinezii* (Figure S1) is an unexpected result of the present study. The divergence time was estimated to 2.2 Ma and requires trans-Andean dispersal and cannot be explained by vicariance due to Andean orogeny (Scott *et al.* 2018) nor by the northward migration theory proposed for *Viviana* (Palazzesi *et al.* 2012). The precise position of the peculiar drought-adapted *Metharme lanata* within the *Larrea* clade as well as its biogeographic history remains elusive, although the placement in that clade is well supported (Godoy-Bürki *et al.* 2018). However, if *Metharme* in fact dates back to the early Miocene or even to the Oligocene (see HPD intervals in Figure 2) it might be indeed one of the oldest representatives of the Atacama Desert, supporting the "Oligocene-Miocene age of aridity in the Atacama Desert" (Dunai *et al.* 2005). Clarifying the biogeographic history of *Metharme* requires a phylogenetic analysis with a better-supported placement of *Metharme*.

3.4.3 Recent biogeographic events in Larreoideae

Our results reveal four recent biogeographic events in the Larreoideae. One is the LDD of *Larrea tridentata* to North America. Hunziker *et al.* (1972) proposed a south-to-north migration for *L. tridentata* based on the assumption that *L. cuneifolia* is "a relatively old" species and sister to the rest of the genus. While the proposed directionality is confirmed, the basis of this assumption is not: *Larrea cuneifolia* diverged from its sister *L. nitida* in the Pleistocene. *L. divaricata* + *L. tridentata* diverged from the remainder of the genus in the late Miocene, parallel to the split between the Atacama endemic *Pintoa chilensis* + Bolivian *B. rivas-martinezii* from the remainder of *Bulnesia*. Porter (1974) argued in favor of a southwards migration of the genus, while in contrast Lia *et al.* (2001) proposed a late Neogene dispersal of a diploid ancestor of *L. tridentata* from South America to North America, which agrees with our results of a northwards dispersal during the Pliocene-Pleistocene transition. However, the sister species *L. divaricata,* is also found in southern Peru and we were not able to include a sample from this population in our analysis. The disjunct pattern of *L. divaricata* might parallel the very recent disjunctions in *B. retama* and *Porlieria hygrometra*. *Bulnesia retama* is a xerophytic shrub widely distributed in Argentina from Catamarca in the North to La Pampa in the South, but with an exclave over 1,500 km to the NW in the southern Peruvian coastal region of Ica (Palacios & Hunziker 1984). Our data clearly support a recent dispersal from Argentina into the coastal desert of Peru as proposed by Hunziker (1980). Studies of isozyme variation and DNA content (Poggio *et al.* 1986; Comas & Hunziker 1996) already suggested a close relationship between these two disjunct metapopulations. Similar distribution patterns as well as timing of dispersal (~250K years) are found between the Peruvian *Porlieria hygrometra* from the dry Andean valleys of central Peru and *P. microphylla* from the dry Andean foothills of Argentina and Bolivia. Unfortunately, we were not able to include the fourth species, *Porlieria arida,* from Bolivia. However, those three very recent trans-Andean LDD events are in line with several apparently extremely recent (< 400 years) dispersal events as argued by Schwarzer *et al.* (2010). It remains unclear why those groups have not colonized the Atacama Desert in recent times.

3.4.4 Conclusion

There are several plant groups which diversified in the hyperarid Atacama Desert such as *Cristaria* Cav., *Heliotropium* L., *Nolana* L.f. and *Oxalis* L., all of which represent annuals, short-lived perennials or shrubs and mostly go back to single colonizations with relatively short life cycles (Luebert & Wen 2008; Dillon *et al.* 2009; Heibl & Renner 2012; Böhnert *et al.* 2019a). Conversely, Zygophyllaceae colonized the Atacama Desert five times independently and represents one of the very few families with truly woody representatives in this extreme habitat. Zygophyllaceae, however, entirely failed to diversify in the Atacama Desert - this is in stark contrast to the recent diversification and high species richness of Old World Zygophylloideae (Wu *et al.* 2015; Lauterbach *et al.* 2016) or Tribuloideae (Lauterbach *et al.* 2019). Zygophyllaceae like most other woody genera (e.g. *Balsamocarpon* Clos, *Monttea* Gay, *Prosopis* L., *Skytanthus* Meyen, *Vasconcellea* A.St.-Hil., *Huidobria* Gay) only have one or very few species in the Atacama Desert indicating a relatively low carrying capacity for this life form and/or an elevated extinction rate due to increasing aridity in the Atacama Desert (Rabosky 2013).

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3.6 References

Barnes JB & Ehlers TA (2009) End member models for Andean Plateau uplift. *Earth-Science Reviews* 97 (1-4): 105–132. DOI: https://doi.org/10.1016/j.earscirev.2009.08.003.

Beier B-A, Chase MW & Thulin M (2003) Phylogenetic relationships and taxonomy of subfamily Zygophylloideae (Zygophyllaceae) based on molecular and morphological data. *Plant Systematics and Evolution* 240 (1-4): 11–39. DOI: https://doi.org/10.1007/s00606-003-0007-0.

Beier B-A, Nylander JAA, Chase MW & Thulin M (2004) Phylogenetic relationships and biogeography of the desert plant genus *Fagonia* (Zygophyllaceae), inferred by parsimony and Bayesian model averaging. *Molecular Phylogenetics and Evolution* 33 (1): 91–108. DOI: https://doi.org/10.1016/j.ympev.2004.05.010.

Bell CD, Soltis DE & Soltis PS (2010) The age and diversification of the angiosperms re-revisited. *American Journal of Botany* 97 (8): 1296–1303. DOI: https://doi.org/10.3732/ajb.0900346.

Bell MA (2015) geoscale: Geological Time Scale Plotting. https://CRAN.R-project.org/package=geoscale.

Bell MA & Lloyd GT (2015) strap: an R package for plotting phylogenies against stratigraphy and assessing their stratigraphic congruence. *Palaeontology* 58 (2): 379–389. DOI: https://doi.org/10.1111/pala.12142.

Bellstedt DU, Galley C, Pirie MD & Linder HP (2012) The Migration of the Palaeotropical Arid Flora: Zygophylloideae as an Example. *Systematic Botany* 37 (4): 951–959. DOI: https://doi.org/10.1600/036364412X656608.

Böhnert T, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider JV, Quandt D & Weigend M (2019a) Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert. *Global and Planetary Change* 181: 102992. DOI: https://doi.org/10.1016/j.gloplacha.2019.102992.

Böhnert T, Weigend M, Merklinger FF, Quandt D & Luebert F (2019b) cpDNA alignments and R script for Historical Biogeography of Zygophyllaceae in the Atacama Desert. *CRC1211 Database*. DOI: https://doi.org/10.5880/CRC1211DB.32.

Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C & Barthlott W (2003) Noncoding plastid *trn*T-*trn*F sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* 16 (4): 558–576. DOI: https://doi.org/10.1046/j.1420-9101.2003.00577.x.

Bouckaert RR & Drummond AJ (2017) bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology* 17 (1): 42. DOI: https://doi.org/10.1186/s12862-017-0890-6.

Bouckaert RR, Heled J, Kuhnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A & Drummond AJ (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10 (4): e1003537. DOI: https://doi.org/10.1371/journal.pcbi.1003537.

Chacón J & Renner SS (2014) Assessing model sensitivity in ancestral area reconstruction using Lagrange: A case study using the Colchicaceae family. *Journal of Biogeography* 41 (7): 1414–1427. DOI: https://doi.org/10.1111/jbi.12301.

Comas CI & Hunziker JH (1996) Isozyme variation in *Bulnesia retama*, *B. schickendantzii* and *B. foliosa* (Zygophyllaceae). *Plant Systematics and Evolution* 199 (3-4): 193–202. DOI: https://doi.org/10.1007/BF00984904.

Dillon MO & Hoffmann AE (1997) Lomas formations of the Atacama Desert northern Chile. In: Davis SD, Heywood VH, Herrera-MacBryde O, Villa-Lobos J & Hamilton AC, eds. *Centres of Plant Diversity, a Guide and Strategy for Their Conservation. Volume 3: The Americas*. United Kingdom: The World Wildlife Fund For Nature and IUCN - The World Conservation Union, *pp* 528–535.

Dillon MO, Tu T, Xie L, Quipuscoa Silvestre V & Wen J (2009) Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution* 47 (5): 457–476. DOI: https://doi.org/10.1111/j.1759-6831.2009.00040.x.

Drummond AJ, Ho SYW, Phillips MJ & Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4 (5): e88. DOI: https://doi.org/10.1371/journal.pbio.0040088.

Dunai TJ, González López GA & Juez-Larré J (2005) Oligocene–Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. *Geology* 33 (4): 321–324. DOI: https://doi.org/10.1130/G21184.1.

Eaton DAR (2019) Toytree: A minimalist tree visualization and manipulation library for Python. *Methods in Ecology and Evolution* 12: 1–5. DOI: https://doi.org/10.1111/2041-210X.13313.

Garzione CN, Auerbach DJ, Jin-Sook Smith J, Rosario JJ, Passey BH, Jordan TE & Eiler JM (2014) Clumped isotope evidence for diachronous surface cooling of the Altiplano and pulsed surface uplift of the Central Andes. *Earth and Planetary Science Letters* 393: 173–181. DOI: https://doi.org/10.1016/j.epsl.2014.02.029.

Gengler–Nowak K (2002) Reconstruction of the Biogeographical History of Malesherbiaceae. *The Botanical Review* 68 (1): 171–188. DOI: https://doi.org/10.1663/0006-8101(2002)068[0171:ROTBHO]2.0.CO;2.

Gernhard T (2008) The conditioned reconstructed process. *Journal of Theoretical Biology* 253 (4): 769–778. DOI: https://doi.org/10.1016/j.jtbi.2008.04.005.

Godoy-Bürki AC (2015) Lectotypifications in the genera *Bulnesia* (Zygophyllaceae). *Phytotaxa* 239 (3): 293. DOI: https://doi.org/10.11646/phytotaxa.239.3.11.

Godoy-Bürki AC, Acosta JM & Aagesen L (2018) Phylogenetic relationships within the New World subfamily Larreoideae (Zygophyllaceae) confirm polyphyly of the disjunct genus *Bulnesia*. *Systematics and Biodiversity* 106: 1–16. DOI: https://doi.org/10.1080/14772000.2018.1451406.

Heibl C (2008 onwards) PHYLOCH: R language tree plotting tools and interfaces to diverse phylogenetic software packages. http://www.christophheibl.de/Rpackages.html.

Heibl C & Renner SS (2012) Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. *Systematic Biology* 61 (5): 823–834. DOI: https://doi.org/10.1093/sysbio/sys034.

Hunziker JH (1980) Evolución en *Bulnesia*: un enfoque multidisciplinario. *Actas IV Congreso Latinoamerica Genetica* 2: 313–324.

Hunziker JH, Palacios RA, Valesi AG de & Poggio L (1972) Species disjunction in *Larrea*: Evidence from morphology, cytogentics, phenoloic compounds, and seed albumins. *Annals of the Missouri Botanical Garden* 59: 244-233.

Lauterbach M, van der Merwe PdW, Keßler L, Pirie MD, Bellstedt DU & Kadereit G (2016) Evolution of leaf anatomy in arid environments - A case study in southern African *Tetraena* and *Roepera* (Zygophyllaceae). *Molecular Phylogenetics and Evolution* 97: 129–144. DOI: https://doi.org/10.1016/j.ympev.2016.01.002.

Lauterbach M, Zimmer R, Alexa AC, Adachi S, Sage R, Sage T, MacFarlane T, Ludwig M & Kadereit G (2019) Variation in leaf anatomical traits relates to the evolution of C_4 photosynthesis in Tribuloideae (Zygophyllaceae). *Perspectives in Plant Ecology, Evolution and Systematics* 39: 125463. DOI: https://doi.org/10.1016/j.ppees.2019.125463.

Lia VV, Confalonieri VA, Comas CI & Hunziker JH (2001) Molecular Phylogeny of *Larrea* and Its Allies (Zygophyllaceae): Reticulate Evolution and the Probable Time of Creosote Bush Arrival to North America. *Molecular Phylogenetics and Evolution* 21 (2): 309–320. DOI: https://doi.org/10.1006/mpev.2001.1025.

Löhne C & Borsch T (2005) Molecular evolution and phylogenetic utility of the *petD* group II intron: a case study in basal angiosperms. *Molecular Biology and Evolution* 22 (2): 317–332. DOI: https://doi.org/10.1093/molbev/msi019.

Luebert F (2011) Hacia una fitogeografía histórica del Desierto de Atacama. *Revista de Geografía Norte Grande (*50): 105–133. DOI: https://doi.org/10.4067/S0718-34022011000300007.

Luebert F & Pliscoff P (2017) Sinopsis bioclimática y vegetacional de Chile. Santiago de Chile: *Editorial Universitaria*. 381 pp.

Luebert F & Wen J (2008) Phylogenetic Analysis and Evolutionary Diversification of *Heliotropium* Sect. *Cochranea* (Heliotropiaceae) in the Atacama Desert. *Systematic Botany* 33 (2): 390–402. DOI: https://doi.org/10.1600/036364408784571635.

Magallón S, Gómez-Acevedo S, Sánchez-Reyes LL & Hernández-Hernández T (2015) A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist* 207 (2): 437–453. DOI: https://doi.org/10.1111/nph.13264.

Matzke NJ (2013) Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* 5 (4): 242–248. DOI: https://doi.org/10.21425/F5FBG19694.

Middleton CP, Senerchia N, Stein N, Akhunov ED, Keller B, Wicker T & Kilian B (2014) Sequencing of chloroplast genomes from wheat, barley, rye and their relatives provides a detailed insight into the evolution of the Triticeae tribe. *PLoS ONE* 9 (3): e85761. DOI: https://doi.org/10.1371/journal.pone.0085761.

Miller MA, Pfeiffer W & Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees: *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans*. 8 pp.

Morley RJ (2003) Interplate dispersal paths for megathermal angiosperms. *Perspectives in Plant Ecology, Evolution and Systematics* 6 (1-2): 5–20. DOI: https://doi.org/10.1078/1433-8319-00039.

Müller KF & Borsch T (2005) Phylogenetics of Amaranthaceae based on *mat*K/*trn*K sequence data: evidence from parsimony, likelihood, and Bayesian analyses. *Annals of the Missouri Botanical Garden* 92 (1): 66–102. DOI: https://doi.org/https://www.jstor.org/stable/3298649.

Navarro G (1997) *Izozogia nellii* (Zygophyllaceae), Nuevo Género y Especie del Gran Chaco de Santa Cruz (Bolivia). *Novon* 7 (1): 1–5. https://www.jstor.org/stable/3392062?seq=1&cid=pdf-reference#references_tab_contents.

Palacios RA & Hunziker JH (1984) Revision taxonomica del genero *Bulnesia* (Zygophyllaceae). *Darwiniana* 25 1-4: 299–320.

Palazzesi L, Gottschling M, Barreda V & Weigend M (2012) First Miocene fossils of Vivianiaceae shed new light on phylogeny, divergence times, and historical biogeography of Geraniales. *Biological Journal of the Linnean Society* 107 (1): 67–85. DOI: https://doi.org/10.1111/j.1095-8312.2012.01910.x.

Paradis E & Schliep K (2019) ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35 (3): 526–528. DOI: https://doi.org/10.1093/bioinformatics/bty633.

Poggio L, Wulff AF & Hunziker JH (1986) Chromosome Size, Nuclear Volume and DNA Content in *Bulnesia* (Zygophyllaceae). *Darwiniana* 27 (1-4): 26–38.

Porter DM (1974) Disjunct Distributions in the New World Zygophyllaceae. *Taxon* 23 (2/3): 339–346. DOI: https://doi.org/10.2307/1218714.

Quandt D, Müller KF & Huttunen S (2003) Characterisation of the Chloroplast DNA *psb*T-H Region and the Influence of Dyad Symmetrical Elements on Phylogenetic Reconstructions. *Plant Biology* 5 (4): 400–410. DOI: https://doi.org/10.1055/s-2003-42715.

R Core Team (2018) R: A Language and Environment for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

Rabosky DL (2013) Diversity-Dependence, Ecological Speciation, and the Role of Competition in Macroevolution. *Annual Review of Ecology, Evolution, and Systematics* 44 (1): 481–502. DOI: https://doi.org/10.1146/annurev-ecolsys-110512-135800.

Rambaut A, Drummond AJ, Xie D, Baele G & Suchard MA (2018) Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology* 67 (5): 901–904. DOI: https://doi.org/10.1093/sysbio/syy032.

Ree RH & Sanmartín I (2018) Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography* 45 (4): 741–749. DOI: https://doi.org/10.1111/jbi.13173.

Ree RH & Smith SA (2008) Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. *Systematic Biology* 57 (1): 4–14. DOI: https://doi.org/10.1080/10635150701883881.

Ritter B, Binnie SA, Stuart FM, Wennrich V & Dunai TJ (2018) Evidence for multiple Plio-Pleistocene lake episodes in the hyperarid Atacama Desert. *Quaternary Geochronology* 44: 1–12. DOI: https://doi.org/10.1016/j.quageo.2017.11.002.

RStudio Team (2016) RStudio: Integrated Development Environment for R. Boston, MA. http://www.rstudio.com/.

Rundel PW, Dillon MO, Palma B, Mooney HA, Gulmon SL & Ehrlinger JR (1991) The Phytogeography and Ecology of the Coastal Atacama and Peruvian Desert. *Aliso: A Journal of Systematic and Evolutionary Botany* 13 (1): 1–49.

Schwarzer C, Huamaní FC, Cano A, La Torre MI & Weigend M (2010) 400 years for long-distance dispersal and divergence in the northern Atacama desert – Insights from the Huaynaputina pumice slopes of Moquegua, Peru. *Journal of Arid Environments* 74 (11): 1540–1551. DOI: https://doi.org/10.1016/j.jaridenv.2010.05.034.

Scotese CR, Gahagan LM & Larson RL (1988) Plate tectonic reconstructions of the Cretaceous and Cenozoic ocean basins. *Tectonophysics* 155 (1-4): 27–48. DOI: https://doi.org/10.1016/0040-1951(88)90259-4.

Scott EM, Allen MB, Macpherson CG, McCaffrey KJW, Davidson JP, Saville C & Ducea MN (2018) Andean surface uplift constrained by radiogenic isotopes of arc lavas. *Nature Communications* 9 (1): 969. DOI: https://doi.org/10.1038/s41467-018-03173-4.

Sheahan MC & Chase MW (2000) Phylogenetic Relationships within Zygophyllaceae Based on DNA Sequences of three Plastid Regions, with Special Emphasis on Zygophylloideae. *Systematic Botany* 25 (2): 371–384. DOI: https://doi.org/10.2307/2666648.

Simpson MG, Johnson LA, Villaverde T & Guilliams CM (2017) American amphitropical disjuncts: Perspectives from vascular plant analyses and prospects for future research. *American Journal of Botany* 104 (11): 1600–1650. DOI: https://doi.org/10.3732/ajb.1700308.

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9): 1312–1313. DOI: https://doi.org/10.1093/bioinformatics/btu033.

Vargas OM, Ortiz EM & Simpson BB (2017) Conflicting phylogenomic signals reveal a pattern of reticulate evolution in a recent high-Andean diversification (Asteraceae: Astereae: *Diplostephium*). *New Phytologist* 214 (4): 1736–1750. DOI: https://doi.org/10.1111/nph.14530.

Villagrán C, Arroyo MTK & Marticorena C (1983) Effectos de la desertización en la distribución de la flora andina en Chile. Effects of natural Desertification on the Distribution of the Andean Flora in Chile. *Revista Chilena de Historia Natural* 56: 137–157.

Wang Q, Wu S, Su X, Zhang L, Xu X, Lyu L, Cai H, Shrestha N, Liu Y, Wang W & Wang Z (2018) Niche conservatism and elevated diversification shape species diversity in drylands: evidence from Zygophyllaceae. *Proceedings of the Royal Society B: Biological Sciences* 285 (1890). DOI: https://doi.org/10.1098/rspb.2018.1742.

Worberg A, Quandt D, Barniske A-M, Löhne C, Hilu KW & Borsch T (2007) Phylogeny of basal eudicots: Insights from non-coding and rapidly evolving DNA. *Organisms Diversity & Evolution* 7 (1): 55–77. DOI: https://doi.org/10.1016/j.ode.2006.08.001.

Wu S-D, Lin L, Li H-L, Yu S-X, Zhang L-J & Wang W (2015) Evolution of Asian Interior Arid-Zone Biota: Evidence from the Diversification of Asian *Zygophyllum* (Zygophyllaceae). *PLoS ONE* 10 (9): e0138697. DOI: https://doi.org/10.1371/journal.pone.0138697.

Wu S-D, Zhang L-J, Lin L, Yu S-X, Chen Z-D & Wang W (2018) Insights into the historical assembly of global dryland floras: the diversification of Zygophyllaceae. *BMC Evolutionary Biology* 18 (1): 166. DOI: https://doi.org/10.1186/s12862-018-1277-z.

Zuloaga FO, Morrone O & Belgrano MJB, eds. (2008) Catálogo de las Plantas Vasculares del Cono Sur. Argentina, Sur de Brasil, Chile, Paraguay y Uruguay. Saint Louis Mo.: *Missouri Botanical Garden Press.*

Chapter 4

Atriplex retusa, the correct name for *A. deserticola*

Atriplex retusa Gay, near Las Bombas north of Caldera, can form cushion-like structures of several meters in diameter.

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Atriplex retusa, the correct name for *A. deserticola* (Chenopodiaceae; Amaranthaceae sensu APG)

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Introduction

The genus *Atriplex* Linnaeus (1753: 1052) (Chenopodiaceae Vent.; placed in Amaranthaceae Juss. s. l. in APG IV 2016) comprises about 300 species distributed mainly in subtropical, temperate, and subarctic regions of the world and is mostly adapted to dry conditions on often saline soils (Sukhorukov & Danin 2009, Kadereit *et al.* 2010, Iamonico 2013, APG IV 2016). The genus is highly diverse in Eurasia, Australia and North America. South America is another centre of diversity with ca. 55 species, 45 of which are considered as native (Brignone *et al.* 2016). A recent and exhaustive taxonomical synopsis of *Atriplex* for South America was published by Brignone *et al.* (2016), but there are also regional taxonomic revisions, e.g., for Chile by Rosas (1989), or Argentina by Múlgura de Romero (1981, 1982 & 1984).

Rosas (1989) presented an exhaustive taxonomic work in his revision of the genus *Atriplex* in Chile, but he was not able to locate and examine all type specimens in herbaria outside Chile. In case of the name *Atriplex retusa* Gay (1849: 240) he pointed out that, based on the protologue, it is morphologically similar to *Atriplex deserticola* Philippi (1860: 47). However, due to the fact that he could not find type material at Santiago (SGO) and that he was not able to visit Paris (P), he excluded the name from his revision.

Brignone *et al.* (2016), in their Synopsis on the South American *Atriplex* species, were able to investigate the digital images of the holo- and isotypes preserved at P, and designated an isotype (at SGO) for *A. retusa*, so confirming Rosas' (1989) assumption that *A. retusa* and *A. deserticola* refer to the same taxon (synonyms). However, while they carefully reviewed the documentation history of the name *A. retusa* in Argentina and Chile, and correctly cited the protologues of all names related to *A. deserticola*, they did not apply the priority rule following Art. 11 of ICN (Turland *et al.* 2018): *A. retusa*, published in 1849, takes priority over the currently accepted name *A. deserticola*, published in 1860.

We re-evaluated all type specimens of *Atriplex deserticola*, and *A. retusa*, as well as the related *A. podocarpa* Philippi (1895: 427), and *A. transandina* Johnston (1929: 303) and came to the conclusion that all these names refer to the same species. As discussed in detail by Brignone *et al.* (2016), the type material of all names shows identical characters: leaves elliptic to oblong, shortly petiolate, with a shortly cuneate to truncate base; leaves tips obtuse to retuse; fruiting valves round–rhomboid at maturity.

Some uncertainty remains regarding the type locality of *Atriplex retusa*, which was only mentioned as "in aequis Guamalata" on the label occurring on the isotype at SGO. The locality Guamalata [or Huamalata] was indeed visited by Gay during one of his collecting trips (see Muñoz 1944). We visited Guamalata and the surrounding area in March and October 2017, but failed to find *A. retusa*. Apart from some introduced *Atriplex* species, the only native species found was *A. repanda* Philippi (1895: 425), which has its type locality near Ovalle and Guamalata, and it is readily differentiated from *A. retusa* by its dentate leaf margins and the fusion of the fruiting valves, which has a high taxonomic value in *Atriplex*. The occurrence of *A. retusa* in Guamalata would represent a southern outlier in the distribution of the species in Chile (Fig. 1). However, since Gay also traveled further north, well in the geographical range of most collections of the species, including the Elqui valley along the Rio Turbio until Vegas del Toro (type location of *A. podocarpa*, ca. 150 km north-east of Guamalata; see Fig. 1) we cannot rule out that the specimen at SGO may have been wrongly labeled. Unfortunately, the material at P does not have any precise indication of locality at all. *A. retusa* shows a disjunct distribution east and west of the Andes (Fig. 1). In Chile, the species is distributed from ca. 30°S (ca. La Serena) up to ca. 25°S (ca. Taltal), while in Argentina the distribution extents from ca. 32°30′S (near Mendoza) up to ca. 23°S north of San Salvador de Jujuy. The species occurs between 1000 and 3000 m a.s.l. and it is often found in or near dry washes.

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The fact that both names describe the same taxon has been previously shown by Rosas (1989), and Brignone *et al.* (2016). Based on the principle of priority, we accept the name *Atriplex retusa* and place the currently used name *A. deserticola* in synonymy.

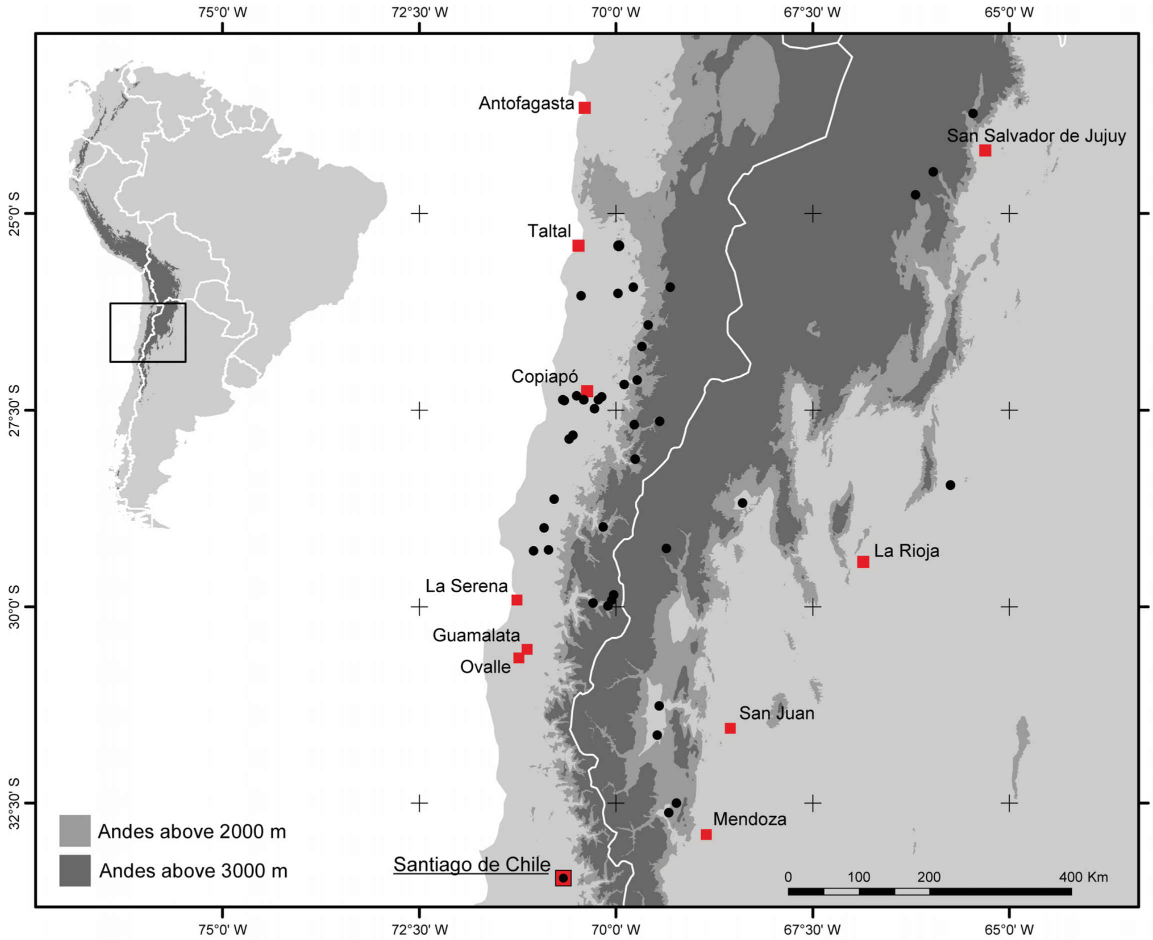

FIGURE 1. Distribution map of *Atriplex retusa* (black dots) in Chile, and Argentina, based on specimens cited and georeferenced in the present paper [localities from Rosas (1989) and Brignone *et al.* (2016)].

Taxonomy

Atriplex retusa Gay (1849: 240) ≡ *Obione retusa* (Gay) Ulbrich (1934: 508).

Type:—CHILE. Coquimbo Province, Guamalata [Huamalata], *Gay 1302* (holotype P barcode 00606466 [photo!], isotypes: P barcode 00606467 [photo!], SGO barcode 000001623 [photo!]).

= *Atriplex deserticola* Philippi (1860: 47) *syn. nov.*

Lectotype (designated by Rosas 1989):—CHILE. Quebrada de la Encantada, 'in deserto atacamensis frequens', February 1854, *Philippi s.n.* (SGO barcode 000001592 [photo!], isolectotype SI barcode 001267 [photo!]).

= *Atriplex podocarpa* Philippi (1895: 427) *syn. nov.* ≡ *Obione podocarpa* (Phil.) Ulbrich (1934: 508).

TYPE:—CHILE. Vegas del Toro, February 1883, *Philippi s.n.* (holotype SGO barcode 000001615 [photo!], isotype SI barcode 001279).

= *Atriplex transandina* Johnston (1929: 303) *syn. nov.* ≡ *Obione transandina* (I.M.Johnst.) Ulbrich (1934: 508).

TYPE:—ARGENTINA. San Juan, Rio de la Tagua below its confluence with the Rio de la Sal, below Vega Cadillo, ca. 3000 m, ca. 29°2′–5′S 69°28′–42′W, 12 January 1926, *Johnston 6148* (holotype GH barcode 00037167 [photo!], isotype K barcode 000583238 [photo!], US barcode 00102653 [photo!]).

Note:—Philippi (1860) and Ulbrich (1934) erroneously cited "Remy" as the author of *Atriplex retusa*. While Remy did contribute several treatments for Gay's Flora Chilena, including the families Amaranthaceae and Phytolacaceae, he did not write the treatment for Chenopodiaceae (Gay 1849, Marticorena 1992), which was written by Gay himself.

Additional specimens examined:—CHILE. **II Región de Antofagasta**: Antofagasta, Agua Verde, behind the Gas Station, 1550 m, 25°24′06.1″S 69°57′38.8″W, 26 March 2017, *Luebert & Böhnert 3669* (BONN, ULS); **III Región de Atacama**, Provincia de Chañaral, Las Bombas, 470 m, 26°01′57.1″S 70°26′09″W, 09 October 2016, *Luebert, Stoll & Böhnert 3307* (BONN, ULS); Quebrada Carrizo, 1500 m, 25°55′14.6″S 69°46′18.2″W, 27 March 2017, *Luebert & Böhnert 3674* (BONN, ULS); Quebrada J.J. Pérez on the road from Altamira to Diego de Almagro, same river system as Quebrada La Encantada, 1100 m, 26°00′07.4″S 69°57′51.6″W, 27 March 2017, *Luebert & Böhnert 3675* (BONN, ULS; Locotype of *Atriplex deserticola* Phil.); Road Llanta–Potrerillos, km 27.5, 1900 m, 26°24′28.7″S 69°34′54.5″W, 28 March 2017, *Luebert & Böhnert 3681* (BONN, ULS); Vegas de Chañaral, 2200 m, 26°40′47.6″S 69°39′40.6″W, 28 March 2017, *Luebert & Böhnert 3685* (BONN, ULS); Copiapó, Quebrada San Andrés, Puquios, 1240 m, 27°09′35.1″S 69°53′16.8″W, 29 March 2017, *Luebert & Böhnert 3690* (BONN, ULS); Road from Ruta 5 to Barranquilla, ca. 1 km S of Ruta 5, 170 m, 27°22′03.7″S 70°39′07.3″W, 30 March 2017, *Luebert & Böhnert 3700* (BONN, ULS); **IV Región de Coquimbo**: Provincia de Elqui, Road from Juntas del Toro to Baños del Toro, near type location of *Atriplex podocarpa* Phil, 2700 m, 29°54′02.4″S 70°02′52.8″W, 01 April 2017, *Luebert & Böhnert 3713* (BONN, ULS); Juntas del Toro, 2100 m, 29°58′29.6″S 70°05′35.7″W, 01 April 2017, *Luebert & Böhnert 3716* (BONN, ULS).

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References

APG IV (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society* 181: 1–20. https://doi.org/10.1111/boj.12385

Brignone, N.F., Denham, S.S. & Pozner, R. (2016) Synopsis of the genus *Atriplex* (Amaranthaceae, Chenopodioideae) for South America. *Australian Systematic Botany* 29: 324–357. https://doi.org/10.1071/SB16026

Gay, C. (1849) *Flora Chilena. Atlas de la historia fisica y politica de Chile. Vol. 5.* Published by the author, Paris, 463 pp. https://doi.org/10.5962/bhl.title.16172

Iamonico, D. (2013) Studies on the genus *Atriplex* (Amaranthaceae) in Italy. V. *Atriplex tornabenei*. *Phytotaxa* 145 (1): 54–60. https://doi.org/10.11646/phytotaxa.145.1.6

Johnston, I.M. (1929) A collection of plants from the high Cordilleras of nothwestern San Juan. *Physis Revista de la Sociedad Argentina de Ciencias Naturales* 9: 297–326.

Kadereit, G., Mavrodiev, E.V., Zacharias, E.H. & Sukhorukov, A.P. (2010) Molecular phylogeny of Atripliceae (Chenopodioideae, Chenopodiaceae): Implications for systematics, biogeography, flower and fruit evolution, and the origin of C4 photosynthesis. *American Journal of Botany* 97: 1664–1687. https://doi.org/10.3732/ajb.1000169

Linnaeus, C. (1753) *Species Plantarum. Vols. 1–2.* L. Salvius, Holmiae [Stockholm], 1200 pp. https://doi.org/10.5962/bhl.title.669

Marticorena, C. (1992) Bibliografía Botánica Taxonómica de Chile. *Monographs in Systematic Botany from the Missouri Botanical Garden* 41: 1–587.

Múlgura de Romero, M.E. (1981) Contribuciones al estudio del género *Atriplex* (Chenopodiaceae) en la Argentina I. *Darwiniana* 23: 119–150.

Múlgura de Romero, M.E. (1982) Contribuciones al estudio del género *Atriplex* (Chenopodiaceae) en la Argentina II. Las especies adventicias. *Darwiniana* 24: 49–68.

Múlgura de Romero, M.E. (1984) Contribuciones al estudio del género *Atriplex* (Chenopodiaceae) en la Argentina III. *Darwiniana* 25: 235–253.

Muñoz, P.C. (1944) El itinerario de Don Claudio Gay. *Boletín del Museo Nacional de Historia Natural Chile* 22: 27–44.

Philippi, R.A. (1860) *Florula Atacamensis seu enumeratio plantarum quas in itinere per desertum Atacamense*. Eduard Anton, Halle, 92 pp.

Philippi, R.A. (1895) Plantas nuevas chilenas de las familias que corresponden al tomo V de la obra de Gay. *Anales de la Universidad de Chile* 91: 415–432.

Rosas, M.R. (1989) El genero *Atriplex* (Chenopodiaceae) en Chile. *Gayana Botánica* 46: 3–82.

Sukhorukov, A.P. & Danin, A. (2009) Taxonomic notes on *Atriplex* sect. *Teutliopsis* and sect. *Atriplex* in Israel and Syria. *Flora Mediterranea* 19: 15–23.

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.J. & Smith, G.F. (Eds.) (2018) *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. [Regnum Vegetabile 159] Koeltz Botanical Books, Glashütten, 254 pp. https://doi.org/10.12705/Code.2018

Ulbrich, E. (1934) Chenopodiaceae. *In:* Engler, A. & Prantl, K. (Eds.) *Die natürlichen Pflanzenfamilien. Vol. 2, 16c*. W. Engelmann, Leipzig, pp. 379–584.

Chapter 5

Evolution of *Atriplex* in the Atacama Desert

Atriplex madariagae Phil. at the mouth of Rio Loa. The species is a common element in Quebradas of the northern Atacama Desert.

The results presented in this chapter form the basis for a manuscript which is currently being prepared for publication as follows:

Böhnert T, Luebert F, Merklinger FF, Harpke D, Stoll A, Blattner F, Quandt D, Weigend M (***in prep.***) Biogeography and Evolution of *Atriplex* (Amaranthaceae) in the Atacama Desert.

Own contribution: Study design, field work, lab work, data analyses, writing.

Data availability: Supplementary material are availbalbe in **Appendix B**.

Abstract

The Atacama Desert in northern Chile is a particularly interesting system to study landscape and biotic evolution and their interaction, due to the long lasting hyperarid conditions combined with comparatively high plant species richness and endemism. Most species in the Atacama Desert are found along the Pacific coast respectively the Andean foothills, only few can exist in its hyperarid core. The genus *Atriplex* is one of the few genera with a surprisingly high species richness in the Atacama Desert, comprising about 15 documented species. They are found almost everywhere in this desert, including the hyperarid core, and most of them are endemic to it. Only few species are found on both sides of the Andes, and there may be a floristic link to Peru. Here, first results on the biogeography and macroevolution of the genus *Atriplex* are present based on the broadest and most comprehensive sampling for the group in the Atacama Desert to date. In a two-step approach we first use Bayesian molecular clock dating to infer divergences time estimations based on plastid DNA sequences, followed by phylogenetic reconstruction using genotyping-by-sequencing (GBS) data. We show that the Atacama Desert has been colonized at least three times by *Atriplex* species since the late Pliocene and early Pleistocene. Two clades show clear floristic connectivity towards Peru, while the third clade has a close link to the eastern side of the Andes. The results presented here suggest a complex history with multiple colonizations and subsequent diversifications under the hostile conditions of the Atacama Desert.

Keywords: Chenopodiaceae — disjunction — Genotyping-by-sequencing — long distance dispersal — South America — trans-Andean Dispersal

5.1 Introduction

The Atacama Desert on the west coast of northern Chile is considered as one of the driest, but also oldest deserts on earth (Garreaud 2009). It is generally agreed that the combination of its location in the subtropical high-pressure belt, the rain shadow effect of the Andes and the influence of the cold Humboldt current are the main causes for its hyperaridity (Hartley *et al.* 2005). Nevertheless, the exact timing of the onset of hyperaridity and its effect in different parts of the Atacama Desert are still under debate (Dunai *et al.* 2005; Ritter *et al.* 2019). The Peruvian coastal desert is located directly north of the Atacama Desert, but is generally considered as a separate ecogeographical region (Rauh 1986; Dillon & Hoffmann 1997; Luebert & Pliscoff 2017). The so-called climatic or Dry Diagonal divides the South American continent in general and the Chilean Atacama Desert in particular from the north-west to the south-east (Eriksen 1983; Houston & Hartley 2003; Houston 2006). The western Andean slopes to the north-east of the desert region receive rainfall from the Amazon basin during Austral Summer (easterlies), while the south-western coastal region receives sporadic precipitation from the Pacific Ocean in Austral Winter (westerlies; Houston & Hartley 2003; Garreaud 2009). In spite of the extreme aridity, the flora of the Atacama is relatively rich with approximately 550 species of vascular plants and a considerable degree of endemism at species level (~60 %; Dillon & Hoffmann 1997). Four floristic elements have been identified, namely (1) neotropical, (2) central Chilean, (3) trans-Andean and (4) anti-/amphitropical (Luebert 2011; see also chapter 3). In the ten genera considered to be species rich (≥ 10 accepted species in the Atacama Desert) the diversity appears to be partly due to *in situ* diversification (Dillon *et al.* 2009; Heibl & Renner 2012). So far, only few studies have been able to address fundamental evolutionary questions, largely due to the lack of highly resolved phylogenetic trees (Luebert & Wen 2008).

Typically, diversifications of Atacama clades appear to be relatively recent and to have progressed from south to north, with the most closely related clades found in Mediterranean Chile. In some cases, Peruvian clades are nested in Atacama clades as shown for e.g., *Malesherbia* Ruiz & Pav. (Gengler–Nowak 2002) or *Oxalis* L. (Heibl & Renner 2012). A similar pattern has also been identified in *Cristaria*, where the transition zone between the Mediterranean region and the southern Atacama Desert has been identified as the ancestral area for the genus, followed by individual colonizations of the central Pampa, the coast and the Andes as well as Peru (see chapter 7 and Böhnert *et al.* 2019). Other genera have a much more complex biogeographic history: The genus *Cryptantha* from the borage family has colonized South America, and the Atacama Desert in particular, several times (Guilliams *et al.* 2017). Thus, both *in situ* diversification and multiple colonizations have played a role in the formation of the Atacama Desert flora (Luebert 2011; Heibl & Renner 2012; chapter 3).

The genus *Atriplex* L., one of the largest genera in the Atacama Desert, represents a particularly interesting case. Unlike the other diverse genera in the Atacama, *Atriplex* (Chenopodiaceae or Amaranthaceae sensu APG IV 2016) is a highly successful genus in many saline and semi-arid habitats across the globe (Kadereit *et al.* 2010). With approx. 300 species, the genus is characterized by wind-pollinated, unisexual flowers and monoecious or dioecious gender distribution (Blackwell & Powell 1981; Stutz *et al.* 1990; Kühn 1993; Mering *et al.* 2019). Species of *Atriplex* are ecophysiologically adapted to often hostile habitats and include a large number of C_4 plants. C_4 photosynthesis has developed multiple times in Amaranthaceae (Kadereit *et al.* 2003). In *Atriplex*, however, it seems that the evolution of C_4 metabolism has been instrumental for the extraordinary success of the genus under (hyper-)arid conditions (Bielig & Brownell 1990; Kadereit *et al.* 2010; Kadereit *et al.* 2012).

The taxonomy of *Atriplex* is notoriously difficult – the flowers are extremely reduced and provide very few useful morphological characters, leaf morphology is subject to considerable ontogenetic plasticity and much of the species delimitation is based on leaf shapes and the morphology of fruiting bracteoles (Figure 2B; Múlgura de Romero 1981; 1982, 1984; Rosas 1989; Flores-Olvera *et al.* 2011; Brignone *et al.* 2016; Böhnert *et al.* 2018). In the literature, a total of 55 species of *Atriplex* are documented for South America, some ten of them are considered as introduced (Brignone *et al.* 2016; Krak *et al.* 2019). However, species delimitation is often poorly understood, especially in Peruvian taxa, which have never been critically revised (Standley 1938; Brako & Zarucchi 1993). Across Chile, a total of 21 species of *Atriplex* is reported, with 15 species found in the Atacama Desert, making *Atriplex* one of the most species-rich genera in this region (Rosas 1989; Brignone *et al.* 2016). *Atriplex* is found with several species north and south of the Dry Diagonal, both along in the winter-rain region on the coast and the summer-rain region in the Andes (Houston 2006).

The present study addresses the hypotheses that the origin of the genus *Atriplex* coincides with climatic and geological factors responsible for (hyper-) aridity in the Atacama Desert during the Miocene and that the diversification of *Atriplex* correlates with increasing aridity during the Miocene and Pliocene as well as the Quaternary climate oscillation. Particular research questoions are: What is the age of *Atriplex* in the Atacama Desert and is the current diversity the product of single or multiple colonization events? Further, how did the spatiotemporal diversification of *Atriplex* took place, what is the influence of the Quaternary climate oscillation on diversification of *Atriplex* and does the current taxonomic concept adequately reflect the recent speciation processes? Aims are (1) to resolve the biogeography of *Atriplex* in the Atacama Desert, (2) identify the timing and patterns of diversification and (3) to investigate the biogeographic connection to central Chile, southwestern Peru and Argentina. The study employs a two-step analyses approach using (a) a two-plastid marker Sanger sequencing approach based on the work of Kadereit *et al.* (2010) and (b) a dataset obtained from Next Generation Sequencing using genotyping-by-sequencing (GBS; Elshire *et al.* 2011).

5.2 Materials and Methods

5.2.1 Taxon sampling and plant identification

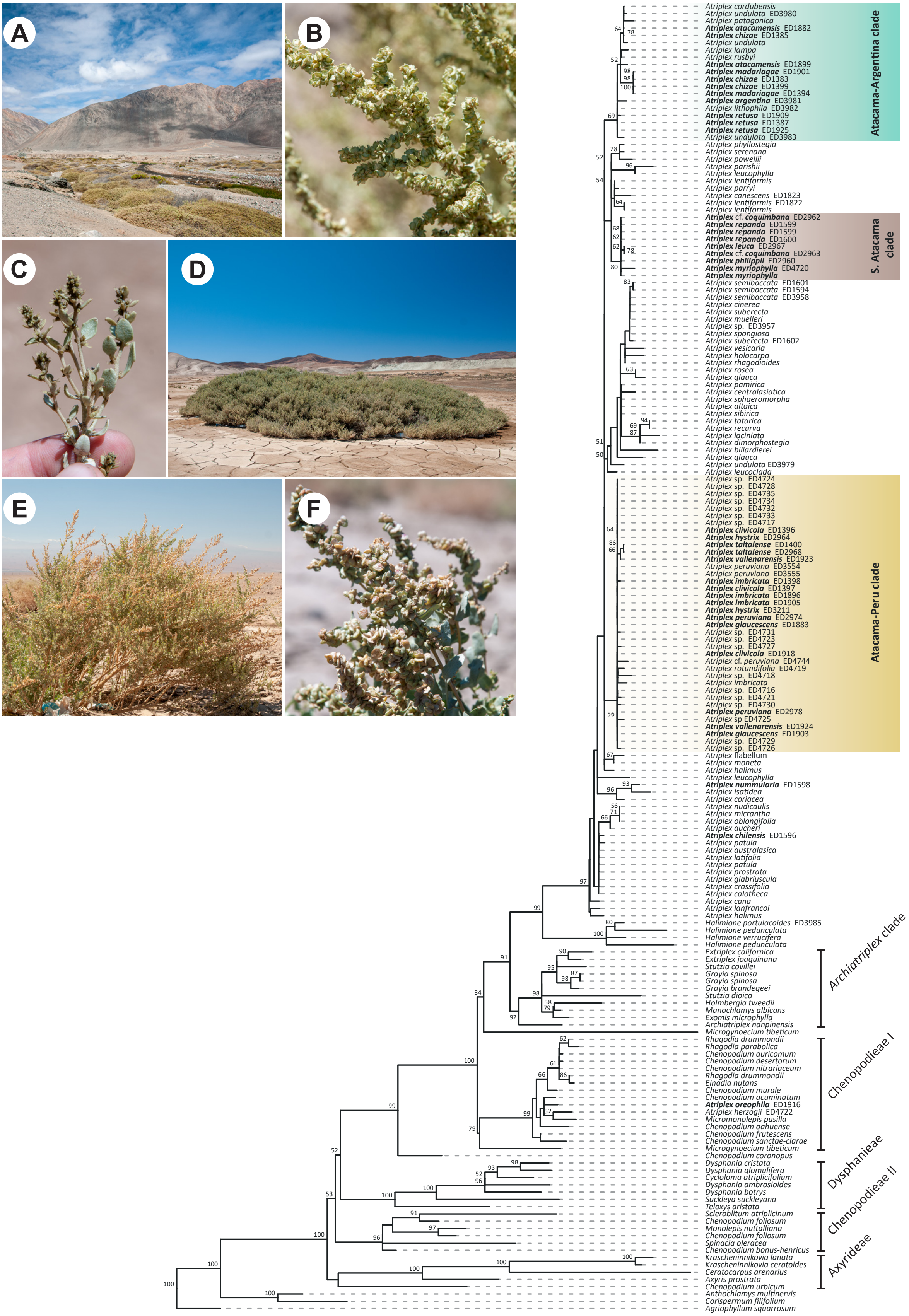
For the present study a complete taxon sampling of all 15 *Atriplex* species documented for the Atacama Desert and 17 of the 21 accepted species of *Atriplex* in Chile (Rosas 1989; Brignone *et al.* 2016; Böhnert *et al.* 2018) is presented, complemented by a comprehensive sampling from Peru and Argentina. For Chile, we were unable to obtain samples of *Atriplex chapinii* I.M.Johnst. from San Felix (Desventuradas Islands, 850 km off the Chilean coast in the Pacific Ocean) as well as *Atriplex coquimbana* Phil. and *Atriplex costellata* Phil., two prostate and apparently very rare and probably closely related species from the area between Ovalle, Coquimbo and Algarrobo (Brignone *et al.* 2016; 2019). The last missing Chilean species, *Atriplex vulgatissima* Speg., is distributed in Argentina and only reaches the Chilean part of Patagonia. Taxon sampling is mainly based on our own material collected between 2016 and 2019 in the Atacama Desert, Peru and Argentina. Herbarium vouchers are stored in local herbaria in Chile (ULS), Peru (USM, HUSA), Argentina (LP) and Germany (BONN). Identification of collected plant material is based on taxonomic revisions (Standley 1938; Múlgura de Romero 1981; 1982, 1984; Rosas 1989; Brignone *et al.* 2016) and digitally available type specimens. Additionally, we made use of extracted DNA from herbarium vouchers from Lima (USM) and Arequipa (HUSA) and additional sequences from online resources based on the study by Kadereit *et al.* (2010). A comprehensive list of all taxa used for this study, including GenBank accession numbers and voucher information are provided in Appendix C (Table S1).

5.2.2 DNA extraction, amplification, library preparation and sequencing

The NucleoSpin Plant II kit (Machery-Nagel, Germany) was used to extract genomic DNA from silica dried leaf tissue or herbarium specimens. We followed the manufacturer protocol but increased the lysate incubation time to 90 minutes to increase DNA yield. For the dating approach, the Ribulose bisphosphate carboxylase large subunit gene (*rbc*L) as well as the spacer between the ATP synthase subunit beta and rbcL (*atp*B-*rbc*L) were amplified. For *rbc*L we used the primer combination rbcL-F1 and rbcL-1460R (Olmstead *et al.* 1992) for amplification and the internal primer rbcL_636Fn and rbcL_724R (Lindqvist & Albert 2002) for sequencing. For the *atp*B-*rbc*L intergenic spacer we made use of the primer combination atpB-rbcLR and atpB-rbcLF1 (Barniske *et al.* 2012). Cycling conditions for rbcL amplification started with an initial denaturation phase at 96° C for 90 s, 60 s of 50° C and 120 s of 68° C, followed by 38 cycles of 94° C for 30 s, 48° C for 60 s and 68° C for 120 s. For the *atp*B-*rbc*L region amplification started with an initial denaturation phase at 96° C for 90 s followed by nine cycles of 60 s at 94° C, then 60 s at 55° C and 180 s at 72° C, finally, 24 cycles with 60 s at 94° C and 60 s at 50° C. Amplicons were purified through gel electrophoresis and further processed using the NucleoSpin Gel and PCR Clean-up kit (Machery-Nagel, Germany) following manufacturer's protocol. PCR products of plastid regions were sent to a sequencing service (eurofins Genomics, Germany) to be sequenced on a 3730XL DNA Analyzer (Applied Bioscience).

For the Next Generation Sequencing approach, library preparation for genotyping-by-sequencing was carried out following the protocol of Wendler *et al.* (2014) and Merklinger *et al.* (2020) using 200 ng of genomic DNA and the restriction enzymes *Pst*I-HF (New England Biolabs, R3140S) and *Msp*I (New England Biolabs, R0106S). After a fragment size selection and barcode ligation step, processed DNA fragments were single-end sequenced on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA) at the Genome Center of the Leibniz Institute of Plant Genetics and Crop Plant Research (Germany).

Figure 1: Maximum Likelihood RAxML plastid phylogeny of *Atriplex* and images of species from the Atacama-Argentina clade (A-F). The three Atacama clades are marked with colored rectangles. Sample names accompanied by ED-numbers are from the present study and species names in bold italics indicate own samples collected in the Atacama Desert. Bootstrap support is only shown for nodes with support ≥ 50. Annotations on the tree are based on Kadereit *et al.* (2010). **A**+**B**) *Atriplex madariagae*; population at the mouth of Rio Loa (**A**) and part of an infructescence (**B**). **C**+**D**) *Atriplex retusa*; female flowers (**C**) and one individual at Las Bombas north of Chañeral (**D**). **E**+**F**) *Atriplex atacamensis*; at the type locality near San Pedro de Atacama (**E**) and part of an infructescence (**F**). ▶


A
B
C
D
E
F
Atriplex cordubensis
Atriplex undulata ED3980
Atriplex patagonica
Atriplex atacamensis ED1882
Atriplex chizae ED1385
Atriplex undulata
Atriplex lampa
Atriplex rusbyi
Atriplex atacamensis ED1899
Atriplex madariagae ED1901
Atriplex chizae ED1383
Atriplex chizae ED1399
Atriplex madariagae ED1394
Atriplex argentina ED3981
Atriplex lithophila ED3982
Atriplex retusa ED1909
Atriplex retusa ED1387
Atriplex retusa ED1925
Atriplex undulata ED3983
Atacama-Argentina clade
Atriplex phyllostegia
Atriplex serenana
Atriplex powellii
Atriplex parishii
Atriplex leucophylla
Atriplex lentiformis
Atriplex parryi
Atriplex canescens ED1823
Atriplex lentiformis ED1822
Atriplex lentiformis
Atriplex cf. **coquimbana** ED2962
Atriplex repanda ED1599
Atriplex repanda ED1599
Atriplex repanda ED1600
Atriplex leuca ED2967
Atriplex cf. **coquimbana** ED2963
Atriplex philippii ED2960
Atriplex myriophylla ED4720
Atriplex myriophylla
S. Atacama clade
Atriplex semibaccata ED1601
Atriplex semibaccata ED1594
Atriplex semibaccata ED3958
Atriplex cinerea
Atriplex suberecta
Atriplex muelleri
Atriplex sp. ED3957
Atriplex spongiosa
Atriplex suberecta ED1602
Atriplex vesicaria
Atriplex holocarpa
Atriplex rhagodioides
Atriplex rosea
Atriplex glauca
Atriplex pamirica
Atriplex centralasiatica
Atriplex sphaeromorpha
Atriplex altaica
Atriplex sibirica
Atriplex tatarica
Atriplex recurva
Atriplex laciniata
Atriplex dimorphostegia
Atriplex billardierei
Atriplex glauca
Atriplex undulata ED3979
Atriplex leucoclada
Atriplex sp. ED4724
Atriplex sp. ED4728
Atriplex sp. ED4735
Atriplex sp. ED4734
Atriplex sp. ED4732
Atriplex sp. ED4733
Atriplex sp. ED4717
Atriplex clivicola ED1396
Atriplex hystrix ED2964
Atriplex taltalense ED1400
Atriplex taltalense ED2968
Atriplex vallenarensis ED1923
Atriplex peruviana ED3554
Atriplex peruviana ED3555
Atriplex imbricata ED1398
Atriplex clivicola ED1397
Atriplex imbricata ED1896
Atriplex imbricata ED1905
Atriplex hystrix ED3211
Atriplex peruviana ED2974
Atriplex glaucescens ED1883
Atriplex sp. ED4731
Atriplex sp. ED4723
Atriplex sp. ED4727
Atriplex clivicola ED1918
Atriplex cf. peruviana ED4744
Atriplex rotundifolia ED4719
Atriplex sp. ED4718
Atriplex imbricata
Atriplex sp. ED4716
Atriplex sp. ED4721
Atriplex sp. ED4730
Atriplex peruviana ED2978
Atriplex sp ED4725
Atriplex vallenarensis ED1924
Atriplex glaucescens ED1903
Atriplex sp. ED4729
Atriplex sp. ED4726
Atacama-Peru clade
Atriplex flabellum
Atriplex moneta
Atriplex halimus
Atriplex leucophylla
Atriplex nummularia ED1598
Atriplex isatidea
Atriplex coriacea
Atriplex nudicaulis
Atriplex micrantha
Atriplex oblongifolia
Atriplex aucheri
Atriplex chilensis ED1596
Atriplex patula
Atriplex australasica
Atriplex latifolia
Atriplex patula
Atriplex prostrata
Atriplex glabriuscula
Atriplex crassifolia
Atriplex calotheca
Atriplex cana
Atriplex lanfrancoi
Atriplex halimus
Halimione portulacoides ED3985
Halimione pedunculata
Halimione verrucifera
Halimione pedunculata
Extriplex californica
Extriplex joaquinana
Stutzia covillei
Grayia spinosa
Grayia spinosa
Grayia brandegeei
Stutzia dioica
Holmbergia tweedii
Manochlamys albicans
Exomis microphylla
Archiatriplex nanpinensis
Archiatriplex clade
Microgynoecium tibeticum
Rhagodia drummondii
Rhagodia parabolica
Chenopodium auricomum
Chenopodium desertorum
Chenopodium nitrariaceum
Rhagodia drummondii
Einadia nutans
Chenopodium murale
Chenopodium acuminatum
Atriplex oreophila ED1916
Atriplex herzogii ED4722
Micromonolepis pusilla
Chenopodium oahuense
Chenopodium frutescens
Chenopodium sanctae-clarae
Microgynoecium tibeticum
Chenopodieae I
Chenopodium coronopus
Dysphania cristata
Dysphania glomulifera
Cycloloma atriplicifolium
Dysphania ambrosioides
Dysphania botrys
Suckleya suckleyana
Teloxys aristata
Dysphanieae
Scleroblitum atriplicinum
Chenopodium foliosum
Monolepis nuttalliana
Chenopodium foliosum
Spinacia oleracea
Chenopodium bonus-henricus
Chenopodieae II
Kraschenninikovia lanata
Kraschenninikovia ceratoides
Ceratocarpus arenarius
Axyris prostrata
Chenopodium urbicum
Axyrideae
Anthochlamys multinervis
Corispermum filifolium
Agriophyllum squarrosum

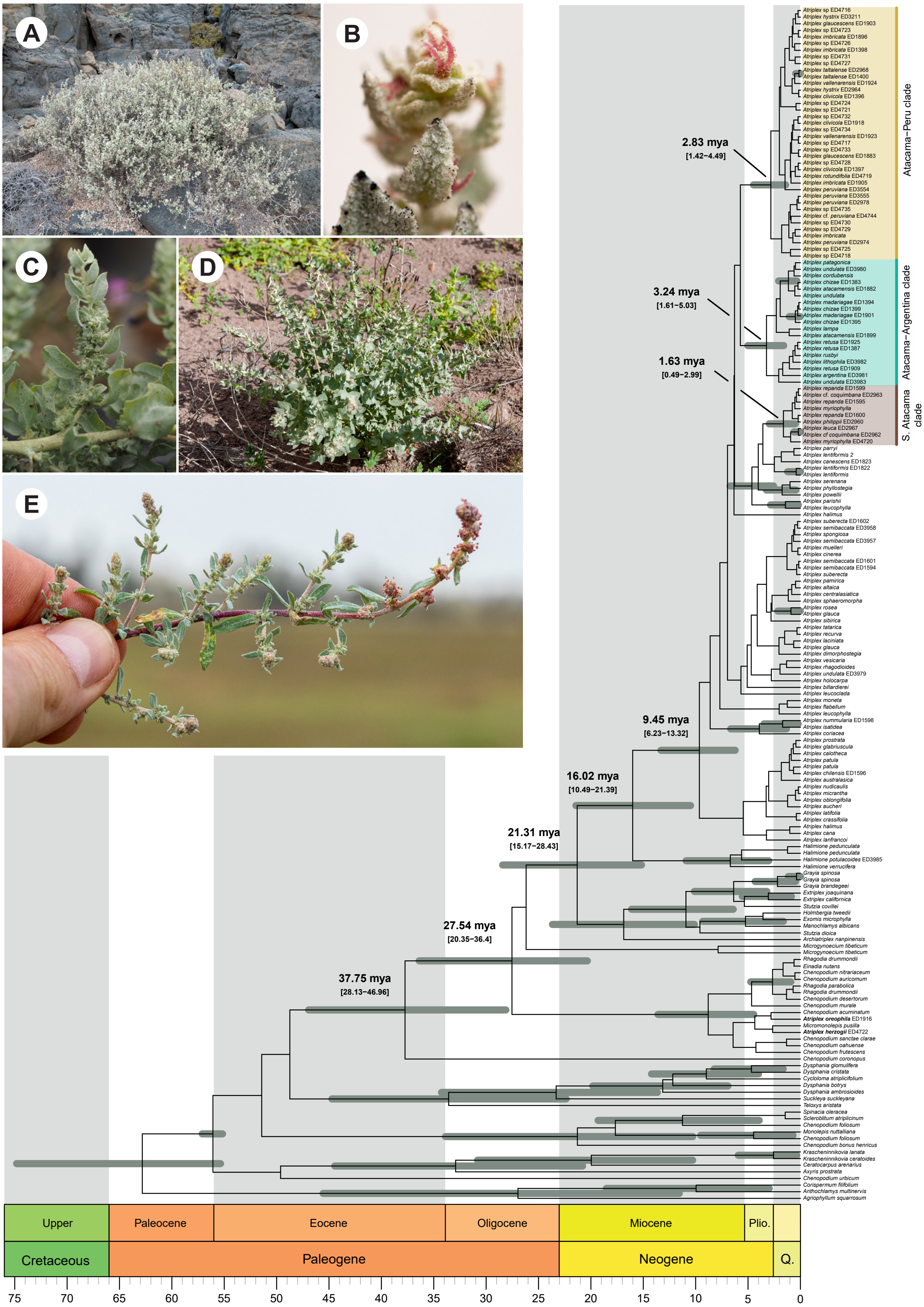
5.2.3 Plastid sequence alignment and phylogenetic analyses

Plastid DNA sequence data were quality trimmed, edited and manually aligned using PhyDe 0.9971 (Müller *et al.* 2005). In the *atp*B-*rbc*L alignment a single poly-A/T hotspot of 5 base pairs (bp) with overlapping microstructural mutations was detected and deleted for downstream analyses (Borsch *et al.* 2003; Löhne & Borsch 2005; Worberg *et al.* 2007). In order to evaluate phylogenetic relationships of the group prior to the divergence times estimation a Maximum Likelihood analysis using RAxML 8.2.10 (Stamatakis 2014) was conducted. We specified the recommended GTRCAT substitution model treating both markers as single partitions and conducted 1000 rapid bootstrap replicates to receive statistical support to tree nodes. The tree was plotted using the python package toytree 1.0.0 (Eaton 2019).

5.2.4 Molecular clock dating

In order to estimate divergence times of *Atriplex* in the Atacama Desert, we applied the Bayesian relaxed clock dating approach using BEAST 2.5.1 (Bouckaert *et al.* 2014). From the BEAST environment we used BEAUTI 2.5 (Bouckaert *et al.* 2014) to set up a XML files prior to the actual analysis. We linked the clock and the tree model but unlinked the site model for the two plastid DNA regions. A relaxed lognormal clock including an estimated clock rate as well as a birth-death model as a tree prior was set (Drummond *et al.* 2006; Gernhard 2008). Instead of specifying a specific substitution model we used the recently published BEAST2 package bModeltest (Bouckaert & Drummond 2017), which allows switching between substitution models during Markov Chain Monte Carlo (MCMC) estimation. For the calibration we followed the secondary calibration approach of Kadereit *et al.* (2010) by applying a normal distribution prior at the crown node of the Amaranthaceae/Chenopodiaceae alliance with a mean age of 56 Ma, a sigma value of 0.5 and an offset of 0.1. As described in Kadereit *et al.* (2010), a member of Corispermoideae were chosen as outgroup according to Müller & Borsch (2005). The actual analysis was performed with a length of 100 Million generations sampling every 10,000 trees. To see if the MCMC reached convergence, ESS values were checked in Tracer 1.7.1 (Rambaut *et al.* 2018) and a maximum clade credibility tree (MCCT) was summarized in TreeAnnotator with a 10 % burn-in, mean node heights and a posterior probability limit of 0.95. The final MCCT was plotted in R 3.5.1 (R Core Team 2018) using RStudio 1.1.463 (RStudio Team 2016), ape 5.0 (Paradis & Schliep 2019), phyloch 1.5-5 (Heibl 2008 onwards), strap 1.4 (Bell & Lloyd 2015) and geoscale 2.0 (Bell 2015).

Figure 2: Plastid BEAST2 Maximum clade credibility tree of *Atriplex* with outgroups from the Chenopodiaceae (A-E). Images are from species of the Atacama-Peru and the South Andean clade. Only for nodes with posterior probability support ≥ 95% HPD confidence intervals are plotted. The three Atacama clades are marked with colored rectangles. Sample names accompanied by ED-number are from the present study. **A**+**B**) *Atriplex clivicola*; from its type location at the coast north of Paposo (**A**) and close up of a female inflorescence (**B**). **C**) *Atriplex hystrix* at the coast near Los Chorros. **D**) *Atriplex glaucescens* in the Andes above Pica. **E**) *Atriplex philippii* from its type location near Batuco north of Santiago de Chile. ▶


A
B
C
D
E
2.83 mya
[1.42–4.49]
3.24 mya
[1.61–5.03]
1.63 mya
[0.49–2.99]
9.45 mya
[6.23–13.32]
16.02 mya
[10.49–21.39]
21.31 mya
[15.17–28.43]
27.54 mya
[20.35–36.4]
37.75 mya
[28.13–46.96]
Atacama–Peru clade
Atacama–Argentina clade
S. Atacama clade
Atriplex sp ED4716
Atriplex hystrix ED3211
Atriplex glaucescens ED1903
Atriplex sp ED4723
Atriplex imbricata ED1896
Atriplex sp ED4726
Atriplex imbricata ED1398
Atriplex sp ED4731
Atriplex sp ED4727
Atriplex taltalense ED2968
Atriplex taltalense ED1400
Atriplex vallenarensis ED1924
Atriplex hystrix ED2964
Atriplex clivicola ED1396
Atriplex sp ED4724
Atriplex sp ED4721
Atriplex sp ED4732
Atriplex clivicola ED1918
Atriplex sp ED4734
Atriplex vallenarensis ED1923
Atriplex sp ED4717
Atriplex sp ED4733
Atriplex glaucescens ED1883
Atriplex sp ED4728
Atriplex clivicola ED1397
Atriplex rotundifolia ED4719
Atriplex imbricata ED1905
Atriplex peruviana ED3554
Atriplex peruviana ED3555
Atriplex peruviana ED2978
Atriplex sp ED4735
Atriplex cf. *peruviana* ED4744
Atriplex sp ED4730
Atriplex sp ED4729
Atriplex imbricata
Atriplex peruviana ED2974
Atriplex sp ED4725
Atriplex sp ED4718
Atriplex patagonica
Atriplex undulata ED3980
Atriplex cordubensis
Atriplex chizae ED1383
Atriplex atacamensis ED1882
Atriplex undulata
Atriplex madariagae ED1394
Atriplex chizae ED1399
Atriplex madariagae ED1901
Atriplex chizae ED1395
Atriplex lampa
Atriplex atacamensis ED1899
Atriplex retusa ED1925
Atriplex retusa ED1387
Atriplex rusbyi
Atriplex lithophila ED3982
Atriplex retusa ED1909
Atriplex argentina ED3981
Atriplex undulata ED3983
Atriplex repanda ED1599
Atriplex cf. *coquimbana* ED2963
Atriplex repanda ED1595
Atriplex myriophylla
Atriplex repanda ED1600
Atriplex philippii ED2960
Atriplex leuca ED2967
Atriplex cf coquimbana ED2962
Atriplex myriophylla ED4720
Atriplex parryi
Atriplex lentiformis 2
Atriplex canescens ED1823
Atriplex lentiformis ED1822
Atriplex lentiformis
Atriplex serenana
Atriplex phyllostegia
Atriplex powellii
Atriplex parishii
Atriplex leucophylla
Atriplex halimus
Atriplex suberecta ED1602
Atriplex semibaccata ED3958
Atriplex spongiosa
Atriplex semibaccata ED3957
Atriplex muelleri
Atriplex cinerea
Atriplex semibaccata ED1601
Atriplex semibaccata ED1594
Atriplex suberecta
Atriplex pamirica
Atriplex altaica
Atriplex centralasiatica
Atriplex sphaeromorpha
Atriplex rosea
Atriplex glauca
Atriplex sibirica
Atriplex tatarica
Atriplex recurva
Atriplex laciniata
Atriplex glauca
Atriplex dimorphostegia
Atriplex vesicaria
Atriplex rhagodioides
Atriplex undulata ED3979
Atriplex holocarpa
Atriplex billardierei
Atriplex leucoclada
Atriplex moneta
Atriplex flabellum
Atriplex leucophylla
Atriplex nummularia ED1598
Atriplex isatidea
Atriplex coriacea
Atriplex prostrata
Atriplex glabriuscula
Atriplex calotheca
Atriplex patula
Atriplex patula
Atriplex chilensis ED1596
Atriplex australasica
Atriplex nudicaulis
Atriplex micrantha
Atriplex oblongifolia
Atriplex aucheri
Atriplex latifolia
Atriplex crassifolia
Atriplex halimus
Atriplex cana
Atriplex lanfrancoi
Halimione pedunculata
Halimione pedunculata
Halimione potulacoides ED3985
Halimione verrucifera
Grayia spinosa
Grayia spinosa
Grayia brandegeei
Extriplex joaquinana
Extriplex californica
Stutzia covillei
Holmbergia tweedii
Exomis microphylla
Manochlamys albicans
Stutzia dioica
Archiatriplex nanpinensis
Microgynoecium tibeticum
Microgynoecium tibeticum
Rhagodia drummondii
Einadia nutans
Chenopodium nitrariaceum
Chenopodium auricomum
Rhagodia parabolica
Rhagodia drummondii
Chenopodium desertorum
Chenopodium murale
Chenopodium acuminatum
Atriplex oreophila ED1916
Micromonolepis pusilla
Atriplex herzogii ED4722
Chenopodium sanctae clarae
Chenopodium oahuense
Chenopodium frutescens
Chenopodium coronopus
Dysphania glomulifera
Dysphania cristata
Cycloloma atriplicifolium
Dysphania botrys
Dysphania ambrosioides
Suckleya suckleyana
Teloxys aristata
Spinacia oleracea
Scleroblitum atriplicinum
Chenopodium foliosum
Monolepis nuttalliana
Chenopodium foliosum
Chenopodium bonus henricus
Krascheninnikovia lanata
Krascheninnikovia ceratoides
Ceratocarpus arenarius
Axyris prostrata
Chenopodium urbicum
Corispermum filifolium
Anthochlamys multinervis
Agriophyllum squarrosum
Upper
Paleocene
Eocene
Oligocene
Miocene
Plio.
Cretaceous
Paleogene
Neogene
Q.
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0

5.2.5 Assembly and phylogenetic analyses of GBS data

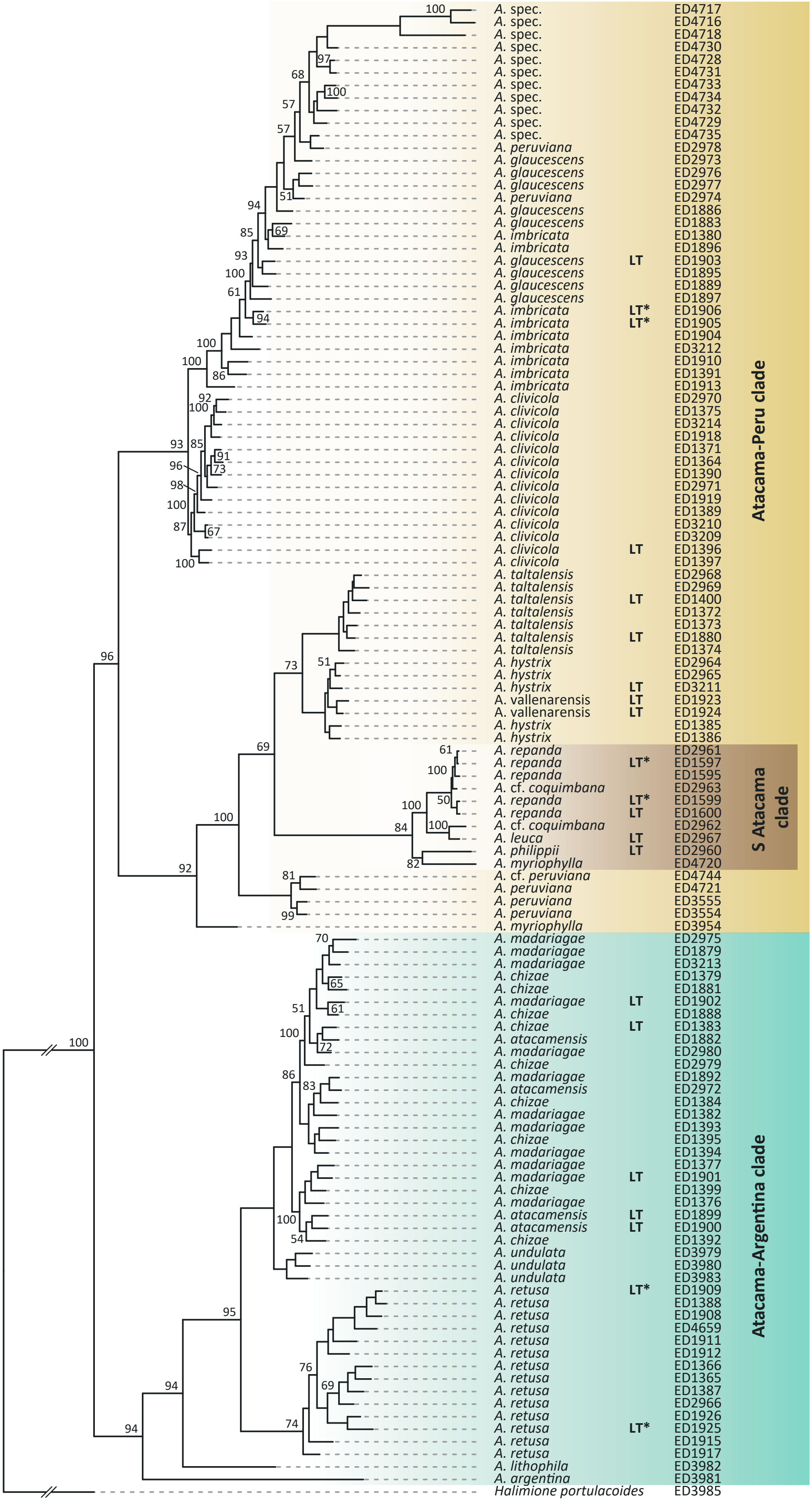
Barcoded raw reads were first de-multiplexed using CASAVA pipeline 1.8 (Illumina, Inc.), than adapter removed (-a AGATCGGAAGAGC), filtered for minimum read length (-m 50) and quality trimmed (-q 25) using CUTADAPT v.1.16 (Martin 2011). Finally, reads were quality checked using FastQC (Andrews *et al.* 2012). Preprocessed raw reads were *de novo* assembled using ipyrad v0.9.15 (Eaton & Overcast 2020), an interactive python assembly and analyses toolkit. Datatype was specified as 'ddRAD' as our GBS approach includes two restriction enzymes in contrast to the original method (Elshire *et al.* 2011). Three different data sets were assembled allowing always 70 % missing data in relation to the number of samples for the ingroup of each assembly (Huang & Knowles 2016): (1) a broad set comprising all *Atriplex* samples (Atri_pops30), (2) all samples from the South Andean and the Atacama-Peru clade (Atri_AtaPeru_leuca) and (3) the Atacama-Argentina clade (Atri_AtaArg). These clades were all retrieved in the phylogenetic analysis with plastid data (see above). *Halimione portulacoides* (L.) Aellen was always set as an outgroup due to its sister relationship to *Atriplex* (Kadereit *et al.* 2010). Assembly and phylogenetic reconstruction using RAxML 8.2.12 (Stamatakis 2014) was conducted within the ipyrad API (Eaton & Overcast 2020) written in a Jupyter-Notebook. Assembly statistics, the code for the assembly and phylogenetic reconstruction is available in Appendix C.

5.3 Results

5.3.1 Plastid phylogeny and divergence times estimation

For the initial plastid Maximum Likelihood (ML) and subsequent BEAST2 analyses a set of 72 samples was complemented with additional 98 samples from online resources. The final alignment consisted of 2,499 bp and 766 distinct alignment patterns after hotspot regions were removed. Topology of the backbone is congruent between ML and BEAST2 trees (Figure 1 & 2). The monophyly of *Halimione* Aellen and *Atriplex*, respectively, is fully supported as well as their sister relationship to each other. The last common ancestor of both genera is dated to the mid-Miocene at approx. 16.02 Ma (95% HPD: 10.49–21.39 Ma), while *Atriplex* started to diversify in the late Miocene (9.45 Ma, 95% HPD: 6.23–13.32 Ma). *Atriplex* is monophyletic, except for the two Andean taxa *Atriplex oreophila* Phil. from the Chilean and *Atriplex herzogii* Standl. from the Peruvian Andes. These two taxa group together with *Micromonolepis pusilla* (Torr. ex S.Watson) Ulbr. from North America in the Chenopodieae II clade (Figure 1) with low statistical support. *Atriplex chilensis* Colla from the coastal region of central Chile comes out in a clade comprising Eurasian and North American taxa. The statistical support for nodes

Figure 3: GBS Maximum Likelihood phylogeny of all *Atriplex* samples from the Atacama Desert and adjacent countries with *Halimione portulacoides* as outgroup. Bootstrap support is only shown for nodes with support ≥ 50. Specimen collected at type locations (locotypes) are marked by LT. Locotypes of a taxonomic synonym are marked additionally by an asterisk (LT*). ▶


A. spec. ED4717
A. spec. ED4716
A. spec. ED4718
A. spec. ED4730
A. spec. ED4728
A. spec. ED4731
A. spec. ED4733
A. spec. ED4734
A. spec. ED4732
A. spec. ED4729
A. spec. ED4735
A. peruviana ED2978
A. glaucescens ED2973
A. glaucescens ED2976
A. glaucescens ED2977
A. peruviana ED2974
A. glaucescens ED1886
A. glaucescens ED1883
A. imbricata ED1380
A. imbricata ED1896
A. glaucescens LT ED1903
A. glaucescens ED1895
A. glaucescens ED1889
A. glaucescens ED1897
A. imbricata LT* ED1906
A. imbricata LT* ED1905
A. imbricata ED1904
A. imbricata ED3212
A. imbricata ED1910
A. imbricata ED1391
A. imbricata ED1913
A. clivicola ED2970
A. clivicola ED1375
A. clivicola ED3214
A. clivicola ED1918
A. clivicola ED1371
A. clivicola ED1364
A. clivicola ED1390
A. clivicola ED2971
A. clivicola ED1919
A. clivicola ED1389
A. clivicola ED3210
A. clivicola ED3209
A. clivicola LT ED1396
A. clivicola ED1397
A. taltalensis ED2968
A. taltalensis ED2969
A. taltalensis LT ED1400
A. taltalensis ED1372
A. taltalensis ED1373
A. taltalensis LT ED1880
A. taltalensis ED1374
A. hystrix ED2964
A. hystrix ED2965
A. hystrix LT ED3211
A. vallenarensis LT ED1923
A. vallenarensis LT ED1924
A. hystrix ED1385
A. hystrix ED1386
A. repanda ED2961
A. repanda LT* ED1597
A. repanda ED1595
A. cf. coquimbana ED2963
A. repanda LT* ED1599
A. repanda LT ED1600
A. cf. coquimbana ED2962
A. leuca LT ED2967
A. philippii LT ED2960
A. myriophylla ED4720
A. cf. peruviana ED4744
A. peruviana ED4721
A. peruviana ED3555
A. peruviana ED3554
A. myriophylla ED3954
A. madariagae ED2975
A. madariagae ED1879
A. madariagae ED3213
A. chizae ED1379
A. chizae ED1881
A. madariagae LT ED1902
A. chizae ED1888
A. chizae LT ED1383
A. atacamensis ED1882
A. madariagae ED2980
A. chizae ED2979
A. madariagae ED1892
A. atacamensis ED2972
A. chizae ED1384
A. madariagae ED1382
A. madariagae ED1393
A. chizae ED1395
A. madariagae ED1394
A. madariagae ED1377
A. madariagae LT ED1901
A. chizae ED1399
A. madariagae ED1376
A. atacamensis LT ED1899
A. atacamensis LT ED1900
A. chizae ED1392
A. undulata ED3979
A. undulata ED3980
A. undulata ED3983
A. retusa LT* ED1909
A. retusa ED1388
A. retusa ED1908
A. retusa ED4659
A. retusa ED1911
A. retusa ED1912
A. retusa ED1366
A. retusa ED1365
A. retusa ED1387
A. retusa ED2966
A. retusa ED1926
A. retusa LT* ED1925
A. retusa ED1915
A. retusa ED1917
A. lithophila ED3982
A. argentina ED3981
Halimione portulacoides ED3985
Atacama-Peru clade
S Atacama clade
Atacama-Argentina clade
100
97
68
100
57
57
51
94
69
85
93
100
61
94
100
100
86
92
100
93
85
91
96
73
98
100
87
67
100
96
51
73
69
61
100
100
50
84
100
82
92
81
99
100
70
65
51
61
100
72
86
83
100
54
95
76
69
94
74
94

within *Atriplex* is, however, relatively low in both trees. The South Andean clade receives moderate Bootstrap support (80 BS) in the ML tree, while the Atacama-Argentina clade is only weakly supported and the Atacama-Peru clade is not supported at all. In contrast, the BEAST tree retrieves four clades with posterior probabilities ≥ 0.95. The oldest among the three Atacama clades comprises taxa from Argentina and the Atacama Desert (Atacama-Argentina clade) with a crown node age of 3.24 Ma (95% HPD: 1.62–5.03 Ma). It comprises a group of taxa of large shrubs found mainly in the dry central part of the Atacama Desert (e.g., *A. atacamensis* Phil. and *A. retusa* Gay; Figure 1 A–F) and the dry regions of Argentina (e.g. ,*A. argentina* Speg. and *A. undulata* (Moq.) D.Dietr.). The Atacama-Peru clade with a crown node age of 2.83 Ma (95% HPD: 1.42–4.49 Ma), comprises taxa from the coast (e.g., *A. hystrix* Phil., *A. clivicola* I.M.Johnston and *A. taltalensis* I.M.Johnston; Figure 2 A–C) and the Andean part of the Atacama Desert (e.g., *A. glaucescens* Phil. or *A. imbricate* D.Dietr.; Figure 2 D), as well as from Peru (e.g., *A. peruviana* Moq. and *A. rotundifolia* Dombey ex Moq.). The South Andean clade (1.63 Ma, 95% HPD: 0.49–2.99 Ma) is the youngest clade embedded in a clade with taxa from North America. It includes the local endemic *A. philippii* R.E.Fr. (Figure 2 E) from the Santiago Basin, *A. repanda* Phil. from South of La Serena as well as *A. leuca* Phil. from the area between Vallenar and Copiapó.

5.3.2 GBS phylogeny and distribution of clades

The backbone of the GBS phylogeny comprising all 119 samples is well supported (≥ 90 BS) resulting in two major clades, having the Atacama-Argentina clade placed as sister to the remaining samples (Figure 3). Topologies of the Atacama-Argentina clade in Figure 3 and the reduced assembly with only samples from that clade (Figure 4) are highly congruent, but receive higher support in the latter. In both trees, *A. argentina* and *A. lithophila* A.Soriano ex Múlgura (both from Argentina), are consecutive sisters to the remaining samples. The position of *A. retusa* as sister to the remaining species is fully supported in Figure 4, while *A. undulata* from Argentina is again sister to a clade comprising large shrubby taxa from the northern part of the Atacama Desert, namely *A. atacamensis*, *A. chizae* Rosas and *A. madariagae* Phil. (ACM clade; Figure 4). However, support in this group is rather low and while *A. retusa* is geographically clearly circumscribed, the other three species do not show any geographical signal as shown in the distribution map of Figure 4.

The topology of the Atacama-Peru and the South Andean clade in Figure 5 is largely congruent with the topology in Figure 3 and retrieves two main clades. The first one comprises taxa mainly from the southern part of the Atacama Desert, e.g. *A. taltalensis, A. hystrix* and *A. vallenarensis* Rosas, as well as from further south (S Atacama clade). *Atriplex myriophylla* Phil. and a clade including *A. peruviana* are sister to those taxa. *Atriplex vallenarensis*, found only in the vicinity of Vallenar, is nested in *A. hystrix*. In Figure 3, the monophyly of the South Andean clade is only weakly supported and it is retrieved as sister to the coastal taxa *A. taltalensis*, *A. hystrix* and *A. vallenarensis*. In Figure 5, however, those coastal taxa are

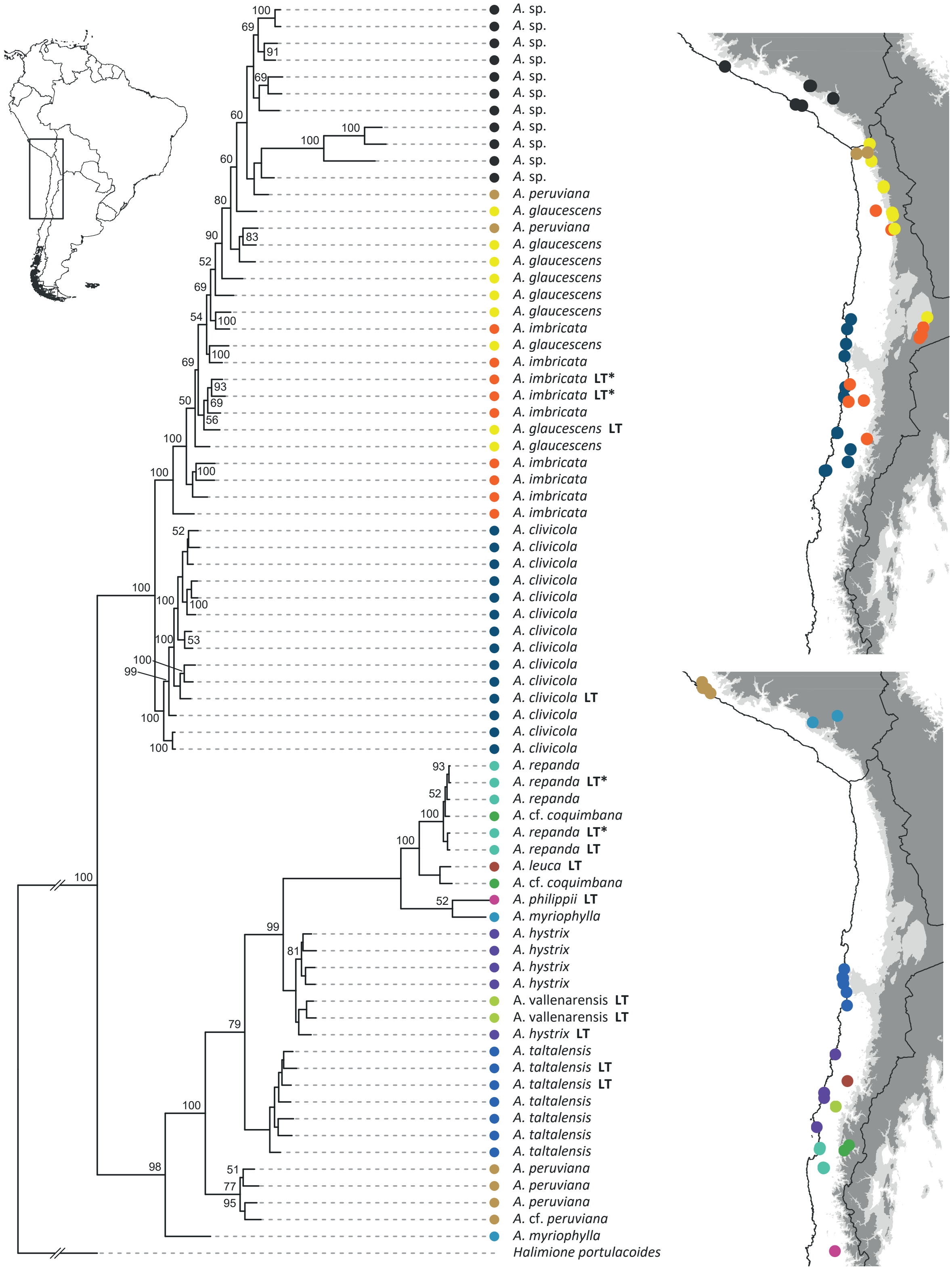


Figure 4: Reduced GBS Maximum Likelihood phylogeny of the *Atriplex* Atacama-Peru clade and its distribution plotted on a map. *Halimione portulacoides* was used as outgroup. Bootstrap support is only shown for nodes with support ≥ 50. Colored dots at the tips of the phylogeny correspond to collection localities in the Atacama Desert and Argentina shown in the two maps on the right. The maps cover the area of northern Chile and parts of southern Peru; bright and dark gray areas indicate Andean elevations above 2000 m and 3000 m, respectively. Specimens collected at type locations (locotypes) are marked by LT. Locotypes of a taxonomic synonym are marked additionally by an asterisk (LT*).

consecutive sister groups to the South Andean clade which receives less support compared to Figure 3, although the topology is congruent. The local endemic *A. philippii* groups together with *A. myriophylla* from Peru and both are sister to a clade comprising *A. leuca* and *A.* cf. *coquimbana*. The remaining taxa, *A. repanda* and another sample of *A.* cf. *coquimbana*, are monophyletic in both trees (Figure 3 & 5), but receives support in Figure 5. The second clade consists of one coastal species (*A. clivicola*) and the two mainly Andean taxa, *A. imbricata* and *A. glaucescens*. *Atriplex clivicola* receives highest support also on internal branches. In contrast, the Andean clade of *A. imbricata* and *A. glaucescens* and the undetermined taxa from southern Peru show more latitudinal/geographical signal than morphological signal. Specimens of *A. peruviana* were retrieved in both clades, rendering the species polyphyletic under its current taxonomic concept.

5.4 Discussion

5.4.1 Plastid phylogeny, molecular dating and GBS trees

The present dataset includes all accepted species of *Atriplex* from the Atacama Desert and the majority of species documented for Chile and Peru (Rosas 1989; Brako & Zarucchi 1993; Brignone *et al.* 2016). It is thus the most comprehensive dataset for the genus from this region to date. A South America-wide study of the genus *Atriplex*, including the majority of all native species, was recently presented by Brignone *et al.* (2019). Our plastid dataset is based on the sampling of Kadereit *et al.* (2010) and we expanded their dataset with 72 additional samples covering a wide geographical range from Chile, Peru and Argentina. We sequenced two plastid markers (*rbc*L & *atp*B-*rbc*L), but – unlike Kadereit *et al.* (2010) – did not include the nuclear marker (ITS), yet. Different from Kadereit *et al.* (2010) we analyzed both plastid regions together in a concatenated alignment approach, rather than separately, taking into account a certain proportion of missing data (Crawley & Hilu 2012), but also receiving higher branch support. The topology of our plastid phylogenies (Figures 1 & 2) is largely congruent with recent publications on the genus *Atriplex* (Kadereit *et al.* 2010; Zacharias & Baldwin 2010; Brignone *et al.* 2019): The genus *Halimione* is retrieved as sister to *Atriplex* and the members of the C_3 clade are sister to the C_4 crown group (Brignone *et al.* 2019). Resolution within *Atriplex* is, however, weakly supported as in other published studies. Nevertheless, we retrieved three clades within the Atacama Desert, which received moderate support in the ML tree, but high support in the BEAST tree (≥ 0.95 pp). Support is high enough to use the phylogeny for age estimations for the relevant nodes. Brignone *et al.* (2019) identified three South American clades which are, however, not fully congruent with our results: The South America 1 clade including *A. argentina* is sister to the remaining taxa from South and North America, although its position receives low support. Conversely, *A. argentina* is retrieved in the Atacama-Argentina clade in our phylogenies (Figure 1 & 2). We further found incongruences in the South America 2 & 3 clades. For example, *A. atacamensis* and

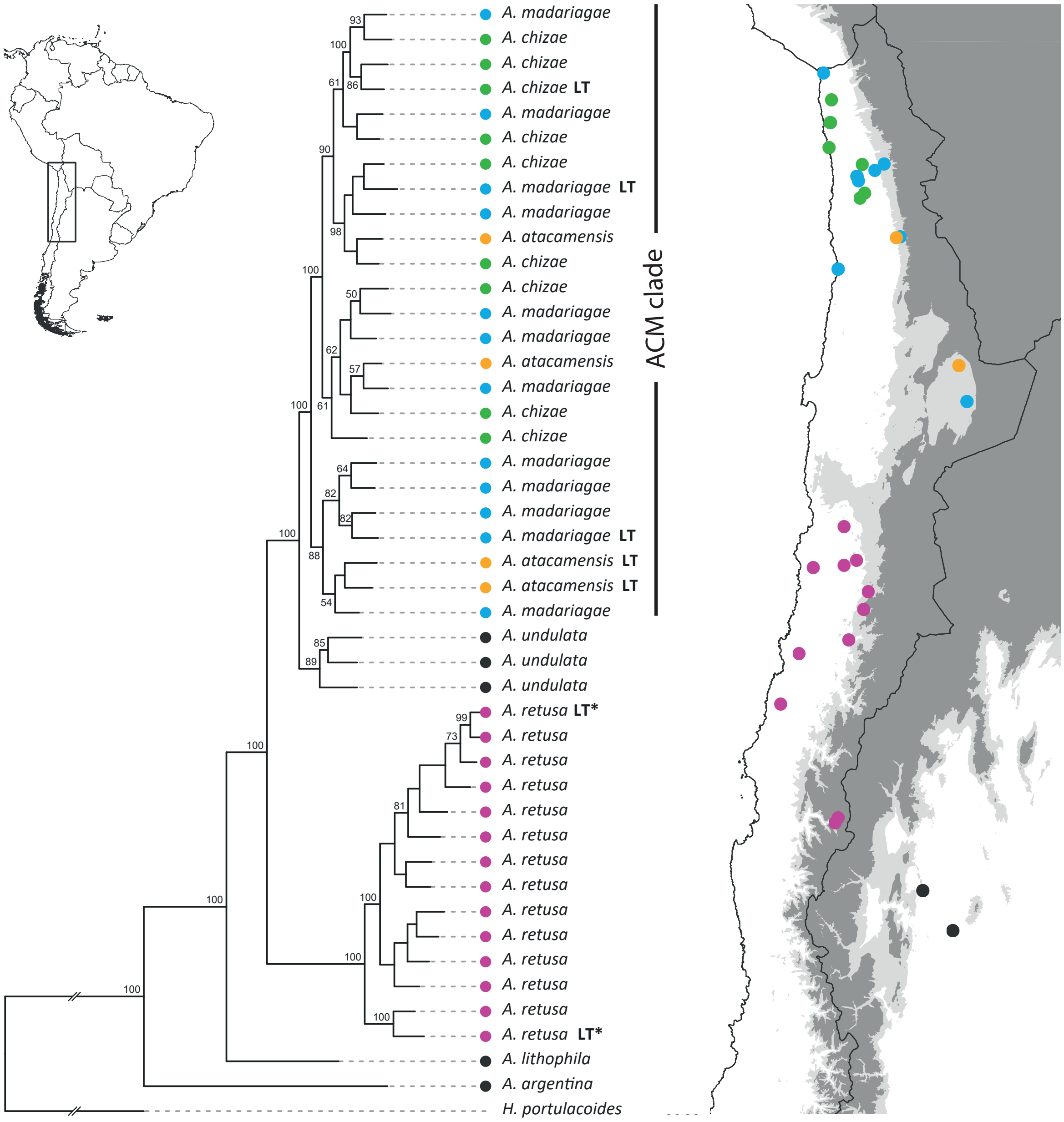


Figure 5: Reduced GBS Maximum Likelihood phylogeny of the Atacama-Argentina clade of *Atriplex* and the distribution of species plotted on a map. *Halimione portulacoides* was used as outgroup. Bootstrap support is only shown for nodes with support ≥ 50. Colored dots at the tips of the phylogeny indicate their collection localities in the Atacama Desert and Argentina as shown in the map on the right. The map covers the area of northern Chile; the bright and dark grey areas indicate Andean elevations above 2000 m and 3000 m, respectively. Specimen collected at type locations (Locotypes) are marked by LT. Locotypes of a taxonomic synonym are marked additionally by an asterisk (LT*).

A. chizae are retrieved on two different clades in Brignone *et al.* (2019), but are retrieved in a highly supported clade together with *A. madariagae* in our GBS phylogeny (Figure 4). Another and even more striking incongruence between our results and the study of Brignone *et al.* (2019) is the position of *Atriplex oreophila*. In our phylogenies it is found in a clade with *Atriplex herzogii* (synonymized by Brignone *et al.* (2016) under *A. myriophylla*) and the North American *Micromonolepis pusilla*, while placed in the South America 2 clade (Brignone *et al.*

2019). We are fairly certain of our determination, since material of *A. oreophila* was collected at the type location in the Quebrada Codocedo in the Province of Copiapó (Rosas 1989). Evidently, a careful reexamination of the taxa in this clade and their relation to the North American monotypic *Micromonolepis* is required. Unfortunately, the sequences generated by Brignone and colleagues (2019) were not available on GenBank during the preparation of this manuscript and could not be included in our analysis for purposes of comparison.

Compared to the plastid phylogenies where three individual clades find support, we retrieved only two clades in the total GBS analysis, with the South Atacama clade nested in what we call the Atacama-Peru clade. However, its position within this clade varies between the overall GBS tree (Figure 3) and the reduced sampling (Figure 5), but in both cases receives only moderate support. A further reduced sampling might lead to a better understanding of the Atacama taxa (Huang & Knowles 2016). Here, especially three clades need further attention: the two morphologically closely related, but geographically well separated taxa *A. taltalensis* and *A. hystrix*, the clade comprising the Andean taxa *A. imbricata*, *A. glaucescens*, *A. peruviana* as well as not yet identified specimens from Peru, and finally the ACM clade, which encompasses three partly sympatric taxa with high morphological similarities.

A recent taxonomic revision is available for *Atriplex* (Brignone *et al.* 2016), but our current data show that several taxonomic questions in South American *Atriplex* are still open (Böhnert *et al.* 2018; Chapter 3). In Chile, the delimitation of *A. oreophila* from *A. herzogii* remains enigmatic and it must be seen if these two species really belong into *Atriplex* or need to be placed into *Micromonolepis*. Similarly, we find *A. vallenarensis* (Rosas 1989) nested in a clade with *A. hystrix*, but with low support. The situation is considerably more complex in Peru where our data are at odds with species limits, e.g., in *Atriplex peruviana*.

5.4.2 Biogeography and Floristic elements

Most genera from the Atacama Desert can be readily assigned to floristic elements, e.g., *Fagonia chilensis* Hook. & Arn (Zygophyllaceae) has its closest relatives in North America (amphitropical disjunction; Simpson *et al.* 2017), while *Bulnesia chilensis* Gay (Zygophyllaceae) has its closest relative on the east side of the Andes (chapter 3). The pattern for *Atriplex* is, however not that simple: representatives of the genus are found in all neighboring regions, the historical biogeography has been enigmatic in the past and the genus meets the criteria defining the four floristic elements presented by Luebert (2011). Our data clearly underscore the multiple external connections of *Atriplex* in the Atacama Desert. Floristic connectivity of Atacama taxa to Mediterranean Central Chile is represented by the South Andean clade with central Chilean of *A. phillipii* as sister to Atacama *A. leuca*. A neotropical connection to the southern Peruvian coast is documented by Peruvian *A. peruviana* (four samples) sister to the coastal Atacama species *A. hystrix* and *A. taltalense*. Several trans-Andean connections, probably in both directions, are documented with Argentinian *A. argentina* and *A. lithophila* sister to the Atacama clade comprising *A. retusa* and the ACM clade. However, trans-Andean dispersal must have happened more frequently than previously thought as *A. retusa* appears to be amphi-Andean (also known from Argentina; Brignone *et al.* 2016; Böhnert *et al.* 2018; Brignone *et al.* 2019) and *A. undulata* from Argentina is sister to the ACM clade. Finally, regarding amphitropical patterns, Brignone *et al.* (2019) have already shown that North America was colonized by *Atriplex* coming from South America followed by a recolonization from North to South America.

For *Atriplex* at least four biogeographic patterns within the Atacama Desert can be recognized, two of which are represented in the Atacama-Argentina clade. Here, the sister groups *A. retusa*, found in the southern pampa region, and the ACM clade, distributed in northern part of the Atacama Desert, are separated by the Dry Diagonal (Houston 2006), although both groups occupy similar habitats. In addition to those two patterns, in the Atacama-Peru clade some taxa occur in the southern portion of the Atacama Desert with few taxa (e.g., *A. leuca*) found in the southern pampa (South Atacama clade), while others are distributed along the coast (e.g., *A. hystrix* & *A. taltalensis*).

5.4.3 Critical remarks and outlook

It has been pointed out that the approach of molecular clock dating with plastid data alone might be problematic (Middleton *et al.* 2014; Vargas *et al.* 2017). Unfortunately, we were not able to incorporate an identical sampling of the nuclear marker ITS. In order to increase methodical congruence with other datasets discussed in this paper (e.g. Kadereit *et al.* 2010; Brignone *et al.* 2019) an inclusion of nuclear data would be desirable. Regarding the GBS data, however, as can be seen in some of the resulting phylogenies, internal support of some clades is very low. In order to increase phylogenetic resolution, it is possible to further reduce the sampling in additional assemblies including only those clades with low resolution (Huang & Knowles 2016; Tripp *et al.* 2017). Molecular clock dating methods might shed further light on the influence of quaternary climate oscillation on the coastal taxa (see for example *Cristaria* in chapter 7). Methods such as species delimitation tools in a coalescent framework (Bryant *et al.* 2012; Stange *et al.* 2018) or D-statistics (Eaton *et al.* 2015) can be used to gain a better understanding of the evolutionary history in the ACM clade.

5.4.4 Conclusion

Although the results presented here for the genus *Atriplex* must be regarded as preliminary, they provide by far the most comprehensive overview of phylogenetic relationship and evolution of the group in the Atacama Desert to date. It has been shown that the species diversity of *Atriplex* in the Atacama Desert is not the result of a single colonization event followed by *in situ* diversification. Rather, it is the result of a recent and quite complex history of multiple incursions into the Atacama Desert followed by divergence in individual clades. Finally, this is the third example providing evidence of a direct floristic connections between the Atacama Desert and Peru along the Andes (see chapter 2 for a general floristic approach and chapter 7 for an example in the genus *Cristaria*).

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5.6 References

Andrews S, Krueger F, Segonds-Pichon A, Biggins L, Krueger C & Wingett S (2012) FastQC. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

APG IV (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society* 181 (1): 1–20. DOI: https://doi.org/10.1111/boj.12385.

Barniske A-M, Borsch T, Müller KF, Krug M, Worberg A, Neinhuis C & Quandt D (2012) Phylogenetics of early branching eudicots: Comparing phylogenetic signal across plastid introns, spacers, and genes. *Journal of Systematics and Evolution* 50 (2): 85–108. DOI: https://doi.org/10.1111/j.1759-6831.2012.00181.x.

Bell MA (2015) geoscale: Geological Time Scale Plotting. https://CRAN.R-project.org/package=geoscale.

Bell MA & Lloyd GT (2015) strap: an R package for plotting phylogenies against stratigraphy and assessing their stratigraphic congruence. *Palaeontology* 58 (2): 379–389. DOI: https://doi.org/10.1111/pala.12142.

Bielig LM & Brownell PF (1990) Hybridization Studies Using Australian C_4 *Atriplex* Species and the C_3 Species *A. prostrata*. *Botanical Gazette* 151 (2): 151–157.

Blackwell WH & Powell MJ (1981) A Preliminary Note on Pollination in the Chenopodiaceae. *Annals of the Missouri Botanical Garden* 68 (4): 524. DOI: https://doi.org/10.2307/2398886.

Böhnert T, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider JV, Quandt D & Weigend M (2019) Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert. *Global and Planetary Change* 181: 102992. DOI: https://doi.org/10.1016/j.gloplacha.2019.102992.

Böhnert T, Luebert F & Weigend M (2018) *Atriplex retusa*, the correct name for *A. deserticola* (Chenopodiaceae; Amaranthaceae sensu APG). *Phytotaxa* 373 (1): 95–98. DOI: https://doi.org/10.11646/phytotaxa.373.1.6.

Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C & Barthlott W (2003) Noncoding plastid *trn*T-*trn*F sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology* 16 (4): 558–576. DOI: https://doi.org/10.1046/j.1420-9101.2003.00577.x.

Bouckaert RR & Drummond AJ (2017) bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology* 17 (1): 42. DOI: https://doi.org/10.1186/s12862-017-0890-6.

Bouckaert RR, Heled J, Kuhnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A & Drummond AJ (2014) BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10 (4): e1003537. DOI: https://doi.org/10.1371/journal.pcbi.1003537.

Brako L & Zarucchi JL (1993) Catalogue of the flowering plants and gymnosperms of Peru. St. Louis, Mo.: *Missouri Botanical Garden*. 1286 pp.

Brignone NF, Denham SS & Pozner R (2016) Synopsis of the genus *Atriplex* (Amaranthaceae, Chenopodioideae) for South America. *Australian Systematic Botany* 29 (5): 324. DOI: https://doi.org/10.1071/SB16026.

Brignone NF, Pozner RE & Denham SS (2019) Origin and evolution of *Atriplex* (Amaranthaceae s.l.) in the Americas: Unexpected insights from South American species. *Taxon* 18 (8): 71. DOI: https://doi.org/10.1002/tax.12133.

Bryant D, Bouckaert R, Felsenstein J, Rosenberg NA & RoyChoudhury A (2012) Inferring species trees directly from biallelic genetic markers: bypassing gene trees in a full coalescent analysis. *Molecular Biology and Evolution* 29 (8): 1917–1932. DOI: https://doi.org/10.1093/molbev/mss086.

Crawley SS & Hilu KW (2012) Impact of missing data, gene choice, and taxon sampling on phylogenetic reconstruction: the Caryophyllales (angiosperms). *Plant Systematics and Evolution* 298 (2): 297–312. DOI: https://doi.org/10.1007/s00606-011-0544-x.

Dillon MO & Hoffmann AE (1997) Lomas formations of the Atacama Desert northern Chile. In: Davis SD, Heywood VH, Herrera-MacBryde O, Villa-Lobos J & Hamilton AC, eds. *Centres of Plant Diversity, a Guide and Strategy for Their Conservation. Volume 3: The Americas*. United Kingdom: The World Wildlife Fund For Nature and IUCN - The World Conservation Union, *pp* 528–535.

Dillon MO, Tu T, Xie L, Quipuscoa Silvestre V & Wen J (2009) Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution* 47 (5): 457–476. DOI: https://doi.org/10.1111/j.1759-6831.2009.00040.x.

Drummond AJ, Ho SYW, Phillips MJ & Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology* 4 (5): e88. DOI: https://doi.org/10.1371/journal.pbio.0040088.

Dunai TJ, González López GA & Juez-Larré J (2005) Oligocene–Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. *Geology* 33 (4): 321–324. DOI: https://doi.org/10.1130/G21184.1.

Eaton DAR (2019) Toytree: A minimalist tree visualization and manipulation library for Python. *Methods in Ecology and Evolution* 12: 1–5. DOI: https://doi.org/10.1111/2041-210X.13313.

Eaton DAR, Hipp AL, González-Rodríguez A & Cavender-Bares J (2015) Historical introgression among the American live oaks and the comparative nature of tests for introgression. *Evolution* 69 (10): 2587–2601. DOI: https://doi.org/10.1111/evo.12758.

Eaton DAR & Overcast I (2020) ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*. DOI: https://doi.org/10.1093/bioinformatics/btz966.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES & Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* 6 (5): e19379. DOI: https://doi.org/10.1371/journal.pone.0019379.

Flores-Olvera H, Vrijdaghs A, Ochoterena H & Smets E (2011) The need to re-investigate the nature of homoplastic characters: An ontogenetic case study of the ‘bracteoles’ in Atripliceae (Chenopodiaceae). *Annals of Botany* 108 (5): 847–865. DOI: https://doi.org/10.1093/aob/mcr203.

Garreaud RD (2009) The Andes climate and weather. *Advances in Geosciences* 22: 3–11. DOI: https://doi.org/10.5194/adgeo-22-3-2009.

Gengler–Nowak K (2002) Reconstruction of the Biogeographical History of Malesherbiaceae. *The Botanical Review* 68 (1): 171–188. DOI: https://doi.org/10.1663/0006-8101(2002)068[0171:ROTBHO]2.0.CO;2.

Gernhard T (2008) The conditioned reconstructed process. *Journal of Theoretical Biology* 253 (4): 769–778. DOI: https://doi.org/10.1016/j.jtbi.2008.04.005.

Guilliams CM, Hasenstab-Lehman KE, Mabry ME & Simpson MG (2017) Memoirs of a frequent flier: Phylogenomics reveals 18 long-distance dispersals between North America and South America in the popcorn flowers (Amsinckiinae). *American Journal of Botany* 104 (11): 1717–1728. DOI: https://doi.org/10.3732/ajb.1700369.

Hartley AJ, Chong G, Houston J & Mather AE (2005) 150 million years of climatic stability: Evidence from the Atacama Desert, northern Chile. *Journal of the Geological Society* 162 (3): 421–424. DOI: https://doi.org/10.1144/0016-764904-071.

Heibl C (2008 onwards) PHYLOCH: R language tree plotting tools and interfaces to diverse phylogenetic software packages. http://www.christophheibl.de/Rpackages.html.

Heibl C & Renner SS (2012) Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. *Systematic Biology* 61 (5): 823–834. DOI: https://doi.org/10.1093/sysbio/sys034.

Houston J (2006) Variability of precipitation in the Atacama Desert: Its causes and hydrological impact. *International Journal of Climatology* 26 (15): 2181–2198. DOI: https://doi.org/10.1002/joc.1359.

Houston J & Hartley AJ (2003) The central Andean west-slope rainshadow and its potential contribution to the origin of hyper-aridity in the Atacama Desert. *International Journal of Climatology* 23 (12): 1453–1464. DOI: https://doi.org/10.1002/joc.938.

Huang H & Knowles LL (2016) Unforeseen Consequences of Excluding Missing Data from Next-Generation Sequences: Simulation Study of RAD Sequences. *Systematic Biology* 65 (3): 357–365. DOI: https://doi.org/10.1093/sysbio/syu046.

Kadereit G, Ackerly D & Pirie MD (2012) A broader model for C_4 photosynthesis evolution in plants inferred from the goosefoot family (Chenopodiaceae s.s.). *Proceedings. Biological Sciences* 279 (1741): 3304–3311. DOI: https://doi.org/10.1098/rspb.2012.0440.

Kadereit G, Borsch T, Weising K & Freitag H (2003) Phylogeny of Amaranthaceae and Chenopodiaceae and the Evolution of C 4 Photosynthesis. *International Journal of Plant Sciences* 164 (6): 959–986. DOI: https://doi.org/10.1086/378649.

Kadereit G, Mavrodiev EV, Zacharias EH & Sukhorukov AP (2010) Molecular phylogeny of Atripliceae (Chenopodioideae, Chenopodiaceae): Implications for systematics, biogeography, flower and fruit evolution, and the origin of C4 photosynthesis. *American Journal of Botany* 97 (10): 1664–1687. DOI: https://doi.org/10.3732/ajb.1000169.

Krak K, Habibi F, Douda J, Vít P, Lomonosova MN, Wang L & Mandák B (2019) Human-mediated dispersal of weed species during the Holocene: A case study of *Chenopodium album* agg. *Journal of Biogeography* 46 (5): 1007–1019. DOI: https://doi.org/10.1111/jbi.13545.

Kühn U (1993) Chenopodiaceae. In: Kubitzki K, Rohwer JG & Bittrich V, eds. *The Families and genera of vascular plants*. *II Flowering plants · dicotyledons*. Magnoliid, Hamamelid and Caryophyllid Families. Berlin, New York: Springer-Verlag.

Lindqvist C & Albert VA (2002) Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). *American Journal of Botany* 89 (10): 1709–1724. DOI: https://doi.org/10.3732/ajb.89.10.1709.

Löhne C & Borsch T (2005) Molecular evolution and phylogenetic utility of the *petD* group II intron: a case study in basal angiosperms. *Molecular Biology and Evolution* 22 (2): 317–332. DOI: https://doi.org/10.1093/molbev/msi019.

Luebert F (2011) Hacia una fitogeografía histórica del Desierto de Atacama. *Revista de Geografía Norte Grande (*50): 105–133. DOI: https://doi.org/10.4067/S0718-34022011000300007.

Luebert F & Pliscoff P (2017) Sinopsis bioclimática y vegetacional de Chile. Santiago de Chile: *Editorial Universitaria*. 381 pp.

Luebert F & Wen J (2008) Phylogenetic Analysis and Evolutionary Diversification of *Heliotropium* Sect. *Cochranea* (Heliotropiaceae) in the Atacama Desert. *Systematic Botany* 33 (2): 390–402. DOI: https://doi.org/10.1600/036364408784571635.

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal* 17 (1): 10–12. DOI: https://doi.org/10.14806/ej.17.1.200.

Mering S von, Akhani H, Arias S, Berendsohn WG, Borsch T, Castañeda-Noa I, Vos J de, Dillenberger M, Eggli U, Fleischmann A, Flores-Olvera MH, Hernández-Ledesma P, Kadereit G, Klak C, Korotkova N, Malekmohammadi M, Moore A, Nyffeler R, Ocampo G, Ortiz Díaz JJ, Oxelman B, Rabeler R, Schlumpberger B, Schuster T, Timaná M, Uotila P & Zuloaga F (2019) The Global Caryophyllales Initiative: Towards an updated taxonomic backbone and a dynamic monograph of a major plant group. *Biodiversity Information Science and Standards* 3. DOI: https://doi.org/10.3897/biss.3.35357.

Merklinger FF, Zheng Y, Luebert F, Harpke D, Böhnert T, Stoll A, Koch MA, Blattner FR, Wiehe T & Quandt D (2020) Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert. *Global and Planetary Change* 184: 103076. DOI: https://doi.org/10.1016/j.gloplacha.2019.103076.

Middleton CP, Senerchia N, Stein N, Akhunov ED, Keller B, Wicker T & Kilian B (2014) Sequencing of chloroplast genomes from wheat, barley, rye and their relatives provides a detailed insight into the evolution of the Triticeae tribe. *PLoS ONE* 9 (3): e85761. DOI: https://doi.org/10.1371/journal.pone.0085761.

Múlgura de Romero ME (1981) Contribuciones al estudio del género *Atriplex* (Chenopodiaceae) en la Argentina I. *Darwiniana* 23: 119–150.

Múlgura de Romero ME (1982) Contribuciones al estudio del género *Atriplex* (Chenopodiaceae) en la Argentina II. Las especies adventicias. *Darwiniana* 24: 49–68.

Múlgura de Romero ME (1984) Contribuciones al estudio del género *Atriplex* (Chenopodiaceae) en la Argentina III. *Darwiniana* 25: 235–253.

Müller KF & Borsch T (2005) Phylogenetics of Amaranthaceae based on *mat*K/*trn*K sequence data: evidence from parsimony, likelihood, and Bayesian analyses. *Annals of the Missouri Botanical Garden* 92 (1): 66–102. DOI: https://doi.org/https://www.jstor.org/stable/3298649.

Müller KF, Quandt D, Müller J & Neinhuis C (2005) PhyDe (0.9971) - Phylogenetic Data Editor. http://www.phyde.de/.

Olmstead RG, Michaels HJ, Scott KM & Palmer JD (1992) Monophyly of the Asteridae and Identification of Their Major Lineages Inferred From DNA Sequences of *rbc*L. *Annals of the Missouri Botanical Garden* 79 (2): 249. DOI: https://doi.org/10.2307/2399768.

Paradis E & Schliep K (2019) ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35 (3): 526–528. DOI: https://doi.org/10.1093/bioinformatics/bty633.

R Core Team (2018) R: A Language and Environment for Statistical Computing. Vienna, Austria. DOI: https://doi.org/https://www.R-project.org/.

Rambaut A, Drummond AJ, Xie D, Baele G & Suchard MA (2018) Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology* 67 (5): 901–904. DOI: https://doi.org/10.1093/sysbio/syy032.

Rauh W (1986) The Peruvian-Chilean Deserts. Chapter 7. In: Evenari M, Noy-Meir I & Goodall DW, eds. *Hot deserts and arid shrublands*. Amsterdam: Elsevier, *pp* 239–267.

Ritter B, Wennrich V, Medialdea A, Brill D, King G, Schneiderwind S, Niemann K, Fernández-Galego E, Diederich J, Rolf C, Bao R, Melles M & Dunai TJ (2019) "Climatic fluctuations in the hyperarid core of the Atacama Desert during the past 215 ka". *Scientific Reports* 9 (1): 5270. DOI: https://doi.org/10.1038/s41598-019-41743-8.

Rosas MR (1989) El genero *Atriplex* (Chenopodiaceae) en Chile. *Gayana. Botánica* 46 1-2: 3–82.

RStudio Team (2016) RStudio: Integrated Development Environment for R. Boston, MA. D. http://www.rstudio.com/.

Simpson MG, Johnson LA, Villaverde T & Guilliams CM (2017) American amphitropical disjuncts: Perspectives from vascular plant analyses and prospects for future research. *American Journal of Botany* 104 (11): 1600–1650. DOI: https://doi.org/10.3732/ajb.1700308.

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9): 1312–1313. DOI: https://doi.org/10.1093/bioinformatics/btu033.

Standley PC (1938) Chenopodiaceae - Goosefoot Family. In: Macbride JF, ed. *Flora of Peru / by J. Francis Macbride*. Chicago, (Ill.): Field Museum of Natural History, *pp* 469–478.

Stange M, Sánchez-Villagra MR, Salzburger W & Matschiner M (2018) Bayesian Divergence-Time Estimation with Genome-Wide SNP Data of Sea Catfishes (Ariidae) Supports Miocene Closure of the Panamanian Isthmus. *Systematic Biology*. DOI: https://doi.org/10.1093/sysbio/syy006.

Stutz HC, Chu G-L & Sanderson SC (1990) Evolutionary studies of *Atriplex*: Phylogenetic relationships of *Atriplex pleiantha*. *American Journal of Botany* 77 (3): 364–369. DOI: https://doi.org/10.1002/j.1537-2197.1990.tb13565.x.

Tripp EA, Tsai Y-HE, Zhuang Y & Dexter KG (2017) RADseq dataset with 90% missing data fully resolves recent radiation of *Petalidium* (Acanthaceae) in the ultra-arid deserts of Namibia. *Ecology and Evolution* 7 (19): 7920–7936. DOI: https://doi.org/10.1002/ece3.3274.

Vargas OM, Ortiz EM & Simpson BB (2017) Conflicting phylogenomic signals reveal a pattern of reticulate evolution in a recent high-Andean diversification (Asteraceae: Astereae: *Diplostephium*). *New Phytologist* 214 (4): 1736–1750. DOI: https://doi.org/10.1111/nph.14530.

Wendler N, Mascher M, Nöh C, Himmelbach A, Scholz U, Ruge-Wehling B & Stein N (2014) Unlocking the secondary gene-pool of barley with next-generation sequencing. *Plant Biotechnology Journal* 12 (8): 1122–1131. DOI: https://doi.org/10.1111/pbi.12219.

Worberg A, Quandt D, Barniske A-M, Löhne C, Hilu KW & Borsch T (2007) Phylogeny of basal eudicots: Insights from non-coding and rapidly evolving DNA. *Organisms Diversity & Evolution* 7 (1): 55–77. DOI: https://doi.org/10.1016/j.ode.2006.08.001.

Zacharias EH & Baldwin BG (2010) A Molecular Phylogeny of North American Atripliceae (Chenopodiaceae), with Implications for Floral and Photosynthetic Pathway Evolution. *Systematic Botany* 35 (4): 839–857. DOI: https://doi.org/10.1600/036364410X539907.

Chapter 6

Origin and diversification of *Cristaria*

Cristaria viridiluteola Gay in the coastal dunes between Huasco and Carrizal Bajo.

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Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert

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ABSTRACT

The Atacama Desert in western South America is considered as one of the driest places on earth, but is nevertheless characterized by surprisingly high species richness and levels of endemism. The plant genus *Cristaria* (Malvaceae), with ca. 21 species, is one of the most diverse genera of the Atacama Desert, while the much less diverse sister genus *Lecanophora* (7 species) is found east of the Andes. Here, we use DNA sequence data and divergence time estimates in order to investigate the biogeographical history of the Atacama species of *Cristaria*. We further investigate a possible influence of Andean uplift and the subsequent onset of hyperaridity in the Atacama Desert on diversification times in *Cristaria*. We sequenced three plastid markers (*ndh*F, *trn*K(*mat*K) & *rpl*16) for 19 species of *Cristaria* and two species of *Lecanophora* from the Atacama Desert and Argentina, respectively. Further, we included sequences of the same plastid regions from GenBank in order to get a comprehensive dataset of Malvoideae. Phylogenetic relationships were inferred using maximum likelihood and Bayesian analyses, and divergence times were estimated with BEAST2. Our results place the monophyletic genera *Cristaria* and *Lecanophora* as sister groups in a clade sister to the rest of Malveae. The split between these two lineages (~20 Ma) correlates with Andean uplift during the early Miocene, indicating a vicariant event. During the late Miocene, two Mediterranean members of *Cristaria* separated from the major Atacama clade. The subsequent diversification of the latter one correlates with the onset and subsequent temporal expansion of hyperarid conditions in the Atacama Desert since the late Miocene and during the Quaternary climate oscillations.

1. Introduction

The Atacama Desert of northern Chile and southern Peru is one of the driest places on earth, with a modern hyperarid core, receiving < 2 mm a^{-1} of precipitation (Houston, 2006). It is delimited by the high Andes to the east, the Pacific Ocean to the west and reaches from southern Peru in the north (ca. 18° S) to the area around La Serena in the south (ca. 30° S). Three major factors are responsible for the (hyper-)aridity of the Atacama Desert: (1) its position at the subtropical high-pressure belt, which has been stable since the late Jurassic (Hartley et al., 2005); (2) a significant rain-shadow effect for Atlantic air masses due to the evolution and uplift of the Andes (Houston and Hartley, 2003); (3) moisture uptake by onshore winds is restricted due to the cold Humboldt current along the coast (Rundel et al., 1991). In spite of the extreme drought, the coastal range harbors surprisingly high plant species richness (Dillon and Hoffmann, 1997) which likely has been shaped by abiotic factors such as the onset of hyperaridity and the rise of the Andes, including the creation of peculiar fog oases (Rauh, 1986; Rundel et al., 1991; Luebert and Wen, 2008).

Due to its rain-shadow effect, Andean orogeny is a crucial factor for understanding the climatic history as well as its impact on plant evolution in the Atacama Desert. Andean orogeny created novel habitats and likely acted as a biogeographic barrier for East-West dispersal, causing vicariance. Andean orogeny has been shown to play a major

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role for the development of the exceptionally high biodiversity in South America in general (Hughes and Eastwood, 2006; Antonelli et al., 2009, 2018; Hoorn et al., 2010; Luebert and Weigend, 2014), and some studies have discussed the influence of aridity in the Atacama Desert on plant evolution (Gengler–Nowak, K., 2002; Luebert and Wen, 2008; Dillon et al., 2009; Heibl and Renner, 2012). Orogeny was initiated by the subduction of the oceanic Nazca Plate under the South American Plate starting ~30–50 Ma ago (Barnes and Ehlers, 2009; Armijo et al., 2015). However, quantifying the exact amount, timing and spacing across the various uplift phases along the Andes remains tentative. Several studies have tried to quantify Andean uplift phases using a wide range of different paleo-altimetry proxies, such as climate-sensitive altitude proxies, e.g., stable isotopes (Garzione et al., 2006), paleobotany (Gregory-Wodzicki, 2000, 2002), fluvial incision rates (Lamb, 2016) or climate-independent proxies such as cosmogenic nuclides (Evenstar et al., 2009) or arc geochemistry (Scott et al., 2018). There is still no consensus on the timing of Andean orogeny, but two main scenarios are widely discussed (Fig. 2D): An early Miocene (or earlier) steady uplift initiated by crustal shortening and thickening (McQuarrie et al., 2005; Barke and Lamb, 2006; Evenstar et al., 2009; Scott et al., 2018) versus a rapid, late Miocene uplift since ~10–16 Ma, controlled by large-scale mantle delamination, magmatic thickening or lower crustal flow (Garzione et al., 2008, 2014; Hoke and Garzione, 2008; Lamb, 2011, 2016; Kar et al., 2016; Perkins et al., 2016).

Andean uplift and the onset of hyperaridity in the Atacama Desert are a matter of ongoing debate (Ritter et al., 2018b). The Andes have a major impact on the distribution of available moisture through a combination of orographic precipitation and deflection of atmospheric circulation in the Andean foothills (Houston, 2006; Jordan et al., 2010). Hyperaridity is considered to have been characteristic for parts of the Atacama Desert since the Miocene, possibly even dating back to the Oligocene or Eocene (Dunai et al., 2005; Ritter et al., 2018a). The onset of hyperarid conditions in the Andean foothills is, however, believed to post-date the onset of hyperaridity in the "dry core" between 19° and 22°S, (Hartley and Chong, 2002; Evenstar et al., 2017). The predominantly arid to hyperarid Miocene climate was repeatedly interrupted by slightly wetter phases that were synchronous with climate-induced changes recorded on the Andean foothills (Evenstar et al., 2017). These wetter episodes largely coincide with globally warmer periods (Zachos et al., 2001; Ritter et al., 2018b; Evenstar et al., 2009; Jordan et al., 2014). Current climatic heterogeneity in the Atacama is clearly reflected in plant occurrence, which is mostly restricted to the Coastal Cordillera (Rundel et al., 1991; Schulz et al., 2011) and the Andean foothills (Luebert and Pliscoff, 2017).

Among the ~550 vascular plant species in the Atacama Desert > 10 genera are represented with > 10 species (Dillon and Hoffmann, 1997) with a general level of endemism of 60% (Rundel et al., 1991). In spite of the highly peculiar flora, very little is known about the evolution of these Atacama plants. Molecular phylogenetic studies have been conducted, for example, in *Nolana* (Solanaceae; Tu et al., 2008, Dillon et al., 2009), *Palaua* (Malvaceae; Huertas et al., 2007, Schneider et al., 2009; Schneider et al., 2011) or *Tarasa* (Malvaceae; Tate and Simpson, 2003) to unravel the relationships between species of the Peruvian and Chilean coastal deserts. Few studies have used molecular clock models to estimate the origin and potential causes of the diversification of the desert plants. However, Heibl and Renner (2012) in *Oxalis* (Oxalidaceae) as well as Luebert and Wen (2008) in *Heliotropium* sect. *Cochranea* (Boraginaceae) found some correlation of the colonization of the coastal desert with the onset of hyperaridity and the Andean uplift. However, clearly, more data are needed in order to better understand the evolution of the Atacama flora in the context of hyperaridity and Andean uplift. In the present study we seek to understand the diversification and biogeographic history of Atacama plants using the herbaceous genus *Cristaria* (Malvaceae, ~21 spp.; Fig. 1) and its closest relative *Lecanophora* (~7 spp.; Krapovickas, 2008). Both genera have been taxonomically revised (Krapovickas, 1950; Muñoz-Schick, 1995; Schneider, 2013). *Cristaria* predominantly occurs west of the Andes (including the Desventuradas Islands) in the coastal desert ranging from northern Peru to central Chile (Muñoz-Schick, 1995), with individual records of two species from the Argentinian side of the Andes (Krapovickas, 1988, 2008). In turn, *Lecanophora* is distributed exclusively east of the Andes in Argentina, with only one species also found in the Chilean part of Patagonia (Domínguez and Elvebakk, 2002).

Our understanding of the timing of geological events has grown tremendously over the past years, however, additional biological information from diverse sources like dated phylogenies of taxa restricted to particular biomes adds valuable knowledge to our overall understanding of earth history (Antonelli et al., 2018). Here, we aim at elucidating the historical biogeography of *Cristaria* in the context of the most comprehensive dated phylogeny for Malvoideae and Malveae to date. We (1) evaluate the monophyly of the genera *Cristaria* and *Lecanophora* and confirm their position in Malvoideae, and (2) infer the divergence times of the desert clades of *Cristaria* and its separation from *Lecanophora* to investigate the role of Andean uplift, the onset of hyperaridity and its temporal expansion for the diversification times in the Atacama Desert.

2. Materials and methods

2.1. Taxon sampling

Our taxon sampling comprises 19 of the 21 accepted species of *Cristaria* (Muñoz-Schick, 1995; Schneider, 2013). We were unable to sample *Cristaria insularis* Phil. from the Desventuradas Islands and *C. cordato-rotundifolia* Gay from near Copiapó. The genus *Lecanophora* is represented with three out of the seven accepted species (Krapovickas, 2008). All samples are from our collections in the Atacama Desert or from the DNA Bank of the Senckenberg Research Institute (Frankfurt am Main, Germany). Voucher data including GenBank accession numbers are provided in the supplementary material (Table S1). Taxon sampling of other Malvoideae is based on Areces-Berazain and Ackerman (2017) (see below) with additional sequences of the *Plagianthus* clade from Wagstaff et al. (2010).

2.2. DNA extraction, amplification and sequencing

Genomic DNA was extracted from silica dried leaf tissue or in few cases from herbarium specimens using the NucleoSpin Plant II kit (Macherey-Nagel, Germany) following the manufacturer's protocol with an increased amount of lysis buffer (600 µl) and an incubation time of 90 min. Three plastid DNA regions (*ndh*F gene, *trn*K(*mat*K) region, *rpl*16 group I intron) were amplified using the primer given in supplementary material (Table S2). PCR conditions are shown in the supplementary material (Table S3–S5). PCR products were purified by gel extraction using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany) following the manufacturer's protocol. Sequencing was performed on a 3730XL DNA Analyzer (Applied Biosciences). DNA sequences were edited and manually aligned using PhyDe 0.9971 (Müller et al., 2005).

2.3. Alignment and phylogenetic analysis

The concatenated plastid DNA alignment from Areces-Berazain and Ackerman (2017) of the same three regions as described above was downloaded from the Dryad digital repository and complemented with our own sequences. Six inversion sites comprising 48 bp were detected in the combined alignments. The inverse fragments were reverse-complemented and aligned for analysis following Quandt et al. (2003). Additionally, in the *trn*K(*mat*K) region as well as in the *rpl*16 alignment 13 hotspot regions comprising a total of 84 bp were detected and excluded from the analysis. The position of these hotspots and inversions

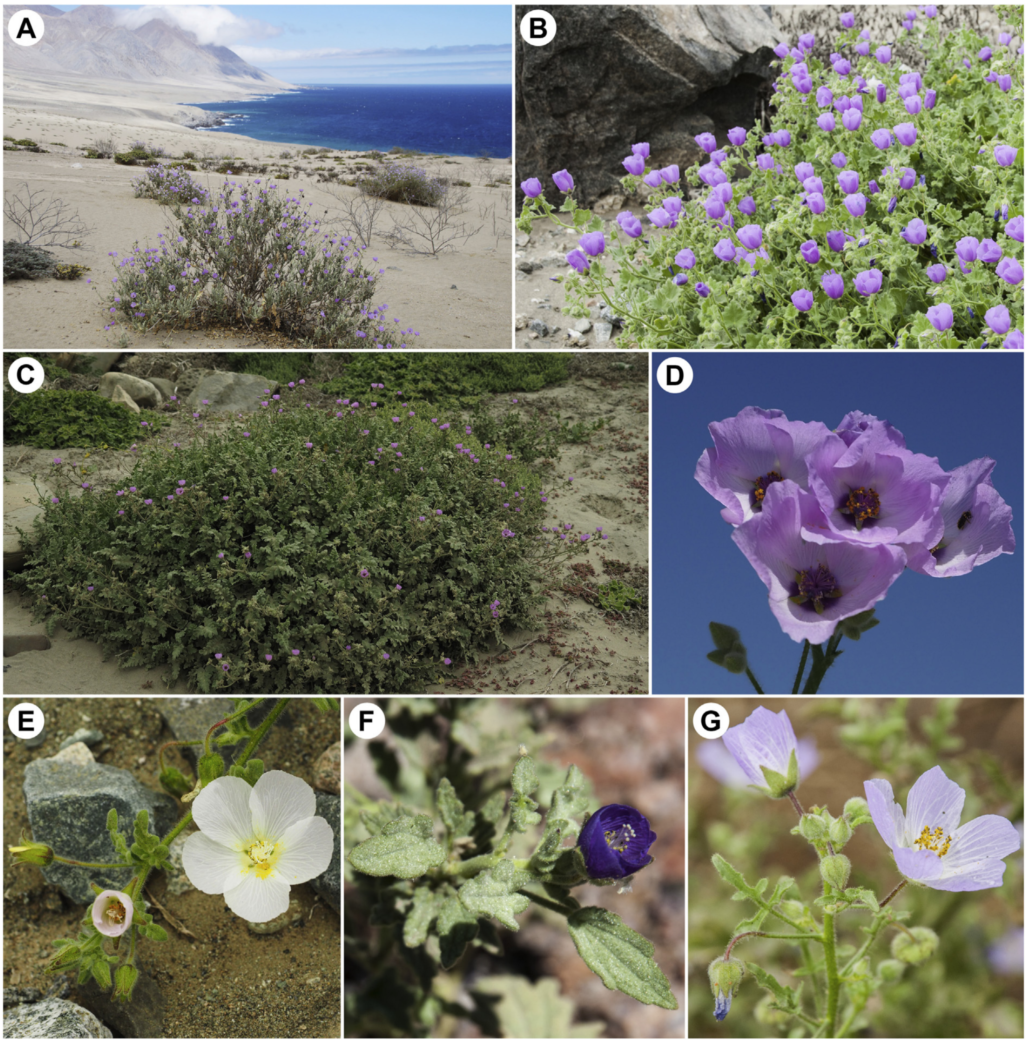

Fig. 1. Images of selected *Cristaria* species with habitat (A–C) and flower details (D–G). (A) *Cristaria viridiluteola* in its natural habitat; (B) *C. integerrima*; (C) *C. glaucophylla*; (D) *C. glaucophylla* flowers; (E) *C. leucantha*; (F) *C. andicola*; (G) *C. dissecta*. (© of the images by the Authors).

in the alignments is reported in the supplementary material (available from the CRC1211-database). Substitution models for all three DNA regions were evaluated using JModeltest 2.1.10 (Darriba et al., 2012). Maximum Likelihood (ML) and Bayesian Inference (BI) methods were applied to examine phylogenetic relationships within Malvoideae and the phylogenetic position of *Cristaria* and *Lecanophora*. The ML analysis was carried out using RAxML 8.2.10 (Stamatakis, 2014) with the GTRGAMMA substitution model and 1000 rapid bootstrap replicates, treating each gene region as a single partition. BI was conducted using MrBayes v3.2.6 (Ronquist et al., 2012) with four runs and four heated chains, a heating parameter of 0.2 and a random starting tree. The analyses were run with 10 million generations and the GTR + G + I substitution model for the concatenated alignment. The first 25% of the sampled generations were discarded as the burn-in. Autocorrelation among data points of the runs was examined using the Effective Sample Sizes as determined with Tracer1.7.1 (Rambaut et al., 2018). All phylogenetic analyses were run on the CIPRES Gateway (Miller et al., 2010). The final trees were visualized and edited in TreeGraph2 (Stöver and Müller, 2010) available in the supplementary material (CRC1211-database).

2.4. Molecular dating

Divergence time estimates were obtained with BEAST 2.5.1 (Bouckaert et al., 2014) using a Bayesian relaxed clock model. We used the same alignment as for the MrBayes analysis to set up an XML file in BEAUTi 2.5 (Bouckaert et al., 2014) with the following specifications: The partitions of the three cpDNA regions were set to link the clock and the tree model and unlinked site model. We used bModelTest (Bouckaert and Drummond, 2017) as implemented in BEAST 2 instead of defining substitution models for each partition. We specified a relaxed clock log normal model with an estimated clock rate as well as a Birth-Death model as tree prior (Drummond et al., 2006; Gernhard, 2008). We used four fossils, which are the oldest known fossils for the respective clades (Table 1 and Fig. 2B), as calibration points, based on the revision of known fossils of Malvoideae by Areces-Berazain and

Table 1
Fossils used for the node dating incl. Taxon names, ages in Mya, hyperparameters associated with the probability distributions: mean and standard deviation (stdev), the corresponding clades as well as references. See Fig. 2 and supplementary Fig. S3 for fossil position along the backbone phylogeny.

Fossil taxon	Age	Mean	stdev	Clade	Reference
Hibiscoxylon nyloticum	88–66	18	1.0	Core Malvoideae	Kräusel, 1939
Malvaciphyllum macondicus	60–58	2	1.25	Eumalvoideae	Carvalho et al., 2011
Malvacearumpollis sp.	37–30	7.5	1.25	Malveae	MacPhail and Truswell, 1989
Echiperiporites estelae	45–34	12	1.25	Hibisceae	Germeraad et al., 1968

Ackerman (2016).

The Markov Chain Monte Carlo (MCMC) was set to 300 million generations, sampling every 10,000th generation. The analyses were conducted twice with identical settings to secure that runs converge on the same results. Final log files were checked in Tracer 1.7.1 (Rambaut et al., 2018), TreeAnnotator was used to produce a Maximum clade credibility tree with mean heights, a 20% burn-in and a posterior probability limit of 0.95. BEAST2 output tree file is available in the supplementary material from the CRC1211-database. Finally, FigTree v1.4.3 as well as the R packages ape 5.0 (Paradis and Schliep, 2019), phylooch 1.5–5 (Heibl, 2008 onwards), strap 1.4 (Bell and Lloyd, 2014) and geoscale 2.0 (Bell, 2015) were used in R 3.5.1 (R Core Team, 2018) and RStudio 1.1.463 (RStudio Team, 2016) to plot and annotate the dated trees.

3. Results

3.1. Phylogenetic position of Cristaria and Lecanophora

The final alignment comprised 339 accessions corresponding to 329 taxa of Malvoideae. The dataset has a length of 7396 characters and 2979 distinct alignment patterns after the deletion of hotspot regions and the inversions replaced by their reverse complement. All Core Malvoideae form a well-supported clade (Supplementary Fig. S2). *Pentaplaris* and *Radyera* as well as a clade encompassing *Howitia* are sister clades to Eumalvoideae. Hibisceae is a highly supported sister clade to Gossypieae + Malveae. Phylogenetic relationships along the Malvoideae backbone were largely congruent between RAxML, MrBayes and BEAST2 analyses, receiving high bootstrap support (BS) and posterior probabilities (PP). The backbone of Malveae received good to moderate support, but the position of some clades remains uncertain. However, eight clades can be considered as well-supported (Fig. 2C & Supplementary Fig. S1). The *Cristaria* clade (100 BS, 1 PP), encompassing *Lecanophora* and *Cristaria*, is sister to the remainder of the tribe. The genera *Cristaria* and *Lecanophora* are retrieved as monophyletic and sister to each other with high branch support. The two coastal, southern Atacama species *C. glaucophylla* Cav. and *C. multiflora* are sister to all other *Cristaria* species, which in turn form a well-supported clade with *C. multifidia* Cav., the only Peruvian member of the genus, sister to the remaining species of the genus, albeit with weak support.

3.2. Molecular dating

The inferred divergence times of the whole Malvoideae (Supplementary Fig. S3) suggest a late Upper Cretaceous and a Paleocene origin for the Core Malvoideae (71 Ma, 95% HPD: 66.5–76) and Eumalvoideae (59.6 Ma, 95% HPD: 58–62) respectively. The origin of the tribe Malveae is dated to the Eocene at ca. 47.8 Ma (95% HPD: 40.4–53.6). The divergence of the crown node of *Cristaria* + *Lecanophora* (Fig. 2D) is dated to the early Miocene (20.7 Ma; 95% HPD: 10.6–31.2). For the crown node of *Cristaria* an age of 7.3 Ma (95% HPD: 3.6–11) is inferred. Two well supported clades of *Cristaria* are dated to the Pliocene-Quaternary boundary. The crown node age of the early diverging clade is dated to the Pliocene-Pleistocene transition (2.92 Ma, 95% HPD: 0.4–6.2) comprising two species from more Mediterranean-like habitats at the southern edge of the Atacama (indicated in Fig. 2D as *Med.*), namely *Cristaria glaucophylla* and *C. multiflora*. The crown node of the second major *Cristaria* clade is dated to 3.79 Ma (95% HPD: 2.4–6; Atacama clade in Fig. 2D) comprising all other species distributed in the Atacama and one species from the Peruvian coastal desert.

4. Discussion

4.1. Phylogenetic relationships of Cristaria and Lecanophora

In the current study we present the most comprehensive dated phylogeny of Malvoideae to date in order to infer phylogenetic relationships and diversification pattern. The major formally named clades, Core Malvoideae, Eumalvoideae as well as the three tribes Gossypieae, Hibisceae, and Malveae receive high support and are congruent with the current classification of the group (Alverson et al., 1999; Bayer et al., 1999; Baum et al., 2004). Several well supported clades can be identified in Malveae and are largely congruent with a phylogeny based on the nuclear ITS region by Tate et al. (2005). Nevertheless, none of those results coincides with the morphological classification of Bayer and Kubitzki (2003). However, several systematic issues remain unsolved, such as the relationship between *Tarasa* and *Sphaeralcea* and the position of the monotypic *Urocarpidium* relative to *Fuertesimalva* (Tate et al., 2005; Tate, 2011). Further, the position of the *Plagianthus* clade also remains unsolved. Areces-Berazain and Ackerman (2017) as well as our BEAST2 tree retrieve the *Plagianthus* clade as sister to all other Malveae, while in our ML and BI analyses the *Cristaria* clade is sister to all Malveae while the *Plagianthus* clade is the second branching clade after *Cristaria*. Our results are, however, able to confirm the monophyly and sister relationship of *Cristaria* and *Lecanophora* suggested in previous studies with very limited taxon sampling for these two genera (Tate et al., 2005; Takeuchi et al., 2018).

4.2. Origin of Cristaria and Lecanophora correlates with Andean uplift in the early Miocene

Ages retrieved for Core Malvoideae are similar to those found by Richardson et al. (2015). However, the crown node as well as internal nodes in Malvoideae are not supported, therefore we consider age estimates as tentative. Although taxon sampling presented here is based on the work of Areces-Berazain and Ackerman (2017), their age estimates are on average ca. 10 Ma older. However, our sampling incorporates more species and we used different prior assumptions. On the other hand, the age estimates in Hoorn et al. (2019) are similar to our results. Stem and crown node ages of Eumalvoideae are retrieved as slightly younger in Hoorn et al. (2019), but the age of 7 Ma for the crown node of *Cristaria* exactly matches our results in spite of the much smaller sampling there (two members of *Cristaria* only).

Andean orogeny along with the increasing aridity since the Oligocene-Eocene (Dunai et al., 2005; Ritter et al., 2018a) caused geographical isolation, biome shifts and the evolution of drought-adapted plants (Stebbins, 1952; Donoghue and Edwards, 2014). Our

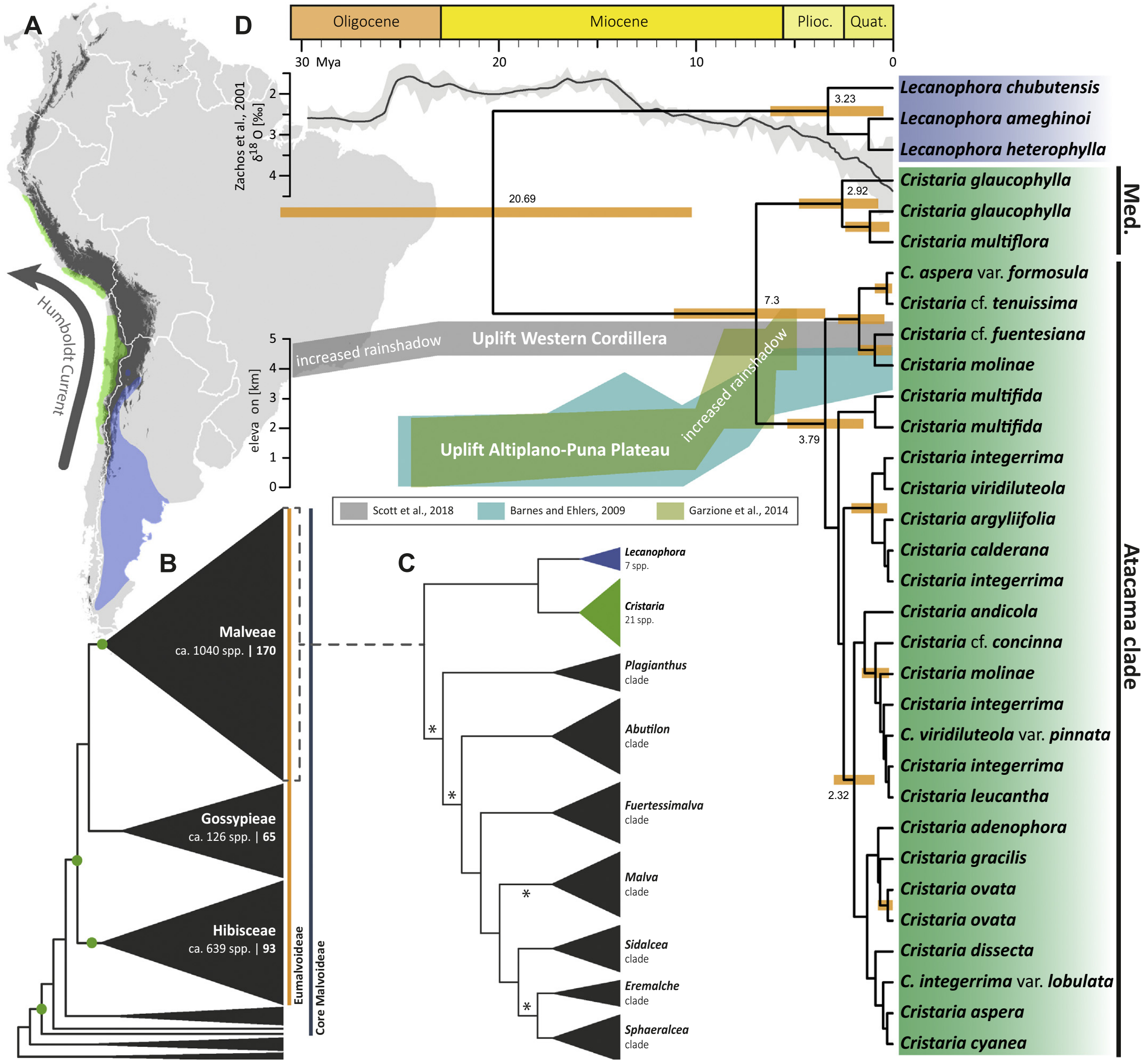

Fig. 2. Geographic distribution and dated phylogeny of the *Cristaria* clade showing coincidence of divergence events with Andean uplift and aridification. A) Distribution map of South America (grey = Andes above 2000 m) showing the distribution of *Cristaria* (green) and *Lecanophora* (blue) based on point distribution data of our own collection and three taxonomic revisions (Krapovickas, 1950; Muñoz-Schick, 1995; Schneider, 2013). Color scheme correspond with the C & D. B) backbone phylogeny of Malvoideae with four fossil calibration points (green dots; see Table 1), species numbers per tribe according to Bayer and Kubitzki (2003) and the number of species incorporated in this study are indicated on the triangle of each tribe. C) Simplified backbone phylogeny from the RAxML phylogeny of Malveae. Branch support ≥60 BS and ≥0.95 PP are not shown, while branches with lower support are indicated by asterisks (from RAxML above branch and MrBayes below branch). The full tree including support values is shown in Fig. S1–S2. D) Dated phylogeny of the *Cristaria* clade with *Cristaria* (green) and *Lecanophora* (blue); two clades within *Cristaria* are differentiated (Med. = clade with species from Mediterranean-like habitats; Atacama clade = species mainly found in the Atacama Desert); the complete dated phylogeny is shown in Fig. S3. The paleo-climate as derived from global deep-sea oxygen isotope records based on Zachos et al. (2001) is indicated as grey line. Two uplift scenarios of the Central Andes from the Oligocene to present are shown. The early uplift scenarios of the Western Cordillera based on Paleo-altimetry reconstructions by Scott et al. (2018) and the late Mid to Late Miocene rapid rise model of the Altiplano-Puna Plateau based on reconstructions from Garzione et al. (2014) and Barnes and Ehlers (2009). The uplift of the Andes resulted in an enhanced rain-shadow effect and probably in an increased aridification of the Atacama Desert. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

data provide evidence that these processes also shaped the vicariant distributions of *Cristaria* and *Lecanophora*. We dated the separation of both genera to 20.69 ± 10 Ma ago. This result predates the Late Miocene uplift of the Andes (Lamb, 2016) and is best explained by an early Miocene or earlier segregation in agreement with the constant uplift model of the Western Cordillera and an increased rain shadow (Barnes and Ehlers, 2009; Fig. 2D). By that time the Central Andes might have reached altitudes above 2000 m as inferred from other dated phylogenies (e.g. Picard et al., 2008). Elevations exceeding 2000 m are here considered as a dispersal barrier that effectively separated both genera. Taxa from both genera have subsequently colonized higher altitudes up to 4000 m and above (e.g. *C. andicola* Gay, *C.*

adenophora I.M.Johnston, *L. jarae* (Phil.) Krapov.) and two species of *Cristaria* (*C. andicola* and *C. dissecta* Hook. & Arn.) have crossed the Andes (Krapovickas, 2008). However, the early branching taxa as well as the majority of *Cristaria* species are coastal or mid elevation taxa and we consider this as the ancestral condition in the genus. An alternative scenario might be that the Most Recent Common Ancestor of *Cristaria* and *Lecanophora* originated in southern South America. Subsequent northward migration and colonization of areas west and east of the emerging Andes might have led to the separation into the two extant genera. Such a scenario has been suggested for *Vivianía* (Vivianiaceae) based on fossil pollen records (Palazzesi et al., 2012). However, such a pollen record is not available for our study group.

4.3. Diversification of Cristaria and onset of hyperaridity in the Atacama Desert

The increasing aridity in the Atacama and increasing precipitation to near modern levels in the Eastern Cordillera (luv side of orographic rain-shadow) during the middle to late Miocene (Barnes et al., 2012) indicate that the impact of the rain-shadow is largely controlled by the height and especially the width of the Central Andes and not exclusively by the height of the Western Cordillera (Rech et al., 2019). Rapid surface uplift of the Altiplano-Puna plateau between 6.8 and 10.3 Ma ± 0.4 Ma to an altitude of 2.5–3.5 km has been suggested by many studies (Lamb, 2016; and citations therein). This interval coincides with the split between the “Mediterranean” and Atacama species of *Cristaria* (see Fig. 2D). Increasing and expanding aridification in the Atacama Desert is also connected to the establishment of the Humboldt current (Fig. 2A) since the Mid-Miocene (Houston and Hartley, 2003), along with the build-up of permanent East Antarctic Ice Sheets since ~12 Ma (Zachos et al., 2001). However, climate models by Garreaud et al. (2010) indicate that Pacific Ocean temperatures were the primary controlling factor for humidity in the Atacama Desert since the late Miocene, rather than Andean uplift. The southern clade of *Cristaria* (coastal *Cristaria glaucophylla* and coastal to mid elevation *C. multiflora*: 0–2000 m) is found around La Serena (ca. 30° S) as well as further south in the transition zone to a more Mediterranean-type climate (Luebert and Pliscoff, 2017). Similar diversification times were inferred, e.g., for *Heliotropium* sect. *Cochranea* (Luebert and Wen, 2008; Luebert et al., 2011) or *Oxalis* (*Carnosa* clade; Heibl and Renner, 2012), pointing towards a general influence of above discussed abiotic factors on plant evolution in the Atacama Desert.

The crown node age of the Atacama clade at ~3.7 ± 2 Ma coincides with the end of the Early Pliocene warm period (Zachos et al., 2001) and the inferred onset and extension of hyperarid conditions towards higher elevations of the Precordillera and Pre-Andean basins (Hartley and Chong, 2002; Placzek et al., 2010). Expansion of the hyperarid conditions in the Atacama most likely reduced the range of *Cristaria* to isolated populations in, e.g., the coastal fog-oasis (“lomas”) and suitable habitats in the Andes, an effect exacerbated by the extremely dissected topography in the Pre- and Western Cordillera (Latorre et al., 2002; Díaz et al., 2012). A similar timing and similar range contractions and fragmentation have been suggested for other plant groups, e.g. *Heliotropium* sect. *Cochranea* (clade F in Luebert and Wen, 2008), *Nolana* (Dillon et al., 2009) and *Oxalis* (Heibl and Renner, 2012).

Global climate models indicate that the reinforcement of the Humboldt Current, especially at the Plio-Pleistocene transition, was an important factor for the establishment of widespread hyperarid conditions within the Atacama Desert (Garreaud et al., 2010). The increased diversification within *Cristaria* correlates with the high amplitude of climate variability with cold and warm cycles during the Quaternary and short-term wetter periods (Latorre et al., 2002, 2003). The repeated fragmentation of habitats with the recurrent closure and re-opening of migration corridors likely controlled diversification in *Cristaria*. This would be expected to lead to the subsimultaneous divergence of populations along the range of the genus. The lack of (phylogenetic and spatial) resolution in interspecific relationships shown in our analysis, but also in previous studies (Luebert and Wen, 2008) would be in agreement with this scenario. However, it may just as well be an artefact of the limited resolution of the markers here employed and will have to be investigated with more highly resolving molecular tools in the future.

4.4. Conclusion

Our data clearly confirm the monophyly of *Cristaria* as well as its sister relationship to *Lecanophora*. The inferred divergence times indicate an early Miocene split of both genera, corresponding to an early Miocene Andean uplift scenario and may imply trans-Andean vicariance. The crown node age of *Cristaria* indicates that diversification was concurrent with the expansion and intensification of hyperarid conditions in the Atacama Desert, inevitably promoting habitat fragmentation. Most modern *Cristaria* taxa evolved during a period of high climatic oscillations during the Quaternary, implying cycles of recurrent isolation and secondary contact of populations. This might have been a trigger for speciation, but also for genetic processes such as reticulate evolution as documented for the closely related genus *Palaua* (Schneider et al., 2011). There is only a single Peruvian species, sister to the Atacama clade, which has likely not exposed to comparable cycles of isolation and secondary context due to the overall much more coherent vegetation zones of the Peruvian coastal desert. Strikingly, no diversification took place in this clade. Ongoing research uses next generation sequencing data in order to gain deeper insights into species relationships and macro-evolutionary processes which drove speciation in *Cristaria* and also provide a better understanding of geological and climatic processes during the Pleistocene climatic cycles shaping the unique plant species richness in the Atacama Desert.

Data availability

Additional data including alignments and results are available from the CRC1211-database (https://crc1211-db.uni-koeln.de; DOI: https://doi.org/10.5880/CRC1211DB.26).

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gloplacha.2019.102992.

References

Alverson, W.S., Whitlock, B.A., Nyffeler, R., Bayer, C., Baum, D.A., 1999. Phylogeny of the Core Malvales: evidence from *ndhF* sequence data. Am. J. Bot. 86 (10), 1474.

https://doi.org/10.2307/2656928.

Antonelli, A., Nylander, Johan A.A., Persson, C., Sanmartín, I., 2009. Tracing the impact of the Andean uplift on neotropical plant evolution. Proc. Natl. Acad. Sci. U. S. A. 106 (24), 9749–9754. https://doi.org/10.1073/pnas.0811421106.

Antonelli, A., Kissling, W.D., Flantua, S.G.A., Bermúdez, M.A., Mulch, A., Muellner-Riehl, A.N., Kreft, H., Linder, H.P., Badgley, C., Fjeldså, J., Fritz, S.A., Rahbek, C., Herman, F., Hooghiemstra, H., Hoorn, C., 2018. Geological and climatic influences on mountain biodiversity. Nat. Geosci. 11 (10), 718–725. https://doi.org/10.1038/s41561-018-0236-z.

Areces-Berazain, F., Ackerman, J.D., 2016. Phylogenetics, delimitation and historical biogeography of the pantropical tree genus *Thespesia* (Malvaceae, Gossypieae). Bot. J. Linn. Soc. 181 (2), 171–198. https://doi.org/10.1111/boj.12414.

Areces-Berazain, F., Ackerman, J.D., 2017. Diversification and fruit evolution in Eumalvoids (Malvaceae). Bot. J. Linn. Soc. 184 (4), 401–417. https://doi.org/10.1093/botlinnean/box035.

Armijo, R., Lacassin, R., Coudurier-Curveur, A., Carrizo, D., 2015. Coupled tectonic evolution of Andean orogeny and global climate. Earth Sci. Rev. 143, 1–35. https://doi.org/10.1016/j.earscirev.2015.01.005.

Barke, R., Lamb, S., 2006. Late Cenozoic uplift of the Eastern Cordillera, Bolivian Andes. Earth Planet. Sci. Lett. 249 (3–4), 350–367. https://doi.org/10.1016/j.epsl.2006.07.012.

Barnes, J.B., Ehlers, T.A., 2009. End member models for Andean Plateau uplift. Earth Sci. Rev. 97 (1–4), 105–132. https://doi.org/10.1016/j.earscirev.2009.08.003.

Barnes, J.B., Ehlers, T.A., Insel, N., McQuarrie, N., Poulsen, C.J., 2012. Linking orography, climate, and exhumation across the Central Andes. Geology 40 (12), 1135–1138. https://doi.org/10.1130/G33229.1.

Baum, D.A., Dewitt Smith, S., Yen, A., Alverson, W.S., Nyffeler, R., Whitlock, B.A., Oldham, R.L., 2004. Phylogenetic relationships of *Malvatheca* (Bombacoideae and Malvoideae; Malvaceae sensu lato) as inferred from plastid DNA sequences. Am. J. Bot. 91 (11), 1863–1871. https://doi.org/10.3732/ajb.91.11.1863.

Bayer, C., Kubitzki, K., 2003. Malvaceae. In: Kubitzki, K., Bayer, C. (Eds.), The Families and Genera of Vascular Plants. V Flowering Plants – Dicotyledons. Malvales, Capparales and Non-betalain Caryophyllales. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 225–311.

Bayer, C., Fay, M.F., Bruijn, A.Y., Savolainen, V., Morton, C.M., Kubitzki, K., Alverson, W.S., Chase, M.W., 1999. Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: a combined analysis of plastid *atp*B and *rbc*L DNA sequences. Bot. J. Linn. Soc. 129 (4), 267–303. https://doi.org/10.1111/j.1095-8339.1999.tb00505.x.

Bell, M.A., 2015. Geoscale: Geological Time Scale Plotting. https://CRAN.R-project.org/package=geoscale.

Bell, M.A., Lloyd, G.T., 2014. Strap: Stratigraphic Tree Analysis for Palaeontology. https://CRAN.R-project.org/package=strap.

Bouckaert, R.R., Drummond, A.J., 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC Evol. Biol. 17 (1), 42. https://doi.org/10.1186/s12862-017-0890-6.

Bouckaert, R., Heled, J., Kuhnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Comput. Biol. 10 (4), e1003537. https://doi.org/10.1371/journal.pcbi.1003537.

Carvalho, M.R., Herrera, F.A., Jaramillo, C.A., Wing, S.L., Callejas, R., 2011. Paleocene Malvaceae from northern South America and their biogeographical implications. Am. J. Bot. 98 (8), 1337–1355. https://doi.org/10.3732/ajb.1000539.

Core Team, R., 2018. R: A Language and Environment for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9 (8), 772. https://doi.org/10.1038/nmeth.2109.

Díaz, F.P., Latorre, C., Maldonado, A., Quade, J., Betancourt, J.L., 2012. Rodent middens reveal episodic, long-distance plant colonizations across the hyperarid Atacama Desert over the last 34,000 years. J. Biogeogr. 39 (3), 510–525. https://doi.org/10.1111/j.1365-2699.2011.02617.x.

Dillon, M.O., Hoffmann, A.E., 1997. Lomas formations of the Atacama Desert northern Chile. In: Davis, S.D., Heywood, V.H., Herrera-MacBryde, O., Villa-Lobos, J., Hamilton, A.C. (Eds.), Centres of Plant Diversity, a Guide and Strategy for Their Conservation. The Americas Vol. 3. The World Wildlife Fund for Nature – The World, UK, pp. 528–535.

Dillon, M.O., Tu, T., Xie, L., Quipuscoa Silvestre, V., Wen, J., 2009. Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. J. Syst. Evol. 47 (5), 457–476. https://doi.org/10.1111/j.1759-6831.2009.00040.x.

Domínguez, E., Elvebakk, A., 2002. *Lecanophora subacaule* Krapov. (Malvaceae): Nuevo registro para la Flora de Chile. Gayana Bot. 59 (1), 43–47. https://doi.org/10.4067/S0717-66432002000100007.

Donoghue, M.J., Edwards, E.J., 2014. Biome Shifts and Niche Evolution in Plants. Annu. Rev. Ecol. Evol. Syst. 45 (1), 547–572. https://doi.org/10.1146/annurev-ecolsys-120213-091905.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4 (5), e88. https://doi.org/10.1371/journal.pbio.0040088.

Dunai, T.J., González López, G.A., Juez-Larré, J., 2005. Oligocene–Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. Geology 33 (4), 321. https://doi.org/10.1130/G21184.1.

Evenstar, L.A., Hartley, A.J., Stuart, F.M., Mather, A.E., Rice, C.M., Chong, G., 2009. Multiphase development of the Atacama Planation Surface recorded by cosmogenic 3He exposure ages: implications for uplift and Cenozoic climate change in western South America. Geology 37 (1), 27–30. https://doi.org/10.1130/G25437A.1.

Evenstar, L.A., Mather, A.E., Hartley, A.J., Stuart, F.M., Sparks, R.S.J., Cooper, F.J., 2017. Geomorphology on geologic timescales: evolution of the late Cenozoic Pacific paleosurface in Northern Chile and Southern Peru. Earth Sci. Rev. 171, 1–27. https://doi.org/10.1016/j.earscirev.2017.04.004.

Garreaud, R.D., Molina, A., Farias, M., 2010. Andean uplift, ocean cooling and Atacama hyperaridity: a climate modeling perspective. Earth Planet. Sci. Lett. 292 (1–2), 39–50. https://doi.org/10.1016/j.epsl.2010.01.017.

Garzione, C.N., Molnar, P., Libarkin, J.C., MacFadden, B.J., 2006. Rapid late Miocene rise of the Bolivian Altiplano: evidence for removal of mantle lithosphere. Earth Planet. Sci. Lett. 241 (3–4), 543–556. https://doi.org/10.1016/j.epsl.2005.11.026.

Garzione, C.N., Hoke, G.D., Libarkin, J.C., Withers, S., MacFadden, B., Eiler, J., Ghosh, P., Mulch, A., 2008. Rise of the Andes. Science 320 (5881), 1304–1307. https://doi.org/10.1126/science.1148615.

Garzione, C.N., Auerbach, D.J., Jin-Sook Smith, J., Rosario, J.J., Passey, B.H., Jordan, T.E., Eiler, J.M., 2014. Clumped isotope evidence for diachronous surface cooling of the Altiplano and pulsed surface uplift of the Central Andes. Earth Planet. Sci. Lett. 393, 173–181. https://doi.org/10.1016/j.epsl.2014.02.029.

Gengler–Nowak, K., 2002. Reconstruction of the biogeographical history of Malesherbiaceae. Bot. Rev. 68 (1), 171–188. https://doi.org/10.1663/0006-8101(2002)068[0171:ROTBHO]2.0.CO;2.

Germeraad, J.H., Hopping, C.A., Muller, J., 1968. Palynology of tertiary sediments from tropical areas. Rev. Palaeobot. Palynol. 6 (3–4), 189–348. https://doi.org/10.1016/0034-6667(68)90051-1.

Gernhard, T., 2008. The conditioned reconstructed process. J. Theor. Biol. 253 (4), 769–778. https://doi.org/10.1016/j.jtbi.2008.04.005.

Gregory-Wodzicki, K.M., 2000. Uplift history of the Central and Northern Andes: a review. Geol. Soc. Am. Bull. 112 (7), 1091–1105. https://doi.org/10.1130/0016-7606(2000)112<1091:UHOTCA>2.3.CO;2.

Gregory-Wodzicki, K.M., 2002. A late Miocene subtropical-dry flora from the northern Altiplano, Bolivia. Palaeogeogr. Palaeoclimatol. Palaeoecol. 180 (4), 331–348. https://doi.org/10.1016/S0031-0182(01)00434-5.

Hartley, A.J., Chong, G., 2002. Late Pliocene age for the Atacama Desert: Implications for the desertification of western South America. Geology 30 (1), 43. https://doi.org/10.1130/0091-7613(2002)030<0043:LPAFTA>2.0.CO;2.

Hartley, A.J., Chong, G., Houston, J., Mather, A.E., 2005. 150 million years of climatic stability: evidence from the Atacama Desert, northern Chile. J. Geol. Soc. Lond. 162 (3), 421–424. https://doi.org/10.1144/0016-764904-071.

Heibl, C., 2008. PHYLOCH: R Language Tree Plotting Tools and Interfaces to Diverse Phylogenetic Software Packages. onwards. http://www.christophheibl.de/Rpackages.html.

Heibl, C., Renner, S.S., 2012. Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. Syst. Biol. 61 (5), 823–834. https://doi.org/10.1093/sysbio/sys034.

Hoke, G.D., Garzione, C.N., 2008. Paleosurfaces, paleoelevation, and the mechanisms for the late Miocene topographic development of the Altiplano plateau. Earth Planet. Sci. Lett. 271 (1–4), 192–201. https://doi.org/10.1016/j.epsl.2008.04.008.

Hoorn, C., Wesselingh, F.P., ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Särkinen, T., Antonelli, A., 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. Science 330 (6006), 927–931. https://doi.org/10.1126/science.1194585.

Hoorn, C., van der Ham, R., de La Parra, F., Salamanca, S., Ter Steege, H., Banks, H., Star, W., van Heuven, B.J., Langelaan, R., Carvalho, F.A., Rodriguez-Forero, G., Lagomarsino, L.P., 2019. Going north and south: the biogeographic history of two Malvaceae in the wake of Neogene Andean uplift and connectivity between the Americas. Rev. Palaeobot. Palynol. https://doi.org/10.1016/j.revpalbo.2019.01.010.

Houston, J., 2006. Variability of precipitation in the Atacama Desert: its causes and hydrological impact. Int. J. Climatol. 26 (15), 2181–2198. https://doi.org/10.1002/joc.1359.

Houston, J., Hartley, A.J., 2003. The central Andean west-slope rainshadow and its potential contribution to the origin of hyper-aridity in the Atacama Desert. Int. J. Climatol. 23 (12), 1453–1464. https://doi.org/10.1002/joc.938.

Huertas, M.L., Schneider, J.V., Zizka, G., 2007. Phylogenetic Analysis of *Palaua* (Malveae, Malvaceae) based on Plastid and Nuclear Sequences. Syst. Bot. 32 (1), 157–165. https://doi.org/10.1600/036364407780360157.

Hughes, C., Eastwood, R., 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. Proc. Natl. Acad. Sci. U. S. A. 103 (27), 10334–10339. https://doi.org/10.1073/pnas.0601928103.

Jordan, T.E., Nester, P.L., Blanco, N., Hoke, G.D., Dávila, F., Tomlinson, A.J., 2010. Uplift of the Altiplano-Puna plateau: a view from the West. Tectonics 29 (5), 1–31. https://doi.org/10.1029/2010TC002661.

Jordan, T.E., Kirk-Lawlor, N.E., Blanco, N.P., Rech, J.A., Cosentino, N.J., 2014. Landscape modification in response to repeated onset of hyperarid paleoclimate states since 14 Ma, Atacama Desert, Chile. Geol. Soc. Am. Bull. 126 (7–8), 1016–1046. https://doi.org/10.1130/B30978.1.

Kar, N., Garzione, C.N., Jaramillo, C., Shanahan, T., Carlotto, V., Pullen, A., Moreno, F., Anderson, V., Moreno, E., Eiler, J., 2016. Rapid regional surface uplift of the northern Altiplano plateau revealed by multiproxy paleoclimate reconstruction. Earth Planet. Sci. Lett. 447, 33–47. https://doi.org/10.1016/j.epsl.2016.04.025.

Krapovickas, A., 1950. Revisión del género *Lecanophora* (Malvaceae). Darwiniana 9, 248–279.

Krapovickas, A., 1988. Malvaceae. In: Correa, M.N. (Ed.), Flora Patagonica Parte V. Dicotyledones dialipétalas (Oxilidaceae a Cornaceae). Coleccion Cientifica del INTA, Buenos Aires, pp. 126–153.

Krapovickas, A., 2008. Malvaceae. In: Zuloaga, F.O., Morrone, O., Belgrano, M.J.B. (Eds.), Catálogo de las plantas vasculares del Cono Sur. Argentina, Sur de Brasil, Chile, Paraguay y Uruguay, vol. 3. Missouri Botanical Garden Press, Saint Louis Mo, pp. 2463–2520.

Kräusel, R., 1939. Ergebnisse der Forschungsreisen Prof. E. Stromers in den Wüsten Ägyptens – IV. Die fossilen Floren Ägyptens – Abhandlungen der Bayerischen Akademie der Wissenschaften – Mathematisch-naturwissenschaftliche Klasse – NF. 47 (1), 1–140.

Lamb, S., 2011. Did shortening in thick crust cause rapid late Cenozoic uplift in the northern Bolivian Andes? J. Geol. Soc. Lond. 168 (5), 1079–1092. https://doi.org/10.1144/0016-76492011-008.

Lamb, S., 2016. Cenozoic uplift of the Central Andes in northern Chile and Bolivia—reconciling paleoaltimetry with the geological evolution. Can. J. Earth Sci. 53 (11), 1227–1245. https://doi.org/10.1139/cjes-2015-0071.

Latorre, C., Betancourt, J.L., Rylander, K.A., Quade, J., 2002. Vegetation invasions into absolute desert: a 45 000 yr rodent midden record from the Calama–Salar de Atacama basins, northern Chile (lat 22°–24°S). Geol. Soc. Am. Bull. 114 (3), 349–366. https://doi.org/10.1130/0016-7606(2002)114<0349:VIIADA>2.0.CO;2.

Latorre, C., Betancourt, J.L., Rylander, K.A., Quade, J., Matthei, O., 2003. A vegetation history from the arid prepuna of northern Chile (22–23°S) over the last 13 500 years. Palaeogeogr. Palaeoclimatol. Palaeoecol. 194 (1–3), 223–246. https://doi.org/10.1016/S0031-0182(03)00279-7.

Luebert, F., Pliscoff, P., 2017. Sinopsis bioclimática y vegetacional de Chile. Editorial Universitaria, Santiago de Chile 381 pp.

Luebert, F., Weigend, M., 2014. Phylogenetic insights into Andean plant diversification. Front. Ecol. Evol. 2 (192), 85. https://doi.org/10.3389/fevo.2014.00027.

Luebert, F., Wen, J., 2008. Phylogenetic analysis and evolutionary diversification of *Heliotropium* Sect. *Cochranea* (Heliotropiaceae) in the Atacama Desert. Syst. Bot. 33 (2), 390–402. https://doi.org/10.1600/036364408784571635.

Luebert, F., Hilger, H.H., Weigend, M., 2011. Diversification in the Andes: age and origins of South American Heliotropium lineages (Heliotropiaceae, Boraginales). Mol. Phylogenet. Evol. 61 (1), 90–102. https://doi.org/10.1016/j.ympev.2011.06.001.

Macphail, M.K., Truswell, E.M., 1989. Palynostratigraphy of the central West Murray Basin. J. Aust. Geol. Geophys. 11 (2–3), 301–331.

McQuarrie, N., Horton, B.K., Zandt, G., Beck, S., DeCelles, P.G., 2005. Lithospheric evolution of the Andean fold–thrust belt, Bolivia, and the origin of the central Andean plateau. Tectonophysics 399 (1–4), 15–37. https://doi.org/10.1016/j.tecto.2004.12.013.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp. 1–8.

Müller, K., Quandt, D., Müller, J., Neinhuis, C., 2005. PhyDe (0.9971) – Phylogenetic Data Editor. www.phyde.de/.

Muñoz-Schick, M., 1995. Revision del género *Cristaria* (Malvaceae) en Chile. Bol. Mus. Nac. Hist. Nat. Chile 45, 45–110.

Palazzesi, L., Gottschling, M., Barreda, V., Weigend, M., 2012. First Miocene fossils of Vivianiaceae shed new light on phylogeny, divergence times, and historical biogeography of Geraniales. Biol. J. Linn. Soc. Lond. 107 (1), 67–85. https://doi.org/10.1111/j.1095-8312.2012.01910.x.

Paradis, E., Schliep, K., 2019. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35 (3), 526–528. https://doi.org/10.1093/bioinformatics/bty633.

Perkins, J.P., Ward, K.M., de Silva, S.L., Zandt, G., Beck, S.L., Finnegan, N.J., 2016. Surface uplift in the Central Andes driven by growth of the Altiplano Puna Magma Body. Nat. Commun. 7, 13185. https://doi.org/10.1038/ncomms13185.

Picard, D., Sempere, T., Plantard, O., 2008. Direction and timing of uplift propagation in the Peruvian Andes deduced from molecular phylogenetics of highland biotaxa. Earth Planet. Sci. Lett. 271 (1–4), 326–336. https://doi.org/10.1016/j.epsl.2008.04.024.

Placzek, C.J., Matmon, A., Granger, D.E., Quade, J., Niedermann, S., 2010. Evidence for active landscape evolution in the hyperarid Atacama from multiple terrestrial cosmogenic nuclides. Earth Planet. Sci. Lett. 295 (1–2), 12–20. https://doi.org/10.1016/j.epsl.2010.03.006.

Quandt, D., Müller, K., Huttunen, S., 2003. Characterisation of the chloroplast DNA *psb*T-H region and the influence of Dyad symmetrical elements on phylogenetic reconstructions. Plant Biol. 5 (4), 400–410. https://doi.org/10.1055/s-2003-42715.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using tracer 1.7. Syst. Biol. 67 (5), 901–904. https://doi.org/10.1093/sysbio/syy032.

Rauh, W., 1986. The Peruvian-Chilean Deserts. Chapter 7. In: Evenari, M., Noy-Meir, I., Goodall, D.W. (Eds.), Hot Deserts and Arid Shrublands, 12B. Elsevier, Amsterdam, pp. 239–267.

Rech, J.A., Currie, B.S., Jordan, T.E., Riquelme, R., Lehmann, S.B., Kirk-Lawlor, N.E., Li, S., Gooley, J.T., 2019. Massive middle Miocene gypsic paleosols in the Atacama Desert and the formation of the Central Andean rain-shadow. Earth Planet. Sci. Lett. 506, 184–194. https://doi.org/10.1016/j.epsl.2018.10.040.

Richardson, J.E., Whitlock, B.A., Meerow, A.W., Madriñán, S., 2015. The age of chocolate: a diversification history of *Theobroma* and Malvaceae. Front. Ecol. Evol. 3 (130), 716. https://doi.org/10.3389/fevo.2015.00120.

Ritter, B., Binnie, S.A., Stuart, F.M., Wennrich, V., Dunai, T.J., 2018a. Evidence for multiple Plio-Pleistocene lake episodes in the hyperarid Atacama Desert. Quat. Geochronol. 44, 1–12. https://doi.org/10.1016/j.quageo.2017.11.002.

Ritter, B., Stuart, F.M., Binnie, S.A., Gerdes, A., Wennrich, V., Dunai, T.J., 2018b. Neogene fluvial landscape evolution in the hyperarid core of the Atacama Desert. Sci. Rep. 8 (1), 13952. https://doi.org/10.1038/s41598-018-32339-9.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61 (3), 539–542. https://doi.org/10.1093/sysbio/sys029.

RStudio Team, 2016. RStudio: Integrated Development Environment for R. MA, Boston. http://www.rstudio.com/.

Rundel, P.W., Dillon, M.O., Palma, B., Mooney, H.A., Gulmon, S.L., Ehrlinger, J.R., 1991. The phytogeography and ecology of the Coastal Atacama and Peruvian Desert. Alsio 13 (1), 1–49.

Schneider, J.V., 2013. The Peruvian species of *Cristaria* (Malveae, Malvaceae): taxonomic revision, chromosome counts, and breeding system. Phytotaxa 110 (1), 31. https://doi.org/10.11646/phytotaxa.110.1.3.

Schneider, J.V., Klie, D., Kacza, J., Huertas, M.L., 2009. Infrageneric variability of pollen morphology in *Palaua* (Malveae, Malvaceae) and the taxonomic utility of quantitative pollen characters. Grana 48 (4), 258–269. https://doi.org/10.1080/00173130903148860.

Schneider, J.V., Schulte, K., Aguilar, J.F., Huertas, M.L., 2011. Molecular evidence for hybridization and introgression in the neotropical coastal desert-endemic *Palaua* (Malveae, Malvaceae). Mol. Phylogenet. Evol. 60 (3), 373–384. https://doi.org/10.1016/j.ympev.2011.05.010.

Schulz, N., Aceituno, P., Richter, M., 2011. Phytogeographic divisions, climate change and plant dieback along the coastal desert of northern Chile. Erdkunde 65 (2), 169–187. https://doi.org/10.3112/erdkunde.2011.02.05.

Scott, E.M., Allen, M.B., Macpherson, C.G., McCaffrey, K.J.W., Davidson, J.P., Saville, C., Ducea, M.N., 2018. Andean surface uplift constrained by radiogenic isotopes of arc lavas. Nat. Commun. 9 (1), 969. https://doi.org/10.1038/s41467-018-03173-4.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30 (9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.

Stebbins, G.L., 1952. Aridity as a Stimulus to Plant Evolution. Am. Nat. 86 (826), 33–44. https://doi.org/10.1086/281699.

Stöver, B.C., Müller, K.F., 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. BMC Bioinform. 11, 7. https://doi.org/10.1186/1471-2105-11-7.

Takeuchi, C., Kano, C.H., Tate, J.A., Esteves, G.L., 2018. Molecular Phylogenetics and character Evolution of *Gaya* and Related Genera (Malvoideae, Malvaceae). Syst. Bot. 43 (3), 676–688. https://doi.org/10.1600/036364418X697391.

Tate, J.A., 2011. The status of *Urocarpidium* (Malvaceae): insight from nuclear and plastid-based phylogenies. Taxon 60 (5), 1330–1338.

Tate, J.A., Simpson, B.B., 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. Syst. Bot. 28 (4), 723–737.

Tate, J.A., Fuertes Aguilar, J., Wagstaff, S.J., La Duke, J.C., Bodo Slotta, T.A., Simpson, B.B., 2005. Phylogenetic relationships within the tribe Malveae (Malvaceae, subfamily Malvoideae) as inferred from ITS sequence data. Am. J. Bot. 92 (4), 584–602. https://doi.org/10.3732/ajb.92.4.584.

Tu, T., Dillon, M.O., Sun, H., Wen, J., 2008. Phylogeny of *Nolana* (Solanaceae) of the Atacama and Peruvian deserts inferred from sequences of four plastid markers and the nuclear LEAFY second intron. Mol. Phylogenet. Evol. 49 (2), 561–573. https://doi.org/10.1016/j.ympev.2008.07.018.

Wagstaff, S.J., Molloy, B.P.J., Tate, J.A., 2010. Evolutionary significance of long-distance dispersal and hybridisation in the New Zealand endemic genus *Hoheria* (Malvaceae). Aust. Syst. Bot. 23 (2), 112–130. https://doi.org/10.1071/SB09017.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292 (5517), 686–693. https://doi.org/10.1126/science.1059412.

Chapter 7

Evolution of *Cristaria* in the Atacama Desert

Cristaria integerrima Phil. (right) and *Nolana* sp. (left). At first glance, both genera look very similar in terms of habit and flower color.

The results presented here form the basis for a manuscript which is currently being prepared for publication:

Böhnert T, Luebert F, Merklinger FF, Harpke D, Stoll A, Schneider JV, Blattner F, Quandt D & Weigend M (***in prep.***) Historical Biogeography and Evolution of *Cristaria* (Malvaceae) in the Atacama Desert.

Own contribution: Study design, field work, lab work, data analyses, writing.

Data availability: Supplementary material are availbalbe in **Appendix D**.

Abstract

The climatic history in the Atacama Desert since the late Miocene has been suggested as a driving force for the diversification in some genera in the Atacama Desert. However, the molecular methods available until recently were unable to provide a detailed picture of the phylogenetic relationships and evolutionary history of the speciose groups studied. Using the genotyping-by-sequencing approach we sequenced over one hundred samples of the genus *Cristaria*, with ~21 species one of the most speciose groups in the Atacama Desert. Alpha-taxonomy in the group is notoriously difficult, and a previous phylogenetic study was unable to resolve species limits or relationships. By using thousands of genomic SNP's (single nucleotide polymorphisms) we are here able to present a nearly fully resolved tree. Current species limits are largely confirmed by our data, but some incongruences will need to be addressed in the future with an adjustment of species limits. We also date the tree using a Maximum Likelihood method and analyze the historical biogeography of the group by applying the Dispersal Extinction Cladogenesis approach. We retrieve eleven well-supported clades harboring one to four accepted species. *Cristaria* appears to have originated in the Mediterranean environment of Central Chile and colonized the Atacama Desert from the south from the late Miocene onwards. Mediterranean and Andean clades arose prior to the Quaternary climate oscillation, whereas the coastal clades show high morphological plasticity. An exemplary analysis of the coastal species *Cristaria integerrima* Phil. indicates a genetic separation into at least four distinct groups corresponding to previously identified clusters of coastal vegetation underscoring the apparently divergent evolution in these vegetation islands.

Keywords: Chile — Dispersal Extrinction Cladogenesis — Dry Diagonal — Genotyping-by-sequencing — Malveae — South America

7.1 Introduction

The arid to hyperarid conditions in the north Chilean Atacama Desert have probably prevailed since the early Miocene or even the Oligocene (Dunai *et al.* 2005). Since then, the rain shadow effect of the Andes has been blocking moisture from the Amazon Basin, while the Humboldt Current prevents precipitation by transporting cold water from the Arctic Sea to the coast of northern Chile (Houston & Hartley 2003). Two dominating atmospheric wind circulations, the southern westerlies and the tropical easterlies, are responsible for occasional winter rainfall in the south-western and summer rainfall in the north-eastern Atacama Desert, respectively. In between lies the virtually rainless Atacama dry core or Dry Diagonal (Lamy *et al.* 2000; Maldonado *et al.* 2005; Houston 2006). However, while the trend of increasing aridity since the Miocene in the Atacama Desert is widely accepted, it has been shown that throughout the Pleistocene this general trend has been interrupted by wetter periods (Baker & Fritz 2015; Ritter *et al.* 2019). These geological findings are supported by pollen records from rodent middens in the dry core (Latorre *et al.* 2002; Nester *et al.* 2007; Gayo *et al.* 2012) and in the southern Atacama Desert (Maldonado *et al.* 2005). These more humid phases correspond to the interglacial periods over the last 250k years (Ritter *et al.* 2019).

A total of 225 vascular plant genera are documented from the Atacama Desert lomas with a total of ~550 species, but only a handful of the genera can be considered as speciose (Dillon & Hoffmann 1997). Well known examples are *Nolana* L.f. (Tu *et al.* 2008), *Heliotropium* L. (Luebert & Wen 2008), *Copiapoa* Britton & Rose (Larridon *et al.* 2015) and *Cristaria* Cav. (Böhnert *et al.* 2019). Phylogenetic studies of these genera are based on concatenated alignments of few nuclear or plastid marker sequences, providing only limited insights into their evolutionary history. While some speciose genera seem to be the result of *in situ* diversification, others are the product of multiple colonization events (e.g., *Atriplex* L., see chapter 5 or Brignone *et al.* 2019; *Cryptantha* Lehm. ex G.Don, Guilliams *et al.* 2017; *Oxalis* L., Heibl & Renner 2012). Not much is known about the processes responsible for diversifications in some groups, while other groups fail to diversify. The macroevolutionary concept of 'carrying capacity' provides a theoretical frame work to approach this question (Alroy 2010; Rabosky 2013). The constantly changing climatic conditions throughout the Pleistocene might have caused populations to contract repeatedly during dry phases, fragmenting them into several populations, followed by expansion and secondary contact in wetter phases (Stebbins 1952). Such metapopulation dynamics (Hanski 1998) can then facilitate further processes such as introgression as shown for the genus *Palaua* Cav. (Malvaceae) in the coastal deserts of Peru and Chile (Schneider *et al.* 2011) or result in incomplete lineage sorting (Avise *et al.* 1987).

Previous phylogenetic studies on South American Malveae (Malvaceae) based on nuclear (Tate *et al.* 2005) as well plastid sequence data (Areces-Berazain & Ackerman 2017) included only a few samples of *Cristaria* and were unable to fully resolv phylogenetic relationships within the genus. However, as shown in chapter 6 (Böhnert *et al.* 2019) the high number of Atacama species in the genus *Cristaria* (Malvaceae) is not the consequence of multiple colonization events (cf. chapter 3 & 5), but the result of vicariance and *in situ* diversification in the Atacama Desert. The genera *Cristaria* and *Lecanophora* Speg. diverged in response to Andean uplift ca. 20 Mya, while the radiation of *Cristaria* is correlated to the onset of hyperarid conditions in the Atacama Desert. However, even the multi-marker plastid phylogeny used in chapter 6, together with a comprehensive sampling, provides only limited insights into the phylogenetic relationships within *Cristaria* (Böhnert *et al.* 2019). A lack of phylogenetic resolution is, unfortunately, a common phenomenon among speciose clades in the Atacama Desert (Dillon *et al.* 2009; Luebert *et al.* 2011; Heibl & Renner 2012; Larridon *et al.* 2015). In order to overcome such limitations, next-generation sequencing techniques are increasingly applied to provide deeper insights into phylogenetic relationships and speciation processes (Lemmon & Lemmon 2013; McCormack *et al.* 2013; Bravo *et al.* 2019).

Two taxonomic treatments of *Cristaria* are available, one for the species distributed in Chile (19 ssp.; Muñoz-Schick 1995) and a second for the single species native to Peru (*Cristaria* *multifida* Cav.; Schneider 2013). Most species of *Cristaria* are restricted to the Atacama Desert, while two range into the Andes and even to Argentina (i.e., *Cristaria andicola* Gay and *Cristaria dissecta* Hook. & Arn.; Muñoz-Schick 1995). A single species is known from the Desventuradas Islands in the Pacific Ocean. The facts that a recent species concept for *Cristaria* is available and that the genus evidently underwent diversification since the late Miocene (Böhnert *et al.* 2019) makes it an excellent model organism to study the impact of geological and climatological factors on plant diversification in the Atacama Desert by applying next-generation sequencing techniques.

We hypothesize that the diversification of the genus *Cristaria* and its geographical range within the Atacama Desert were both caused by the increasing aridity since the Middle Miocene and the climatic oscillations during the Quaternary. Accordingly, the following specific questions will be addressed: How did the spatiotemporal diversification of *Cristaria* take place? Did the Quaternary climate oscillation influence the diversification processes as well as the biogeographic history of *Cristaria*? What is the current status of speciation processes and is this adequately reflected in the current taxonomic concepts? In order to address these questions, the phylogenetic relationships within *Cristaria* were analyzed using next-generation sequencing techniques and molecular clock dating methods. Further, ancestral areas of *Cristaria* were analyzed in the context of the recent climatic history of the Atacama Desert.

7.2 Materials and Methods

7.2.1 Taxon sampling

In her revision Muñoz-Schick (1995) accepted 19 species of *Cristaria* for Chile, but also listed six taxonomic names as doubtful species ("especies dudosas"). Since we were able to recollect and thereby confirm the existence of *Cristaria concinna* Phil., one of the doubtful species according to Muñoz-Schick (1995), we provisionally accept 20 species for Chile, plus a single species from Peru (Schneider 2013). Our taxon sampling comprises 19 of the 21 accepted species of *Cristaria* and is based on the work of Böhnert *et al.* (2019), but the dataset was significantly increased to 128 specimens with up to 42 individuals per species. As in Böhnert *et al.* (2019) we were unable to sample *Cristaria insularis* Phil. and *Cristaria cordato-rotundifolia* Gay. The sister genus *Lecanophora*, represented with two species, was used as the outgroup. Sampling is mainly based on our own collecting trips between 2016 and 2019 and was supplemented with samples from the DNA Bank of the Senckenberg Research Institute (Frankfurt am Main, Germany). Herbarium vouchers are stored in local herbaria in Chile (ULS) and Germany (BONN, FR). Voucher information are provided in Appendix D as a supplementary material (Table S1). Identification of specimens is based on Muñoz-Schick (1995) and her taxonomic concept for the genus, as well as on Schneider (2013) with regard to the Peruvian representatives of the genus.

7.2.2 DNA extraction, library preparation and sequencing

In order to obtain genomic DNA from silica dried leaf samples, we followed the modified extraction protocol of Böhnert *et al.* (2019) using the NucleoSpin Plant II kit (Macherey-Nagel, Germany) with an increased lysis buffer of 600 µl. Quality and quantity of extracted DNA was checked on 1 % agarose gels using Lonza GelStar Nucleic Acid Gel Stain (100x) with 20 ng of double stranded Lambda DNA (New England Biolabs, N3011S) as well as on a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, United States). Finally, samples were standardized to 20 ng/µl and 15 µl were used for library preparation and sequencing. The library preparation protocol for genotyping-by-sequencing by Elshire *et al.* (2011) with modifications suggested by Wendler *et al.* (2014) and Merklinger *et al.* (2020) was applied. To reduce DNA fragment size, 200 µl genomic DNA per sample were digested using the rare-cutting enzymes *PstI-HF®* (New England Biolabs, R3140S; recognition site: CTGCA'G) and the methylation-sensitive *Msp*I (New England Biolabs, R0106S, recognition site: C'CGG). Subsequently, DNA fragments were size-selected with a SYBR gold stained electrophoresis gels to 200-600 bp. Individual samples were barcoded and then single-end sequenced (reads of 100 bp) on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA) at the Genome Center of the Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany).

7.2.3 Assembly and phylogenetic analyses of GBS data

After de-multiplexing of barcoded reads (CASAVA pipeline 1.8, Illumina, Inc.) raw sequences were adapter- (-a AGATCGGAAGAGC) and quality- (-q 25, -m 50) trimmed using CUTADAPT v1.16 (Martin 2011). Finally, we checked read quality as well as for remaining adapter sequences using FastQC (Andrews *et al.* 2012). Quality-trimmed and checked GBS data were *de novo* assembled using ipyrad v0.9.15 (Eaton & Overcast 2020). The parameter specifying the datatype was set to ddRAD as our approach uses two restriction enzymes in contrast to the original GBS approach (Elshire *et al.* 2011). The threshold allowing sequence similarity was set to 85 % and all other parameters were left at their default values. During the assembly at the final step (step seven), the previously separately treated samples were combined to produce a final alignment with a single consensus sequence per sample.

Missing data can have significant impact on tree topology and support (Huang & Knowles 2016; Tripp *et al.* 2017). Here, 70 % missing data were allowed regarding the actual number of ingroup samples for three different datasets. First, an assembly of a dataset including all 117 *Cristaria* samples, covering the whole genus and using two *Lecanophora* samples as outgroup. Second, a reduced dataset including 42 samples of the species *C. integerrima* and thrid an identical dataset but additionally using *C. molinae* Gay as an outgroup. For phylogenetic reconstruction we employed a Maximum Likelihood approach using RAxML v.8.2.10 (Stamatakis 2014) with the GTRGAMMA substitution model and 500 rapid bootstrap replicates followed by a full search for the best tree on both datasets.

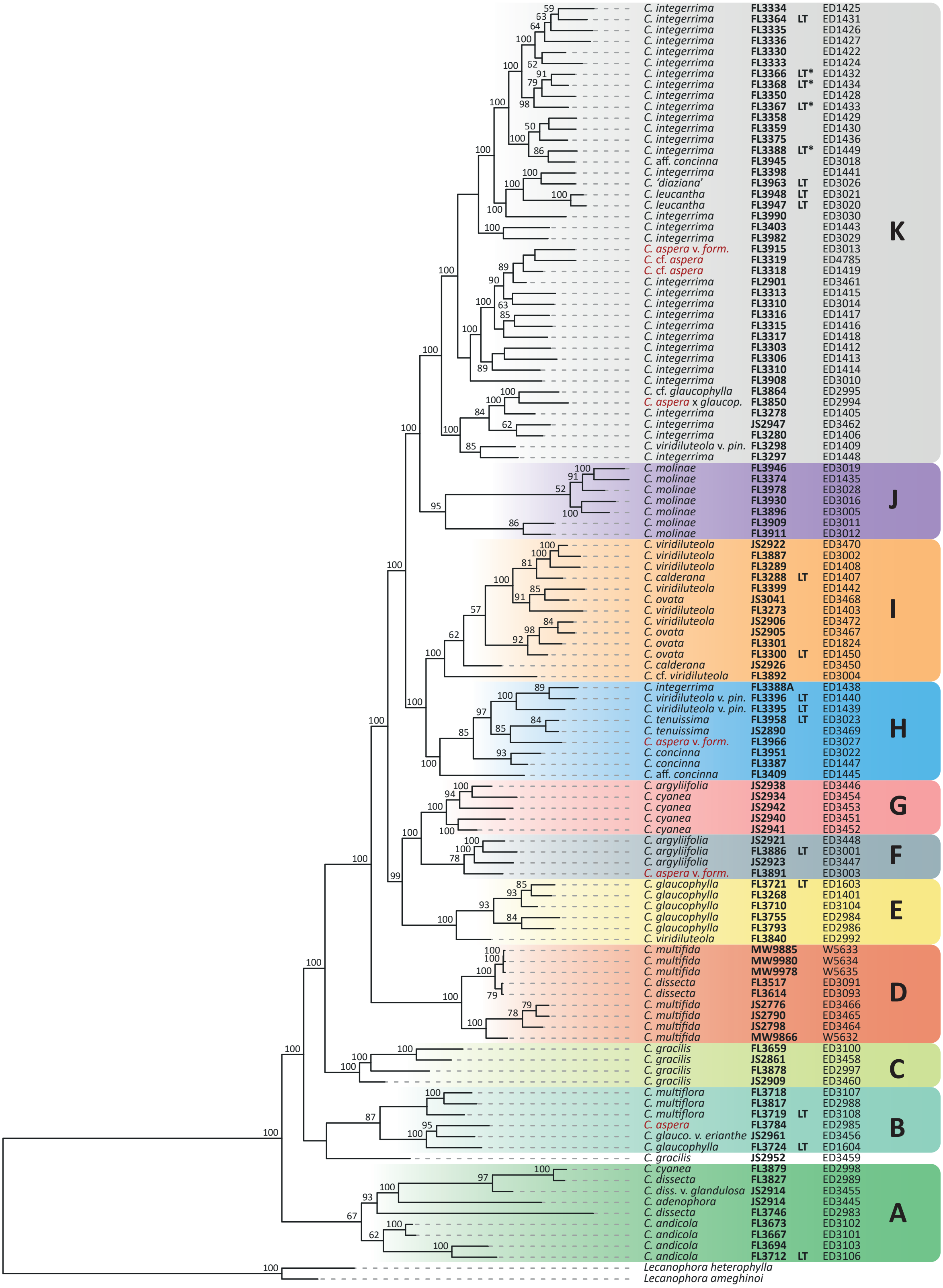


Figure 1: RAxML tree of the genus *Cristaria* based on SNP data from 117 samples using the pop30 dataset. *Lecanophora* was used as an outgroup. Only bootstrap support values ≥ 50 are shown. Specimens identified as *C. aspera* are shown in red. For each sample the collector (FL = Federico Luebert; JS = Julio Schneider; MW = Maximilian Weigend), collection number and lab IDs are given. Samples marked with LT are locotypes and an asterisk (LT*) indicates a locotype of a nomenclatural synonym. Major clades representing accepted species or taxa with considerable support are labeled from A to K. Each of them is marked in different colors which correspond to the color code for the distribution map in Figure 2.

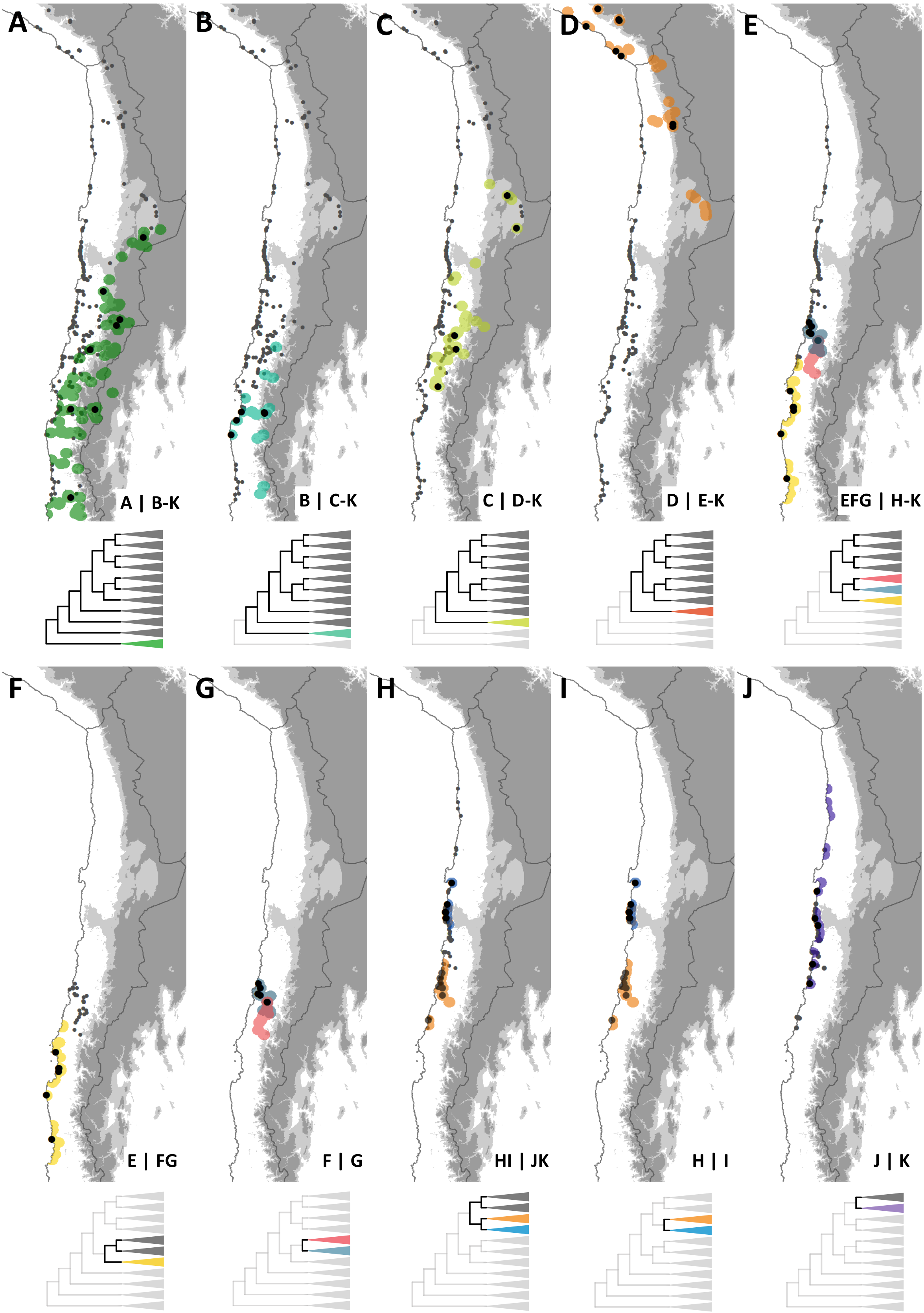
As the number of samples included for the species *C. integerrima* does allow to infer population structure, the Bayesian clustering algorithm STRUCTURE v2.3.4 (Pritchard *et al.* 2000) was applied. A clustering scenario of up to 6 groups was considered (K = 2–6) and for each values of *K* 10 replicates with 100,000 generations were run, while 10,000 were discarded as burn-in. To combine replicate runs and evaluate convergence as well as delta K the software CLUMPP (Evanno *et al.* 2005; Jakobsson & Rosenberg 2007) was used. Samples within the resulting bar plots were sorted to match the ladderized orientation of the RAxML tree. Assembly as well as phylogenetic and population analyses have been done within the ipyrad-analysis toolkit in a Python interface written in a Jupyter-Notebook (for full documentation see Appendix D).

7.2.4 Molecular clock dating and ancestral area reconstruction

The stem and crown node ages of *Cristaria* estimated by Böhnert *et al.* (2019) were used to date the here generated GBS Maximum Likelihood tree using a Penalized Likelihood approach (Sanderson 2002) as implemented in the R (v3.5.1; R Core Team 2018) package ape 5.3 (Paradis & Schliep 2019). By applying a cross-validation criterion the best fitting smoothing parameter was identified as lambda = 1.0 (Appendix D, supplementary Figure S1). Afterwards, the number of tips was reduced down to one tip per clade and outgroups were excluded. Subsequently, the Dispersal Extinction Cladogenesis (DEC) approach described by Ree & Smith (2008) was applied for the eleven main clades using the R-package BioGeoBEARS 1.1.1 (Matzke 2013; chapter 3) without the DEC+J model (Ree & Sanmartín 2018).

In order to assign meaningful distribution areas to the eleven *Cristaria* clades prior to the ancestral area reconstruction, a complete overview of the current distribution of each species or clade was needed. Therefore, in addition to the here presented taxon sampling, as many specimens cited in Muñoz-Schick (1995) were entered into a database and then georeferenced until a comprehensive overview of the actual distribution of the species was achieved (423 specimens incl. own collections; for full list see Appendix D, Table S2). Based on this dataset every sister relationship between clades (see Figure 1) was plotted on ten different maps (see Figure 2A-J). This step-by-approach made it possible to identify six distinct geographic areas of current species distribution which were then used for the ancestral area reconstruction (areas A to F, see Figure 3A): First, southern Peru (area A) and the northern part of the Atacama Desert including the northern Chilean Andes (area B) were separated from the southern part along the Dry Diagonal (Houston 2006). This separation is reflected by the occurrence of clade D in areas A and B (Figure 2D) in contrast to the distri-

Figure 2: Distribution maps of all *Cristaria* clades. Each specimen was plotted with a 20 km buffer in the color of the respective clade in Figure 1. The distribution of specimens included in the current phylogenetic analyses are additionally marked with black dots. The maps (**A**–**J**) show the distribution of a specific clade in relation to its sister clade (shown in small gray dots). The phylogenetic trees beneath each map are simplified versions of Figure 1 and indicate which clades are shown. Clades or parts of the tree shown in light gray are not shown in the map. The letters in the bottom right corner of each map also indicate which clades are shown in relation to each other separated by a vertical line. ▶


A
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B | C-K
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EFG | H-K
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H
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J | K

bution of the basal clades A to C in the Atacama south of the climate diagonal (Figure 2A-C). Further, the southern Atacama Desert above 2000 m a.s.l. including the Mediterranean Andes (area C) was separated from the southern Atacama Pampa (area D) taking into account the widely distributed clades A and C as well as the clades F and G. Finally, two coastal areas (area E: southern Atacama Coast; area F: northern Atacama Coast) were separated from the southern Atacama Pampa, taking into account the distribution of clade E in relation to the clades H–K (Figure 2E-J).

7.3 Results

7.3.1 GBS assembly and phylogenetic reconstruction

We sequenced 128 samples in total, of which 11 had to be excluded due to low read numbers or sequencing failure, including *Cristaria fuentesiana* I.M.Johnst., thereby reducing the number of species analyzed to 18. The remaining 117 samples, however, include a total of 153M reads (mean = 1.3M; *stdev* = 987K) of the genus *Cristaria* and two outgroup taxa of the genus *Lecanophora*. The main assembly covering all samples including outgroups resulted in 308K reads in total and 2546 reads per sample on average (*stdev* = 710; supplementary Material Appendix D Table S2).

The RAxML analyses including all samples that passed the assembly criteria resulted in a phylogenetic tree with a fully supported backbone and eleven highly supported clades with one to four distinct taxa each (Figure 1, clades labeled from A to K). The known distribution of each clade is given in Figure 2. The names assigned to the samples represented in the tree are based on the morphological characteristics commonly applied to this genus. However, we labelled one specimen as *Cristaria diaziana* I.M.Johnst. even though it is currently treated as a synonym of *C. integerrima*. The respective specimen was collected at the type location of *C. diaziana* and showed aberrant morphological characters compared to *C. leucantha* I.M.Johnst. and *C. integerrima*. The tree shows a number of taxonomic inconsistencies with regard to the phylogenetic signal of the GBS data. The most obvious example is *Cristaria aspera* Gay (tip labels marked in red, Figure 1) of which seven samples are placed in four different clades. Apart from *C. aspera*, most incongruences were found in the morphologically highly variable *C. integerrima* clade (Figure 1, clade K). Further, minor incongruences were found in clades A, B, E, G and H. While only the clades C and J appear to be monotypic, six clades harbor at least two or even more species (see clades A, B, D, H, I & K). In clade A the Andean species *C. andicola* and *Cristaria adenophora* I.M.Johnst. are grouped together with *Cristaria cyanea* Phil. ex Baker f. and *C. dissecta*, the latter of which is also found in clade D. Further inconsistencies were found in clade B where *Cristaria multiflora* Gay and two specimens identified as *Cristaria glaucophylla* Cav. are grouped together, although the majority of specimens of *C. glaucophylla* form a clade (clade E). Clades H and I comprise two respectively three species, but with only moderately supported internal resolution.

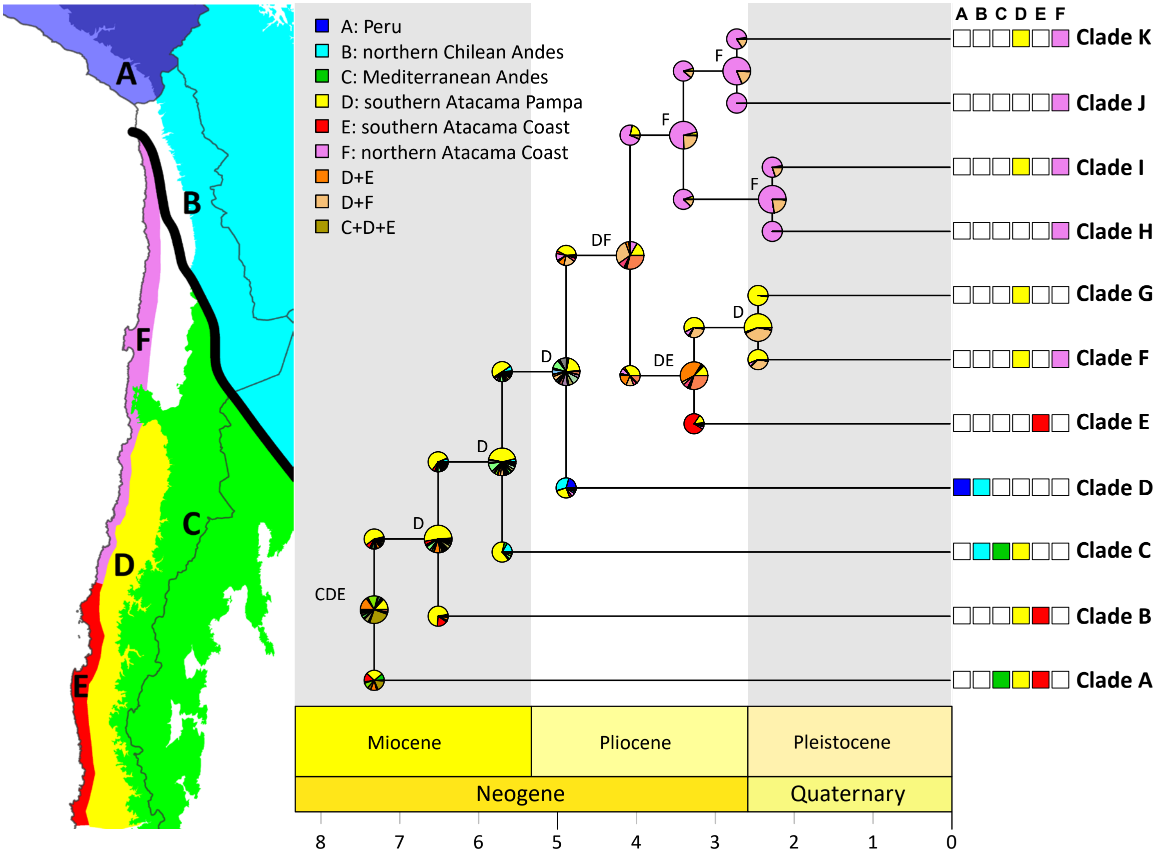

Figure 3: Ancestral area reconstruction of *Cristaria* plotted on a reduced and time calibrated RAxML tree. Tips of the tree represent the eleven clades from Figure 1. Squares between tips and tip labels indicate the distribution assigned to each clade (area A-F) with color codes corresponding to the areas indicated on the map of the Atacama Desert and the legend. Pie charts at the nodes depict relative probabilities of ancestral areas as estimated from the Dispersal-Extinction-Cladogenesis (DEC) analysis with BioGeoBEARS. Letters next to the pie charts indicate the areas with highest relative probabilities.

7.3.2 Dating and ancestral area reconstruction

A simplified dated phylogeny together with the results of the ancestral area reconstruction is provided in Figure 3, while the full dated GBS phylogeny is provided in Appendix D (supplementary Figure S2). The earliest branching clade within *Cristaria* diverged approximately 7 Mya, i.e. during the late Miocene, from an apparently widely distributed last common ancestor covering the majority of the southern Atacama Desert ecosystems. Clades B and C both diverged during the latest Miocene period (6.5 Mya and 5.5 Mya, respectively) from a common ancestral area in the southern Atacama Pampa (area D). From this region *Cristaria* appears to have colonized the southern Atacama Coast (area E) in case of clade B as well as the Mediterranean Andes (area C) and the northern Chilean Andes (area B) in case of clade C. Clade D, the *C. multifida* clade, colonized Peru via the northern Chilean Andes during the early Pliocene coming from the southern Atacama Pampa area, but this relationship receives only limited support. During the mid-Pliocene (~4 Mya) two major lineages diverged from a last common ancestor distributed in the southern Atacama Pampa (area D) and the northern Atacama Coast (area F): First, a lineage comprising the clades E (*C. glaucophylla*), F (*Cristaria argyliifolia* Phil.) and G (*C. cyanea*) with a last common geographical distribution in the southern Atacama Pampa and Coast (areas D and E). *Cristaria glaucophylla* split from the remaining two clades in the late Pliocene and colonized the southern Atacama Coast (area E), while the two partially sympatric species *C. argyliifolia* (clade F) and *C. cyanea* (clade G) diverged in the early Pleistocene. The second mid-Pliocene lineage encompasses the remaining four clades (H–K), which are endemic to the northern Atacama Coast where they are largely sympatric (Figure 2 H–J). A few individual records of particular species are here found so far inland that they had to be included in the southern Atacama Pampa area (cf. clades I & K).

7.3.3 Phylogeny and population structure of *Cristaria integerrima*

Within the *C. integerrima* clade we found highest support for four genetic clusters with a delta *K* of 7.2 followed by 1.9 in favor of three genetic clusters (Figure 4). Compared to the overall phylogeny in Figure 1 the reduced RAxML tree lacks some of the statistical support, although the four genetic clusters from the STRUCTURE analysis correspond with the topology of the tree (note that the orientation of the tree does not represent a latitudinal distribution of the samples). These four clusters are geographically well separated by three border regions: (i) between Caldera and Chañeral, (ii) between Cifuncho and Taltal and (iii) between Paposo and Miguel Diaz. The border region between Paposo and Miguel Diaz is less pronounced as individuals from the two northern cluster occur sympatrically, which is also reflected in comparably less genetic separation of those two clusters (Figure 4 B). A single individual collected at the very northern distribution limit of the species near Tocopilla (ED3030) clusters within the northern cluster but in the phylogeny in the Taltal/Paposo cluster (green and yellow tips, respectively).

7.4 Discussion

7.4.1 Biogeographic history of *Cristaria* from the Miocene to Pliocene

Restriction site associated DNA sequencing techniques such as GBS (Elshire *et al.* 2011; Wendler *et al.* 2014) are increasingly applied to gain insights into complex diversification processes (e.g., Andrews *et al.* 2016; Meier *et al.* 2017; Pérez-Escobar *et al.* 2017; Vargas *et al.* 2017; Ahmed *et al.* 2019), because classic Sanger sequencing methods often fail to resolve shallow relationships, as demonstrated for some of the speciose Atacama genera (Luebert & Wen 2008; Dillon *et al.* 2009; Larridon *et al.* 2015; Böhnert *et al.* 2019). This is the first study successfully applying GBS to resolve phylogenetic relationships in *Cristaria*, one of the most species rich genera in the Atacama Desert with 20 species currently accepted, as well as to any other Atacama clade in a phylogenetic context. As shown by Böhnert *et al.* (2019) the diversity of *Cristaria* is the result of *in situ* diversification in response to increasing hyperaridity in the Atacama Desert as has been previously shown for e.g., *Heliotropium*

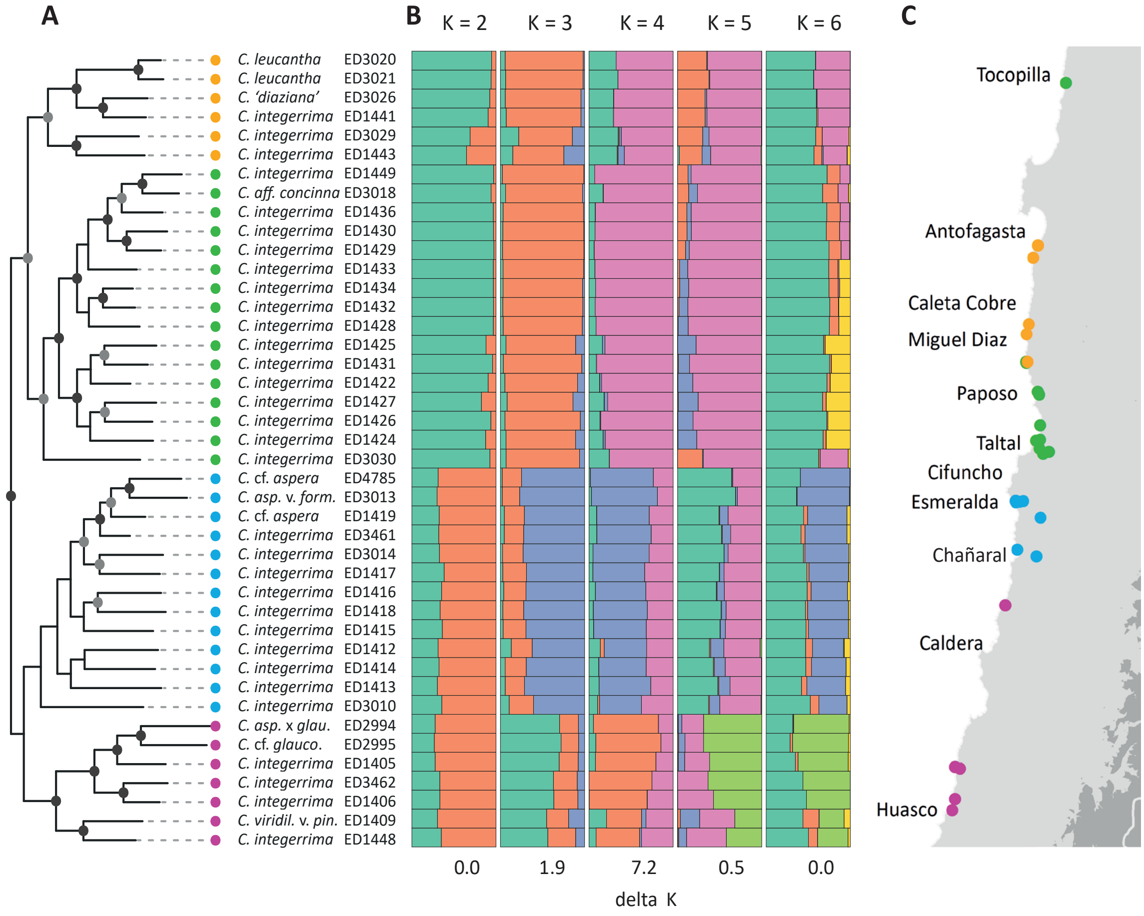

Figure 4: Phylogenetic inference and STRUCTURE analysis of 42 samples of *C. integerrima*. Assignment to this clade is based on the phylogenetic reconstruction in Figure 1. **A**) Maximum Likelihood reconstruction with RAxML of the *C. integerrima* clade with outgroup (*C. molinae*) not shown. Bootstrap support is indicated with dots at the nodes. Gray dots indicate moderate support of ≥ 50, black dots show high support of ≥ 90, nodes without dots are considered as not supported (< 50). Lab IDs are shown alongside taxonomic identifications. Colored dots at node tips correspond to the distribution in the map. **B**) Results of the STRUCTURE analysis are shown as bar plots given the assignment of individual ancestry from two to six genetic clusters (K2 – K6), corresponding delta K values are given below the plots. **C**) Distribution map of all *C. integerrima* samples according to their clades. For orientation, a set of localities and settlements along this part of the coast line of the Atacama Desert is given. The map extents from 22° S to 28.5° S.

sect. *Cochranea* (Luebert *et al.* 2011) or *Oxalis* (Heibl & Renner 2012). In *Cristaria*, however, it seems that the oldest and most widespread clades (Figure 2A-D), which originated during the late Miocene, are absent from the hyperarid core of the Atacama Desert. This might be explained by phylogenetic niche conservatism (Pyron *et al.* 2015) as the ancestral area inferred in this study points towards an origin of the genus in Mediterranean central Chile. As climatic conditions in the Andean Atacama were most likely wetter compared to the core desert, the colonization and subsequent adaptation to Andean climates by *C. andicola* seems plausible (Houston 2006). The increasing climatic fluctuations during the Pliocene probably allowed cyclic incursions of ancient *Cristaria* into the desert, whereas the Miocene in the Atacama Desert was characterized by harsh hyperarid conditions that had possibly prevented such incursions (Zachos *et al.* 2001). Therefore, the presence of the single northern Andean and Peruvian species, *C. multifida*, is best explained by vicariance due to an intensification of the Dry Diagonal after a wetter period during the Quaternary (Lamy *et al.* 2000; Houston 2006). Shortly after the split from a common ancestral population, possibly occupying an ecosystem similar to the present-day southern Atacama Pampa, all remaining clades originated in the late Pliocene or early Pleistocene (Figure 3, clades E-K). Unfortunately, a more detailed understanding of climatic history, that would allow a better correlation between the diversification and the inferred ages, is not yet available.

7.4.2 Quaternary Diversification

As shown above, the most recently diverged clades H to K are currently sympatric along the northern Atacama Coast. Even though those four clades are phylogenetically well separated, morphological plasticity is very high pointing towards repeated reticulate processes, likely along with Quaternary climate oscillations (Stebbins 1952; Degnan & Rosenberg 2009; Avise *et al.* 2016; Ritter *et al.* 2019). Due to the comprehensive sampling of *C. integerrima* – nearly at population level across the entire range – four genetic clusters can be identified, with a single outlier. These four clusters are geographically well structured and are highly congruent with floristic clusters identified for the Loma vegetation along the coastal region between Huasco and Antofagasta (Schulz *et al.* 2011). It seems plausible that in wetter phases during the Quaternary these clusters experienced expansion and gene exchange and became isolated during dry phases. In order to confirm whether such repeated genetic contact has left traces in the genetic signatures, future studies may include testing for introgression (e.g., D-statistics; Eaton *et al.* 2015; Federman *et al.* 2018) or population splits and mixture (Pickrell & Pritchard 2012).

7.4.3 Taxonomic notes

Due to the high morphological plasticity and likely continuous adaptation to constantly changing environments species delimitation is notoriously difficult in *Cristaria*. Therefore, the – purely morphological – treatment by Muñoz-Schick (1995) turns out to be a surprisingly accurate treatment of the genus *Cristaria* for Chile in general and the Atacama Desert in particular. Nevertheless, the results presented here highlight some problems, with regard to *C. aspera*, *C. leucantha* and a few others. The highly resolved GBS phylogeny here provided can certainly be seen as a solid basis and the data generated for this study still bear a great potential to further unravel the evolutionary history of *Cristaria*.

7.4.4 Conclusion

The results shown here for *Cristaria* give the most comprehensive overview on the evolutionary and biogeographic history for any of the species-rich genera of the Atacama Desert to date. We are able to provide a highly resolved phylogeny for *Cristaria* providing deep insights into the evolutionary history of this group and the Atacama Desert flora in general, showing the utility of GBS to address macroevolutionary questions of recently diverged clades. Most importantly, our highly resolved phylogeny shows a very strong geographical and paleoclimatic signal: It could be demonstrated that the major clades diverged during the late Miocene and Pliocene, likely under climatic conditions prevailing to this day in the ancestral area, the Mediterranean-climate region south of the Atacama Desert. More recent climate oscillations are reflected in the structure of the northern coastal clusters and an Andean connection can be demonstrated for Chilean and Peruvian taxa. The data here provided also represent a solid basis for a critical revision of the genus and the adjustment of species limits to improve its classification. With the genetic data set compiled for this study and the sequencing methods used here, a large number of additional research questions can be addressed. First in-depth analyses indicate that especially the sympatric coastal species will provide further insights into the recent climatic and biological history of the Atacama Desert.

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7.6 References

Ahmed D, Comte A, Curk F, Costantino G, Luro F, Dereeper A, Mournet P, Froelicher Y & Ollitrault P (2019) Genotyping by sequencing can reveal the complex mosaic genomes in gene pools resulting from reticulate evolution: a case study in diploid and polyploid citrus. *Annals of Botany* 123 (7): 1231–1251. DOI: https://doi.org/10.1093/aob/mcz029.

Alroy J (2010) The shifting balance of diversity among major marine animal groups. *Science* 329 (5996): 1191–1194. DOI: https://doi.org/10.1126/science.1189910.

Andrews KR, Good JM, Miller MR, Luikart G & Hohenlohe PA (2016) Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics* 17 (2): 81–92. DOI: https://doi.org/10.1038/nrg.2015.28.

Andrews S, Krueger F, Segonds-Pichon A, Biggins L, Krueger C & Wingett S (2012) FastQC. DOI: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Areces-Berazain F & Ackerman JD (2017) Diversification and fruit evolution in Eumalvoids (Malvaceae). *Botanical Journal of the Linnean Society* 184 (4): 401–417. DOI: https://doi.org/10.1093/botlinnean/box035.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA & Saunders NC (1987) Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. *Annual Review of Ecology and Systematics* 18 (1): 489–522. DOI: https://doi.org/10.1146/annurev.es.18.110187.002421.

Avise JC, Bowen BW & Ayala FJ (2016) In the light of evolution X: Comparative phylogeography. *Proceedings of the National Academy of Sciences* 113 (29): 7957–7961. DOI: https://doi.org/10.1073/pnas.1604338113.

Baker PA & Fritz SC (2015) Nature and causes of Quaternary climate variation of tropical South America. *Quaternary Science Reviews* 124: 31–47. DOI: https://doi.org/10.1016/j.quascirev.2015.06.011.

Böhnert T, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider JV, Quandt D & Weigend M (2019) Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert. *Global and Planetary Change* 181: 1–8. DOI: https://doi.org/10.1016/j.gloplacha.2019.102992.

Bravo GA, Antonelli A, Bacon CD, Bartoszek K, Blom MPK, Huynh S, Jones G, Knowles LL, Lamichhaney S, Marcussen T, Morlon H, Nakhleh LK, Oxelman B, Pfeil B, Schliep A, Wahlberg N, Werneck FP, Wiedenhoeft J, Willows-Munro S & Edwards SV (2019) Embracing heterogeneity: Coalescing the Tree of Life and the future of phylogenomics. *PeerJ* 7: e6399. DOI: https://doi.org/10.7717/peerj.6399.

Brignone NF, Pozner RE & Denham SS (2019) Origin and evolution of *Atriplex* (Amaranthaceae s.l.) in the Americas: Unexpected insights from South American species. *Taxon* 18 (8): 71. DOI: https://doi.org/10.1002/tax.12133.

Degnan JH & Rosenberg NA (2009) Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* 24 (6): 332–340. DOI: https://doi.org/10.1016/j.tree.2009.01.009.

Dillon MO & Hoffmann AE (1997) Lomas formations of the Atacama Desert northern Chile. In: Davis SD, Heywood VH, Herrera-MacBryde O, Villa-Lobos J & Hamilton AC, eds. *Centres of Plant Diversity, a Guide and Strategy for Their Conservation. Volume 3: The Americas*. United Kingdom: The World Wildlife Fund For Nature and IUCN - The World Conservation Union, *pp* 528–535.

Dillon MO, Tu T, Xie L, Quipuscoa Silvestre V & Wen J (2009) Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution* 47 (5): 457–476. DOI: https://doi.org/10.1111/j.1759-6831.2009.00040.x.

Dunai TJ, González López GA & Juez-Larré J (2005) Oligocene–Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. *Geology* 33 (4): 321–324. DOI: https://doi.org/10.1130/G21184.1.

Eaton DAR, Hipp AL, González-Rodríguez A & Cavender-Bares J (2015) Historical introgression among the American live oaks and the comparative nature of tests for introgression. *Evolution* 69 (10): 2587–2601. DOI: https://doi.org/10.1111/evo.12758.

Eaton DAR & Overcast I (2020) ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*. DOI: https://doi.org/10.1093/bioinformatics/btz966.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES & Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* 6 (5): e19379. DOI: https://doi.org/10.1371/journal.pone.0019379.

Evanno G, Regnaut S & Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14 (8): 2611–2620. DOI: https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Federman S, Donoghue MJ, Daly DC & Eaton DAR (2018) Reconciling species diversity in a tropical plant clade (*Canarium*, Burseraceae). *PLoS ONE* 13 (6): e0198882. DOI: https://doi.org/10.1371/journal.pone.0198882.

Gayo EM, Latorre C, Jordan TE, Nester PL, Estay SA, Ojeda KF & Santoro CM (2012) Late Quaternary hydrological and ecological changes in the hyperarid core of the northern Atacama Desert (~21°S). *Earth-Science Reviews* 113 (3-4): 120–140. DOI: https://doi.org/10.1016/j.earscirev.2012.04.003.

Guilliams CM, Hasenstab-Lehman KE, Mabry ME & Simpson MG (2017) Memoirs of a frequent flier: Phylogenomics reveals 18 long-distance dispersals between North America and South America in the popcorn flowers (Amsinckiinae). *American Journal of Botany* 104 (11): 1717–1728. DOI: https://doi.org/10.3732/ajb.1700369.

Hanski I (1998) Metapopulation dynamics. *Nature* 396 (6706): 41–49. DOI: https://doi.org/10.1038/23876.

Heibl C & Renner SS (2012) Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. *Systematic Biology* 61 (5): 823–834. DOI: https://doi.org/10.1093/sysbio/sys034.

Houston J (2006) Variability of precipitation in the Atacama Desert: Its causes and hydrological impact. *International Journal of Climatology* 26 (15): 2181–2198. DOI: https://doi.org/10.1002/joc.1359.

Huang H & Knowles LL (2016) Unforeseen Consequences of Excluding Missing Data from Next-Generation Sequences: Simulation Study of RAD Sequences. *Systematic Biology* 65 (3): 357–365. DOI: https://doi.org/10.1093/sysbio/syu046.

Jakobsson M & Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23 (14): 1801–1806. DOI: https://doi.org/10.1093/bioinformatics/btm233.

Lamy F, Klump J, Hebbeln D & Wefer G (2000) Late Quaternary rapid climate change in northern Chile. *Terra Nova* 12 (1): 8–13. DOI: https://doi.org/10.1046/j.1365-3121.2000.00265.x.

Larridon I, Walter HE, Guerrero PC, Duarte M, Cisternas MA, Hernández CP, Bauters K, Asselman P, Goetghebeur P & Samain M-S (2015) An integrative approach to understanding the evolution and diversity of *Copiapoa* (Cactaceae), a threatened endemic Chilean genus from the Atacama Desert. *American Journal of Botany* 102 (9): 1506–1520. DOI: https://doi.org/10.3732/ajb.1500168.

Latorre C, Betancourt JL, Rylander KA & Quade J (2002) Vegetation invasions into absolute desert. A 45 000 yr rodent midden record from the Calama–Salar de Atacama basins, northern Chile (lat 22°–24°S). *Geological Society of America Bulletin* 114 (3): 349–366. DOI: https://doi.org/10.1130/0016-7606(2002)114<0349:VIIADA>2.0.CO;2.

Lemmon EM & Lemmon AR (2013) High-Throughput Genomic Data in Systematics and Phylogenetics. *Annual Review of Ecology, Evolution and Systematics* 44 (1): 99–121. DOI: https://doi.org/10.1146/annurev-ecolsys-110512-135822.

Luebert F, Hilger HH & Weigend M (2011) Diversification in the Andes: Age and origins of South American *Heliotropium* lineages (Heliotropiaceae, Boraginales). *Molecular Phylogenetics and Evolution* 61 (1): 90–102. DOI: https://doi.org/10.1016/j.ympev.2011.06.001.

Luebert F & Wen J (2008) Phylogenetic Analysis and Evolutionary Diversification of *Heliotropium* Sect. *Cochranea* (Heliotropiaceae) in the Atacama Desert. *Systematic Botany* 33 (2): 390–402. DOI: https://doi.org/10.1600/036364408784571635.

Maldonado A, Betancourt JL, Latorre C & Villagran C (2005) Pollen analyses from a 50 000-yr rodent midden series in the southern Atacama Desert (25° 30′ S). *Journal of Quaternary Science* 20 (5): 493–507. DOI: https://doi.org/10.1002/jqs.936.

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal* 17 (1): 10–12. DOI: https://doi.org/10.14806/ej.17.1.200.

Matzke NJ (2013) Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* 5 (4): 242–248. DOI: https://doi.org/10.21425/F5FBG19694.

McCormack JE, Hird SM, Zellmer AJ, Carstens BC & Brumfield RT (2013) Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution* 66 (2): 526–538. DOI: https://doi.org/10.1016/j.ympev.2011.12.007.

Meier JI, Marques DA, Mwaiko S, Wagner CE, Excoffier L & Seehausen O (2017) Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nature Communications* 8: 14363. DOI: https://doi.org/10.1038/ncomms14363.

Merklinger FF, Zheng Y, Luebert F, Harpke D, Böhnert T, Stoll A, Koch MA, Blattner FR, Wiehe T & Quandt D (2020) Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert. *Global and Planetary Change* 184: 103076. DOI: https://doi.org/10.1016/j.gloplacha.2019.103076.

Muñoz-Schick M (1995) Revision del género *Cristaria* (Malvaceae) en Chile. *Boletín del Museo Nacional de Historia Natural Chile* 45: 45–110.

Nester PL, Gayó E, Latorre C, Jordan TE & Blanco N (2007) Perennial stream discharge in the hyperarid Atacama Desert of northern Chile during the latest Pleistocene. *Proceedings of the National Academy of Sciences* 104 (50): 19724–19729. DOI: https://doi.org/10.1073/pnas.0705373104.

Paradis E & Schliep K (2019) ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35 (3): 526–528. DOI: https://doi.org/10.1093/bioinformatics/bty633.

Pérez-Escobar OA, Chomicki G, Condamine FL, Karremans AP, Bogarín D, Matzke NJ, Silvestro D & Antonelli A (2017) Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. *New phytologist* 215 (2): 891–905. DOI: https://doi.org/10.1111/nph.14629.

Pickrell JK & Pritchard JK (2012) Inference of population splits and mixtures from genome-wide allele frequency data. *PLoS Genetics* 8 (11): e1002967. DOI: https://doi.org/10.1371/journal.pgen.1002967.

Pritchard JK, Stephens M & Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 155 (2): 945–959. DOI: https://doi.org/https://www.genetics.org/content/genetics/155/2/945.full.pdf.

Pyron RA, Costa GC, Patten MA & Burbrink FT (2015) Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. *Biological Reviews* 90 (4): 1248–1262. DOI: https://doi.org/10.1111/brv.12154.

R Core Team (2018) R: A Language and Environment for Statistical Computing. Vienna, Austria. DOI: https://www.R-project.org/.

Rabosky DL (2013) Diversity-Dependence, Ecological Speciation, and the Role of Competition in Macroevolution. *Annual Review of Ecology, Evolution, and Systematics* 44 (1): 481–502. DOI: https://doi.org/10.1146/annurev-ecolsys-110512-135800.

Ree RH & Sanmartín I (2018) Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography* 45 (4): 741–749. DOI: https://doi.org/10.1111/jbi.13173.

Ree RH & Smith SA (2008) Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. *Systematic Biology* 57 (1): 4–14. DOI: https://doi.org/10.1080/10635150701883881.

Ritter B, Wennrich V, Medialdea A, Brill D, King G, Schneiderwind S, Niemann K, Fernández-Galego E, Diederich J, Rolf C, Bao R, Melles M & Dunai TJ (2019) “Climatic fluctuations in the hyperarid core of the Atacama Desert during the past 215 ka”. *Scientific Reports* 9 (1): 5270. DOI: https://doi.org/10.1038/s41598-019-41743-8.

Sanderson MJ (2002) Estimating Absolute Rates of Molecular Evolution and Divergence Times: A Penalized Likelihood Approach. *Molecular Biology and Evolution* 19 (1): 101–109. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a003974.

Schneider JV (2013) The Peruvian species of *Cristaria* (Malveae, Malvaceae). Taxonomic revision, chromosome counts, and breeding system. *Phytotaxa* 110 (1): 31. DOI: https://doi.org/10.11646/phytotaxa.110.1.3.

Schneider JV, Schulte K, Aguilar JF & Huertas ML (2011) Molecular evidence for hybridization and introgression in the neotropical coastal desert-endemic *Palaua* (Malveae, Malvaceae). *Molecular Phylogenetics and Evolution* 60 (3): 373–384. DOI: https://doi.org/10.1016/j.ympev.2011.05.010.

Schulz N, Aceituno P & Richter M (2011) Phytogeographic divisions, climate change and plant dieback along the coastal desert of northern Chile. *Erdkunde* 65 (2): 169–187. DOI: https://doi.org/10.3112/erdkunde.2011.02.05.

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30 (9): 1312–1313. DOI: https://doi.org/10.1093/bioinformatics/btu033.

Stebbins GL (1952) Aridity as a Stimulus to Plant Evolution. *The American Naturalist* 86 (826): 33–44. DOI: https://doi.org/10.1086/281699.

Tate JA, Fuertes Aguilar J, Wagstaff SJ, La Duke JC, Bodo Slotta TA & Simpson BB (2005) Phylogenetic relationships within the tribe Malveae (Malvaceae, subfamily Malvoideae) as inferred from ITS sequence data. *American Journal of Botany* 92 (4): 584–602. DOI: https://doi.org/10.3732/ajb.92.4.584.

Tripp EA, Tsai Y-HE, Zhuang Y & Dexter KG (2017) RADseq dataset with 90% missing data fully resolves recent radiation of *Petalidium* (Acanthaceae) in the ultra-arid deserts of Namibia. *Ecology and Evolution* 7 (19): 7920–7936. DOI: https://doi.org/10.1002/ece3.3274.

Tu T, Dillon MO, Sun H & Wen J (2008) Phylogeny of *Nolana* (Solanaceae) of the Atacama and Peruvian deserts inferred from sequences of four plastid markers and the nuclear LEAFY second intron. *Molecular Phylogenetics and Evolution* 49 (2): 561–573. DOI: https://doi.org/10.1016/j.ympev.2008.07.018.

Vargas OM, Ortiz EM & Simpson BB (2017) Conflicting phylogenomic signals reveal a pattern of reticulate evolution in a recent high-Andean diversification (Asteraceae: Astereae: *Diplostephium*). *New Phytologist* 214 (4): 1736–1750. DOI: https://doi.org/10.1111/nph.14530.

Wendler N, Mascher M, Nöh C, Himmelbach A, Scholz U, Ruge-Wehling B & Stein N (2014) Unlocking the secondary gene-pool of barley with next-generation sequencing. *Plant Biotechnology Journal* 12 (8): 1122–1131. DOI: https://doi.org/10.1111/pbi.12219.

Zachos J, Pagani M, Sloan L, Thomas E & Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292 (5517): 686–693. DOI: https://doi.org/10.1126/science.1059412.

Chapter 8

Final Discussion and Conclusion

In the far north of Chile the famous Pan-American Highway crosses the absoulte desert.

„I am tired of repeating the epithets barren and sterile. These words, however, as commonly used, are comparative; I have always applied them to the plains of Patagonia, which can boast of spiny bushes and some tufts of grass; and this is absolute fertility, as compared with Northern Chile.“

Charles R. Darwin
Journal of Researches into the Natural History and Geology of the Countries Visited during the Voyage of HMS Beagle round the World, under the Command of Capt. Fitz Roy, R.N. (1st publ. 1839)

8.1 Phytogeography of the Atacama Desert

The sparse vegetation of the Atacama Desert in northern Chile is unevenly distributed due to varying climatic conditions (Houston 2006; Luebert & Pliscoff 2017). Along the Andean foothills the vegetation is subject to a stronger seasonality and is therefore characterized by more therophytes and geophytes and less shrubs compared to the coast (chapter 2), where, especially in the central part, large shrubs are the dominating elements (Rauh 1986). While the vegetation of the coastal desert has received much attention throughout the years (e.g., Johnston 1929; Dillon & Hoffmann 1997; Schulz *et al.* 2011), the vegetation along the Andean foothills has so far only been studied fragmentarily (e.g., Gutiérrez *et al.* 1998; Díaz *et al.* 2019). In chapter 2, the first study of the Andean Atacama vegetation over a latitudinal range is presented based on four altitudinal transects, which has strongly increased our understanding of this vegetation type. Comparison of these results with available data from the coast provides for the first time statistical support for the assumption that the floristic composition and spatial variability of the vegetation of the Atacama Desert (see Question 1; chapter 1) is basically characterized by two vegetational belts separated by a hyperarid core (chapter 2; Rauh 1986; Rundel *et al.* 1991; Dillon & Hoffmann 1997; Schulz *et al.* 2011; Luebert & Pliscoff 2017). Floristic composition along the Atacama Desert coast is mainly influenced by the interplay of the topography of the coastal cordillera and the gradually decreasing influence of fog and sporadic precipitation towards the north as well as by erratic El Niño events. (Schulz *et al.* 2011). Along the Andean foothills, in turn, vegetation is feed by summer rainfall due to enhanced moisture transport by easterly winds from the Amazon basin (Garreaud 2009).

As discussed above, the hyperarid core in the northern Atacama Desert effectively separates two dissimilar floristic entities. The Peruvian coastal desert, which connects directly in the north of Chile, shows great similarity with the Atacama Desert in terms of the climatic factors and geological history responsible for the prevailing hyperarid conditions. However, differences do exist: Compared to the Atacama, there is no floristic separation between the coast and the Andean foothills and the vegetation of the coastal lomas reaches far into the Peruvian Andes (Rauh 1986; Rundel *et al.* 1991). Vegetation has been shown to be very different and in fact the existence of an actual floristic barrier along the Chilean-Peruvian border has been discussed for some time (Pinto & Luebert 2009). However, this seems to be the case mainly along the coastal and lower inland part in this area. Here, the coastal range is rather low and prevents fog condensation (Madella *et al.* 2018). This allows the formation of species-poor communities of plants adapted to the fog which penetrates further inland (Merklinger *et al.* 2020). In fact, the results presented in chapter 2 show that a barrier does exist between the coastal vegetation of the Atacama Desert and Peru but, on the other hand, that this barrier is less pronounced towards the Andes, where a floristic connection between Chile and Peru could be documented. Those results are supported by Schwarzer *et al.* (2010), who suggested that the actually observed floristic composition is under constant change and that dispersal is much more frequent than previously assumed. Therefore, the question (Q2) whether a floristic connection exists between the Atacama Desert and the Peruvian desert must be answered with yes. However, further fine scale floristic studies are required in order to get a deeper understanding of the floristic composition and connection along the desert part of the Andes of Chile and Peru.

Based on the findings regarding a floristic connection the next logical question is whether also an active genetic connection exists between the Atacama Desert and the Peruvian desert. As shown in chapters 5 and 7 for the genera *Atriplex* L. and *Cristaria* Cav., respectively, a genetic connection between the Atacama Desert and the Peruvian desert does exist. In both genera at least one clade has a continuous distribution along the Andean foothills from Chile towards Peru. In case of *Cristaria* it was even possible to provide evidence that Peru was colonized from the south resulting in a continuous distribution of *Cristaria multifida* Cav., which formerly was believed to be restricted to Peru (Muñoz-Schick 1995; Schneider 2013). Further evidence of existing geneflow along the Andean foothills, which supports the here presented findings, is provided based on population genetics of wild tomatoes (Beddows *et al.* 2017).

With regard to the first hypothesis laid out in the General Introduction (see H1 in chapter 1), it can be concluded that the coastal and the Andean desert belt of the Atacama as well as the Peruvian desert are indeed three floristically separated units as shown in chapter 2. Furthermore, apart from a more pronounced floristic connection between the Atacama Desert and Peru along the Andes it was possible to provide evidence of a genetic connection along the Andes, a phenomenon that seems to be more common than previously known.

8.2 Origin of the Atacama Desert flora

The ages of endemic lineages in the Atacama Desert are as diverse as its abiotic and biotic history. Ages for the groups studied in this thesis are ranging from approx. 20 Ma in case of *Metharme lanata* Phil. (stem group age; chapter 3), over 7.3 Ma in case of *Cristaria* (crown group age; chapter 6) to only ~1.6 Ma in case of the South Atacama clade in *Atriplex* (crown group age; chapter 5). The here estimated heterogeneity regarding the ages of the endemic lineages is clearly reflected in all other groups for which comparable studies exist. The divergence of *Nolana* L.f. is estimated at approx. 4 Ma (Dillon *et al.* 2009). Even younger ages were estimated for the columnar cacti genus *Eulychnia* Phil. (Hernández-Hernández *et al.* 2014; Merklinger *et al. in prep*.) or the Boraginaceae genus *Cryptantha* Lehm. ex G.Don, which has colonized South America and the Atacama Desert in particular multiple times between mid-Pleistocene and mid Pliocene followed by *in situ* diversification (Guilliams *et al.* 2017). As shown in Figure 1, apart from few exceptions (note differences regarding stem or crown group ages), the majority of clades present today in the Atacama Desert are rather young

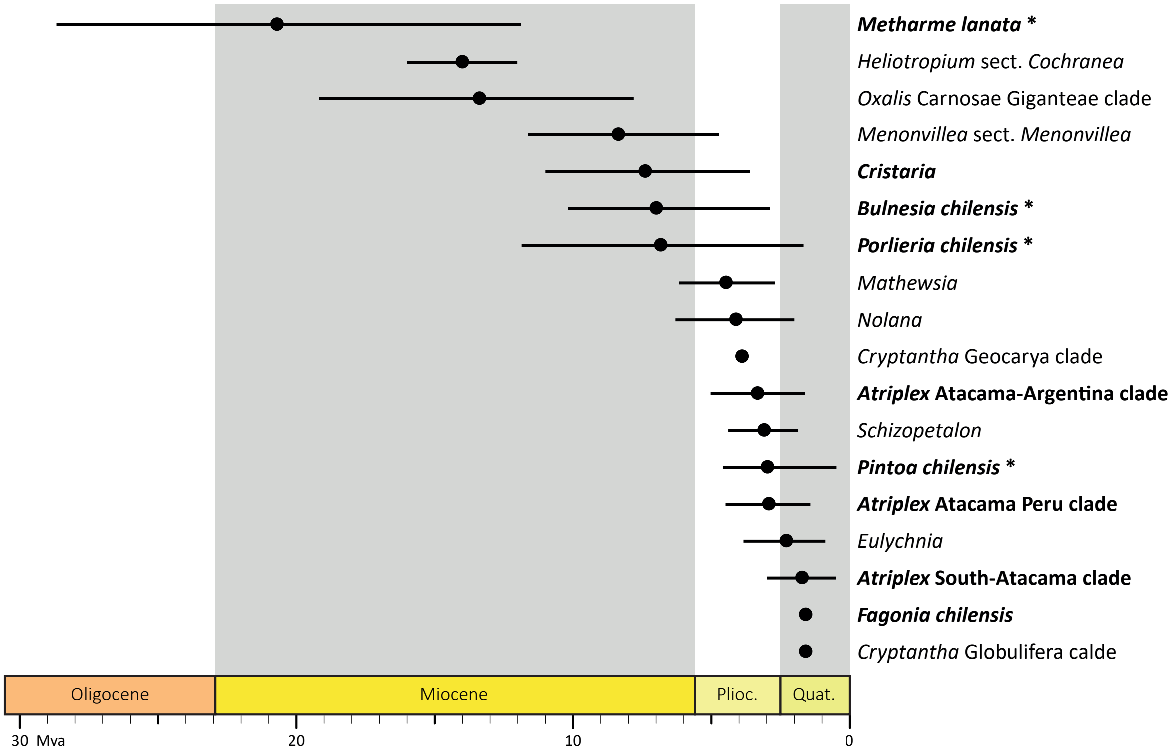


Figure 1: Ages of Atacama Desert plant groups presented in this study (species/clade names in bold) and from literature. Dots represent mean age values and lines are confidence intervals (highest posterior density). For taxa marked by an asterisk (*) stem node ages are given while all other values are crown node ages. Age estimates were taken from chapter 3, 5 and 6; as well as Heibl & Renner (2012), Salariato *et al.* (2016), Luebert & Wen (2008), Dillon *et al.* (2009), Guilliams *et al.* (2017), Merklinger *et al.* (*in prep.*).

and post-date the onset of hyperaridity during the Miocene (Dunai *et al.* 2005).

If two or more species of a certain taxon are present in the Atacama Desert, it raises the question (Q5) whether Atacama lineages are the product of single colonization events or multiple incursions into the desert. For Zygophyllaceae (chapter 3) it could be shown that the desert has been colonized individually five times since the Miocene. However, none of those events was followed by subsequent *in situ* diversification, which contrasts with the increased diversification of Zygophyllaceae in Africa and the Central Asian deserts (Wang *et al.* 2018; Wu *et al.* 2018). In case of *Atriplex* (chapter 5), however, the current diversity of 15 accepted species (Rosas 1989; Brignone *et al.* 2016) is also the product of multiple incursions but in this case was followed by some diversification processes. Other taxa with a similar colonization history are, e.g., *Oxalis* L. (Heibl & Renner 2012), *Cryptantha* (Guilliams *et al.* 2017) or representatives from the clades Cremolobeae, Eudemeae and Schizopetaleae of Brassicaceae (Salariato *et al.* 2016). On the other hand, species diversity of other endemic groups is the product of diversification from just a single common ancestor as shown for the genus *Cristaria* (chapter 6 & 7). This pattern has been documented also for *Heliotropium* L. sect. *Cochranea* (Miers) Kuntze (Luebert & Wen 2008). Also the most speciose genus, *Nolana*, has probably originated and diversified in the Atacama Desert, although its biogeographic history seems to be more complex, involving colonization events towards Peru and even the Galapagos islands (Dillon *et al.* 2009; Ossa *et al.* 2013). In conclusion, based on the data presented in this work and in previous studies, it can be stated that there is no common history of colonization and diversification of plants in the Atacama. Instead, the picture is evenly heterogeneous as the ages of endemic plant lineages.

The question (Q6), if the timing of the colonization of the Atacama Desert is correlated to climatic and geological events, can only be answered on a preliminary basis due to incomplete and inconclusive data. Apparently, most clades are younger (Figure 1) than the postulated onset of hyperaridity in the Atacama Desert (Dunai *et al.* 2005; Ritter *et al.* 2018). In fact, the majority of the study groups have colonized the Atacama in more recent times. Therefore, it seems appropriate to assume that the more recent climatic development during the Pliocene and Pleistocene had a stronger influence on the vegetation rather than the onset of hyperaridity. Furthermore, I was able to provide strong evidence that the rise of the Andes played a major role in the early evolutionary history of the genus *Cristaria* (chapter 6 or Böhnert *et al.* 2019). As shown in chapter 7, again for the genus *Cristaria*, the last common ancestor of this genus had possibly occupied a more Mediterranean climate as it is found today in central Chile south of the Atacama Desert. This implies a more recent colonization of the Atacama Desert by *Cristaria*, probably facilitated by stronger climatic fluctuations during the Pliocene and Pleistocene. However, studies on the historical biogeography of Vivianaceae revealed, based on fossil records in Patagonia, a possible origin of arid-adapted ancestors much further south (Palazzesi *et al.* 2012), but corresponding fossil records are missing for the groups studied here.

In summary, it can be said that the origin of endemic plant lineages does not fully coincide with climatic and geological factors responsible for (hyper-) aridity in the Atacama Desert during the middle to late Miocene (H2). While the Atacama itself and its hyperaridity are rather old (Dunai *et al.* 2005; Hartley *et al.* 2005) the flora seems to be much younger, which is at odds with the times estimated for, e.g., floristic elements of North American deserts (Axelrod 1979; Vásquez-Cruz & Sosa 2019). However, multiple climatic and geological factors have played different roles regarding the colonization of the different groups studied in this thesis. Luebert (2011) already provided a broad picture regarding the different floristic elements found in the Atacama Desert. The present work complements this approach by adding temporal and historical dimensions to our understanding of the biogeographic history of the Atacama Desert.

8.3 Macroevolution of the Atacama Desert flora

As discussed above, a general pattern of a rather recent colonization history of the Atacama Desert has been documented. With the here presented exemplary studies on *Atriplex* and *Cristaria* (chapter 5 and 7, respectively) it could be shown that the spatiotemporal diversification of speciose plant lineages cannot be generalized (Q7). The genus *Cristaria* for example has colonized the southern central pampa of the Atacama Desert from central Chile probably during wetter phases in the Pliocene. Major clades have formed before the Quaternary, but those lineages which show the highest morphological and possibly also genetic diversity have developed only within the last 2.5 Ma and are distributed mainly along the coastal cordillera. In the genus *Atriplex* in turn, the situation is different (chapter 5): The genus colonized the Atacama multiple times (see chapter 8.2). While two clades subsequently diversified in the south and along the coastal part of the Atacama Desert, the ACM clade is mainly found in and around the dry core. The biogeographic history of *Heliotropium* sect. *Cochranea* (Luebert & Wen 2008; Luebert *et al.* 2011), *Nolana* (Dillon *et al.* 2009) or *Oxalis* (Heibl & Renner 2012) is equally heterogeneous.

The increasingly arid conditions of the Atacama Desert since the Miocene (Dunai *et al.* 2005; Ritter *et al.* 2018) were repeatedly interrupted by several more humid episodes throughout the Quaternary (Ritter *et al.* 2019). At the end of the last glacial maximum, the nowadays hyperarid core of the Atacama Desert had developed into a wetland fed by increased rainfall in the high Andes due to more intense El Niño and La Niña events (Lamy *et al.* 2000; Latorre *et al.* 2002; Stuut & Lamy 2004; Gayo *et al.* 2012). The data on the coastal clades of *Cristaria* (chapter 7) presented here as well as phylogeographic studies on closely related species of *Nolana*, clearly indicate a population expansion due to Quaternary climate fluctuations (Ossa *et al.* 2013). Geneflow among sympatric Atacama species does still exist as shown by Luebert *et al.* (2014) for *Heliotropium* sect. *Cochranea*. It remains to be tested if this holds true for the sympatric coastal taxa of *Cristaria*. Furthermore, population structure within the coastal *Cristaria integerrima* Phil. clade even revealed four well separated geographically clusters corresponding to floristic clusters identified by Schulz *et al.* (2011). Therefore, it can be assumed that Quaternary climate oscillations have had major influence on the diversification processes of the speciose Atacama plant clades (Q8). In conclusion, the initial hypothesis regarding the influence of increasing aridity during Miocene towards a stronger influence of a more recent climatic history during Pliocene and Pleistocene (H3) needs to be rejected.

As laid out above, our current understanding of species diversity in the rather young Atacama plant lineages seems to reflect a snapshot of still ongoing speciation processes. The current taxonomic concepts for those clades are, however, not always fully in line with the genetic patterns (Q9). For example, the here studied speciose genera *Atriplex* and *Cristaria* (chapter 5 and 7, respectively) are both characterized by diversification in relation to rather recent climatic change. For both genera taxonomic revisions are available (Rosas 1989; Muñoz-Schick 1995; Brignone *et al.* 2016). However, several taxonomic questions arose based on the results of the genotyping-by-sequencing approach applied in chapters 5 and 7. The here presented phylogenetic data will lay a foundation for several taxonomic changes in *Atriplex* and *Cristaria* in the near future.

8.4 Conclusion and outlook

Studying the origin and diversification of a flora in a certain geographic area allows only limited insights into the past. We are bound to extrapolate from the present inventory and the fossil record. Unfortunately, the fossil record of the Atacama is very sparse. Pollen found in fossil rodent middens allow insights into the past of about 50K years (Latorre *et al.* 2002; Maldonado *et al.* 2005). Dispersal and also long-distance dispersal is much more common as previously assumed (Schwarzer *et al.* 2010). Spatiotemporal heterogeneity is also reflected in a constant adaptation to changing climatic conditions, where even slight and temporal climatic changes result in a floristic response (Díaz *et al.* 2019). Such climatic variability and the resulting floristic responses are then driving forces for evolutionary process. Populations become separated, acquire new genetic traits by adaptation or genetic drift developing into new species (Stebbins 1952). Closely related species that occur sympatrically today may nevertheless be the result of allopatric speciation, but in order to unravel such recent speciation processes a population level sampling as well as sophisticated sequencing and analyzing methods are required.

During the last decade, new methods in the field of molecular genomics have become important tools for phylogenetics and phytogeography (Lemmon & Lemmon 2013). In particular, restriction site-associated DNA sequencing (RADseq; Baird *et al.* 2008), to which the GBS method used in this thesis can be assigned, became very popular due to low cost and wide applicability from macroevolutionary studies (e.g., Eaton *et al.* 2015) down to the population level (e.g., Merklinger *et al.* 2020). However, the large amount of data generated in the context of such studies requires a more profound understanding of data structure and the impact of different analytical methods (Andrews *et al.* 2016). In addition to some complex decisions that need to be made prior to the sequencing step (e.g., library preparation or number of samples per lane), several thresholds (e.g., the amount of missing data in the inter species assembly) need to be set during the final assembly prior to the actual phylogenetic reconstruction (Huang & Knowles 2016). A conservative assembly allowing only a limited amount or no missing data at all might result in a strong reduction of phylogenetic resolution and support for species relationships, as shown by Huang & Knowles (2016) based on simulated RAD datasets (see also Wagner *et al.* 2013). Based on this information and own experiences, *inter alia* with the genera *Atriplex* and *Cristaria* (chapter 5 and 7, respectively), a 70 % clustering threshold was applied. However, as can be seen in some of the resulting phylogenies, internal support of some clades is still rather low. In order to increase phylogenetic resolution, it is possible to further reduce the sampling and run additional assemblies including only those clades with low resolution (Tripp *et al.* 2017).

Another approach for future research on the evolutionary history of the Atacama Desert are so-called species distribution models or ecological niche modeling. Such models allow, based on current day distribution patter and the knowledge about past climatic conditions, to extrapolate distribution patterns based on the assumption that the taxa studied had the same ecological requirements as they have today, also known as niche conservatism (Pyron *et al.* 2015). It has been shown that such approaches provide valuable insights into the ecological and evolutionary history of Atacama clades (Heibl & Renner 2012; Luebert *et al.* 2014). The data and knowledge gained in the framework of this thesis will be fundamental for future research applying a wider spectrum of methods in order to better understand the biological history of the Atacama Desert. Such extended knowledge might also help to extrapolate possible future adaptations of life in the Atacama Desert and other deserts under different climate change scenarios. Although the first consequences of man-made global climate change are omnipresent, it is not yet fully understood how climate change will affect specific ecosystems. Among the most important factors in the Atacama region are the cyclically occurring El Niño events (Dillon & Rundel 1990). If those cyclic events change their frequency or intensity, major impacts on ecosystems throughout the whole Pacific region are expected (Holmgren *et al.* 2006; Wang *et al.* 2019). However, more and more methods are being developed to increase our understanding how global climate change will affect plant life (e.g., Waldvogel *et al.* 2020).

8.5 References

Andrews KR, Good JM, Miller MR, Luikart G & Hohenlohe PA (2016) Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics* 17 (2): 81–92. DOI: https://doi.org/10.1038/nrg.2015.28.

Axelrod DI (1979) Age and origin of Sonoran Desert vegetation. *Occasional papers of the California Academy of Sciences* 132: 1–74.

Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA & Johnson EA (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* 3 (10): e3376. DOI: https://doi.org/10.1371/journal.pone.0003376.

Beddows I, Reddy A, Kloesges T & Rose LE (2017) Population Genomics in Wild Tomatoes—The Interplay of Divergence and Admixture. *Genome Biology and Evolution* 9 (11): 3023–3038. DOI: https://doi.org/10.1093/gbe/evx224.

Böhnert T, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider JV, Quandt D & Weigend M (2019) Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert. *Global and Planetary Change* 181: 102992. DOI: https://doi.org/10.1016/j.gloplacha.2019.102992.

Brignone NF, Denham SS & Pozner R (2016) Synopsis of the genus *Atriplex* (Amaranthaceae, Chenopodioideae) for South America. *Australian Systematic Botany* 29 (5): 324. DOI: https://doi.org/10.1071/SB16026.

Díaz FP, Latorre C, Carrasco-Puga G, Wood JR, Wilmshurst JM, Soto DC, Cole TL & Gutiérrez RA (2019) Multiscale climate change impacts on plant diversity in the Atacama Desert. *Global Change Biology* 25 (5): 1733–1745. DOI: https://doi.org/10.1111/gcb.14583.

Dillon MO & Hoffmann AE (1997) Lomas formations of the Atacama Desert northern Chile. In: Davis SD, Heywood VH, Herrera-MacBryde O, Villa-Lobos J & Hamilton AC, eds. *Centres of Plant Diversity, a Guide and Strategy for Their Conservation. Volume 3: The Americas*. United Kingdom: The World Wildlife Fund For Nature and IUCN - The World Conservation Union, *pp* 528–535.

Dillon MO & Rundel PW (1990) The Botanical Response of the Atacama and Peruvian Desert Floras to the 1982-83 El Niño Event. In: Glynn PW, ed. *Global Ecological Consequences of the 1982–83 El Nino—Southern Oscillation*: Elsevier, *pp* 487–504.

Dillon MO, Tu T, Xie L, Quipuscoa Silvestre V & Wen J (2009) Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America. *Journal of Systematics and Evolution* 47 (5): 457–476. DOI: https://doi.org/10.1111/j.1759-6831.2009.00040.x.

Dunai TJ, González López GA & Juez-Larré J (2005) Oligocene–Miocene age of aridity in the Atacama Desert revealed by exposure dating of erosion-sensitive landforms. *Geology* 33 (4): 321–324. DOI: https://doi.org/10.1130/G21184.1.

Eaton DAR, Hipp AL, González-Rodríguez A & Cavender-Bares J (2015) Historical introgression among the American live oaks and the comparative nature of tests for introgression. *Evolution* 69 (10): 2587–2601. DOI: https://doi.org/10.1111/evo.12758.

Garreaud RD (2009) The Andes climate and weather. *Advances in Geosciences* 22: 3–11. DOI: https://doi.org/10.5194/adgeo-22-3-2009.

Gayo EM, Latorre C, Jordan TE, Nester PL, Estay SA, Ojeda KF & Santoro CM (2012) Late Quaternary hydrological and ecological changes in the hyperarid core of the northern Atacama Desert (~21°S). *Earth-Science Reviews* 113 (3-4): 120–140. DOI: https://doi.org/10.1016/j.earscirev.2012.04.003.

Guilliams CM, Hasenstab-Lehman KE, Mabry ME & Simpson MG (2017) Memoirs of a frequent flier: Phylogenomics reveals 18 long-distance dispersals between North America and South America in the popcorn flowers (Amsinckiinae). *American Journal of Botany* 104 (11): 1717–1728. DOI: https://doi.org/10.3732/ajb.1700369.

Gutiérrez JR, López-Cortes F & Marquet PA (1998) Vegetation in an altitudinal gradient along the Río Loa in the Atacama Desert of northern Chile. *Journal of Arid Environments* 40 (4): 383–399. DOI: https://doi.org/10.1006/jare.1998.0462.

Hartley AJ, Chong G, Houston J & Mather AE (2005) 150 million years of climatic stability: Evidence from the Atacama Desert, northern Chile. *Journal of the Geological Society* 162 (3): 421–424. DOI: https://doi.org/10.1144/0016-764904-071.

Heibl C & Renner SS (2012) Distribution models and a dated phylogeny for Chilean *Oxalis* species reveal occupation of new habitats by different lineages, not rapid adaptive radiation. *Systematic Biology* 61 (5): 823–834. DOI: https://doi.org/10.1093/sysbio/sys034.

Hernández-Hernández T, Brown JW, Schlumpberger BO, Eguiarte LE & Magallón S (2014) Beyond aridification: Multiple explanations for the elevated diversification of cacti in the New World Succulent Biome. *New Phytologist* 202 (4): 1382–1397. DOI: https://doi.org/10.1111/nph.12752.

Holmgren M, Stapp P, Dickman CR, Gracia C, Graham S, Gutiérrez JR, Hice C, Jaksic F, Kelt DA, Letnic M, Lima M, López BC, Meserve PL, Milstead WB, Polis GA, Previtali MA, Richter M, Sabaté S & Squeo FA (2006) Extreme climatic events shape arid and semiarid ecosystems. *Frontiers in Ecology and the Environment* 4 (2): 87–95. DOI: https://doi.org/10.1890/1540-9295(2006)004[0087:ECESAA]2.0.CO;2.

Houston J (2006) Variability of precipitation in the Atacama Desert: Its causes and hydrological impact. *International Journal of Climatology* 26 (15): 2181–2198. DOI: https://doi.org/10.1002/joc.1359.

Huang H & Knowles LL (2016) Unforeseen Consequences of Excluding Missing Data from Next-Generation Sequences: Simulation Study of RAD Sequences. *Systematic Biology* 65 (3): 357–365. DOI: https://doi.org/10.1093/sysbio/syu046.

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

Lamy F, Klump J, Hebbeln D & Wefer G (2000) Late Quaternary rapid climate change in northern Chile. *Terra Nova* 12 (1): 8–13. DOI: https://doi.org/10.1046/j.1365-3121.2000.00265.x.

Latorre C, Betancourt JL, Rylander KA & Quade J (2002) Vegetation invasions into absolute desert: A 45 000 yr rodent midden record from the Calama-Salar de Atacama basins, northern Chile (lat 22°-24°S). *Geological Society of America Bulletin* 114 (3): 349–366. DOI: https://doi.org/10.1130/0016-7606(2002)114<0349:VIIADA>2.0.CO;2.

Lemmon EM & Lemmon AR (2013) High-Throughput Genomic Data in Systematics and Phylogenetics. *Annual Review of Ecology, Evolution and Systematics* 44 (1): 99–121. DOI: https://doi.org/10.1146/annurev-ecolsys-110512-135822.

Luebert F (2011) Hacia una fitogeografía histórica del Desierto de Atacama. *Revista de Geografía Norte Grande (*50): 105–133. DOI: https://doi.org/10.4067/S0718-34022011000300007.

Luebert F, Hilger HH & Weigend M (2011) Diversification in the Andes: Age and origins of South American *Heliotropium* lineages (Heliotropiaceae, Boraginales). *Molecular Phylogenetics and Evolution* 61 (1): 90–102. DOI: https://doi.org/10.1016/j.ympev.2011.06.001.

Luebert F, Jacobs P, Hilger HH & Muller LAH (2014) Evidence for nonallopatric speciation among closely related sympatric *Heliotropium* species in the Atacama Desert. *Ecology and Evolution* 4 (3): 266–275. DOI: https://doi.org/10.1002/ece3.929.

Luebert F & Pliscoff P (2017) Sinopsis bioclimática y vegetacional de Chile. Santiago de Chile: *Editorial Universitaria*. 381 pp.

Luebert F & Wen J (2008) Phylogenetic Analysis and Evolutionary Diversification of *Heliotropium* Sect. *Cochranea* (Heliotropiaceae) in the Atacama Desert. *Systematic Botany* 33 (2): 390–402. DOI: https://doi.org/10.1600/036364408784571635.

Madella A, Delunel R, Audin L & Schlunegger F (2018) Why is there no Coastal Cordillera at the Arica Bend (Western Central Andes)? *Basin Research* 30: 248–268. DOI: https://doi.org/10.1111/bre.12218.

Maldonado A, Betancourt JL, Latorre C & Villagran C (2005) Pollen analyses from a 50 000-yr rodent midden series in the southern Atacama Desert (25° 30′ S). *Journal of Quaternary Science* 20 (5): 493–507. DOI: https://doi.org/10.1002/jqs.936.

Merklinger FF, Zheng Y, Luebert F, Harpke D, Böhnert T, Stoll A, Koch MA, Blattner FR, Wiehe T & Quandt D (2020) Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert. *Global and Planetary Change* 184: 103076. DOI: https://doi.org/10.1016/j.gloplacha.2019.103076.

Muñoz-Schick M (1995) Revision del género *Cristaria* (Malvaceae) en Chile. *Boletín del Museo Nacional de Historia Natural Chile* 45: 45–110.

Ossa PG, Pérez F & Armesto JJ (2013) Phylogeography of two closely related species of *Nolana* from the coastal Atacama Desert of Chile: post-glacial population expansions in response to climate fluctuations. *Journal of Biogeography* 40 (11): 2191–2203. DOI: https://doi.org/10.1111/jbi.12152.

Palazzesi L, Gottschling M, Barreda V & Weigend M (2012) First Miocene fossils of Vivianiaceae shed new light on phylogeny, divergence times, and historical biogeography of Geraniales. *Biological Journal of the Linnean Society* 107 (1): 67–85. DOI: https://doi.org/10.1111/j.1095-8312.2012.01910.x.

Pinto R & Luebert F (2009) Datos sobra la flora vascular del desierto costero de Arica y Tarapacá, Chile, y sus relaciones fitogeográficas con el sur de Perú. *Gayana. Botánica* 66 (1): 28–49. DOI: https://doi.org/10.4067/S0717-66432009000100004.

Pyron RA, Costa GC, Patten MA & Burbrink FT (2015) Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. *Biological Reviews* 90 (4): 1248–1262. DOI: https://doi.org/10.1111/brv.12154.

Rauh W (1986) The Peruvian-Chilean Deserts. Chapter 7. In: Evenari M, Noy-Meir I & Goodall DW, eds. *Hot deserts and arid shrublands*. Amsterdam: Elsevier, *pp* 239–267.

Ritter B, Stuart FM, Binnie SA, Gerdes A, Wennrich V & Dunai TJ (2018) Neogene fluvial landscape evolution in the hyperarid core of the Atacama Desert. *Scientific Reports* 8 (1): 13952. DOI: https://doi.org/10.1038/s41598-018-32339-9.

Ritter B, Wennrich V, Medialdea A, Brill D, King G, Schneiderwind S, Niemann K, Fernández-Galego E, Diederich J, Rolf C, Bao R, Melles M & Dunai TJ (2019) “Climatic fluctuations in the hyperarid core of the Atacama Desert during the past 215 ka”. *Scientific Reports* 9 (1): 5270. DOI: https://doi.org/10.1038/s41598-019-41743-8.

Rosas MR (1989) El genero *Atriplex* (Chenopodiaceae) en Chile. *Gayana. Botánica* 46 1-2: 3–82.

Rundel PW, Dillon MO, Palma B, Mooney HA, Gulmon SL & Ehrlinger JR (1991) The Phytogeography and Ecology of the Coastal Atacama and Peruvian Desert. *Aliso: A Journal of Systematic and Evolutionary Botany* 13 (1): 1–49.

Salariato DL, Zuloaga FO, Franzke A, Mummenhoff K & Al-Shehbaz IA (2016) Diversification patterns in the CES clade (Brassicaceae tribes Cremolobeae, Eudemeae, Schizopetaleae) in Andean South America. *Botanical Journal of the Linnean Society* 181 (4): 543–566. DOI: https://doi.org/10.1111/boj.12430.

Schneider JV (2013) The Peruvian species of *Cristaria* (Malveae, Malvaceae). Taxonomic revision, chromosome counts, and breeding system. *Phytotaxa* 110 (1): 31. DOI: https://doi.org/10.11646/phytotaxa.110.1.3.

Schulz N, Aceituno P & Richter M (2011) Phytogeographic divisions, climate change and plant dieback along the coastal desert of northern Chile. *Erdkunde* 65 (2): 169–187. DOI: https://doi.org/10.3112/erdkunde.2011.02.05.

Schwarzer C, Huamaní FC, Cano A, La Torre MI & Weigend M (2010) 400 years for long-distance dispersal and divergence in the northern Atacama desert – Insights from the Huaynaputina pumice slopes of Moquegua, Peru. *Journal of Arid Environments* 74 (11): 1540–1551. DOI: https://doi.org/10.1016/j.jaridenv.2010.05.034.

Stebbins GL (1952) Aridity as a Stimulus to Plant Evolution. *The American Naturalist* 86 (826): 33–44. DOI: https://doi.org/10.1086/281699.

Stuut J-BW & Lamy F (2004) Climate variability at the southern boundaries of the Namib (southwestern Africa) and Atacama (northern Chile) coastal deserts during the last 120,000 yr. *Quaternary Research* 62 (3): 301–309. DOI: https://doi.org/10.1016/j.yqres.2004.08.001.

Tripp EA, Tsai Y-HE, Zhuang Y & Dexter KG (2017) RADseq dataset with 90% missing data fully resolves recent radiation of *Petalidium* (Acanthaceae) in the ultra-arid deserts of Namibia. *Ecology and Evolution* 7 (19): 7920–7936. DOI: https://doi.org/10.1002/ece3.3274.

Vásquez-Cruz M & Sosa V (2019) Assembly and origin of the flora of the Chihuahuan Desert: The case of sclerophyllous Rosaceae. *Journal of Biogeography* 12 (3): e0168933. DOI: https://doi.org/10.1111/jbi.13745.

Wagner CE, Keller I, Wittwer S, Selz OM, Mwaiko S, Greuter L, Sivasundar A & Seehausen O (2013) Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. *Molecular Ecology* 22 (3): 787–798. DOI: https://doi.org/10.1111/mec.12023.

Waldvogel A-M, Feldmeyer B, Rolshausen G, Exposito-Alonso M, Rellstab C, Kofler R, Mock T, Schmid K, Schmitt I, Bataillon T, Savolainen O, Bergland A, Flatt T, Guillaume F & Pfenninger M (2020) Evolutionary genomics can improve prediction of species' responses to climate change. *Evolution Letters* 31: 434. DOI: https://doi.org/10.1002/evl3.154.

Wang B, Luo X, Yang Y-M, Sun W, Cane MA, Cai W, Yeh S-W & Liu J (2019) Historical change of El Niño properties sheds light on future changes of extreme El Niño. *Proceedings of the National Academy of Sciences of the United States of America* 116 (45): 22512–22517. DOI: https://doi.org/10.1073/pnas.1911130116.

Wang Q, Wu S, Su X, Zhang L, Xu X, Lyu L, Cai H, Shrestha N, Liu Y, Wang W & Wang Z (2018) Niche conservatism and elevated diversification shape species diversity in drylands: evidence from Zygophyllaceae. *Proceedings. Biological sciences* 285 (1890). DOI: https://doi.org/10.1098/rspb.2018.1742.

Wu S-D, Zhang L-J, Lin L, Yu S-X, Chen Z-D & Wang W (2018) Insights into the historical assembly of global dryland floras: the diversification of Zygophyllaceae. *BMC Evolutionary Biology* 18 (1): 166. DOI: https://doi.org/10.1186/s12862-018-1277-z.

Summary

Böhnert T (2020) ***Historical Biogeography and Macroevolution of the Atacama Desert Flora***.
Dissertation, Rheinische Friedrich-Wilhelms-Universität Bonn. 231 pp.

The aim of this doctoral thesis is to gain a broader understanding of the biogeographic and evolutionary history of the Atacama Desert flora. This work is embedded in an interdisciplinary research project (CRC1211, ‘Earth – Evolution at the Dry Limit’) which studies the biological and landscape evolution of hyperarid environments. The Atacama Desert is part of a coastal desert system which reaches along the west coast of South America from approx. 30° S around the city of La Serena in northern Chile towards the north of Peru at approx. 5° S. The Chilean Atacama Desert and Peruvian desert have much in common regarding reasons for the prevailing hyperarid conditions. However, both are clearly separated in terms of their floristic compositions although a certain floristic and genetic connectivity possibly does exist. Located in northern Chile, the Atacama Desert is not only one of the oldest deserts but also one of the driest places on earth with hyperarid conditions prevailing since the Miocene or earlier. Despite the extreme conditions documented for the desert, its flora is surprisingly rich in terms of species diversity and degree of endemism.

One objective of this thesis is to better understand the floristic composition of the coastal and Andean vegetation of the desert and to evaluate a floristic and genetic link between the Atacama Desert and the coastal desert of Peru. A further objective is to explore whether the origin of plant lineages coincides with climatic and geological factors responsible for the hyperaridity in the Atacama Desert during the middle and late Miocene. And finally, to find out if the diversification of speciose plant lineages in the Atacama Desert correlates with increasing aridity during the Miocene and Pliocene as well as with the Quaternary climate oscillation.

The present work consists of eight chapters including a general introduction and discussion as well as six research chapters. The floristic composition of the Andean foothills was studied based on plot-based vegetation assessments along four altitudinal transects. Similarity and cluster analyses were applied to gain insights into the floristic connectivity between the coast and the Andean foothills as well as Peru. A nomenclatural correction of the genus *Atriplex* L. (Amaranthaceae) is presented applying the priority rule of the botanical code of nomenclature for plants. In order to understand the colonization history for the genera *Atriplex* and *Cristaria* Cav. (Malvaceae) as well as the five representatives of Zygophyllaceae in the Atacama Desert, phylogenic studies have been conducted using plastid sequencing information. These datasets were analyzed using Maximum Likelihood and Bayesian molecular clock dating and an ancestral area reconstruction approach under the Dispersal Extinction Cladogenesis. Two chapters are devoted to the results of a modified restriction site associated DNA sequencing approach, termed genotyping-by-sequencing (GBS).

Assembly of the GBS data and subsequent phylogenetic and population genetic analyses were conducted in the Python programming language environment.

Core results of this thesis are, that the vegetation of the Andean foothills is highly dissimilar to the vegetation at coastal range of the Atacama Desert. In addition, the floras of the Peruvian and Chilean coast are equally dissimilar, while a floristic connection along the Andes between those two countries does exist. This floristic connection is underpinned by genetic connection shown for *Atriplex* and *Cristaria*. Furthermore, for both genera relatively young crown node ages have been estimated. In fact, member of the genus *Atriplex* has colonized the Atacama Desert at least three times in the late Pliocene and Pleistocene, while the genus *Cristaria* evolved through a vicariant separation from its sister genus due to Andean uplift during early and mid-Miocene. Ancestral area reconstruction suggests a possible origin in what is called today Mediterranean Chile, with a colonization of the Atacama Desert during the Pliocene and Pleistocene, similar to what was estimated for the genus *Atriplex* and other speciose genera in the Atacama Desert. In fact, most of the diversification processes seem to be the result of recent climatic history during the Quaternary, which contrasts with the calculated age of plant groups of other deserts. In summary, it can be said that the onset and persistence of hyperaridity in the Atacama Desert had little effect on the present flora and its spatiotemporal development, compared to the major impact of the Quaternary climate oscillation. In order to further understand the influence of the more recent climatic history on plant life, future research might focus on macro-evolutionary process which have been responsible for current species richness of the Atacama Desert.

Curriculum Vitae

Tim Böhnert

List of Publications

Peer-Reviewed Publications

***Böhnert T**, Weigend M, Merklinger FF, Quandt D & Luebert F (2020) Historical assembly of Zygophyllaceae in the Atacama Desert. *Frontiers of Biogeography* 12.3, e45197. DOI: https://doi.org/10.21425/F5FBG45197

*Ruhm J, **Böhnert T**, Weigend M, Merklinger FF, Stoll A, Quandt D & Luebert F (2020) Plant life at the dry limit-Spatial patterns of floristic diversity and composition around the hyperarid core of the Atacama Desert. *PLoS ONE* 15 (5), e0233729. DOI: https://doi.org/10.1371/journal.pone.0233729

Merklinger FF, Zheng Y, Luebert F, Harpke D, **Böhnert T**, Stoll A, Koch MA, Blattner FR, Wiehe T & Quandt D (2020) Population genomics of *Tillandsia landbeckii* reveals unbalanced genetic diversity and founder effects in the Atacama Desert. *Global and Planetary Change* 184: 103076. DOI: https://doi.org/10.1016/j.gloplacha.2019.103076.

***Böhnert T**, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider JV, Quandt D & Weigend M (2019) Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert. *Global and Planetary Change* 181: 102992. DOI: https://doi.org/10.1016/j.gloplacha.2019.102992.

***Böhnert T**, Luebert F & Weigend M (2018) *Atriplex retusa*, the correct name for *A. deserticola* (Chenopodiaceae; Amaranthaceae sensu APG). *Phytotaxa* 373 (1): 95–98. DOI: https://doi.org/10.11646/phytotaxa.373.1.6.

Mutke J, **Böhnert T** & Weigend M (2017) Save last cloud forests in western Andes. *Nature* 541 (7636): 157. DOI: https://doi.org/10.1038/541157e.

Böhnert T & Weigend M (2017) *Brunellia* (Brunelliaceae) in Peru—distribution, a new species and a key. *Phytotaxa* 329 (2): 101–113. DOI: https://doi.org/10.11646/phytotaxa.329.2.1.

Böhnert T & Lobin W (2017) *Leopoldia neumannii* sp. nov. (Asparagaceae, Scilloideae): a new species of *Muscari* sensu lato from Greece. *Willdenowia* 47 (2): 179–185. DOI: https://doi.org/10.3372/wi.47.47210.

Böhnert T & Weigend M (2016) Three new species records of *Symplocos* (Symplocaceae) from northern Peru. *Revista Peruana de Biología* 23 (3): 339–342. DOI: https://doi.org/10.15381/rpb.v23i3.12873.

Böhnert T, Wenzel A, Altenhövel C, Beeretz L, Tjitrosoedirdjo SS, Meijide A, Rembold K & Kreft H (2016) Effects of land-use change on vascular epiphyte diversity in Sumatra (Indonesia). *Biological Conservation* 202: 20–29. DOI: https://doi.org/10.1016/j.biocon.2016.08.008.

Data Publications

***Böhnert T**, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider J, Quandt D & Weigend M (2019) cpDNA alignments and phylogenetic trees of *Cristaria* (Malvaceae) from the Atacama Desert. *CRC1211 Database*. DOI: https://doi.org/ 10.5880/CRC1211DB.26

***Böhnert T**, Weigend M, Merklinger FF, Quandt D & Luebert F (2019) cpDNA alignments and R-skript for Historical Biogeography of Zygophyllaceae in the Atacama Desert. *CRC1211 Database*. DOI: https://doi.org/10.5880/CRC1211DB.32.

Publications marked by an asterisk *
are part of this Dissertation.

*Ruhm J, **Böhnert T**, Weigend M, Stoll A, Merklinger FF, Quandt D & Luebert F (2019) Spatial floristic data from the Atacama and Peruvian Desert. *CRC1211 Database*. DOI: https://doi.org/10.5880/CRC1211DB.29.

Merklinger FF, Zheng Y, Luebert F, Harpke D, **Böhnert T**, Stoll A, Koch M, Blattner FR, Wiehe T & Quandt D (2019) Sample locations and GBS output files for analysing the population genomics of *Tillandsia landbeckii*. *CRC1211 Database*. DOI: https://doi.org/10.5880/CRC1211DB.27.

Conference contributions

Merklinger FF, Zheng Y, Luebert F, Harpke D, **Böhnert T**, Stoll A, Koch MA, Blattner FR, Wiehe T & Quandt D (2020) Population genetics of isolated *Tillandsia* lomas in the Atacama Desert of northern Chile. *General Assembly of the European Geosciences Union (EGU)*, Vienna, Austria. [**Talk**]

***Böhnert T**, Luebert F, Schneider J, Merklinger FF, Stoll A, Quandt D & Weigend M (2019) Biogeography and evolution of the Atacama genus *Cristaria* (Malvaceae). *IX. Biennial Conference of the International Biogeography Society*, Malaga, Spain. [**Poster**]

***Böhnert T**, Merklinger FF, Böker S, Harpke D, Stoll A, Blattner F, Weigend M, Quandt D & Luebert F (2018) Biogeography and Evolution of the Atacama Desert Flora. *XII Congreso Latinoamericano de Botanico*, Quito, Ecuador. [**Poster**]

Rembold K, Altenhövel C, **Böhnert T** & Kreft H (2013) Vascular epiphyte diversity in different transformation systems in Sumatra, Indonesia. *Flora Malesiana*, Bogor, Indonesia. [**Talk**]

Rembold K, Altenhövel C & **Böhnert T** (2013) Vascular epiphyte diversity in different transformation systems in Sumatra. *SFB Poster workshop*, Göttingen, Germany. [**Poster**]

Publications marked by an asterisk *
are part of this Dissertation.

Abbreviations

µl	microlitre
a.s.l.	above sea level
ACM	*Atriplex atacamensis*, *A. chizae* & *A. madariagae* clade
bp	base pairs
cf.	compare or to be confirmed
CRC	Colaborative Research Center
DEC	Dispersal Extinction Cladogenesis
DFG	Deutsche Forschungsgemeinschaft
DNA	deoxyribonucleic acid
e.g.	exempli gratia, for example
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 preperation
in rev.	in review
incl.	including
K	thousands (1,000)
m	meter
M	millions (1,000,000)
Ma	million years
ML	Maximum Likelihood
mya	million years ago
ng	nano gram
PP	posterior probabilities
RAD	Restriction site Associated DNA
SFB	Sonderforschungs Bereich
ssp.	two or more species
stdev	standard deviation
submit.	submitted
Tab.	Table
var.	variety

Appendices

Appendix A: Supplementary material of chapter 3

Appendix B: Supplementary material of chapter 5

Appendix C: Supplementary material of chapter 6

Appendix D: Supplementary material of chapter 7

Appendix A

— supplementary material of chapter 3 —

Figure S1: Maximum Likelihood phylogeny of Zygophyllaceae.
Figure S2: BEAST2 MCCT of Zygophyllaceae with four secondarily calibrated nodes.
Figure S3: BEAST2 MCCT of Zygophyllaceae with one secondarily calibrated node.
Figure S4: Global ancestral area reconstruction of Zygophyllaceae with maximum area = 3.
Figure S5: Ancestral area reconstruction of Larreoideae with maximum area = 3.

Table S1: List of species, voucher information and GenBank accession numbers.
Table S2: List of primer for three cp DNA regions.
Table S3: PCR programm *rbc*L.
Table S4: PCR programm *trn*L-*trn*F.
Table S5: PCR programm *trn*L-*trn*F.
Table S6: BioGeoBEARS species distribution matrix for the global Zygophyllaceae dataset.
Table S7: BioGeoBEARS species distribution matrix for Larreoideae dataset.

SUPLLEMENTARY MATERIAL – FIGURES S1 - S5

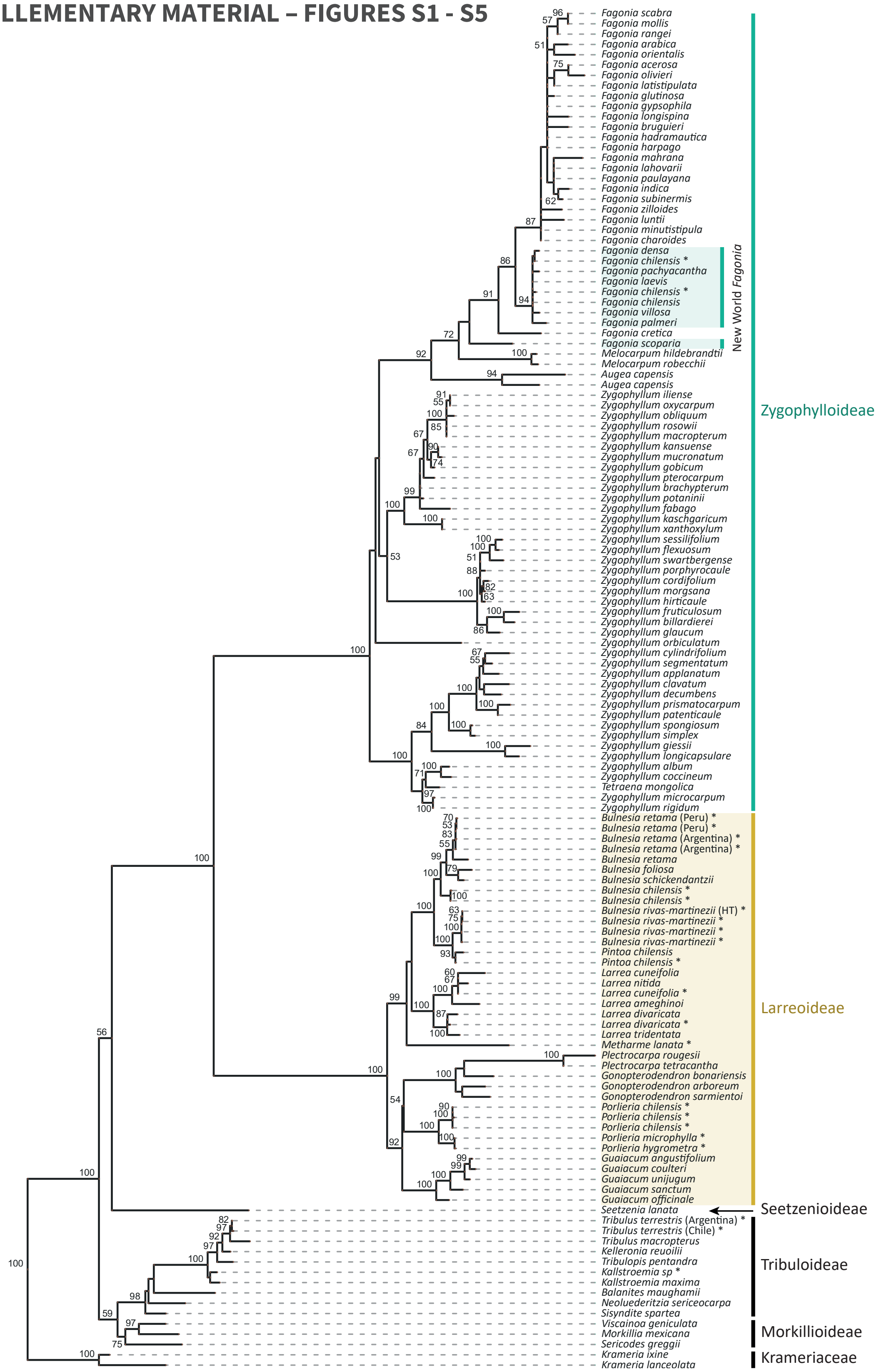


Figure S1: Maximum Likelihood RAxML tree of Zygophyllaceae with Krameriaceae as outgroup and bootstrap support > 50. Subfamilies and individual clades relevant for the study are highlighted. Sequences of samples marked with and asterisk (*) were generated for the present study.

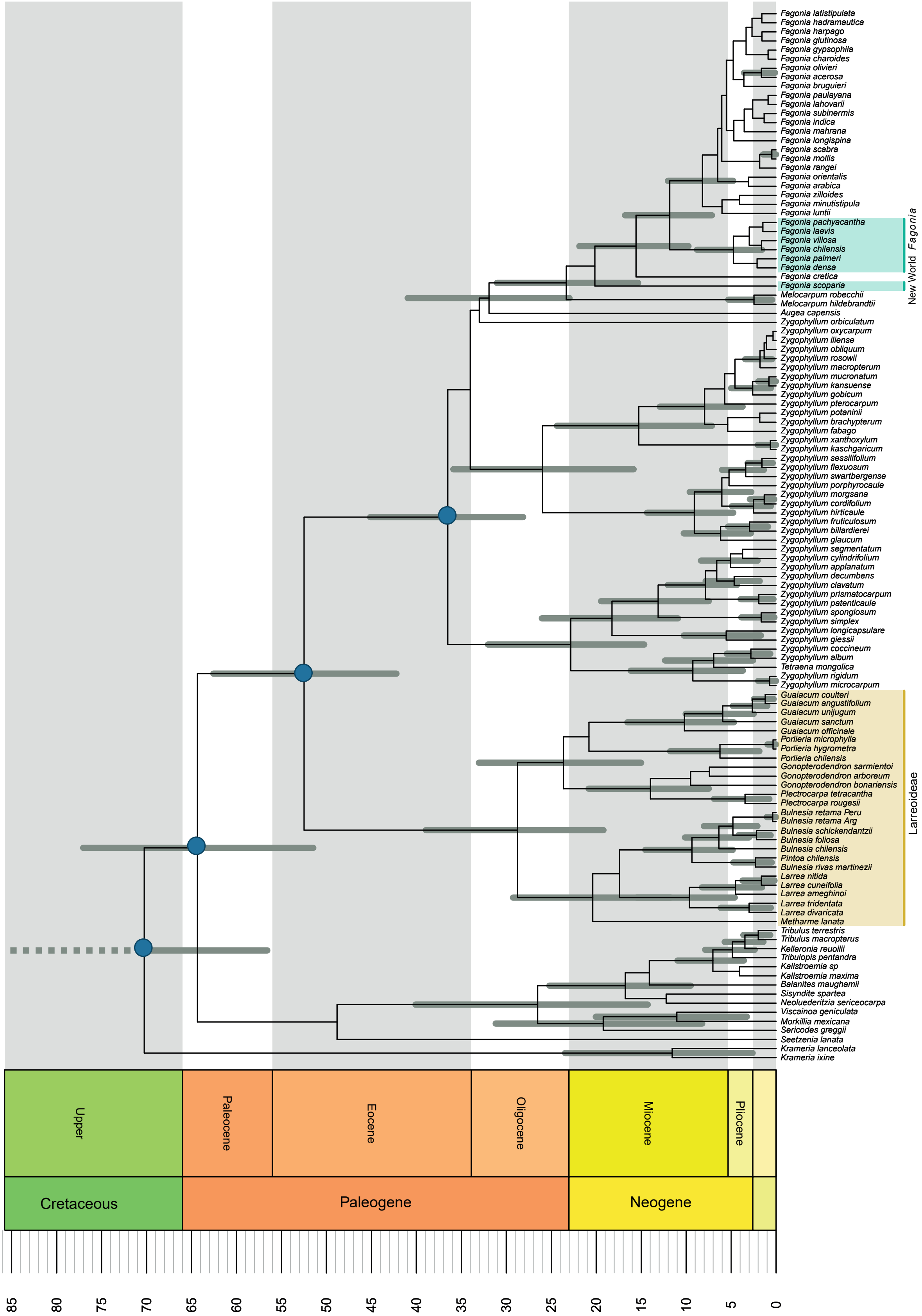


Figure S2: BEAST2 Maximum clade credibility tree of Zygophyllaceae with Krameriaceae as outgroup and four secondarily calibrated nodes. Only for nodes with posterior probability support ≥ 95% HPD confidence intervals are plot-ted. Position of the four calibrated nodes are indicated with blue dots and listed in table 1. Main clades of relevance for the study are indicated.

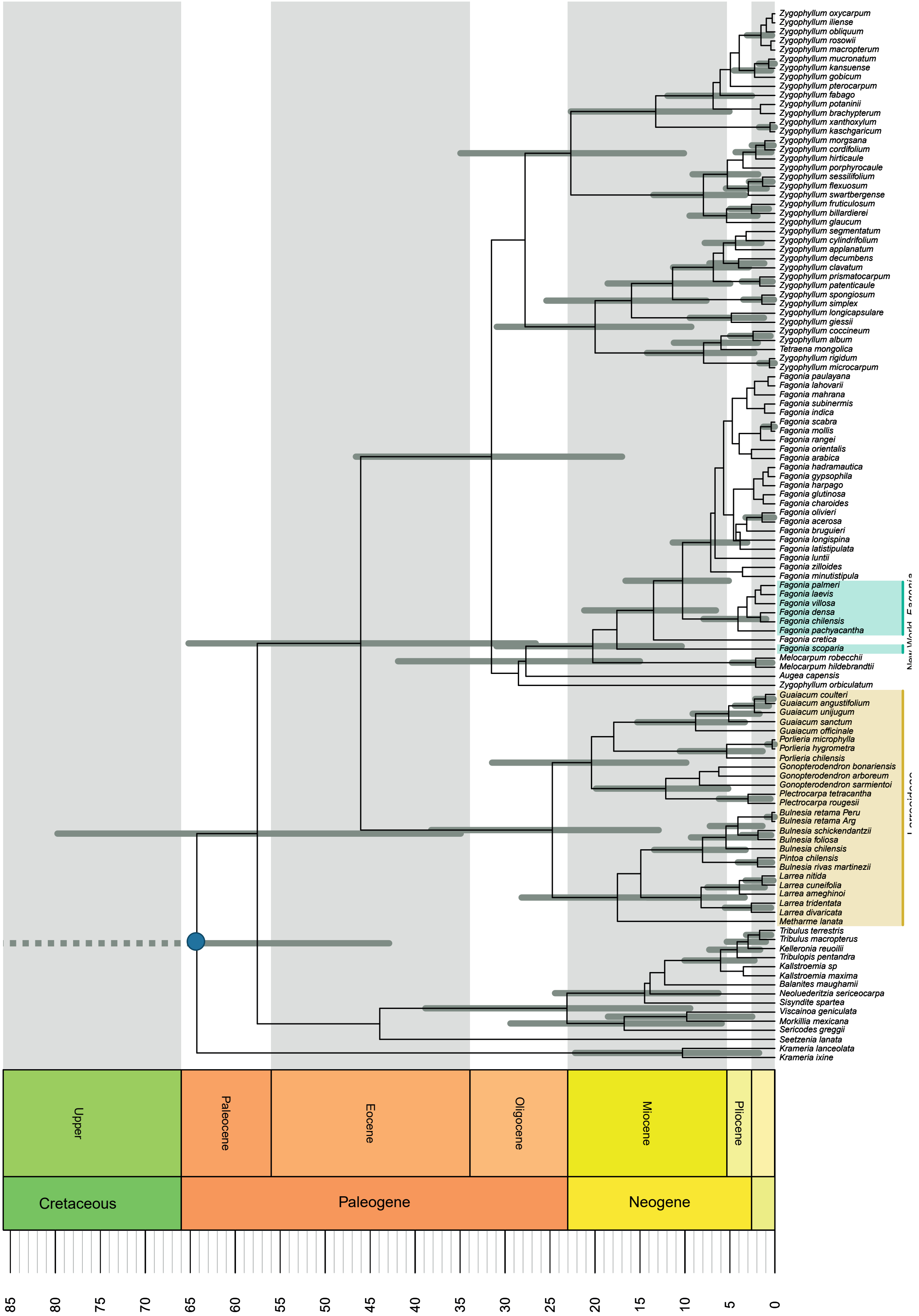


Figure S3: BEAST2 Maximum clade credibility tree of Zygophyllaceae with Krameriaceae as outgroup and one secondarily calibrated node. Only for nodes with posterior probability support ≥ 95% HPD confidence intervals are plotted. Position of the single secondarily calibrated node is indicated with a blue dot and listed in table 1. Main clades of relevance for the study are indicated.

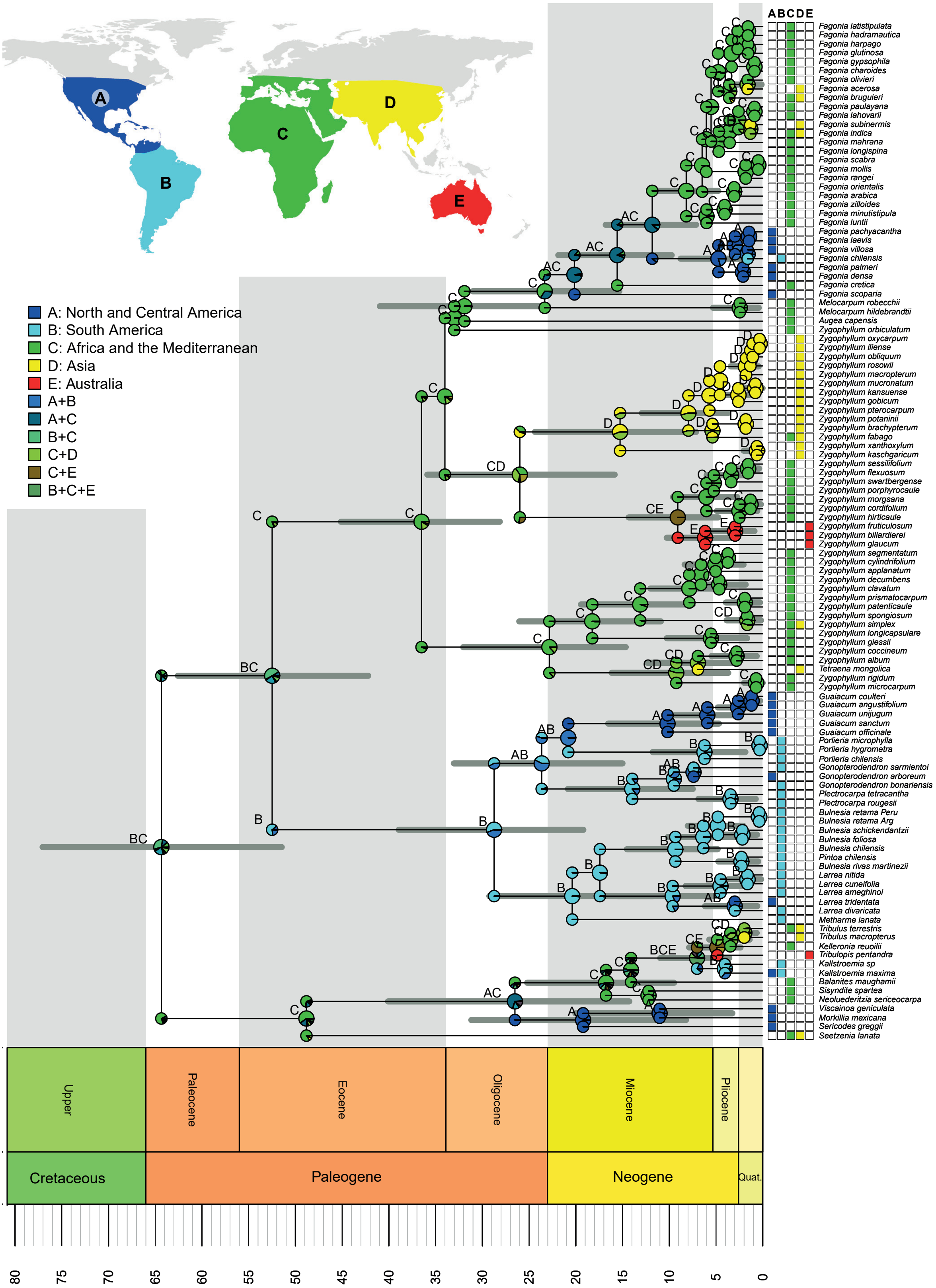


Figure S4: Global ancestral area reconstruction of Zygophyllaceae plotted on BEAST MCCT with outgroups removed and maximum area set to 3. Only for nodes with posterior probability support ≥ 0.95 confidence intervals are shown. Squares between tips and tip labels indicate distributions assigned to each single species (A-E) with color codes corresponding to the areas indicated on the map and the legend. Pie charts depict relative probabilities of areas as estimated from the Dispersal-Extinction-Cladogenesis (DEC) analysis with BioGeoBEARS. Letters next to the pie charts indicate the areas with highest relative probabilities.

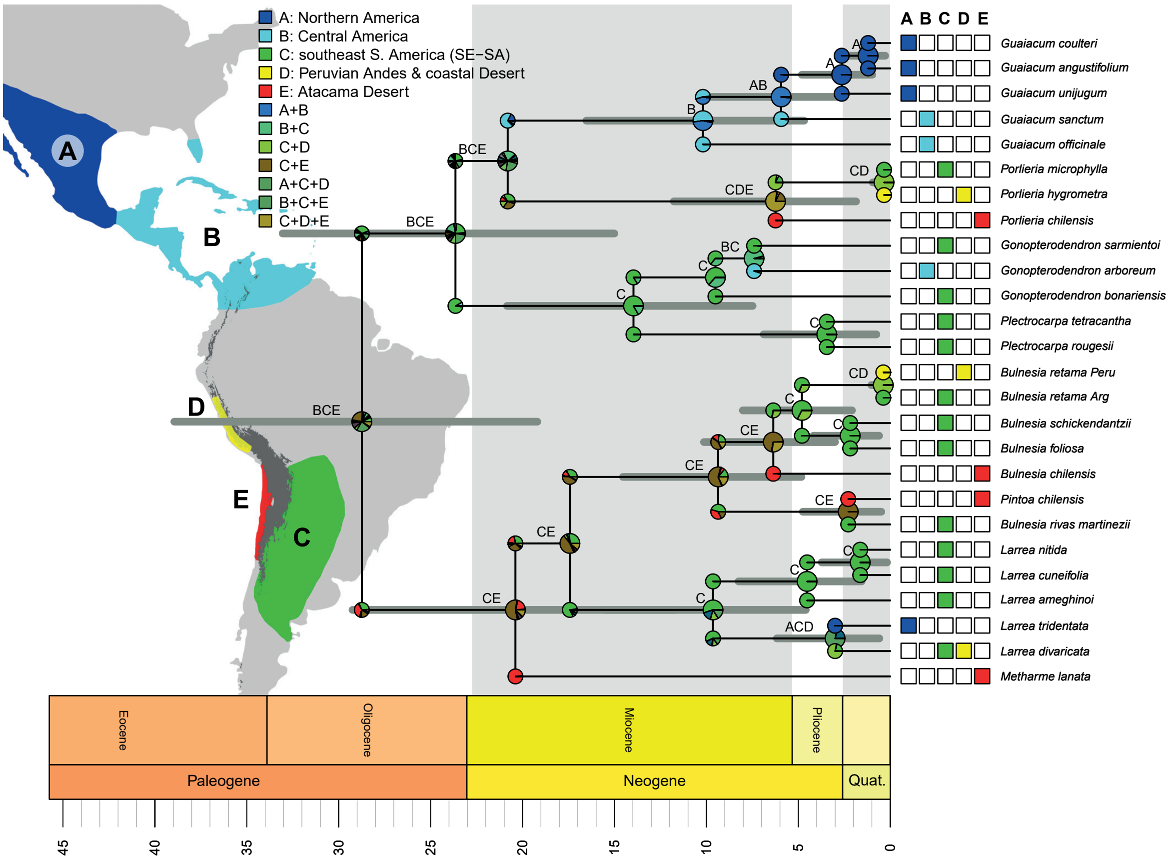


Figure S5: Ancestral area reconstruction of Larreoideae for the Americas plotted on the reduced BEAST tree from figure 1 with maximum area set to 3. Only for nodes with posterior probability support ≥ 0.95 confidence intervals are shown. Squares between tips and tip labels indicate distributions assigned to each single species (A-E) with color codes corresponding to the areas indicated on the map and the legend. Pie charts at the nodes depict relative pro-babilities of areas as estimated from the Dispersal-Extinction-Cladogenesis (DEC) analysis with BioGeoBEARS. Letters next to the pie charts indicate the areas with highest relative probabilities.

Table S1: List of species, voucher information and GenBank accession numbers. Samples marked with an asterisk (*) indicate the samples which were used for phylogenetic maximum likelihood reconstruction but excluded for the BEAST analyses.

Subfamily	Species	Voucher	*rbc*L	*trn*L-*trn*F	*trn*S-*trn*G	Reference
Zygophylloideae	*Augea capensis* Thunb.		EF655978	EF655998	---	Bellstedt *et al*. 2012
Zygophylloideae	*Augea capensis* Thunb. *		Y15015	AJ387945	---	Sheahan & Chase 1996, 2000
Tribuloideae	*Balanites maughamii* Sprague		Y15016	AJ387946	---	Sheahan & Chase 1996, 2000
Larreoideae	*Bulnesia chilensis* Gay *	F. Luebert *et al*. 2981 (ULS, BONN)	MN525775	MN587211	MN786772	this study
Larreoideae	*Bulnesia chilensis* Gay	F. Luebert *et al*. 3687 (ULS, BONN)	MN525781	MN587217	MN786775	this study
Larreoideae	*Bulnesia foliosa* Griseb.		MH357632	MH357621	---	Godoy-Bürki & Aagesen 2018
Larreoideae	*Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb. (Arg.)	G. Sancho *et al*. 363 (LP, BONN)	MN525788	MN587223	MN786781	this study
Larreoideae	*Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb. (Peru)	M. Weigend *et al*. 9811 (BONN)	MN525795	MN587230	MN786788	this study
Larreoideae	*Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb. *	T. Boehnert & C. Bayer 462 (BONN; cult. Palm Garten Frankf.)	MN525776	MN587212	MN786773	this study
Larreoideae	*Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb. (Arg.) *		MH357633	MH357622	---	Godoy-Bürki & Aagesen 2018
Larreoideae	*Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb. (Peru) *	M. Weigend *et al*. 9819 (BONN)	MN525796	MN587231	MN786789	this study
Larreoideae	*Bulnesia rivas-martinezii* G. Navarro	G. Navarro 1913 (LPB)	MN525790	MN587225	MN786783	this study
Larreoideae	*Bulnesia rivas-martinezii* G. Navarro *	G Torrico & C. Peca 321 (LPB)	MN525791	MN587226	MN786784	this study
Larreoideae	*Bulnesia rivas-martinezii* G. Navarro *	R. Lopez *et al*. 876 (LPB)	MN525792	MN587227	MN786785	this study
Larreoideae	*Bulnesia rivas-martinezii* G. Navarro *	NM/CF 755 (LPB)	MN525793	MN587228	MN786786	this study
Larreoideae	*Bulnesia schickendantzii* Hieron.		MH357635	MH357624	---	Godoy-Bürki & Aagesen 2018
Zygophylloideae	*Fagonia acerosa* Boiss.		---	AY641579	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia arabica* L.		---	AY641580	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia bruguieri* DC.		---	AY641582	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia charoides* Chiov.		---	AY641583	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia chilensis* Hook. & Arn.	F. Luebert *et al*. 3486 (BONN)	MN525778	MN587214	---	this study
Zygophylloideae	*Fagonia chilensis* Hook. & Arn. *	F. Luebert *et al*. 3704 (ULS, BONN)	MN525777	MN587213	---	this study
Zygophylloideae	*Fagonia chilensis* Hook. & Arn. *		---	AY641584	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia cretica* L.		AJ133855	AJ387942	---	Sheahan & Chase 2000
Zygophylloideae	*Fagonia densa* I.M. Johnst.		---	AY641587	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia glutinosa* Delile		---	AY641588	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia gypsophila* Beier & Thulin		---	AY641589	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia hadramautica* Beier & Thulin		---	AY641590	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia harpago* Emb. & Maire		---	AY641591	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia indica* Burm. f.		Y15018	AJ387943	---	Sheahan & Chase 1996, 2000
Zygophylloideae	*Fagonia laevis* Standl.		---	AY641595	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia lahovarii* Volkens & Schweinf.		---	AY641596	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia latistipulata* Beier & Thulin		---	AY641598	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia longispina* Batt.		---	AY641599	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia luntii* Baker		AJ133856	AJ387944	---	Sheahan & Chase 2000
Zygophylloideae	*Fagonia mahrana* Beier		---	AY641600	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia minutistipula* Engl.		---	AY300771	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia mollis* Delile		---	AY641601	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia olivieri* DC.		---	AY641602	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia orientalis* C. Presl		---	AY641603	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia pachyacantha* Rydb.		---	AY641604	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia palmeri* Vasey & Rose		---	AY641605	---	Beier *et al*. 2004

Subfamily	Species	Voucher	*rbc*L	*trn*L-*trn*F	*trn*S-*trn*G	Reference
Zygophylloideae	*Fagonia paulayana* J. Wagner & Vierh.		---	AY641608	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia rangei* Loes. ex Engl.		---	AY641609	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia scabra* Forssk.		---	AY300768	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia scoparia* Brandegee		---	AY300773	---	Beier *et al*. 2004
Zygophylloideae	*Fagonia subinermis* Boiss.		---	AY641610	---	Beier *et al*. 2004
Zygophylloideae	Fagonia villosa D.M. Porter		---	AY641611	---	Beier *et al*. 2004
Zygophylloideae	Fagonia zilloides Humbert		---	AY641612	---	Beier *et al*. 2004
Larreoideae	*Gonopterodendron arboreum* (Jacq.) A.C. Godoy-Bürki		Y15017	AJ387947	---	Sheahan & Chase 1996, 2000
Larreoideae	*Gonopterodendron bonariensis* (Griseb.) A.C. Godoy-Bürki		MH357631	MH357619	---	Godoy-Bürki & Aagesen 2018
Larreoideae	*Gonopterodendron sarmientoi* (Lorentz ex Griseb.) A.C. Godoy-Bürki		MH357634	MH357623	---	Godoy-Bürki & Aagesen 2018
Larreoideae	*Guaiacum angustifolium* Engelm.		---	JX669512	JX669513	Dertien & Duvall 2014
Larreoideae	*Guaiacum coulteri* A. Gray		---	JX682625	JX682626	Dertien & Duvall 2014
Larreoideae	*Guaiacum officinale* L.		---	EU253467	JX901015	Dertien & Duvall 2014
Larreoideae	*Guaiacum sanctum* L.		---	EU253457	EU253473	Dertien & Duvall 2014
Larreoideae	*Guaiacum unijugum* Brandegee		---	JX682627	JX682628	Dertien & Duvall 2014
Tribuloideae	*Kallstroemia maxima* (L.) Hook. & Am.		Y15020	AJ387949	---	Sheahan & Chase 1996, 2000
Tribuloideae	*Kallstroemia* sp.	M. Weigend *et al*. 9981 (BONN)	MN525797	MN587232	---	this study
Tribuloideae	*Kelleronia revoilii* Franch.		Y15021	AJ387950	---	Sheahan & Chase 1996, 2000
	Krameria ixine L.		EU644679	---	---	Bellstedt *et al*. 2012
	Krameria lanceolata Torr.		Y15032	---	---	Wu *et al*. 2015
Larreoideae	*Larrea ameghinoi* Speg.		AF333329	---	---	Lia *et al*. 2001
Larreoideae	*Larrea cuneifolia* Cav.	G. Sancho *et al*. 364 (LP, BONN)	MN525789	MN587224	MN786782	this study
Larreoideae	*Larrea cuneifolia* Cav. *		AF200471	---	---	Lia *et al*. 2001
Larreoideae	*Larrea divaricata* Cav.	G. Sancho *et al*. 358 (LP, BONN)	MN525786	MN587221	MN786780	this study
Larreoideae	*Larrea divaricata* Cav. *		AF200472	---	---	Lia *et al*. 2001
Larreoideae	*Larrea nitida* Cav.		AF200473	---	---	Lia *et al*. 2001
Larreoideae	*Larrea tridentata* (Sessé & Moc. ex DC.) Coville		Y15022	AJ387951	---	Sheahan & Chase 1996, 2000
Zygophylloideae	*Melocarpum hildebrandtii* (Engl.) Beier & Thulin		AJ133868	AJ387971	---	Sheahan & Chase 2000
Zygophylloideae	*Melocarpum robecchii* (Engl.) Beier & Thulin		AJ133870	AJ387972	---	Sheahan & Chase 2000
Larreoideae	*Metharme lanata* Phil.	F. Luebert *et al*. 3628 (ULS, BONN)	MN525780	MN587216	MN786774	this study
Morkillioideae	*Morkillia mexicana* Rose & Painter		AJ133857	AJ387952	---	Sheahan & Chase 2000
Tribuloideae	*Neoluederitzia sericeocarpa* Schinz		Y15023	AJ387953	---	Sheahan & Chase 1996, 2000
Larreoideae	*Pintoa chilensis* Gay	F. Luebert *et al*. 3701 (ULS, BONN)	MN525782	MN587218	MN786776	this study
Larreoideae	*Pintoa chilensis* Gay *		AJ133858	AJ387954	---	Sheahan & Chase 2000
Larreoideae	*Plectrocarpa rougesii* Descole, O'Donell & Lourteig		MH357636	MH357629	---	Godoy-Bürki & Aagesen 2018
Larreoideae	*Plectrocarpa tetracantha* Gillies ex Hook. & Arn.		AF333330	---	---	Lia *et al*. 2001
Larreoideae	*Porlieria chilensis* I.M. Johnst.	F. Luebert *et al*. 3725 (BONN)	MN525774	MN587210	MN786771	this study
Larreoideae	*Porlieria chilensis* I.M. Johnst. *	F. Luebert *et al*. 3030 (BONN)	MN525783	---	MN786777	this study
Larreoideae	*Porlieria chilensis* I.M. Johnst. *	F. Luebert *et al*. 3771 (ULS, BONN)	MN525784	MN587219	MN786778	this study
Larreoideae	*Porlieria hygrometra* Ruiz & Pav.	M. Weigend *et al*. 7303 (BONN)	MN525794	MN587229	MN786787	this study
Larreoideae	*Porlieria microphylla* (Baill.) Descole, O'Donell & Lourteig	G. Sancho *et al*. 357 (LP, BONN)	MN525785	MN587220	MN786779	this study
Seetzenioideae	*Seetzenia lanata* (Willd.) Bullock		Y15025	AJ387956	---	Sheahan & Chase 1996, 2000
Morkillioideae	*Sericodes greggii* A. Gray		AJ133859	AJ387957	---	Sheahan & Chase 2000
Tribuloideae	*Sisyndite spartea* E. Meyer ex. Sonder & Harvey		Y15026	AJ387958	---	Sheahan & Chase 1996, 2000
Zygophylloideae	*Tetraena mongolica* Maxim.		Y15027	AJ387959	---	Sheahan & Chase 1996, 2000
Tribuloideae	*Tribulopis pentandra* R.Br.		AJ133860	AJ387960	---	Sheahan & Chase 2000
Tribuloideae	*Tribulus macropterus* Boiss.		Y15028	AJ387961	---	Sheahan & Chase 1996, 2000
Tribuloideae	*Tribulus terrestris* L.	F. Luebert *et al*. 3592	MN525779	MN587215	---	this study

Subfamily	Species	Voucher	*rbc*L	*trn*L-*trn*F	*trn*S-*trn*G	Reference
Tribuloideae	*Tribulus terrestris* L. *	G. Sancho *et al.* 360 (LP, BONN)	MN525787	MN587222	---	this study
Morkillioideae	*Viscainoa geniculata* (Kellogg) Greene		Y15029	AJ387962	---	Sheahan & Chase 1996, 2000
Zygophylloideae	*Zygophyllum album* L.		AJ133861	AJ387963	---	Sheahan & Chase 2000
Zygophylloideae	*Zygophyllum applanatum* VanZyl		EF655988	EF656012	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum billardierei* DC.		AJ133862	AJ387964	---	Sheahan & Chase 2000
Zygophylloideae	*Zygophyllum brachypterum* Kar. & Kir.		---	KR001987/KR002014	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum clavatum* Schltr. & Diels		EF655986	EF656010	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum coccineum* L.		AJ133863	AJ387965	---	Sheahan & Chase 2000
Zygophylloideae	*Zygophyllum cordifolium* L. f.		EF655993	EF656022	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum cylindrifolium* Schinz		AJ133864	AJ387966	---	Sheahan & Chase 2000
Zygophylloideae	*Zygophyllum decumbens* Del.		AJ133865	AJ387967	---	Sheahan & Chase 2000
Zygophylloideae	*Zygophyllum fabago* L.		Y15030	AJ387968	---	Sheahan & Chase 1996, 2000
Zygophylloideae	*Zygophyllum flexuosum* Eckl. & Zeyh.		EF655995	EF656032	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum fruticulosum* DC.		AJ133866	AJ387969	---	Sheahan & Chase 2000
Zygophylloideae	*Zygophyllum giessii* Merxm. & A. Schreib.		EF655980	EF656000	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum glaucum* F.Muell.		AJ133867	AJ387970	---	Sheahan & Chase 2000
Zygophylloideae	*Zygophyllum gobicum* Maxim.		---	KR001995/KR002008	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum hirticaule* Van Zyl		AJ133869	AJ387973	---	Sheahan & Chase 2000
Zygophylloideae	*Zygophyllum iliense* Popov		---	KR001993/KR002006	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum kansuense* Y. X. Liou		---	KR001996/KR002010	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum kaschgaricum* Boriss.		---	KR001981/KR002000	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum longicapsulare* Schinz		EF655981	EF656001	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum macropterum* C. A. Mey.		---	KR001991/KR002003	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum microcarpum* Licht. ex Cham.		EF655983	EF656002	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum morgsana* L.		EF655994	EF656021	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum mucronatum* Maxim.		---	KR001997/KR002009	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum obliquum* Popov		---	KR001989/KR002002	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum orbiculatum* Welw. ex Oliv.		EF655979	EF655999	---	Bellstedt *et al.* 2008
Zygophylloideae	*Zygophyllum oxycarpum* Popov		---	KR001992/KR002007	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum patenticaule* Van Zyl ined.		EF655989	EF656008	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum porphyrocaule* Van Zyl ined.		EF655992	EF656018	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum potaninii* Maxim.		---	KR001986/KR002013	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum prismatocarpum* Sond.		EF655990	EF656009	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum pterocarpum* Bunge		---	KR001985/KR002012	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum rigidum* Schinz		EF655982	EF656003	---	Bellstedt *et al.* 2012
Zygophylloideae	*Zygophyllum rosowii* Bunge		---	KR001994/KR002005	---	Wu *et al.* 2015
Zygophylloideae	*Zygophyllum segmentatum* Van Zyl		EF655987	EF656015	---	Bellstedt *et al.* 2012

Subfamily	Species	Voucher	*rbc*L	*trn*L-*trn*F	*trn*S-*trn*G	Reference
Zygophylloideae	*Zygophyllum sessilifolium* L.		EF655997	EF656047	---	Bellstedt *et al*. 2012
Zygophylloideae	*Zygophyllum simplex* L.		EF655984	EF656004	---	Bellstedt *et al*. 2012
Zygophylloideae	*Zygophyllum spongiosum* Van Zyl		EF655985	EF656006	---	Bellstedt *et al*. 2012
Zygophylloideae	*Zygophyllum swartbergense* Van Zyl		EF655996	EF656043	---	Bellstedt *et al*. 2012
Zygophylloideae	*Zygophyllum xanthoxylum* Engl.		AJ133872	AJ387975	---	Sheahan & Chase 2000

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

Location	Primer	Sequence	Direct.	Reference
*rbc*L	rbcL-1F	ATGTCACCACAAACAGAAACTAAAGC	F	Olmstead *et al.* 1992
*rbc*L	rbcL-1460R	CTTTTAGTAAAAGATTGGGCCGAG	R	Olmstead *et al.* 1992
*rbc*L	rbcL_636Fn	GCGTTGGAGAGATCGTTTCT	F	Lindqvist & Albert 2002
*rbc*L	rbcL_724R	TCGCATGTACCTGCAGTAGC	R	Lindqvist & Albert 2002
*trn*L$_{\mathrm{UGU}}$	trnL_15	A(AT)TGGTAGACGCTRCGGACT	F	Noben *et al.* 2017
*trn*F$_{\mathrm{GAA}}$	trnF_39	TTTGAACTGGTGACACRAGGA	R	Noben *et al.* 2017
*trn*S-*trn*G	trnGUUC	GAATCGAACCCGCATCGTTAG	F	Shaw *et al.* 2007
*trn*S-*trn*G	trnG2G	GCGGGTATAGTTTAGTGGTAAAA	R	Shaw *et al.* 2005
*trn*S	trnSGCU	GCCGCTTTAGTCCACTCAGC	F	Hamilton 1999
*trn*G	trnGUCC	GAACGAATCACACTTTTACCAC	R	Hamilton 1999

Table S3: PCR programm *rbc*L.

	Temperature [°C]	Time [s]	
1	96	90	
2	50	60	
3	68	120	
4	94	30	
5	48	60	38 x
6	68	120	
7	8	break	

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

	Temperature [°C]	Time [s]	
1	94	120	
2	94	30	
3	52	60	35 x
4	72	60	
5	72	600	
7	8	break	

Table S5: PCR programm *trn*L-*trn*F.

	Temperature [°C]	Time [s]	
1	95	300	
2	95	30	
3	50	60	34 x
4	72	60	
5	72	240	
7	8	break	

Table S6: BioGeoBEARS species distribution matrix for the global Zygophyllaceae dataset. Comprising of 114 samples and 5 geographical categories.

Species	A	B	C	D	E
Augea capensis	0	0	1	0	0
Balanites maughamii	0	0	1	0	0
Bulnesia chilensis	0	1	0	0	0
Bulnesia foliosa	0	1	0	0	0
Bulnesia retama (Argentina)	0	1	0	0	0
Bulnesia retama (Peru)	0	1	0	0	0
Bulnesia rivas-martinezii	0	1	0	0	0
Bulnesia schickendantzii	0	1	0	0	0
Fagonia acerosa	0	0	0	1	0
Fagonia arabica	0	0	1	0	0
Fagonia bruguieri	0	0	1	1	0
Fagonia charoides	0	0	1	0	0
Fagonia chilensis	0	1	0	0	0
Fagonia cretica	0	0	1	0	0
Fagonia densa	1	0	0	0	0
Fagonia glutinosa	0	0	1	0	0
Fagonia gypsophila	0	0	1	0	0
Fagonia hadramautica	0	0	1	0	0
Fagonia harpago	0	0	1	0	0
Fagonia indica	0	0	1	1	0
Fagonia laevis	1	0	0	0	0
Fagonia lahovarii	0	0	1	0	0
Fagonia latistipulata	0	0	1	0	0
Fagonia longispina	0	0	1	0	0
Fagonia luntii	0	0	1	0	0
Fagonia mahrana	0	0	1	0	0
Fagonia minutistipula	0	0	1	0	0
Fagonia mollis	0	0	1	0	0
Fagonia olivieri	0	0	1	0	0
Fagonia orientalis	0	0	1	0	0
Fagonia pachyacantha	1	0	0	0	0
Fagonia palmeri	1	0	0	0	0
Fagonia paulayana	0	0	1	0	0
Fagonia rangei	0	0	1	0	0
Fagonia scabra	0	0	1	0	0
Fagonia scoparia	1	0	0	0	0
Fagonia subinermis	0	0	0	1	0
Fagonia villosa	1	0	0	0	0
Fagonia zilloides	0	0	1	0	0
Gonopterodendron arboreum	1	0	0	0	0
Gonopterodendron bonariensis	0	1	0	0	0
Gonopterodendron sarmientoi	0	1	0	0	0
Guaiacum angustifolium	1	0	0	0	0
Guaiacum coulteri	1	0	0	0	0
Guaiacum officinale	1	0	0	0	0
Guaiacum sanctum	1	0	0	0	0
Guaiacum unijugum	1	0	0	0	0
Kallstroemia maxima	1	1	0	0	0
Kallstroemia sp	0	1	0	0	0
Kelleronia reuoilii	0	0	1	0	0
Larrea ameghinoi	0	1	0	0	0
Larrea cuneifolia	0	1	0	1	0
Larrea divaricata	0	1	0	0	0
Larrea nitida	0	1	0	0	0
Larrea tridentata	1	0	0	0	0
Melocarpum hildebrandtii	0	0	1	0	0
Melocarpum robecchii	0	0	1	0	0
Metharme lanata	0	1	0	0	0
Morkillia mexicana	1	0	0	0	0
Neoluederitzia sericeocarpa	0	0	1	0	0
Pintoa chilensis	0	1	0	0	0
Plectrocarpa rougesii	0	1	0	0	0
Plectrocarpa tetracantha	0	1	0	0	0
Porlieria chilensis	0	1	0	0	0
Porlieria hygrometra	0	1	0	0	0
Porlieria microphylla	0	1	0	0	0
Seetzenia lanata	0	0	1	1	0
Sericodes greggii	1	0	0	0	0
Sisyndite spartea	0	0	1	0	0
Tetraena mongolica	0	0	0	1	0
Tribulopis pentandra	0	0	0	0	1
Tribulus macropterus	0	0	0	1	0
Tribulus terrestris	0	0	1	1	0
Viscainoa geniculata	1	0	0	0	0

Table S6: Continue.

Species	A	B	C	D	E
Zygophyllum album	0	0	1	0	0
Zygophyllum applanatum	0	0	1	0	0
Zygophyllum billardierei	0	0	0	0	1
Zygophyllum brachypterum	0	0	0	1	0
Zygophyllum clavatum	0	0	1	0	0
Zygophyllum coccineum	0	0	1	0	0
Zygophyllum cordifolium	0	0	1	0	0
Zygophyllum cylindrifolium	0	0	1	0	0
Zygophyllum decumbens	0	0	1	0	0
Zygophyllum fabago	0	0	1	1	0
Zygophyllum flexuosum	0	0	1	0	0
Zygophyllum fruticulosum	0	0	0	0	1
Zygophyllum giessii	0	0	1	0	0
Zygophyllum glaucum	0	0	0	0	1
Zygophyllum gobicum	0	0	0	1	0
Zygophyllum hirticaule	0	0	1	0	0
Zygophyllum iliense	0	0	0	1	0
Zygophyllum kansuense	0	0	0	1	0
Zygophyllum kaschgaricum	0	0	0	1	0
Zygophyllum longicapsulare	0	0	1	0	0
Zygophyllum macropterum	0	0	0	1	0
Zygophyllum microcarpum	0	0	1	0	0
Zygophyllum morgsana	0	0	1	0	0
Zygophyllum mucronatum	0	0	0	1	0
Zygophyllum obliquum	0	0	0	1	0
Zygophyllum orbiculatum	0	0	1	0	0
Zygophyllum oxycarpum	0	0	0	1	0
Zygophyllum patenticaule	0	0	1	0	0
Zygophyllum porphyrocaule	0	0	1	0	0
Zygophyllum potaninii	0	0	0	1	0
Zygophyllum prismatocarpum	0	0	1	0	0
Zygophyllum pterocarpum	0	0	0	1	0
Zygophyllum rigidum	0	0	1	0	0
Zygophyllum rosowii	0	0	0	1	0
Zygophyllum segmentatum	0	0	1	0	0
Zygophyllum sessilifolium	0	0	1	0	0
Zygophyllum simplex	0	0	1	1	0
Zygophyllum spongiosum	0	0	1	0	0
Zygophyllum swartbergense	0	0	1	0	0
Zygophyllum xanthoxylum	0	0	0	1	0

Table S7: BioGeoBEARS species distribution matrix for Larreoideae dataset. Comprising of 26 samples and 5 geographical categories.

Species	A	B	C	D	E
Bulnesia chilensis	0	0	0	0	1
Bulnesia foliosa	0	0	1	0	0
Bulnesia retama (Argentina)	0	0	1	0	0
Bulnesia retama (Peru)	0	0	0	1	0
Bulnesia rivas-martinezii	0	0	1	0	0
Bulnesia schickendantzii	0	0	1	0	0
Gonopterodendron arboreum	0	1	0	0	0
Gonopterodendron bonariensis	0	0	1	0	0
Gonopterodendron sarmientoi	0	0	1	0	0
Guaiacum angustifolium	1	0	0	0	0
Guaiacum coulteri	1	0	0	0	0
Guaiacum officinale	0	1	0	0	0
Guaiacum sanctum	0	1	0	0	0
Guaiacum unijugum	1	0	0	0	0
Larrea ameghinoi	0	0	1	0	0
Larrea cuneifolia	0	0	1	0	0
Larrea divaricata	0	0	1	1	0
Larrea nitida	0	0	1	0	0
Larrea tridentata	1	0	0	0	0
Metharme lanata	0	0	0	0	1
Pintoa chilensis	0	0	0	0	1
Plectrocarpa rougesii	0	0	1	0	0
Plectrocarpa tetracantha	0	0	1	0	0
Porlieria chilensis	0	0	0	0	1
Porlieria hygrometra	0	0	0	1	0
Porlieria microphylla	0	0	1	0	0

Table S8: Divergence time estimations for the major nodes of the Zygophyllaceae phylogeny under two different calibrations schemes with one (calibration scheme 1) and four (calibrations scheme 2) secondarily calibrated nodes, respectively.

	calibration scheme 1			calibration scheme 2		
Node	mean	min	max	mean	min	max
stem Zygophyllaceae	64.74	42.89	87.87	70.83	54.79	83.97
crown Zygophyllaceae	58.02	34.93	79.77	64.9	51.15	76.83
stem Morkillioideae + Tribuloideae	23.59	9.41	38.86	26.73	14.7	40.32
crown Morkillioideae	17.23	5.91	29.41	19.55	8.1	30.77
crown Tribuloideae	14.96	6.27	24.45	17.16	9.03	25.19
stem Larreoideae + Zygophylloideae	46.52	26.59	65.19	53.26	42.46	62.78
crown Larreoideae	25.21	12.82	38.21	28.96	18.22	38.45
crown *Larrea* clade	17.98	8.73	28.16	20.61	11.88	28.67
crown *Guaiacum* clade	20.86	9.85	31.45	23.93	15	33.27
crown Zygophylloideae	31.98	16.99	46.59	37.22	27.74	44.86
crown New World *Fagonia* (excl. *F. scoparia*)	4.57	0.96	7.94	5.32	1.41	8.5

References

Beier B-A, Nylander JAA, Chase MW & Thulin M (2004) Phylogenetic relationships and biogeography of the desert plant genus *Fagonia* (Zygophyllaceae), inferred by parsimony and Bayesian model averaging. *Molecular Phylogenetics and Evolution* 33 (1): 91–108. DOI: https://doi.org/10.1016/j.ympev.2004.05.010.

Bellstedt DU, Galley C, Pirie MD & Linder HP (2012) The Migration of the Palaeotropical Arid Flora: Zygophylloideae as an Example. *Systematic Botany* 37 (4): 951–959. DOI: https://doi.org/10.1600/036364412X656608.

Dertien JR & Duvall MR (2014) Perspectives on the systematics and phylogenetics of *Guaiacum* (Zygophyllaceae): Complexities in conservation of endangered hardwoods due to fragmentation, introgression, and intermittent gene flow. *Revista Mexicana de Biodiversidad* 85 (3): 808–822. DOI: https://doi.org/10.7550/rmb.43422.

Godoy-Bürki AC, Acosta JM & Aagesen L (2018) Phylogenetic relationships within the New World subfamily Larreoideae (Zygophyllaceae) confirm polyphyly of the disjunct genus *Bulnesia*. *Systematics and Biodiversity* 106: 1–16. DOI: https://doi.org/10.1080/14772000.2018.1451406.

Hamilton MB (1999) Four primer pairs for the amplification of chloroplast regions with intraspecific variation. *Molecular Ecology* 8 (3): 521–523.

Lia VV, Confalonieri VA, Comas CI & Hunziker JH (2001) Molecular phylogeny of *Larrea* and Its Allies (Zygophyllaceae): Reticulate Evolution and the Probable Time of Creosote Bush Arrival to North America. *Molecular Phylogenetics and Evolution* 21 (2): 309–320. DOI: https://doi.org/10.1006/mpev.2001.1025.

Lindqvist C & Albert VA (2002) Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). *American Journal of Botany* 89 (10): 1709–1724. DOI: https://doi.org/10.3732/ajb.89.10.1709.

Noben S, Kessler M, Quandt D, Weigand A, Wicke S, Krug M & Lehnert M (2017) Biogeography of the Gondwanan tree fern family Dicksoniaceae — A tale of vicariance, dispersal and extinction. *Journal of Biogeography* 44 (11): 2648–2659. DOI: https://doi.org/10.1111/jbi.13056.

Olmstead RG, Michaels HJ, Scott KM & Palmer JD (1992) Monophyly of the Asteridae and Identification of Their Major Lineages Inferred From DNA Sequences of *rbc*L. *Annals of the Missouri Botanical Garden* 79 (2): 249. DOI: https://doi.org/10.2307/2399768.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE & Small RL (2005) The tortoise and the hare II. Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92 (1): 142–166. DOI: https://doi.org/10.3732/ajb.92.1.142.

Shaw J, Lickey EB, Schilling EE & Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany* 94 (3): 275–288. DOI: https://doi.org/10.3732/ajb.94.3.275.

Sheahan MC & Chase MW (1996) A phylogenetic analysis of Zygophyllaceae R.Br. based on morphological, anatomical and *rbc*L DNA sequence data. *Botanical Journal of the Linnean Society* 122 (4): 279–300. DOI: https://doi.org/10.1111/j.1095-8339.1996.tb02077.x.

Sheahan MC & Chase MW (2000) Phylogenetic Relationships within Zygophyllaceae Based on DNA Sequences of three Plastid Regions, with Special Emphasis on Zygophylloideae. *Systematic Botany* 25 (2): 371–384. DOI: https://doi.org/10.2307/2666648.

Wu S-D, Lin L, Li H-L, Yu S-X, Zhang L-J & Wang W (2015) Evolution of Asian Interior Arid-Zone Biota: Evidence from the Diversification of Asian *Zygophyllum* (Zygophyllaceae). *PLoS ONE* 10 (9): e0138697. DOI: https://doi.org/10.1371/journal.pone.0138697.

Appendix B

— supplementary material of chapter 5 —

Table S1: List of *Atriplex* species, voucher information, GenBank accession numbers & GBS assembly statistics.

Python skript: Jupyter-Notebook documenting the assembly of *Atriplex* GBS data, assembly statistics and phylogenetic analyses.

Table S1: List of species, voucher information, GenBank accession numbers & GBS assembly statistics. pops30 = all *Atriplex* GBS samples, AtaArg = *Atriplex* GBS samples from Atacama-Argentina clade, AtaPeru = Atriplex GBS samples from the Atacama-Peru clade.

Species	Country	Voucher	Lab-ID	*rbc*L	*atp*B-*rbc*L	Sequencing ID	pops30	AtaArg	AtaPeru	Reference
Agriophyllum squarrosum (L.) Moq.	Russia	Freitag 28196a (KAS)		AY270051	HM587612					Kadereit *et al.* 2003, 2010
Anthochlamys multinervis Rech.f.	Iran	Freitag 13979 (KAS)		AY270056	HM587613					Kadereit *et al.* 2003, 2010
Archiatriplex nanpinensis G.L. Chu	China	Downie 759 (ILL)		HM587580	HM587614					Kadereit *et al.* 2010
Atriplex argentina Speg.	Argentina	Sancho *et al.* 366 (LP, BONN)	ED3981	MN810506	MN810435	A_arg_ED3981	2653	4742		this study
Atriplex atacamensis Phil.	Chile	Böhnert & Merklinger 440 (BONN, ULS)	ED1882	MN810479	MN810409	A_ata_ED1882	2785	5269		this study
Atriplex atacamensis Phil.	Chile	Luebert & Böhnert 3646 (BONN, ULS)	ED1899	MN810482	MN810412	A_ata_ED1899	2410	4588		this study
Atriplex atacamensis Phil.	Chile	Luebert & Böhnert 3647 (BONN, ULS)	ED1900			A_ata_ED1900	2670	5008		this study
Atriplex aucheri Moq.	Uzbekistan	Freitag 30101 (KAS)		HM587581	HM587616					Kadereit *et al.* 2010
Atriplex australasica Moq.	Australia	Jacobs 9268 (NSW)		HM587582	HM587617					Kadereit *et al.* 2010
Atriplex billardierei Hook. f.	Australia	Buchanan 13863 (NSW)			HM587618					Kadereit *et al.* 2010
Atriplex cana C.A. Mey.	Russia	Sukhorukov & Seregin R-64 (MW)			HM587620					Kadereit *et al.* 2010
Atriplex canescens (Pursh) Nutt.	USA	Böhnert 464 (xx-0-BONN-37035)	ED1823	MN810478	MN810408					this study
Atriplex centralasiatica Iljin	Kazakhstan	Lomonosova 190 (MW)		HM587583	HM587621					Kadereit *et al.* 2010
Atriplex chilensis Colla	Chile	Luebert & Böhnert 3734 (BONN, ULS)	ED1596	MN810471	MN810401					this study
Atriplex chizae Rosas	Chile	Luebert & Böhnert 3437 (BONN, ULS)	ED1379			A_chiz_ED1379	2457	4671		this study
Atriplex chizae Rosas	Chile	Luebert & Böhnert 3440 (BONN, ULS)	ED1383	MN810460	MN810390	A_chiz_ED1383	3202	6244		this study
Atriplex chizae Rosas	Chile	Luebert & Böhnert 3445 (BONN, ULS)	ED1384			A_chiz_ED1384	3209	6279		this study
Atriplex chizae Rosas	Chile	Luebert & Böhnert 3435 (BONN, ULS)	ED1392			A_chiz_ED1392	3187	6202		this study
Atriplex chizae Rosas	Chile	Luebert & Böhnert 3446A (BONN, ULS)	ED1395	MN810463	MN810393	A_chiz_ED1395	3216	6203		this study
Atriplex chizae Rosas	Chile	Luebert & Böhnert 3441 (BONN, ULS)	ED1399	MN810467	MN810397	A_chiz_ED1399	3176	6235		this study
Atriplex chizae Rosas	Chile	Böhnert & Merklinger 439 (BONN, ULS)	ED1881			A_chiz_ED1881	2661	5079		this study
Atriplex chizae Rosas	Chile	Luebert *et al.* 3497 (BONN, ULS)	ED1888			A_chiz_ED1888	2687	5109		this study
Atriplex chizae Rosas	Chile	Luebert *et al.* 4012 (BONN, ULS)	ED2979			A_chiz_ED2979	2619	4911		this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3281 (BONN, ULS)	ED1364			A_cli_ED1364	2233		3326	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3339 (BONN, ULS)	ED1371			A_cli_ED1371	2281		3383	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3404 (BONN, ULS)	ED1375			A_cli_ED1375	2994		4274	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3276 (BONN, ULS)	ED1389			A_cli_ED1389	2258		3372	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3311 (BONN, ULS)	ED1390			A_cli_ED1390	3091		4451	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3390 (BONN, ULS)	ED1396	MN810464	MN810394	A_cli_ED1396	3129		4481	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3292 (BONN, ULS)	ED1397	MN810465	MN810395	A_cli_ED1397	3073		4438	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert & Böhnert 3702 (BONN, ULS)	ED1918	MN810488	MN810418	A_cli_ED1918	2303		3417	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert & Böhnert 3703 (BONN, ULS)	ED1919			A_cli_ED1919	2101		3140	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3971 (BONN, ULS)	ED2970			A_cli_ED2970	2373		3504	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3977 (BONN, ULS)	ED2971			A_cli_ED2971	2317		3419	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3293 (BONN, ULS)	ED3209			A_cli_ED3209	2487		3679	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert *et al.* 3294 (BONN, ULS)	ED3210			A_cli_ED3210	2283		3390	this study
Atriplex clivicola I.M.Johnst	Chile	Luebert & Böhnert 3705 (BONN, ULS)	ED3214			A_cli_ED3214	2225		3323	this study
Atriplex cf. *coquimbana* Phil.	Chile	Luebert *et al.* 3818 (BONN, ULS)	ED2962	MN810493	MN810423	A_coq_ED2962	2532		3755	this study
Atriplex cf. *coquimbana* Phil.	Chile	Luebert *et al.* 3821 (BONN, ULS)	ED2963	MN810494	MN810424	A_coq_ED2963	2581		3792	this study
Atriplex cordubensis Gand. & Stuck	Argentina	Kubitzki 08-16 (HBG)			HM587623					Kadereit *et al.* 2010
Atriplex coriacea Forssk.	Egypt	Freitag 19569 (KAS)		AY270045	HM587624					Kadereit *et al.* 2003, 2010
Atriplex crassifolia Ledeb.	Russia	Lomonosova & Sukhorukov (MW)			HM587625					Kadereit *et al.* 2010
Atriplex dimorphostegia Kar. & Kir.	Kuwait	Al-Dosari 1839 (KTUH)			HM587626					Kadereit *et al.* 2010

Species	**Country**	**Voucher**	**Lab-ID**	***rbc*L**	***atp*B-*rbc*L**	**Sequencing ID**	**pops30**	**AtaArg**	**AtaPeru**	**Reference**
Atriplex flabellum Bunge ex Boiss.	Kirghizia	Kaden *et al.* 69500 (MW)			HM587627					Kadereit *et al.* 2010
Atriplex glauca L.	Egypt	Botschantzev (LE)			HM587629					Kadereit *et al.* 2010
Atriplex glauca L.	Spain	Freitag (KAS)		HM587584						Kadereit *et al.* 2010
Atriplex glaucescens Phil.	Chile	Böhnert & Merklinger 443 (BONN, ULS)	ED1883	MN810480	MN810410	A_gla_ED1883	2065		3136	this study
Atriplex glaucescens Phil.	Chile	Luebert *et al.* 3463 (BONN, ULS)	ED1886			A_gla_ED1886	2629		3938	this study
Atriplex glaucescens Phil.	Chile	Luebert *et al.* 3499 (BONN, ULS)	ED1889			A_gla_ED1889	2491		3702	this study
Atriplex glaucescens Phil.	Chile	Luebert *et al.* 3582 (BONN, ULS)	ED1895			A_gla_ED1895	2777		4114	this study
Atriplex glaucescens Phil.	Chile	Luebert *et al.* 3600 (BONN, ULS)	ED1897			A_gla_ED1897	2064		3121	this study
Atriplex glaucescens Phil.	Chile	Luebert & Böhnert 3656 (BONN, ULS)	ED1903	MN810484	MN810414	A_gla_ED1903	2007		2995	this study
Atriplex glaucescens Phil.	Chile	Luebert *et al.* 3998 (BONN, ULS)	ED2973			A_gla_ED2973	2743		4067	this study
Atriplex glaucescens Phil.	Chile	Luebert *et al.* 4007 (BONN, ULS)	ED2976			A_gla_ED2976	2506		3725	this study
Atriplex glaucescens Phil.	Chile	Luebert *et al.* 4009 (BONN, ULS)	ED2977			A_gla_ED2977	2525		3784	this study
Atriplex halimus L.	Spain	Kadereit & Kadereit (MJG)			HM587630					Kadereit *et al.* 2010
Atriplex halimus L.	Spain	Hensen (KAS)		AY270059						Kadereit *et al.* 2003, 2010
Atriplex herzogii Standl.	Peru	Weigend *et al.* 9855 (USM, HUSA, BONN)	ED4722	MN810513	MN810445					this study
Atriplex holocarpa F. Muell.	Australia	Jacobs 9189 (NSW)			HM587631					Kadereit *et al.* 2010
Atriplex hystrix Phil.	Chile	Luebert *et al.* 3271 (BONN, ULS)	ED1385			A_hys_ED1385	1834		3110	this study
Atriplex hystrix Phil.	Chile	Luebert *et al.* 3274 (BONN, ULS)	ED1386			A_hys_ED1386	3071		4846	this study
Atriplex hystrix Phil.	Chile	Luebert *et al.* 3839 (BONN, ULS)	ED2964	MN810495	MN810425	A_hys_ED2964	2143		3624	this study
Atriplex hystrix Phil.	Chile	Luebert *et al.* 3858 (BONN, ULS)	ED2965			A_hys_ED2965	2351		3956	this study
Atriplex hystrix Phil.	Chile	Luebert *et al.* 3295 (BONN, ULS)	ED3211	MN810500	MN810430	A_hys_ED3211	1687		2920	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert & Böhnert 3438 (BONN, ULS)	ED1380			A_imb_ED1380	2484		3718	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert *et al.* 3305 (BONN, ULS)	ED1391			A_imb_ED1391	2468		3634	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert *et al.* 3595 (BONN, ULS)	ED1896	MN810481	MN810411	A_imb_ED1896	2762		4106	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert & Böhnert 3660 (BONN, ULS)	ED1904			A_imb_ED1904	2358		3523	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert & Böhnert 3662 (BONN, ULS)	ED1905	MN810485	MN810415	A_imb_ED1905	2397		3595	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert & Böhnert 3665 (BONN, ULS)	ED1906			A_imb_ED1906	2419		3622	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert & Böhnert 3676 (BONN, ULS)	ED1910			A_imb_ED1910	2855		4124	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert & Böhnert 3688 (BONN, ULS)	ED1913			A_imb_ED1913	2490		3634	this study
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert *et al.* 3332 (BONN, ULS)	ED3212			A_imb_ED3212	2310		3459	this study
Atriplex imbricata (Moq.) D.Dietr.	Bolivia	Beck 21609 (KAS, LPB)			HM587632					Kadereit *et al.* 2010
Atriplex imbricata (Moq.) D.Dietr.	Chile	Luebert *et al.* 3286 (BONN, ULS)	ED1398	MN810466	MN810396					this study
Atriplex isatidea Moq.	Australia	Jacobs 9164 (NSW)			HM587633					Kadereit *et al.* 2010
Atriplex laciniata L.		Oellgaard (MW)			HM587635					Kadereit *et al.* 2010
Atriplex lampa (Gilles ex Moq.) D. Dietr.	Argentina	Kubitzki 08-23		HM587585	HM587634					Kadereit *et al.* 2010
Atriplex lanfrancoi (Brullo & Pavone) G.Kadereit & Sukhor.	Malta	Weber		HM587597	HM587680					Kadereit *et al.* 2010
Atriplex latifolia Wahlenb.	Russia	Timofeeva 1673 (MW)			HM587636					Kadereit *et al.* 2010
Atriplex lentiformis (Torr.) S. Watson	USA	Zacharias 520 (UC)			HM587637					Kadereit *et al.* 2010
Atriplex lentiformis (Torr.) S. Watson	USA	Kadereit 2008/26 (MJG)		HM587586						Kadereit *et al.* 2010
Atriplex lentiformis (Torr.) S. Watson	USA	Böhnert 463 (US-0-BONN-35881)	ED1822	MN810477	MN810407					this study
Atriplex leuca Phil.	Chile	Luebert *et al.* 3873 (BONN, ULS)	ED2967	MN810496	MN810426	A_leu_ED2967	2528		3769	this study
Atriplex leucoclada Boiss.	Iran	Ertter 18793 (UC)			HM587638					Kadereit *et al.* 2010
Atriplex leucophylla D. Dietr.	USA	Greenhouse (JEPS)			HM587639					Kadereit *et al.* 2010
Atriplex leucophylla D. Dietr.		Kadereit 2008/38 (MJG)		HM587587						Kadereit *et al.* 2010
Atriplex lithophila A. Soriano ex Múlgura	Argentina	Sancho *et al.* 373 (LP, BONN)	ED3982	MN810507	MN810436	A_lit_ED3982	2942	5348		this study
Atriplex madariagae Phil.	Chile	Luebert *et al.* 3416 (BONN, ULS)	ED1376			A_mad_ED1376	2736	5105		this study
Atriplex madariagae Phil.	Chile	Luebert *et al.* 3417 (BONN, ULS)	ED1377			A_mad_ED1377	2951	5628		this study

Species	Country	Voucher	Lab-ID	*rbc*L	*atp*B-*rbc*L	Sequencing ID	pops30	AtaArg	AtaPeru	Reference
Atriplex madariagae Phil.	Chile	Luebert & Böhnert 3449 (BONN, ULS)	ED1382			A_mad_ED1382	3184	6176		this study
Atriplex madariagae Phil.	Chile	Luebert & Böhnert 3443 (BONN, ULS)	ED1393			A_mad_ED1393	2579	4864		this study
Atriplex madariagae Phil.	Chile	Luebert & Böhnert 3444 (BONN, ULS)	ED1394	MN810462	MN810392	A_mad_ED1394	2739	5108		this study
Atriplex madariagae Phil.	Chile	Böhnert & Merklinger 438 (BONN, ULS)	ED1879			A_mad_ED1879	2917	5530		this study
Atriplex madariagae Phil.	Chile	Luebert *et al.* 3540 (BONN, ULS)	ED1892			A_mad_ED1892	2723	5127		this study
Atriplex madariagae Phil.	Chile	Luebert & Böhnert 3652 (BONN, ULS)	ED1901	MN810483	MN810413	A_mad_ED1901	2753	5104		this study
Atriplex madariagae Phil.	Chile	Luebert & Böhnert 3653 (BONN, ULS)	ED1902			A_mad_ED1902	2603	4895		this study
Atriplex madariagae Phil.	Chile	Luebert *et al.* 4003 (BONN, ULS)	ED2975			A_mad_ED2975	2271	4328		this study
Atriplex madariagae Phil.	Chile	Luebert *et al.* 4014 (BONN, ULS)	ED2980			A_mad_ED2980	2691	5060		this study
Atriplex madariagae Phil.	Chile	Luebert & Böhnert 3446 (BONN, ULS)	ED3213			A_mad_ED3213	2451	4693		this study
Atriplex moneta Bunge ex Boiss.	Turkmenistan	Efimova & Shatov (MW)			HM587641					Kadereit *et al.* 2010
Atriplex muelleri Benth.	Australia	Jacobs 9083 (NSW)			HM587642					Kadereit *et al.* 2010
Atriplex myriophylla Phil.	Peru	Montesinos 2880 (USM)	ED3954			A_myrio_ED3954	1770		2915	this study
Atriplex myriophylla Phil.	Peru	Montesinos 3780 (HUSA)	ED4720		MN810443	A_myrio_ED4720	1442		2445	this study
Atriplex myriophylla Phil.	Bolivia	Beck 11128 (KAS, LPB)			HM587643					Kadereit *et al.* 2010
Atriplex nudicaulis Bogusl.	Russia	Vekhov (MW)			HM587644					Kadereit *et al.* 2010
Atriplex nummularia Lindl.	Chile	Luebert & Böhnert 3732 (BONN, ULS)	ED1598	MN810472	MN810402					this study
Atriplex oblongifolia Waldst. & Kit.	Russia	Sukhorukov (MW)			HM587645					Kadereit *et al.* 2010
Atriplex oreophila Phil.	Chile	Luebert & Böhnert 3695 (BONN, ULS)	ED1916	MN810487	MN810417					this study
Atriplex parishii S. Watson	USA	Kadereit 2009/13 (MJG)		HM587588	HM587647					Kadereit *et al.* 2010
Atriplex parryi S. Watson	USA	Zacharias 585 (JEPS)		HM587589	HM587648					Kadereit *et al.* 2010
Atriplex patagonica D. Dietr.	Argentina	Schröder			HM587649					Kadereit *et al.* 2010
Atriplex patula L.				X15925						Hudson *et al.* 1990
Atriplex peruviana Moq.	Chile	Luebert *et al.* 4000 (BONN, ULS)	ED2974	MN810498	MN810428	A_per_ED2974	2472		3717	this study
Atriplex peruviana Moq.	Chile	Luebert *et al.* 4010 (BONN, ULS)	ED2978	MN810499	MN810429	A_per_ED2978	2086		3125	this study
Atriplex peruviana Moq.	Peru	Morales & Cuba 945 (USM)	ED3554	MN810501		A_per_ED3554	2219		3514	this study
Atriplex peruviana Moq.	Peru	Orellana *et al.* 212 (USM)	ED3555	MN810502		A_per_ED3555	2686		4292	this study
Atriplex peruviana Moq.	Peru	Weigend *et al.* 9815 (USM, HUSA, BONN)	ED4721	MN810512	MN810444	A_spec_ED4721	2200		3669	this study
Atriplex peruviana Moq.	Peru	Merklinger 2018-147 (BONN)	ED4744	MN810527	MN810459	A_spec_ED4744	2243		3746	this study
Atriplex philippii R.E. Fr.	Chile	Luebert *et al.* 3737 (BONN, ULS)	ED2960	MN810492	MN810422	A_phil_ED2960	2080		3345	this study
Atriplex phyllostegia (Torr. ex S. Watson) S. Watson	USA	Zacharias 992 (UC)		HM587590	HM587651					Kadereit *et al.* 2010
Atriplex powellii S. Watson	USA	Zacharias 529 (UC)		HM587591	HM587652					Kadereit *et al.* 2010
Atriplex prostrata Boucher ex DC.	Russia	Sukhorukov (MW)			HM587653					Kadereit *et al.* 2010
Atriplex repanda Phil.	Chile	Luebert & Böhnert 3729 (BONN, ULS)	ED1595	MN810470	MN810400	A_rep_ED1595	2246		3410	this study
Atriplex repanda Phil.	Chile	Luebert & Böhnert 3731 (BONN, ULS)	ED1597			A_rep_ED1597	2366		3577	this study
Atriplex repanda Phil.	Chile	Luebert & Böhnert 3720 (BONN, ULS)	ED1599	MN810473	MN810403	A_rep_ED1599	2514		3752	this study
Atriplex repanda Phil.	Chile	Luebert & Böhnert 3723 (BONN, ULS)	ED1600	MN810474	MN810404	A_rep_ED1600	2230		3393	this study
Atriplex repanda Phil.	Chile	Luebert *et al.* 3797 (BONN, ULS)	ED2961			A_rep_ED2961	2760		4030	this study
Atriplex retusa Gay	Chile	Luebert *et al.* 3283 (BONN, ULS)	ED1365			A_leu_ED1365	2458	4477		this study
Atriplex retusa Gay	Chile	Luebert *et al.* 3284 (BONN, ULS)	ED1366			A_leu_ED1366	2713	4995		this study
Atriplex retusa Gay	Chile	Luebert *et al.* 3282 (BONN, ULS)	ED1387	MN810461	MN810391	A_ret_ED1387	3037	5714		this study
Atriplex retusa Gay	Chile	Luebert *et al.* 3307 (BONN, ULS)	ED1388			A_ret_ED1388	2328	4304		this study
Atriplex retusa Gay	Chile	Luebert & Böhnert 3674 (BONN, ULS)	ED1908			A_ret_ED1908	2656	4935		this study
Atriplex retusa Gay	Chile	Luebert & Böhnert 3675 (BONN, ULS)	ED1909	MN810486	MN810416	A_ret_ED1909	2553	4739		this study
Atriplex retusa Gay	Chile	Luebert & Böhnert 3681 (BONN, ULS)	ED1911			A_ret_ED1911	2670	4963		this study
Atriplex retusa Gay	Chile	Luebert & Böhnert 3685 (BONN, ULS)	ED1912			A_ret_ED1912	2432	4550		this study
Atriplex retusa Gay	Chile	Luebert & Böhnert 3690 (BONN, ULS)	ED1915			A_ret_ED1915	2610	4832		this study

Species	Country	Voucher	Lab-ID	*rbc*L	*atp*B-*rbc*L	Sequencing ID	pops30	AtaArg	AtaPeru	Reference
Atriplex retusa Gay	Chile	Luebert & Böhnert 3700 (BONN, ULS)	ED1917			A_ret_ED1917	2211	4189		this study
Atriplex retusa Gay	Chile	Luebert & Böhnert 3713 (BONN, ULS)	ED1925	MN810491	MN810421	A_ret_ED1925	2661	4915		this study
Atriplex retusa Gay	Chile	Luebert & Böhnert 3716 (BONN, ULS)	ED1926			A_ret_ED1926	2676	4998		this study
Atriplex retusa Gay	Chile	Luebert *et al.* 3870 (BONN, ULS)	ED2966			A_leu_ED2966	2103	3902		this study
Atriplex retusa Gay	Chile	Luebert *et al.* 3996 (BONN, ULS)	ED2972			A_ret_ED2972	2732	5137		this study
Atriplex retusa Gay	Chile	Luebert & Böhnert 3669 (BONN, ULS)	ED4659			A_ret_ED4659	2985	5666		this study
Atriplex rhagodioides F. Muell.	Australia	Towler & Jacobs 322 (NSW)			HM587655					Kadereit *et al.* 2010
Atriplex rosea L.	USA	Kadereit 2009/11		HM587592	HM587656					Kadereit *et al.* 2010
Atriplex rotundifolia Dombey ex Moq.	Peru	Quipuscoa *et al.* 3596 (HUSA)	ED4719	MN810511	MN810442					this study
Atriplex rusbyi Britton	Bolivia	Beck 11335 (KAS)			HM587657					Kadereit *et al.* 2010
Atriplex semibaccata R.Br.	Peru	Linares & Galan (USM)	ED3958		MN810432					this study
Atriplex semibaccata R.Br.	Chile	Luebert & Böhnert 3728 (BONN, ULS)	ED1594	MN810469	MN810399					this study
Atriplex semibaccata R.Br.	Chile	Luebert & Böhnert 3733 (BONN, ULS)	ED1601	MN810475	MN810405					this study
Atriplex serenana A. Nelson ex Abrams	USA	Zacharias 495 (UC)		HM587593	HM587658					Kadereit *et al.* 2010
Atriplex sibirica L.	Russia	Volosnova (MW)			HM587659					Kadereit *et al.* 2010
Atriplex sp.	Peru	Ortiz *et al.* 110 (HUSA)	ED4716		MN810439	A_spec_ED4716	2520		3690	this study
Atriplex sp.	Peru	Quispe 118 (HUSA)	ED4717	MN810510	MN810440	A_spec_ED4717	2377		3450	this study
Atriplex sp.	Peru	Quipuscoa 3590 (HUSA)	ED4718		MN810441	A_spec_ED4718	1749		2511	this study
Atriplex sp.	Peru	Weigend *et al.* 9886 (USM, HUSA, BONN)	ED4728	MN810519	MN810451	A_spec_ED4728	3123		4557	this study
Atriplex sp.	Peru	Weigend *et al.* 9887 (USM, HUSA, BONN)	ED4729	MN810520	MN810452	A_spec_ED4729	2890		4265	this study
Atriplex sp.	Peru	Weigend *et al.* 9889 (USM, HUSA, BONN)	ED4730	MN810521	MN810453	A_spec_ED4730	3112		4556	this study
Atriplex sp.	Peru	Weigend *et al.* 9890 (USM, HUSA, BONN)	ED4731	MN810522	MN810454	A_spec_ED4731	3047		4492	this study
Atriplex sp.	Peru	Weigend *et al.* 9965 (USM, HUSA, BONN)	ED4732	MN810523	MN810455	A_spec_ED4732	2722		4047	this study
Atriplex sp.	Peru	Weigend *et al.* 9966 (USM, HUSA, BONN)	ED4733	MN810524	MN810456	A_spec_ED4733	3094		4549	this study
Atriplex sp.	Peru	Weigend *et al.* 9967 (USM, HUSA, BONN)	ED4734	MN810525	MN810457	A_spec_ED4734	2872		4254	this study
Atriplex sp.	Peru	Weigend *et al.* 9968 (USM, HUSA, BONN)	ED4735	MN810526	MN810458	A_spec_ED4735	2718		4041	this study
Atriplex sp.	Peru	Montesinos 3122 (USM)	ED3957	MN810503	MN810431					this study
Atriplex sp.	Peru	Weigend *et al.* 9868 (USM, HUSA, BONN)	ED4723	MN810514	MN810446					this study
Atriplex sp.	Peru	Weigend *et al.* 9879 (USM, HUSA, BONN)	ED4724	MN810515	MN810447					this study
Atriplex sp.	Peru	Weigend *et al.* 9880 (USM, HUSA, BONN)	ED4725	MN810516	MN810448					this study
Atriplex sp.	Peru	Weigend *et al.* 9881 (USM, HUSA, BONN)	ED4726	MN810517	MN810449					this study
Atriplex sp.	Peru	Weigend *et al.* 9882 (USM, HUSA, BONN)	ED4727	MN810518	MN810450					this study
Atriplex spongiosa F. Muell.				AY270060	HM587661					Kadereit *et al.* 2003, 2010
Atriplex suberecta I. Verd.	Chile	Luebert & Böhnert 3730 (BONN, ULS)	ED1602	MN810476	MN810406					this study
Atriplex taltalense I.M.Johnst	Chile	Luebert *et al.* 3386 (BONN, ULS)	ED1372			A_tal_ED1372	2873		4624	this study
Atriplex taltalense I.M.Johnst	Chile	Luebert *et al.* 3393 (BONN, ULS)	ED1373			A_tal_ED1373	2251		3761	this study
Atriplex taltalense I.M.Johnst	Chile	Luebert *et al.* 3400 (BONN, ULS)	ED1374			A_tal_ED1374	2591		4288	this study
Atriplex taltalense I.M.Johnst	Chile	Luebert *et al.* 3391 (BONN, ULS)	ED1400	MN810468	MN810398	A_tal_ED1400	2980		4766	this study
Atriplex taltalense I.M.Johnst	Chile	Luebert *et al.* 3923 (BONN, ULS)	ED2968	MN810497	MN810427	A_tal_ED2968	2212		3762	this study
Atriplex taltalense I.M.Johnst	Chile	Böhnert & Merklinger 437 (BONN, ULS)	ED1880			A_tal_ED1880	2699		4416	this study
Atriplex taltalense I.M.Johnst	Chile	Luebert *et al.* 3935 (BONN, ULS)	ED2969			A_tal_ED2969	2319		3897	this study
Atriplex tatarica L.	Russia	Sukhorukov (MW)			HM587664					Kadereit *et al.* 2010
Atriplex undulata (Moq.) D.Dietr.	Argentina	Sancho *et al.* 354 (LP, BONN)	ED3979	MN810504	MN810433	A_und_ED3979	3065	5916		this study
Atriplex undulata (Moq.) D.Dietr.	Argentina	Sancho *et al.* 365 (LP, BONN)	ED3980	MN810505	MN810434	A_und_ED3980	3097	5913		this study
Atriplex undulata (Moq.) D.Dietr.	Argentina	Sancho *et al.* 374 (LP, BONN)	ED3983	MN810508	MN810437	A_und_ED3983	3125	6057		this study
Atriplex undulata (Moq.) D.Dietr.	Argentina	Múlgura 2005 (SI, KAS)		AY270061	HM587665					Kadereit *et al.* 2003, 2010
Atriplex vallenarensis M.Rosas	Chile	Luebert & Böhnert 3708 (BONN, ULS)	ED1923	MN810489	MN810419	A_val_ED1923	2176		3686	this study
Atriplex vallenarensis M.Rosas	Chile	Luebert & Böhnert 3709 (BONN, ULS)	ED1924	MN810490	MN810420	A_val_ED1924	2652		4372	this study

Species	Country	Voucher	Lab-ID	*rbc*L	*atp*B-*rbc*L	Sequencing ID	pops30	AtaArg	AtaPeru	Reference
Atriplex vesicaria Heward ex Benth.	Australia	Jacobs 9202 (NSW)			HM587666					Kadereit *et al.* 2010
Axyris prostrata L.	Mongolia	Miehe 96-140-04 (KAS)		AY270062	HM587667					Kadereit *et al.* 2003, 2010
Ceratocarpus arenarius L.	Russia	Freitag 33017 (KAS)		HM587594						Kadereit *et al.* 2010
Chenopodium acuminatum Willd.	Mongolia	Miehe 96-060-5 (KAS)		AY270077	HM587668					Kadereit *et al.* 2003, 2010
Chenopodium auricomum Lindley	Australia	Jacobs 8655 (NSW)		AY270078	HM587669					Kadereit *et al.* 2003, 2010
Chenopodium bonus-henricus L.		Bot. Gard. Mainz (MJG)		AY270079	HM587670					Kadereit *et al.* 2003, 2010
Chenopodium coronopus Moq.	Spain	Royl 6823 (MJG)		HM587595	HM587671					Kadereit *et al.* 2010
Chenopodium desertorum (J. Black) J. Black	Australia	Jacobs 8650 (NSW)		AY270042	HM587672					Kadereit *et al.* 2003, 2010
Chenopodium foliosum Asch.	Mongolia	Hilbig (HAL)			HM587673					Kadereit *et al.* 2010
Chenopodium foliosum Asch.				AY270081						Kadereit *et al.* 2003
Chenopodium frutescens C.A. Mey.	Russia	Korolyuk (NS)		AY270082	HM587674					Kadereit *et al.* 2003, 2010
Chenopodium murale L.	Italy	Licht 5801 (MJG)			HM587675					Kadereit *et al.* 2010
Chenopodium nitrariaceum (F. Muell.) Benth.	Australia	Jacobs 9212 (NSW, MJG)			HM587676					Kadereit *et al.* 2010
Chenopodium oahuense (Meyren) Aellen	USA	Carlquist 2085 (UC)			HM587677					Kadereit *et al.* 2010
Chenopodium sanctae-clarae Johow				AY270043						Kadereit *et al.* 2003
Chenopodium urbicum L.	Greece	Raus 242 (MJG)		HM587596	HM587678					Kadereit *et al.* 2010
Corispermum filifolium C.A. Mey. ex A.K. Becker	Turkey	Freitag 28702 (KAS)		AY270084	HM587679					Kadereit *et al.* 2003, 2010
Cycloloma atriplicifolium J.M. Coult.		Bot. Garden Kassel (KAS)		HM587598	HM587681					Kadereit *et al.* 2010
Dysphania ambrosioides (L.) Mosyakin & Clemants	Portugal	Royl 6394 (MJG)		HM587599	HM587682					Kadereit *et al.* 2010
Dysphania botrys (L.) Mosyakin & Clemants	Turkey	Freitag & Adigüzel 28769 (KAS)		AY270080	HM587683					Kadereit *et al.* 2003, 2010
Dysphania cristata (F. Muell.) Mosyakin & Clemants	Australia	Jacobs 8653 (NSW)		AY270046	HM587684					Kadereit *et al.* 2003, 2010
Dysphania glomulifera (Nees) Paul G. Wilson	Australia	Jacobs 8738 (NSW)		AY270086	HM587685					Kadereit *et al.* 2003, 2010
Einadia nutans (R. Br.) A.J. Scott	Australia	Towler 325 (NSW)		HM587600	HM587686					Kadereit *et al.* 2010
Exomis microphylla (Thunb.) Aellen	South Africa	Mucina 81200/3 (MJG)		HM587601	HM587687					Kadereit *et al.* 2010
Extriplex californica (Moq.) E.H. Zacharias	USA	Kadereit 2009/37 (MJG)		HM587602	HM587688					Kadereit *et al.* 2010
Extriplex joaquinana (A. Nelson) E.H. Zacharias	USA	Zacharias 306		HM587603	HM587689					Kadereit *et al.* 2010
Grayia brandegeei A. Gray	USA	Coles (COLO)		HM587604	HM587690					Kadereit *et al.* 2010
Grayia spinosa (Hook.) Moq.	USA	Larkesto (BM)			HM587691					Kadereit *et al.* 2010
Grayia spinosa (Hook.) Moq.	USA	Neely 4244 (COLO)		HM587605	HM587693					Kadereit *et al.* 2010
Halimione pedunculata (L.) Aellen	Russia	Freitag 33094 (KAS)			HM587694					Kadereit *et al.* 2010
Halimione pedunculata (L.) Aellen	Denmark	Kadereit 2000/202 (MJG)		AY270093						Kadereit *et al.* 2003
Halimione portulacoides (L.) Aellen	Greece	Raus *et al.* 30895 (B 10 0745204)	ED3985	MN810509	MN810438	OUT_A-port_ED3985	964	1704	1290	this study
Halimione verrucifera (M. Bieb.) Aellen	Russia	Freitag 33092 (KAS)		HM587606	HM587695					Kadereit *et al.* 2010
Holmbergia tweedii Speg.	Argentina	Krapovickas & Cristobal 24545 (P)			HM587696					Kadereit *et al.* 2010
Krascheninnikovia ceratoides (L.) Gueldenst.	Pakistan	Dickoré 12752 (KAS)		AY270105	HM587697					Kadereit *et al.* 2003, 2010
Krascheninnikovia lanata (Pursh) A. Meeuse & A. Smit	USA	Nelson 23554 (USCH, KAS)			HM587698					Kadereit *et al.* 2010
Manochlamys albicans (Soland. in Ait.) Aellen	South Africa	Mucina 200900/4 (MJG)		HM587607	HM587699					Kadereit *et al.* 2010

Species	Country	Voucher	Lab-ID	*rbc*L	*atp*B-*rbc*L	Sequencing ID	pops30	AtaArg	AtaPeru	Reference
Microgynoecium tibeticum Hook.f.	China	Miehe 05-059-11 (KAS)			HM587700					Kadereit *et al.* 2010
Microgynoecium tibeticum Hook.f.	China	Dickor 4284 (KAS)		AY270107						Kadereit *et al.* 2003
Micromonolepis pusilla (Torr. ex S. Watson) Ulbr.	USA	Tiehm 11763 (NSW)		HM587608	HM587701					Kadereit *et al.* 2010
Monolepis nuttalliana Greene		Bot. Garden Kassel (KAS)		AY270108	HM587702					Kadereit *et al.* 2003, 2010
Rhagodia drummondii Moq.	Australia	Jacobs 9131 (NSW)			HM587703					Kadereit *et al.* 2010
Rhagodia drummondii Moq.	Australia	Schmalz 194 (MJG)		AY270124						Kadereit *et al.* 2003
Rhagodia parabolica R. Br.		Jacobs 9208 (NSW)			HM587704					Kadereit *et al.* 2010
Scleroblitum atriplicinum (F. Muell.) Ulbr.				AY270044						Kadereit *et al.* 2003
Spinacia oleracea L.		Kadereit (MJG)			HM587705					Kadereit *et al.* 2010
Stutzia covillei (Standl.) E.H. Zacharias	USA	Zacharias 811 (UC)		HM587609	HM587706					Kadereit *et al.* 2010
Stutzia dioica (Nutt.) E.H. Zacharias		Welp 6269 (NY)		HM587610	HM587707					Kadereit *et al.* 2010
Suckleya suckleyana Rydb.	USA	Larson 6492		HM587611						Kadereit *et al.* 2010
Teloxys aristata (L.) Moq.	Mongolia	Neuffer & Hurka 11727 (KAS)		AY270140	HM587708					Kadereit *et al.* 2003, 2010

References

Hudson GS, Mahon JD, Anderson PA, Gibbs MJ, Badger MR, Andrews TJ & Whitfeld PR (1990) Comparisons of *rbc*L genes for the large subunit of ribulose-bisphosphate carboxylase from closely related C_3 and C_4 plant species. *The Journal of Biological Chemistry* 265 (2): 808–814.

Kadereit G, Borsch T, Weising K & Freitag H (2003) Phylogeny of Amaranthaceae and Chenopodiaceae and the Evolution of C_4 Photosynthesis. *International Journal of Plant Sciences* 164 (6): 959–986. DOI: https://doi.org/10.1086/378649.

Kadereit G, Mavrodiev EV, Zacharias EH & Sukhorukov AP (2010) Molecular phylogeny of Atripliceae (Chenopodioideae, Chenopodiaceae): Implications for systematics, biogeography, flower and fruit evolution, and the origin of C_4 photosynthesis. *American Journal of Botany* 97 (10): 1664–1687. DOI: https://doi.org/10.3732/ajb.1000169.

GBS Phylogeny of *Atriplex* from the Atacama Desert

Here a denovo assembly for an empirical Genotype-By-Sequencing (GBS) data set of the genus Cristaria Cav. (Malvaceae) using the ipyrad Python API is documented. The assembly subsequent phylogenetic reconstruction was conducted in a Linux environment (Ubuntu 18.04) on a computer with an Intel Core i7 CPU and 32 GB RAM.

Setup (software and parallelization)

[1]:
```
## conda install ipyrad -c ipyrad
## conda install toytree -c eaton-lab
## conda install sra-tools -c bioconda
## conda install entrez-direct -c bioconda
```

[2]:
```
## 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. 0.9.15
toytree v. 0.2.3
```

Parallel processes on independent Python kernels

To start a parallel client you must run the command-line program ‘ipcluster’. This will essentially start a number of independent Python processes (kernels) which we can then send bits of work to do. The cluster can be stopped and restarted independently of this notebook, which is convenient for working on a cluster where connecting to many cores is not always immediately available.

Open a terminal and type the following command to start an ipcluster instance with N engines.

[3]:
```
## ipcluster start --n=8
```

```
[3]: ## connect to cluster
     ipyclient = ipp.Client()
     #ipyclient.ids
     print(ip.cluster_info(ipyclient))
     print("{} engines found".format(len(ipyclient)))
```

```
Parallel connection | Eulychnia: 8 cores
None
8 engines found
```

Data Assembly

Create an Assembly object and set parameters in *ipyRAD* params file

This object stores the parameters of the assembly and the organization of data files.

```
[49]: ## you must provide a name for the Assembly
      data = ip.Assembly("Atriplex")
```

```
New Assembly: Atriplex
```

```
[50]: ## set parameters
      data.set_params("project_dir", "Atri_Assembly")
      data.set_params("sorted_fastq_path", "../GBS_Atri_Data/*.fastq.gz")
      data.set_params("clust_threshold", "0.85")
      data.set_params("max_Hs_consens", (0.05))
      data.set_params("restriction_overhang", ('TGCAG', 'GGCC'))
      data.set_params("output_formats", "*")
      data.set_params("datatype", "ddrad")

      ## see / print all parameters
      data.get_params()
```

```
[50]: 0   assembly_name              Atriplex
      1   project_dir                ./Atri_Assembly
      2   raw_fastq_path
      3   barcodes_path
      4   sorted_fastq_path          ~/BioInf/Atriplex_GBS/GBS_Atri_Data/*.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       6
      12  mindepth_majrule           6
      13  maxdepth                   10000
```

```
14  clust_threshold            0.85
15  max_barcode_mismatch       0
16  filter_adapters            0
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          4
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
```

Assemble the data set

First steps 1 & 2

Second steps 3-6

Third step 7 with different `min_sample_locus` setting

[51]:

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

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:01:17 | loading reads        | s1 |
[####################] 100% 0:08:55 | processing reads     | s2 |
```

[52]:

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

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:00:05 | concatenating        | s3 |
[####################] 100% 0:01:31 | dereplicating        | s3 |
[####################] 100% 0:30:15 | clustering/mapping   | s3 |
[####################] 100% 0:00:01 | building clusters    | s3 |
[####################] 100% 0:00:00 | chunking clusters    | s3 |
[####################] 100% 0:48:30 | aligning clusters    | s3 |
[####################] 100% 0:01:01 | concat clusters      | s3 |
[####################] 100% 0:00:10 | calc cluster stats   | s3 |
[####################] 100% 0:08:14 | inferring [H, E]     | s4 |
[####################] 100% 0:00:09 | calculating depths   | s5 |
[####################] 100% 0:00:14 | chunking clusters    | s5 |
```

```
[####################] 100% 0:21:05 | consens calling      | s5 |
[####################] 100% 0:00:20 | indexing alleles     | s5 |
[####################] 100% 0:00:05 | concatenating inputs | s6 |
[####################] 100% 0:04:29 | clustering tier 1    | s6 |
[####################] 100% 0:00:01 | concatenating inputs | s6 |
[####################] 100% 0:03:17 | clustering across    | s6 |
[####################] 100% 0:00:05 | building clusters    | s6 |
[####################] 100% 0:03:08 | aligning clusters    | s6 |
```

[14]:

```
## access the stats of the assembly (so far) from the .stats attribute
data.stats
```

[14]:

```
               state  reads_raw  reads_passed_filter  clusters_total  \
A_arg_ED3981       6    2922983              2922876          110250
A_ata_ED1882       6     988788               988718           28760
A_ata_ED1899       6    1007536              1007462           31862
A_ata_ED1900       6     961274               961198           25685
A_chiz_ED1379      6    3567330              3567080           60856
A_chiz_ED1383      6    1879701              1879662           53997
A_chiz_ED1384      6    1554802              1554783           58095
A_chiz_ED1392      6    2858821              2858770           94016
A_chiz_ED1395      6    2123882              2123847           61818
A_chiz_ED1399      6    2146930              2146900           72432
A_chiz_ED1881      6     733892               733829           23256
A_chiz_ED1888      6     815343               815291           24236
A_chiz_ED2979      6     699773               699733           27098
A_cli_ED1364       6     659749               659705           54399
A_cli_ED1371       6    1140191              1140113          105993
A_cli_ED1375       6    2115991              2115825           83915
A_cli_ED1389       6     974358               974309           77759
A_cli_ED1390       6    1426899              1426871          112591
A_cli_ED1396       6    1940587              1940555           70746
A_cli_ED1397       6    2256051              2256010          128134
A_cli_ED1918       6     689471               689426           66524
A_cli_ED1919       6     718423               718374           84496
A_cli_ED2970       6     983194               983128           70658
A_cli_ED2971       6     714931               714890           63045
A_cli_ED3209       6     805232               805186           39885
A_cli_ED3210       6     727769               727703           44715
A_cli_ED3214       6     663110               663052           63541
A_coq_ED2962       6     720210               720164           37943
A_coq_ED2963       6     718763               718718           27420
A_gla_ED1883       6    1032086              1032018           23026
A_gla_ED1886       6     977116               977052           27231
A_gla_ED1889       6     997055               997000           41022
A_gla_ED1895       6    1120982              1120896           54083
A_gla_ED1897       6     905857               905812           85686
```

A_gla_ED1903	6	857561	857499	25199
A_gla_ED2973	6	1046069	1046005	27368
A_gla_ED2976	6	499540	499497	30170
A_gla_ED2977	6	789208	789144	33693
A_hys_ED1385	6	668716	668673	104041
A_hys_ED1386	6	2079707	2079675	123764
A_hys_ED2964	6	480052	480014	57113
A_hys_ED2965	6	618993	618951	49562
A_hys_ED3211	6	943301	943249	36309
A_imb_CF_ED3553	6	1405497	1405037	75705
A_imb_CF_ED3960	6	768858	768611	130196
A_imb_ED1380	6	625687	625651	27517
A_imb_ED1391	6	735999	735946	69008
A_imb_ED1896	6	1389751	1389648	38984
A_imb_ED1904	6	868190	868132	22805
A_imb_ED1905	6	1051800	1051719	30094
A_imb_ED1906	6	656188	656132	27833
A_imb_ED1910	6	1584135	1584030	33997
A_imb_ED1913	6	704939	704884	22210
A_imb_ED3212	6	773515	773463	33652
A_leu_ED1365	6	1084786	1084714	46030
A_leu_ED1366	6	992792	992724	60949
A_leu_ED2966	6	592476	592439	68360
A_leu_ED2967	6	693190	693151	45523
A_lit_ED3982	6	2953037	2952943	62933
A_mad_ED1376	6	1042536	1042470	31502
A_mad_ED1377	6	1534766	1534656	61574
A_mad_ED1382	6	1785659	1785634	60409
A_mad_ED1393	6	1164822	1164735	41539
A_mad_ED1394	6	832471	832408	30480
A_mad_ED1879	6	1273282	1273196	52506
A_mad_ED1892	6	733911	733853	30930
A_mad_ED1901	6	985829	985736	43702
A_mad_ED1902	6	657142	657093	35294
A_mad_ED2975	6	1074577	1074503	26787
A_mad_ED2980	6	863382	863311	32815
A_mad_ED3213	6	776910	776836	24192
A_myrio_ED3551	6	2976416	2975635	585478
A_myrio_ED3552	6	2309412	2308719	306645
A_myrio_ED3954	6	2013729	2013226	394452
A_myrio_ED4720	6	3847188	3846190	314956
A_per_CF_ED3956	6	739648	739458	202348
A_per_CF_ED3959	6	1344592	1344187	110499
A_per_ED2974	6	822865	822805	22148
A_per_ED2978	6	994178	994113	29968
A_per_ED3554	6	2325945	2325194	237860
A_per_ED3555	6	1598019	1597497	133103

A_per_ED3955	6	2133954	2133282	118439
A_phil_ED2960	6	1178638	1178573	54429
A_port_ED3985	6	2287048	2286949	299279
A_rep_ED1595	6	790210	790144	38085
A_rep_ED1597	6	989337	989267	57469
A_rep_ED1599	6	1063464	1063386	66079
A_rep_ED1600	6	737028	736987	56212
A_rep_ED2961	6	2484448	2484292	55775
A_ret_ED1387	6	1376431	1376407	97478
A_ret_ED1388	6	564016	563977	33218
A_ret_ED1908	6	1236109	1236010	24767
A_ret_ED1909	6	1222044	1221968	31205
A_ret_ED1911	6	941837	941765	27041
A_ret_ED1912	6	633088	633046	21060
A_ret_ED1915	6	680486	680429	29621
A_ret_ED1917	6	732668	732613	35747
A_ret_ED1925	6	1019409	1019349	30086
A_ret_ED1926	6	1062018	1061937	41101
A_ret_ED2972	6	1010169	1010085	29138
A_ret_ED4659	6	2918387	2918261	49529
A_rot_ED3558	6	185248	185195	70410
A_rot_ED3560	6	746438	746172	249694
A_rot_ED4719	6	714624	714366	140776
A_spec_ED3957	6	2112030	2111442	211201
A_spec_ED4716	6	3056344	3055330	81133
A_spec_ED4717	6	3836063	3834880	162180
A_spec_ED4718	6	941638	941343	205666
A_spec_ED4721	6	3108355	3107234	46009
A_spec_ED4728	6	2254912	2254035	179299
A_spec_ED4729	6	3244806	3243745	170214
A_spec_ED4730	6	1933410	1932740	127443
A_spec_ED4731	6	3146064	3145117	62266
A_spec_ED4732	6	4184897	4183642	84589
A_spec_ED4733	6	3752846	3751552	75846
A_spec_ED4734	6	3725571	3724375	59768
A_spec_ED4735	6	3224241	3223198	86326
A_spec_ED4744	6	4245647	4244291	110686
A_tal_ED1372	6	1876582	1876556	210638
A_tal_ED1373	6	928462	928404	102611
A_tal_ED1374	6	1384300	1384214	86154
A_tal_ED1400	6	1763418	1763391	109861
A_tal_ED1880	6	1042482	1042394	33183
A_tal_ED2968	6	611170	611131	63977
A_tal_ED2969	6	889092	889039	80675
A_und_ED3979	6	3842339	3842198	88067
A_und_ED3980	6	3293260	3293136	80265
A_und_ED3983	6	4334500	4334338	65014

```
A_val_ED1923         6     641110               641076           37757
A_val_ED1924         6    1121748              1121686           46761

                 clusters_hidepth  hetero_est  error_est  reads_consens
A_arg_ED3981                24562    0.021225   0.000975          20475
A_ata_ED1882                 8912    0.013036   0.000817           8450
A_ata_ED1899                 7724    0.013729   0.000746           7337
A_ata_ED1900                 8301    0.012900   0.000752           7887
A_chiz_ED1379               10715    0.015359   0.000478           9885
A_chiz_ED1383               11731    0.013697   0.000760          11132
A_chiz_ED1384               11792    0.013273   0.000861          11177
A_chiz_ED1392               13773    0.018172   0.000690          12633
A_chiz_ED1395               11289    0.014201   0.000686          10704
A_chiz_ED1399               11921    0.014287   0.000762          11271
A_chiz_ED1881                8395    0.012751   0.000813           7992
A_chiz_ED1888                8326    0.012724   0.000776           7952
A_chiz_ED2979                8006    0.012916   0.000887           7598
A_cli_ED1364                13228    0.008205   0.001147          12632
A_cli_ED1371                24601    0.009484   0.000969          23510
A_cli_ED1375                13595    0.013190   0.000818          12674
A_cli_ED1389                18443    0.011781   0.001307          17443
A_cli_ED1390                24203    0.009600   0.000960          23226
A_cli_ED1396                19798    0.012113   0.000904          18651
A_cli_ED1397                34171    0.018226   0.001087          30273
A_cli_ED1918                10835    0.011755   0.000968          10247
A_cli_ED1919                21785    0.007254   0.001292          20831
A_cli_ED2970                19772    0.007609   0.000952          19141
A_cli_ED2971                16069    0.006805   0.000929          15624
A_cli_ED3209                 8464    0.010793   0.000987           8033
A_cli_ED3210                10358    0.010176   0.000888           9916
A_cli_ED3214                 8615    0.011393   0.000966           8090
A_coq_ED2962                 8879    0.012314   0.000768           8350
A_coq_ED2963                 9278    0.012528   0.000855           8663
A_gla_ED1883                 7087    0.012692   0.000773           6727
A_gla_ED1886                 8718    0.012491   0.000816           8319
A_gla_ED1889                 8911    0.012922   0.000982           8407
A_gla_ED1895                10555    0.012856   0.000888           9958
A_gla_ED1897                18193    0.010883   0.001222          17035
A_gla_ED1903                 6742    0.013227   0.000735           6418
A_gla_ED2973                 9083    0.012229   0.000769           8662
A_gla_ED2976                 8305    0.012255   0.000985           7880
A_gla_ED2977                10079    0.011557   0.000832           9501
A_hys_ED1385                15402    0.021079   0.001631          13144
A_hys_ED1386                19717    0.016787   0.001020          17991
A_hys_ED2964                 9385    0.017545   0.001005           8418
A_hys_ED2965                 9523    0.013919   0.000849           8926
A_hys_ED3211                14882    0.008639   0.001541          14227
```

A_imb_CF_ED3553	23299	0.006907	0.002084	22391
A_imb_CF_ED3960	26200	0.008570	0.002386	24193
A_imb_ED1380	8149	0.011999	0.000968	7772
A_imb_ED1391	10091	0.012399	0.001014	9492
A_imb_ED1896	10102	0.012038	0.000744	9602
A_imb_ED1904	7880	0.012762	0.000799	7490
A_imb_ED1905	8358	0.012571	0.000832	7907
A_imb_ED1906	8137	0.012213	0.000880	7721
A_imb_ED1910	9152	0.010632	0.000662	8734
A_imb_ED1913	8102	0.011580	0.000851	7737
A_imb_ED3212	7672	0.011220	0.000835	7333
A_leu_ED1365	13404	0.007100	0.000966	12810
A_leu_ED1366	9534	0.010045	0.000797	8814
A_leu_ED2966	14284	0.012623	0.001316	13038
A_leu_ED2967	8946	0.012724	0.000741	8390
A_lit_ED3982	12128	0.007074	0.000722	11670
A_mad_ED1376	8866	0.013594	0.000786	8387
A_mad_ED1377	10059	0.013756	0.000737	9443
A_mad_ED1382	13378	0.012330	0.000802	12715
A_mad_ED1393	9441	0.013898	0.000926	8763
A_mad_ED1394	8361	0.012683	0.000761	7948
A_mad_ED1879	9885	0.013499	0.000851	9271
A_mad_ED1892	8716	0.012923	0.000840	8227
A_mad_ED1901	11144	0.013375	0.000867	10526
A_mad_ED1902	10751	0.015601	0.000905	9855
A_mad_ED2975	7307	0.012736	0.000756	6943
A_mad_ED2980	8382	0.013187	0.000776	7934
A_mad_ED3213	7502	0.012387	0.000728	7196
A_myrio_ED3551	99878	0.036224	0.004766	67702
A_myrio_ED3552	45002	0.015951	0.002310	40246
A_myrio_ED3954	56269	0.035773	0.003903	39616
A_myrio_ED4720	65405	0.022193	0.002774	53718
A_per_CF_ED3956	24881	0.025322	0.004042	19582
A_per_CF_ED3959	21055	0.011596	0.001904	19412
A_per_ED2974	8111	0.011866	0.000750	7733
A_per_ED2978	7541	0.013533	0.000800	7128
A_per_ED3554	33732	0.014272	0.002039	30916
A_per_ED3555	13848	0.019547	0.001431	12266
A_per_ED3955	30521	0.011293	0.001940	28550
A_phil_ED2960	7969	0.005347	0.000746	7700
A_port_ED3985	16690	0.018485	0.001130	14841
A_rep_ED1595	7993	0.013664	0.000776	7471
A_rep_ED1597	15151	0.010784	0.001011	14386
A_rep_ED1599	13398	0.011714	0.000972	12618
A_rep_ED1600	16942	0.009485	0.000951	16240
A_rep_ED2961	10414	0.014911	0.000601	9720
A_ret_ED1387	22645	0.014391	0.000934	20369

```
A_ret_ED1388              6833    0.005343    0.000854             6614
A_ret_ED1908              7783    0.006312    0.000625             7569
A_ret_ED1909              7629    0.005583    0.000718             7397
A_ret_ED1911              7835    0.005800    0.000726             7616
A_ret_ED1912              7122    0.005319    0.000756             6953
A_ret_ED1915              7726    0.005890    0.000773             7489
A_ret_ED1917              8692    0.005506    0.000783             8449
A_ret_ED1925              7774    0.005736    0.000720             7535
A_ret_ED1926             11990    0.007628    0.000853            11565
A_ret_ED2972              8723    0.013168    0.000720             8270
A_ret_ED4659              9978    0.005360    0.000672             9657
A_rot_ED3558              6679    0.016397    0.002814             5931
A_rot_ED3560             19353    0.019772    0.003242            15890
A_rot_ED4719              6822    0.018869    0.001793             6034
A_spec_ED3957            32510    0.022681    0.001987            27416
A_spec_ED4716            18600    0.006688    0.001060            17933
A_spec_ED4717            34087    0.004508    0.000950            33100
A_spec_ED4718            16532    0.009226    0.002227            15265
A_spec_ED4721             9100    0.015592    0.000813             8435
A_spec_ED4728            15820    0.018816    0.001287            13875
A_spec_ED4729            20135    0.012750    0.000944            18633
A_spec_ED4730            13754    0.015995    0.001228            12582
A_spec_ED4731            11741    0.012594    0.000816            11115
A_spec_ED4732            10476    0.012513    0.000699             9810
A_spec_ED4733            13128    0.014793    0.000817            12215
A_spec_ED4734            11191    0.014548    0.000765            10425
A_spec_ED4735            14084    0.012183    0.000820            13338
A_spec_ED4744            25219    0.015130    0.001485            22721
A_tal_ED1372             57994    0.010129    0.001597            54624
A_tal_ED1373             20096    0.018009    0.002141            17798
A_tal_ED1374             19513    0.015020    0.001141            17815
A_tal_ED1400             25556    0.015193    0.001085            22860
A_tal_ED1880             10403    0.015699    0.000825             9658
A_tal_ED2968              9087    0.016395    0.000860             8231
A_tal_ED2969             14668    0.014909    0.001116            13432
A_und_ED3979             15354    0.016796    0.000876            14038
A_und_ED3980             13611    0.013359    0.000683            12682
A_und_ED3983             14500    0.011841    0.000618            13759
A_val_ED1923             10229    0.012902    0.000820             9623
A_val_ED1924             10551    0.016876    0.000878             9682
```

Branch to create several final data sets with different parameter setting

In order to analyse the GBS data using Maximum Likelihood method (RAxML) the assembly will be branched after step 6. Based on the amount of reads per sample, some samples have been removed resulting in 119 out of 130 samples used for analyses. For the final assembly of the three different data taxon sets a population assignment will be used specifing all *Atriplex* samples as ingroup and the *Halimione* as outgroup. Further, `min_samples_locus` will be specified to allow in all sets 70 % missing data.

Load assembly object (until step 6) **to avoid rerunning the assembly until here!**

[4]:

```
## load assembly object from previous assemblies
data = ip.load_json("Atri_Assembly/Atriplex.json")
```

```
loading Assembly: Atriplex
from saved path: ~/BioInf/Atriplex_GBS/GBS_Atri_Anal/Atri_Assembly/Atriplex.json
```

[5]:

```
## make a subsample list of samples you wanna keep in the assembly
## DELETED: A_per_CF_ED3956; A_per_CF_ED3959; A_rot_ED3560;   A_imb_CF_ED3553;
##          A_imb_CF_ED3960; A_per_ED3955;    A_rot_ED3558;   A_rot_ED3560;
##          A_rot_ED4719;    A_spec_ED3957;   A_myrio_ED3551; A_myrio_ED3552;
keep = [i for i in data.samples.keys() if i in [
    'A_arg_ED3981','A_ata_ED1882','A_ata_ED1899','A_ata_ED1900',
    'A_chiz_ED1379','A_chiz_ED1383','A_chiz_ED1384','A_chiz_ED1392',
    'A_chiz_ED1395','A_chiz_ED1399','A_chiz_ED1881','A_chiz_ED1888',
    'A_chiz_ED2979','A_cli_ED1364','A_cli_ED1371','A_cli_ED1375',
    'A_cli_ED1389','A_cli_ED1390','A_cli_ED1396','A_cli_ED1397',
    'A_cli_ED1918','A_cli_ED1919','A_cli_ED2970','A_cli_ED2971',
    'A_cli_ED3209','A_cli_ED3210','A_cli_ED3214','A_coq_ED2962',
    'A_coq_ED2963','A_gla_ED1883','A_gla_ED1886','A_gla_ED1889',
    'A_gla_ED1895','A_gla_ED1897','A_gla_ED1903','A_gla_ED2973',
    'A_gla_ED2976','A_gla_ED2977','A_hys_ED1385','A_hys_ED1386',
    'A_hys_ED2964','A_hys_ED2965','A_hys_ED3211','A_imb_ED1380',
    'A_imb_ED1391','A_imb_ED1896','A_imb_ED1904','A_imb_ED1905',
    'A_imb_ED1906','A_imb_ED1910','A_imb_ED1913','A_imb_ED3212',
    'A_leu_ED1365','A_leu_ED1366','A_leu_ED2966','A_leu_ED2967',
    'A_lit_ED3982','A_mad_ED1376','A_mad_ED1377','A_mad_ED1382',
    'A_mad_ED1393','A_mad_ED1394','A_mad_ED1879','A_mad_ED1892',
    'A_mad_ED1901','A_mad_ED1902','A_mad_ED2975','A_mad_ED2980',
    'A_mad_ED3213','A_myrio_ED3954','A_myrio_ED4720','A_per_ED2974',
    'A_per_ED2978','A_per_ED3554','A_per_ED3555','A_phil_ED2960',
    'A_rep_ED1595','A_rep_ED1597','A_rep_ED1599','A_rep_ED1600',
    'A_rep_ED2961','A_ret_ED1387','A_ret_ED1388','A_ret_ED1908',
    'A_ret_ED1909','A_ret_ED1911','A_ret_ED1912','A_ret_ED1915',
    'A_ret_ED1917','A_ret_ED1925','A_ret_ED1926','A_ret_ED2972',
    'A_ret_ED4659','A_spec_ED4716','A_spec_ED4717','A_spec_ED4718',
    'A_spec_ED4721','A_spec_ED4728','A_spec_ED4729','A_spec_ED4730',
    'A_spec_ED4731','A_spec_ED4732','A_spec_ED4733','A_spec_ED4734',
    'A_spec_ED4735','A_spec_ED4744','A_tal_ED1372','A_tal_ED1373',
    'A_tal_ED1374','A_tal_ED1400','A_tal_ED1880','A_tal_ED2968',
    'A_tal_ED2969','A_und_ED3979','A_und_ED3980','A_und_ED3983',
    'A_val_ED1923','A_val_ED1924','OUT_A-port_ED3985']]
```

[56]:

```
## Assembly with population assignment with all Atriplex samples and 70 %␣
 ↪missing data
Atri_pops30 = data.branch("Atri_pops30", subsamples = keep)
Atri_pops30.populations = {
    "ingroup": (38, [i for i in Atri_pops30.samples if "A_" in i]),
    "outgourp": (0, [i for i in Atri_pops30.samples if "OUT_" in i]),
    }
#Atri_pops30.set_params("min_samples_locus", 38)
Atri_pops30.run("7", force = True)
```

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:00:05 | applying filters     | s7 |
[####################] 100% 0:00:10 | building arrays      | s7 |
[####################] 100% 0:00:03 | writing conversions  | s7 |
[####################] 100% 0:00:15 | indexing vcf depths  | s7 |
[####################] 100% 0:00:38 | writing vcf output   | s7 |
```

Assembly for subsets

Based on the overall phylogeny we seperated the samples in four assemblies.

[6]:

```
## KEEP LIST Ata-Peru & A. leuca clade Zusammen
AtaPeruLeuca_keep = [i for i in data.samples.keys() if i in [
    'A_cli_ED1364','A_cli_ED1371','A_cli_ED1375','A_cli_ED1389',
    'A_cli_ED1390','A_cli_ED1396','A_cli_ED1397','A_cli_ED1918',
    'A_cli_ED1919','A_cli_ED2970','A_cli_ED2971','A_cli_ED3209',
    'A_cli_ED3210','A_cli_ED3214','A_coq_ED2962','A_coq_ED2963',
    'A_gla_ED1883','A_gla_ED1886','A_gla_ED1889','A_gla_ED1895',
    'A_gla_ED1897','A_gla_ED1903','A_gla_ED2973','A_gla_ED2976',
    'A_gla_ED2977','A_hys_ED1385','A_hys_ED1386','A_hys_ED2964',
    'A_hys_ED2965','A_hys_ED3211','A_imb_ED1380','A_imb_ED1391',
    'A_imb_ED1896','A_imb_ED1904','A_imb_ED1905','A_imb_ED1906',
    'A_imb_ED1910','A_imb_ED1913','A_imb_ED3212','A_leu_ED2967',
    'A_myrio_ED3954','A_myrio_ED4720','A_per_ED2974','A_per_ED2978',
    'A_per_ED3554','A_per_ED3555','A_phil_ED2960','A_rep_ED1595',
    'A_rep_ED1597','A_rep_ED1599','A_rep_ED1600','A_rep_ED2961',
    'A_spec_ED4716','A_spec_ED4717','A_spec_ED4718','A_spec_ED4721',
    'A_spec_ED4728','A_spec_ED4729','A_spec_ED4730','A_spec_ED4731',
    'A_spec_ED4732','A_spec_ED4733','A_spec_ED4734','A_spec_ED4735',
    'A_spec_ED4744','A_tal_ED1372','A_tal_ED1373','A_tal_ED1374',
    'A_tal_ED1400','A_tal_ED1880','A_tal_ED2968','A_tal_ED2969',
    'A_val_ED1923','A_val_ED1924','OUT_A-port_ED3985']]
```

[7]:

```
## KEEP LIST Ata-Arg clade
AtaArg_keep = [i for i in data.samples.keys() if i in [
    'A_arg_ED3981','A_ata_ED1882','A_ata_ED1899','A_ata_ED1900',
    'A_chiz_ED1379','A_chiz_ED1383','A_chiz_ED1384','A_chiz_ED1392',
```

```
    'A_chiz_ED1395','A_chiz_ED1399','A_chiz_ED1881','A_chiz_ED1888',
    'A_chiz_ED2979','A_leu_ED1365','A_leu_ED1366','A_leu_ED2966',
    'A_lit_ED3982','A_mad_ED1376','A_mad_ED1377','A_mad_ED1382',
    'A_mad_ED1393','A_mad_ED1394','A_mad_ED1879','A_mad_ED1892',
    'A_mad_ED1901','A_mad_ED1902','A_mad_ED2975','A_mad_ED2980',
    'A_mad_ED3213','A_ret_ED1387','A_ret_ED1388','A_ret_ED1908',
    'A_ret_ED1909','A_ret_ED1911','A_ret_ED1912','A_ret_ED1915',
    'A_ret_ED1917','A_ret_ED1925','A_ret_ED1926','A_ret_ED2972',
    'A_ret_ED4659','A_und_ED3979','A_und_ED3980','A_und_ED3983',
    'OUT_A-port_ED3985']]
```

```
[8]: ## Assembly Atacama-Peru && A. leuca clade
Atri_AtaPeru_leuc = data.branch("Atri_AtaPeru_leuc", subsamples =␣
 ↪AtaPeruLeuca_keep)
Atri_AtaPeru_leuc.populations = {
    "ingroup": (22, [i for i in Atri_AtaPeru_leuc.samples if "A_" in i]),
    "outgroup":(0, [i for i in Atri_AtaPeru_leuc.samples if "OUT_" in i])
}
Atri_AtaPeru_leuc.run("7", force = True)

## Assembly Atacama-Argentina clade
Atri_AtaArg = data.branch("Atri_AtaArg", subsamples = AtaArg_keep)
Atri_AtaArg.populations = {
    "ingroup": (13, [i for i in Atri_AtaArg.samples if "A_" in i]),
    "outgroup":(0, [i for i in Atri_AtaArg.samples if "OUT_" in i])
}
Atri_AtaArg.run("7", force = True)
```

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:00:05 | applying filters     | s7 |
[####################] 100% 0:00:10 | building arrays      | s7 |
[####################] 100% 0:00:03 | writing conversions  | s7 |
[####################] 100% 0:00:13 | indexing vcf depths  | s7 |
[####################] 100% 0:00:30 | writing vcf output   | s7 |
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:00:04 | applying filters     | s7 |
[####################] 100% 0:00:06 | building arrays      | s7 |
[####################] 100% 0:00:03 | writing conversions  | s7 |
[####################] 100% 0:00:07 | indexing vcf depths  | s7 |
[####################] 100% 0:00:14 | writing vcf output   | s7 |
```

Phylogenetic analyses using RAxML

Load assembly files

[9]:
```
## reload assemblies from their JSON files
Atri_pops30 = ip.load_json("Atri_Assembly/Atri_pops30.json")
Atri_AtaPeru_leuc = ip.load_json("Atri_Assembly/Atri_AtaPeru_leuc.json")
Atri_AtaArg = ip.load_json("Atri_Assembly/Atri_AtaArg.json")
```

```
loading Assembly: Atri_pops30
from saved path:
~/BioInf/Atriplex_GBS/GBS_Atri_Anal/Atri_Assembly/Atri_pops30.json
loading Assembly: Atri_AtaPeru_leuc
from saved path:
~/BioInf/Atriplex_GBS/GBS_Atri_Anal/Atri_Assembly/Atri_AtaPeru_leuc.json
loading Assembly: Atri_AtaArg
from saved path:
~/BioInf/Atriplex_GBS/GBS_Atri_Anal/Atri_Assembly/Atri_AtaArg.json
```

[68]:
```
## loop to run RAxML on all Atriplex assemblies
## Atri_pops30 = A full assembl with all samples and A. portulacoides as␣
 ↪outgroup
for dset in [Atri_pops30, Atri_AtaPeru_leuc, Atri_AtaArg]:
    rax = ipa.raxml(
        workdir = "./Atri_RAxML",
        name = dset.name,
        data = dset.outfiles.phy,
        N = 200,
        T = 8,
        o = ["OUT_A-port_ED3985"],
    )
    rax.run(force = True)
```

```
job Atri_pops30 finished successfully
job Atri_leuca finished successfully
job Atri_AtaPeru finished successfully
job Atri_AtaPeru_leuc finished successfully
job Atri_AtaArg finished successfully
```

Plotting results

1. Plotting RAxML Tree of all samples

[81]:
```
## load tree
AtriAll = toytree.tree("./Atri_RAxML/RAxML_bipartitions.Atri_pops30")

## root tree with Halimione portulacoides
rAtriAll = AtriAll.root(wildcard = "OUT_A-port_ED3985")
```

```
## define aesthetics for tree plotting
canvas, axes = rAtriAll.ladderize(1).draw(
    height = 1300, width = 800,
    tip_labels_align = True,
    node_labels = rAtriAll.get_node_values("support"),
    node_sizes = 0,
    node_labels_style = {"font-size": "9px",
                         "baseline-shift": "7px",
                         "-toyplot-anchor-shift": "-11px"},
);

## export trees
import toyplot.pdf
import toyplot.svg
toyplot.pdf.render(canvas, "Atri_RAxML/Atri_ML_Fig/Atri_All_pops30_GBS_RAxML.
 ↪pdf")
toyplot.pdf.render(canvas, "Atri_RAxML/Atri_ML_Fig/Atri_All_pops30_GBS_RAxML.
 ↪svg")
```

2. Plotting RAxML Tree of Atacama Peru & A. leuch clade

[107]:

```
## load tree
AtriAtaPeruleuca = toytree.tree("./Atri_RAxML/RAxML_bipartitions.
 ↪Atri_AtaPeru_leuc")

## root tree with Halimione portulacoides
rAtriAtaPeruleuca = AtriAtaPeruleuca.root(wildcard = "OUT_A-port_ED3985")

## define aesthetics for tree plotting
canvas, axes = rAtriAtaPeruleuca.ladderize(1).draw(
    height = 1100, width = 800,
    tip_labels_align = True,
    node_labels = rAtriAtaPeruleuca.get_node_values("support"),
    node_sizes = 0,
    node_labels_style = {"font-size": "9px",
                         "baseline-shift": "7px",
                         "-toyplot-anchor-shift": "-11px"},
);

## export trees
import toyplot.pdf
import toyplot.svg
toyplot.pdf.render(canvas, "Atri_RAxML/Atri_ML_Fig/
 ↪Atri_AtaPeruleuca_pops30_GBS_RAxML.pdf")
toyplot.pdf.render(canvas, "Atri_RAxML/Atri_ML_Fig/
 ↪Atri_AtaPeruleuca_pops30_GBS_RAxML.svg")
```

3. Plotting RAxML Tree of Atacama Argentina clade

[99]:

```
## load tree
AtriAtaArg = toytree.tree("./Atri_RAxML/RAxML_bipartitions.Atri_AtaArg")

## root tree with Halimione portulacoides
rAtriAtaArg = AtriAtaArg.root(wildcard = "OUT_A-port_ED3985")

## define aesthetics for tree plotting
canvas, axes = rAtriAtaArg.ladderize(1).draw(
    height = 1100, width = 900,
    tip_labels_align = True,
    node_labels = rAtriAtaArg.get_node_values("support"),
    node_sizes = 0,
    node_labels_style = {"font-size": "9px",
                         "baseline-shift": "7px",
                         "-toyplot-anchor-shift": "-11px"},
);

## export trees
import toyplot.pdf
import toyplot.svg
toyplot.pdf.render(canvas, "Atri_RAxML/Atri_ML_Fig/
 ↪Atri_AtriAtaArg_pops30_GBS_RAxML.pdf")
toyplot.pdf.render(canvas, "Atri_RAxML/Atri_ML_Fig/
 ↪Atri_AtriAtaArg_pops30_GBS_RAxML.svg")
```

Appendix C

— supplementary material of chapter 6 —

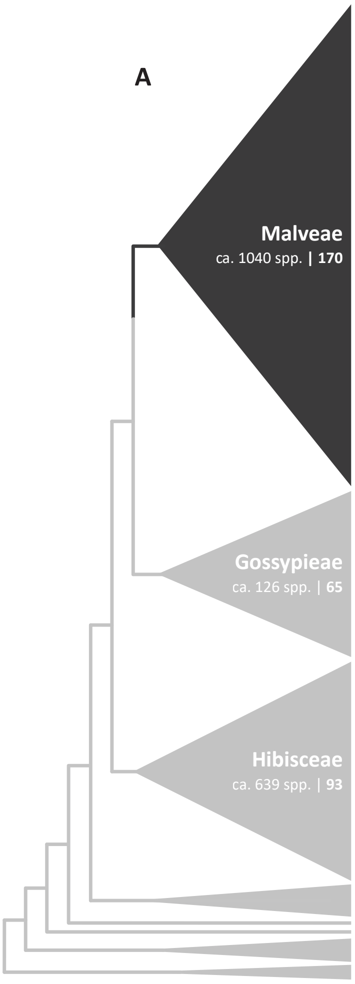
Figure S1: RAxML phylogeny of Malveae with bootstrap and posterior probabilities.

Figure S2: RAxML phylogeny of Malvoideae excluding the tribe Malveae with bootstrap and posterior probabilities.

Figure S3: BEAST2 MCCT of Malvoideae.

Table S1: List of species, voucher information and GenBank accession numbers.

Figure S1



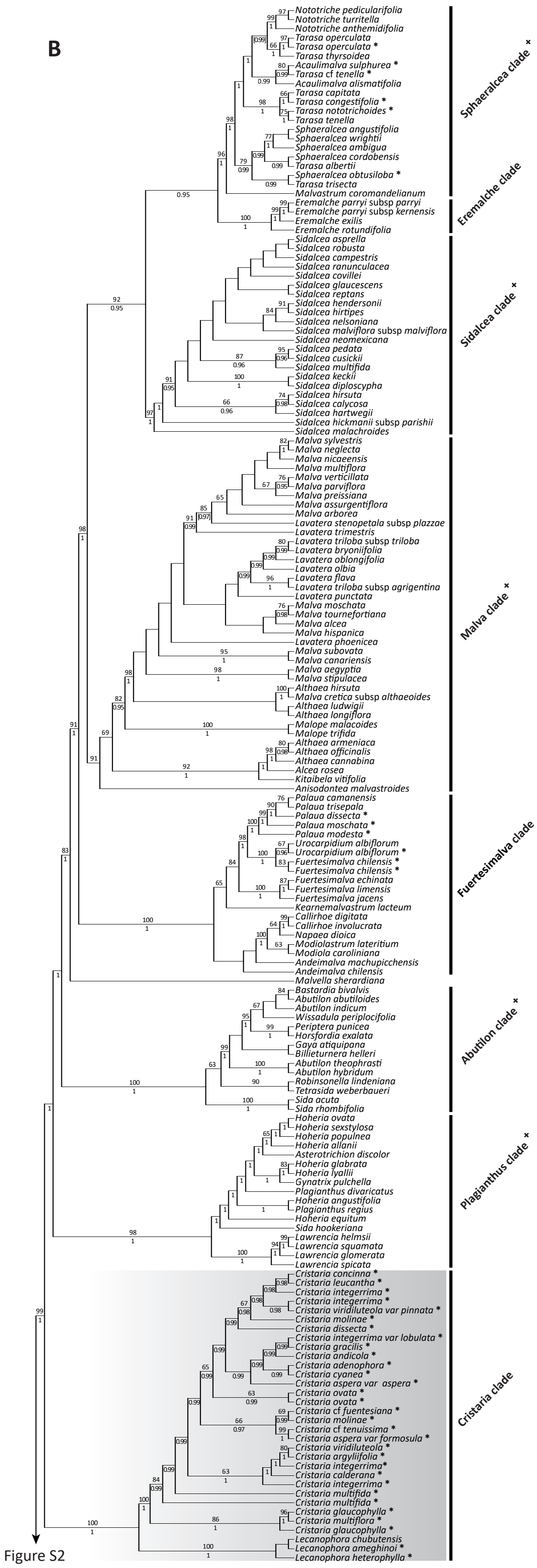


Figure S1: A) Backbone Phylogeny of the subfamily Malvoideae with three tribes. Numbers below tribe names shows the approximate number of species per tribe and the number of species included in this study. **B)** RAxML tree of the tribe Malveae with bootstrap (above branch) and posterior from Bayesian analysis (below branches). Individual clades are highlighted and named according to the largest genus in the clade or in accordance with Bayer and Kubitzki (2003) which are marked by a plus sign (**+**). The *Cristaria* clade is highlighted with grey background. For species marked by an asterisk own sequences were generated.

Figure S2

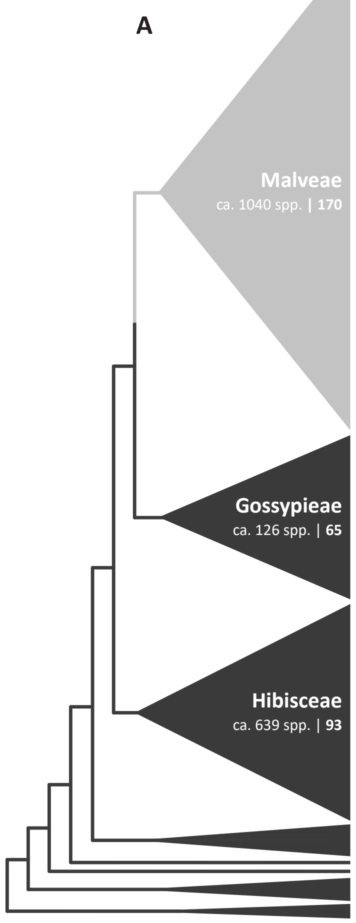


Figure S2: **A)** Backbone Phylogeny of the subfamily Malvoideae with three tribes. Numbers below tribe names shows the approximate number of species per tribe and the number of species included in this study. **B)** RAxML tree of the subfamily Malvoideae excluding the tribe Malveae (see figure S1) with bootstrap (above branch) and posterior from Bayesian analysis (below branches). Individual clades highlighted are the tribes Gossypieae and Hibisceae.

Figure S3

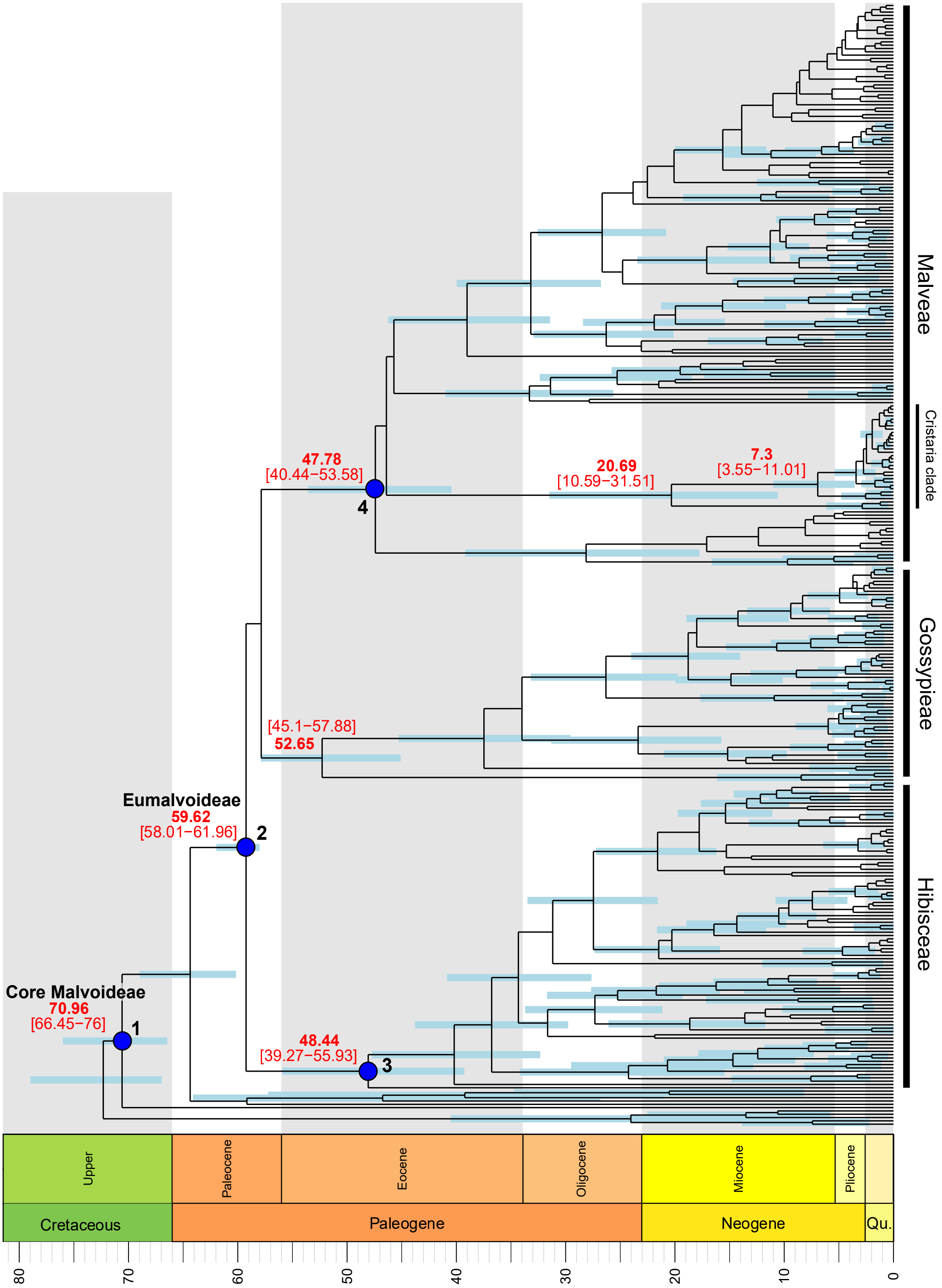


Figure S3: BEAST2 tree of the subfamily Malvoideae showing ages of main clades and 95% HPD confidence intervals in square brackets. Blue circles indicate fossil calibration points (1-4; see table 1). Tribes of Malvoideae are indicated together with the target clade (Cristaria clade; see figure S1). Only for nodes with posterior support of 0.95 or higher 95 % HPD confidence intervals are shown in light blue. Qu = Quaternary (Pleistocene & Holocene).

Table S1: List of species, voucher information of own collections incl. herbaria where vouchers are stored and GenBank accession.

Species	Voucher	*trn*K/*mat*K	*ndh*F	*rpl*16
Abelmoschus caillei (A.Chev.) J.M.C.Stevels				KP222323
Abelmoschus crinitus Wall.				KP222356
Abelmoschus esculentus Moench		JN114733		KP222337
Abelmoschus ficulneus (L.) Wight & Arn.			AF384638	AF384560
Abelmoschus manihot (L.) Medik.		EF562457	AF384639	AF384561
Abutilon abutiloides (Jacq.) Garcke		KT966998	KT967036	KT967074
Abutilon hybridum Voss		AY589058	AF111716	
Abutilon indicum (L.) Sweet		KP093591		
Abutilon theophrasti Medik.		HQ696683		
Acaulimalva alismatifolia (K.Schum. & Hieron.) Krapov.		FJ204708	FJ204727	FJ204768
Acaulimalva sulphurea Krapov.	A. Sagastegui & M. Zapate 16575 (BONN)		MK728870	MN120555
Alcea rosea L.		EU346805	EU346847	
Althaea armeniaca Ten.		EU346763	EU346807	
Althaea cannabina L.		EU346764	EU346810	
Althaea hirsuta L.		EU346794	EU346808	
Althaea longiflora Boiss. & Reut.		EU346795	EU346809	
Althaea ludwigii L.		EU346796	EU346812	
Althaea officinalis L.		EU346765	EU346811	
Alyogyne hakeifolia (Giord.) Alef.		AY589059	AY589083	AF384564
Alyogyne huegelii (Endl.) Fryxell			AF384657	AF384565
Alyogyne pinoniana (Gaudich.) Fryxell			AF384659	AF384566
Andeimalva chilensis (Gay) J.A.Tate		AY213108	FJ204730	FJ204772
Andeimalva machupicchensis (Krapov.) J.A.Tate		AY213109	FJ204735	FJ204778
Anisodontea malvastroides (Baker f.) D.M.Bates		EU346803	EU346848	
Anotea flavida Ulbr.			U55322	
Asterotrichion discolor (Hook.f.) Melville		GU045813		
Azanza lampas (Cav.) Alef.		KT966966	KT967005	KT967042
Azanza thespesioides (Benth.) F.Areces		KT966965	KT967004	KT967041
Bastardia bivalvis (Cav.) Kunth		KT966996	KT967034	KT967072
Billieturnera helleri (Rose ex A.Heller) Fryxell		FJ204699	FJ204742	FJ204758
Callirhoe digitata Nutt.				JQ217458
Callirhoe involucrata (Torr. & A.Gray) A.Gray		JF799575	JF799578	JF799583
Camptostemon schultzii Mast.		AY321162	AF111727	
Cephalohibiscus peekelii Ulbr.		KT966964	KT967003	KT967040
Chiranthodendron pentadactylon Larreat		AY321164	AF111722	
Cienfuegosia heterophylla (Vent.) Garcke		KT966962	KT967001	KT967038
Cienfuegosia tripartita Gürke			U55324	
Cristaria adenophora I.M. Johnst.	F. Luebert, T. Böhnert & F. Merklinger 3900 (BONN, FR, ULS)	MN129829	MK728853	MN120538
Cristaria andicola Gay	F. Luebert & T. Böhnert 3712 (BONN, FR, ULS)	MN129842	MK728866	MN120551
Cristaria argyliifolia Phil.	F. Luebert, T. Böhnert & F. Merklinger 3886 (BONN, FR, ULS)	MN129827	MK728851	MN120536
Cristaria aspera var. *aspera* Gay	F. Luebert, T. Böhnert & F. Merklinger 3849 (BONN, FR, ULS)	MN129824	MK728848	MN120533
Cristaria aspera var. *formosula* (I.M. Johnst.) Muñoz-Schick	F. Luebert, T. Böhnert & F. Merklinger 3891 (BONN, FR, ULS)	MN129828	MK728852	MN120537
Cristaria calderana Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3288 (BONN, FR, ULS)	MN129810	MK728831	MN120516
Cristaria cf. *fuentesiana* I.M. Johnst.	F. Luebert, T. Böhnert & F. Merklinger 3871 (BONN, FR, ULS)	MN129825	MK728849	MN120534
Cristaria cf. *tenuissima* Muñoz-Schick	F. Luebert, T. Böhnert & F. Merklinger 3958 (BONN, FR, ULS)	MN129833	MK728857	MN120542
Cristaria concinna Phil.	F. Luebert, T. Böhnert & F. Merklinger 3951 (BONN, FR, ULS)	MN129832	MK728856	MN120541
Cristaria cyanea Phil. ex Baker f.	F. Luebert, T. Böhnert & F. Merklinger 3880 (BONN, FR, ULS)	MN129826	MK728850	MN120535
Cristaria dissecta Hook. & Arn.	F. Luebert, A. Stoll & T. Böhnert 3409 (BONN, FR, ULS)	MN129818	MK728840	MN120525
Cristaria glaucophylla Cav.	F. Luebert, A. Stoll & T. Böhnert 3268 (BONN, FR, ULS)	MN129809	MK728830	MN120515
Cristaria glaucophylla Cav.	F. Luebert & T. Böhnert 3724 (BONN, FR, ULS)	MN129821	MK728843	MN120528
Cristaria gracilis Gay	F. Luebert & T. Böhnert 3659 (BONN, FR, ULS)	MN129841	MK728865	MN120550
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3331 (BONN, FR, ULS)	MN129812	MK728833	MN120518
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3364 (BONN, FR, ULS)	MN129813	MK728834	MN120519
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3367 (BONN, FR, ULS)	MN129814	MK728835	MN120520

Species	**Voucher**	***trn*K/*mat*K**	***ndh*F**	***rpl*16**
Cristaria integerrima Phil.	F. Luebert, T. Böhnert & F. Merklinger 3963 (BONN, FR, ULS)	MN129836	MK728860	MN120545
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	F. Luebert, T. Böhnert & F. Merklinger 3917 (BONN, FR, ULS)	MN129830	MK728854	MN120539
Cristaria leucantha I.M. Johnst.	F. Luebert, T. Böhnert & F. Merklinger 3947 (BONN, FR, ULS)	MN129831	MK728855	MN120540
Cristaria molinae Gay	F. Luebert, A. Stoll & T. Böhnert 3374 (BONN, FR, ULS)	MN129815	MK728836	MN120521
Cristaria molinae Gay	F. Luebert, A. Stoll & T. Böhnert 3407 (BONN, ULS)	MN129817	MK728839	MN120524
Cristaria multifida Cav.	J. Schneider (FR)		MK728871	
Cristaria multifida Cav.	J. Schneider (FR)		MK728872	
Cristaria multiflora Gay	F. Luebert & T. Böhnert 3719 (BONN, FR, ULS)	MN129843	MK728867	MN120552
Cristaria ovata Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3300 (BONN, FR, ULS)	MN129819	MK728841	MN120526
Cristaria ovata Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3301 (BONN, FR, ULS)	MN129822	MK728844	MN120529
Cristaria viridiluteola Gay	F. Luebert, A. Stoll & T. Böhnert 3289 (BONN, FR, ULS)	MN129811	MK728832	MN120517
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3395 (BONN, FR, ULS)	MN129816	MK728838	MN120523
Decaschistia byrnesii Fryxell		AY589066	AY589079	
Eremalche exilis (A.Gray) Greene		JF799576	JF799579	JF799584
Eremalche parryi (Greene) Greene subsp. kernensis (C.B.Wolf) D.M.Bates				JQ217462
Eremalche parryi (Greene) Greene subsp. parryi				JQ217463
Eremalche rotundifolia (A.Gray) Greene				JQ217464
Fioria vitifolia (L.) Mattei			AF384640	AF384570
Fremontodendron californicum (Torr.) Coville		AY321165	AF111721	-
Fuertesimalva chilensis (A. Braun & C.D. Bouché) Fryxell	F. Luebert, T. Böhnert & F. Merklinger 3961 (BONN, FR, ULS)	MN129835	MK728859	MN120544
Fuertesimalva chilensis (A. Braun & C.D. Bouché) Fryxell	F. Luebert, T. Böhnert, F.F. Merklinger, A. Stoll & D. Quandt 3491 (BONN, FR, ULS)	MN129845	MK728869	MN120554
Fuertesimalva echinata (C.Presl) Fryxell		FJ204697	FJ204719	FJ204755
Fuertesimalva jacens (S.Watson) Fryxell		AY213099	FJ204718	FJ204764
Fuertesimalva limensis (L.) Fryxell		AY213098	FJ204720	FJ204765
Gaya atiquipana Krapov.		FJ204706	FJ204748	FJ204766
Gossypioides kirkii (Mast.) Skovsted		AF403563	U55329	AF403104
Gossypium anapoides J.M.Stewart, Craven, Brubaker & Wendel				AF059436
Gossypium anomalum Wawra & Peyr.		AF403557	U55332	AF403100
Gossypium arboreum L.		HQ325740	U55331	AF031451
Gossypium areysianum Deflers		NC_018112	NC_018112	NC_018112
Gossypium australe F.Muell				AF059433
Gossypium barbadense L.		AP009123	U55339	AF031453
Gossypium bickii Prokh.		AF403562	AF403555	AF059428
Gossypium capitis-viridis Mauer		JN019794	JN019794	JN019794
Gossypium costulatum Tod.				AF059439
Gossypium cunninghamii Tod.			U55333	AF059444
Gossypium darwinii G.Watt		NC_016670	NC_016670	AF031456
Gossypium davidsonii Kellogg		AF520728	AF520733	AF520718
Gossypium enthyle Fryxell, Craven & J.M.Stewart				AF059447
Gossypium exiguum Fryxell, Craven & J.M.Stewart				AF059452
Gossypium gossypioides (Ulbr.) Standl.		AF520727	NC_017894	AF520717
Gossypium herbaceum L.		NC_023215	NC_023215	NC_023215
Gossypium hirsutum L.		HQ901196	U55340	AF031452
Gossypium incanum (O.Schwartz) Hillc.		NC_018109	NC_018109	NC_018109
Gossypium londonderriense Fryxell, Craven & J.M.Stewart				AF059455
Gossypium longicalyx J.B.Hutch. & B.J.S.Lee		AF403561	U55338	AF403103
Gossypium marchantii Fryxell, Craven & J.M.Stewart				AF059458
Gossypium mustelinum Miers ex G.Watt		HQ325743	HQ325743	AF031455
Gossypium nelsonii Fryxell		JN201490.1		AF059430.1
Gossypium nobile Fryxell, Craven & J.M.Stewart				AF059462
Gossypium pilosum Fryxell				AF059467
Gossypium populifolium (Benth.) F.Muell.				AF059473
Gossypium pulchellum (C.A.Gardner) Fryxell				AF059477
Gossypium raimondii Ulbr.		AF403559	U55335	AF403101
Gossypium robinsonii F.Muell.		AF403558	U55334	AF059425
Gossypium rotundifolium Fryxell, Craven & J.M.Stewart				AF059482
Gossypium schwendimanii Fryxell & S.D.Koch		AF520729	AF520734	AF520719

Species	Voucher	*trn*K/*mat*K	*ndh*F	*rpl*16
Gossypium somalense (Gürke) J.B.Hutch., Silow & S.G.Stephens		AF403560	JN019793	AF403102
Gossypium stocksii Mast.		JF317355	U55337	NC_023217
Gossypium sturtianum J.H.Willis		NC_023218	NC_023218	AF059423
Gossypium thurberi Tod.		NC_015204	NC_015204	NC_015204
Gossypium tomentosum Nutt. ex Seem.		NC_016690	NC_016690	AF031454
Gossypium trilobum (Sessé & Moc. ex DC.) Skovsted		AF520730	AF520735	AF520720
Gossypium turneri Fryxell		AF520731	U55336	AF520721
Gynatrix pulchella (Willd.) Alef.		GU045814		
Hampea appendiculata (Donn.Sm) Standl.		KT966970	U55327	KT967046
Hampea mexicana Fryxell		KT966968	KT967007	KT967044
Hampea nutricia Fryxell		KT966967	KT967006	KT967043
Hampea trilobata Standl.		KT966969	KT967008	KT967045
Helicteropsis microsiphon (Baill.) Hochr.		EF207264	EF207296	
Hibiscus arnottianus A.Gray		KX984262	KX984267	KX984271
Hibiscus bojerianus Baill.		EF207275	EF207306	
Hibiscus brachysiphonius F.Muell.			AF384644	AF384575
Hibiscus caerulescens Baill.		EF207265	EF207297	
Hibiscus calyphyllus Cav.		JX517307	AF384655	AF384577
Hibiscus cannabinus L.		EF207259	EF207290	AY727397
Hibiscus clayii O.Deg. & I.Deg.		KX984263	KX984268	KX984272
Hibiscus clypeatus L.		KX984264	KX984269	KX984273
Hibiscus coatesii F.Muell.			AF384645	AF384578
Hibiscus coccineus Walter			AY341395	AY341407
Hibiscus costatus A.Rich.		AY589057	U55323	
Hibiscus cravenii (Fryxell) B.E.Pfeil & Craven			AF384648	AF384563
Hibiscus dasycalyx S.F.Blake & Shiller			AY341397	AY341406
Hibiscus dongolensis Caill. ex Delile		EF207271	EF207303	AF384580
Hibiscus drummondii Turcz.			AF384647	AF384581
Hibiscus engleri K.Schum.			AF384641	AF384582
Hibiscus ferrugineus Cav.		EF207268	EF207300	
Hibiscus fryxellii Mabb.			AF384632	AF384584
Hibiscus furcellatus Lam.			AF384629	AF384585
Hibiscus grandidieri Baill.		EF207263	EF207295	
Hibiscus grandiflorus Michx.		KJ772835	AY341398	AY341400
Hibiscus heterophyllus Vent.		KM894805	AF384631	AF384586
Hibiscus humbertianus Hochr.		EF207266	EF207298	
Hibiscus laevis All.			AY341396	AY341405
Hibiscus ludwigii Eckl. & Zeyh.			AF384656	AF384588
Hibiscus macrogonus Baill.		EF207273	EF562456	
Hibiscus mandrarensis Humbert ex Hochr.		EF207274	EF207305	
Hibiscus mastersianus Hiern				AF384590
Hibiscus mechowii O.Hoffm.		KR137547		AY727395
Hibiscus meeusei Exell			AF384627	AF384591
Hibiscus meraukensis Hochr.			AF384633	AF384592
Hibiscus moscheutos L. subsp. *moscheutos*		KJ772836	AY341399	AY341402
Hibiscus moscheutos subsp. *incanus* (J.C.Wendl.) H.E.Ahles				AY341401
Hibiscus moscheutos subsp. *lasiocarpos* (Cav.) O.J.Blanch.				AY341403
Hibiscus moscheutos subsp. *palustris* (L.) R.T.Clausen				AY341404
Hibiscus nigricaulis Baker f.			AF384628	AF384594
Hibiscus pedunculatus L.f.			AF384649	AF384596
Hibiscus pentaphyllus F.Muell.			AF384651	AF384597
Hibiscus peralbus Fryxell			AF384652	AF384598
Hibiscus physaloides Guill. & Perr.			AF384643	AF384599
Hibiscus radiatus Cav.		JQ693600		AF384600
Hibiscus rosa-sinensis L.		AY321160	AY589075	
Hibiscus rostellatus Guill. & Perr.				AF384601
Hibiscus sabdariffa L.		KX984265	KX984266	
Hibiscus schinzii Gürke ex Schinz			AF384642	AF384604
Hibiscus splendens Fras. ex Graham		KM894510		AF384606
Hibiscus striatus Cav.		KT966990	KT967028	KT967066
Hibiscus sturtii Hook.				AF384608
Hibiscus surattensis L.		EF207258	EF207289	AF384609
Hibiscus syriacus L.		EF207270	EF207302	AF384610
Hibiscus tozerensis Craven & B.E.Pfeil		EF207269	EF207301	
Hibiscus trionum L.		HM850991	AY341394	AF384612
Hibiscus waimeae A.Heller		EF207262	EF207294	AF384613
Hibiscus zonatus F. Muell.			AF384630	AF384614
Hoheria allanii (Cockayne) Heenan		GU045797		
Hoheria angustifolia Raoul		FJ204700	FJ204722	FJ204757

Species	**Voucher**	***trn*K/*mat*K**	***ndh*F**	***rpl*16**
Hoheria equitum Heads		GU045800		
Hoheria glabrata Sprague & Summerh.		AY944613		
Hoheria lyallii Hook.f.		AY944617		
Hoheria ovata G.Simpson & J.S.Thomson		AY944626		
Hoheria populnea A.Cunn. Cultivated		AY944627		
Hoheria sexstylosa Colenso		GU045806		
Horsfordia exalata Fryxell		FJ204707	FJ204747	FJ204767
Howittia trilocularis F.Muell.		AY589065	AY589085	AF384615
Humbertiella decaryi (Hochr.) Dorr.		EF207279	EF207310	
Humbertiella henrici Hochr.		EF207281	EF207312	FJ514219
Humbertiella quararibeoides Hochr.		EF207280	EF207311	
Jumelleanthus perrieri Hochr.		EF207272	EF207304	
Kearnemalvastrum lacteum (Aiton) D.M.Bates		JF799577	JF799580	JF799585
Kitaibela vitifolia Willd.		EU346804	EU346849	
Kokia drynarioides Lewton		AF403564	U55330	AF403105
Kokia kauaiensis (Rock) O.Deg & Duvel		KT966961	KT967000	KT967037
Kosteletzkya depressa (L.) O.J.Blanch., Fryxell & D.M.Bates		KM463406		
Kosteletzkya diplocrater (Hochr.) Hochr.		EF207276	EF207307	
Kosteletzkya pentacarpa (L.) Ledeb.		EF207257	EF207288	
Kosteletzkya reflexiflora Hochr.		EF207283	EF207314	
Kosteletzkya velutina Garcke		EF207282	EF207313	
Kydia calycina Roxb.		EF207261	EF207293	
Lagunaria patersonia (Andrews) G.Don		AY589064	AY589084	AF384616
Lavatera bryoniifolia Mill.		EU346768	EU346815	
Lavatera flava Desf.		EU346772	EU346818	
Lavatera oblongifolia Boiss.		EU346767	EU346825	
Lavatera olbia L.		EU346766	EU346826	
Lavatera phoenicea Willd.		EU346802	EU346828	
Lavatera punctata All.		EU346776	EU346830	
Lavatera stenopetala subsp. *plazzae* (Atzei) Iamonico		EU346773	EU346829	
Lavatera triloba subsp. *agrigentina* (Tineo) R.Fern.		EU346769	EU346814	
Lavatera triloba subsp. *triloba* L.		EU346771	EU346816	
Lavatera trimestris L.		EU346774	EU346832	
Lawrencia glomerata Hook.		GU045815		
Lawrencia helmsii (F.Muell. & Tate) Lander		GU045816		
Lawrencia spicata Hook.		GU045817		
Lawrencia squamata Miq.		GU045818		
Lebronnecia kokioides Fosberg		KT966963	KT967002	KT967039
Lecanophora ameghinoi (Speg.) Speg.	J. Schneider & Huertas 2967 (FR)		MK728846	MN120531
Lecanophora chubutensis (Speg.) Rodrigo		FJ204709	FJ204744	FJ204769
Lecanophora heterophylla (Cav.) Krapov.	J. Schneider & Huertas 2966 (FR)		MK728845	MN120530
Macrostelia grandifolia Fryxell			AF384646	AF384619
Macrostelia laurina (Baill.) Hochr. & Humbert		EF207267	EF207299	
Malachra radiata L.		KT966993	KT967031	KT967069
Malope malacoides L.		EU346800	EU346833	
Malope trifida Cav.		AY589060	AY589076	
Malva aegyptia L.		EU346798	EU346835	
Malva alcea L.		EU346790	EU346840	
Malva arborea (L.) Webb & Berthel.		EU346779	EU346821	
Malva assurgentiflora (Kellogg) M.F.Ray		EU346780	EU346819	
Malva canariensis M.F.Ray		EU346778	EU346820	
Malva cretica subsp. *althaeoides* (Cav.) Dalby		EU346797	EU346837	
Malva hispanica L.		EU346793	EU346838	
Malva moschata L.		EU346792	EU346841	
Malva multiflora (Cav.) Soldano, Banfi & Galasso		EU346783	EU346813	
Malva neglecta Wallr		EU346788	EU346842	AF384620
Malva nicaeensis All.		EU346785	EU346843	
Malva parviflora L.		KT966999	EU346844	FJ204762
Malva preissiana Miq.		EU346784	EU346827	
Malva stipulacea Cav.		EU346799	EU346836	
Malva subovata (DC.) Molero & J.M.Monts.		EU346781	EU346822	
Malva sylvestris L.		EU346787	EU346845	
Malva tournefortiana L.		EU346791	EU346839	
Malva verticillata L.		EU346789	EU346846	
Malvastrum coromandelianum (L.) Garcke		FJ204716	FJ204741	FJ204787
Malvaviscus arboreus Cav.		AY589061	AF111718	AF384621
Malvaviscus penduliflorus DC.		KT966994	KT967032	KT967070
Malvella sherardiana Jaub. & Spach		EU346806		
Matisia palenquiana (A.Robyns) W.S.Alverson		HQ696684		
Megistostegium microphyllum Hochr.		EF207278	EF207309	FJ514238
Megistostegium perrieri Hochr.		EF207277	EF207308	FJ514224

Species	**Voucher**	***trn*K/*mat*K**	***ndh*F**	***rpl*16**
Modiola caroliniana (L.) G.Don		EF207256	EF207287	JF799586
Modiolastrum lateritium (Hook.) Krapov.		AY213095	JF799582	JF799587
Napaea dioica L.				JQ217459
Nototriche anthemidifolia A.W.Hill		AY213107	FJ204728	FJ204770
Nototriche pedicularifolia A.W.Hill		AY213106	FJ204729	FJ204771
Nototriche turritella A.W.Hill		FJ204714	FJ204743	FJ204785
Palaua camanensis Ferreyra & Chanco		FJ204710	FJ204731	FJ204773
Palaua dissecta Benth.	F. Luebert, T. Böhnert & F. Merklinger 3960 (BONN, FR, ULS)	MN129834	MK728858	MN120543
Palaua modesta Reiche	F. Luebert, A. Stoll & T. Böhnert 3385 (BONN, ULS)		MK728837	MN120522
Palaua moschata Cav.	F. Luebert, A. Stoll & T. Böhnert 3381 (BONN, FR, ULS)	MN129820	MK728842	MN120527
Palaua trisepala Hochr.		FJ204695	FJ204721	FJ204753
Pavonia fruticosa (Mill.) Fawc. & Rendle		KT966991	KT967029	KT967067
Pavonia spinifex (L.) Cav.		KT966992	KT967030	KT967068
Pentaplaris doroteae L.O.Williams & Standl.		AY321163	AY326476	
Periptera punicea DC.		FJ204715	FJ204751	FJ204786
Perrierophytum humbertii Hochr.		EF207284	EF207315	
Perrierophytum rubrum Hochr.		EF207285	EF207316	
Phragmotheca ecuadorensis W.S.Alverson		AY589068	AF111725	
Plagianthus divaricatus J.R.Forst. & G.Forst.		GU045819		
Plagianthus regius (Poit.) Hochr.		HM348799	FJ204723	FJ204759
Quararibea costaricensis W.S.Alverson		AY321166	AF111723	
Radyera farragei (F.Muell) Fryxell & S.H.Hashmi		AY589063	AY589078	AF384623
Robinsonella lindeniana Rose & Baker f.		FJ204711	FJ204750	FJ204774
Sida acuta Burm.f.		KJ663774	AF384661	
Sida hookeriana Miq.				AF384624
Sida rhombifolia L.		KT966997	KT967035	KT967073
Sidalcea asprella Greene				JQ217489
Sidalcea calycosa M.E.Jones				JQ217473
Sidalcea campestris Greene				Q217477
Sidalcea covillei Greene				JQ217485
Sidalcea cusickii Piper				JQ217476
Sidalcea diploscypha (Torr. & A.Gray) A.Gray				JQ217467
Sidalcea glaucescens Greene				JQ217481
Sidalcea hartwegii A.Gray				JQ217471
Sidalcea hendersonii S.Watson				JQ217486
Sidalcea hickmanii Greene subsp. *parishii* (B.L.Rob.) C.L.Hitchc.				JQ217466
Sidalcea hirsuta A.Gray				JQ217474
Sidalcea hirtipes C.L.Hitchc.				JQ217482
Sidalcea keckii Wiggins				JQ217468
Sidalcea malachroides A.Gray				JQ217465
Sidalcea malviflora A.Gray subsp. *malviflora*				JQ217494
Sidalcea multifida Greene				JQ217495
Sidalcea nelsoniana Piper				JQ217483
Sidalcea neomexicana A.Gray				JQ217488
Sidalcea pedata A.Gray				JQ217490
Sidalcea ranunculacea Greene				JQ217491
Sidalcea reptans Greene				JQ217475
Sidalcea robusta A.Heller				JQ217484
Sphaeralcea ambigua A.Gray				JQ217460
Sphaeralcea angustifolia (Cav.) G.Don		EF207255	EF207286	
Sphaeralcea cordobensis Krapov.		AY213101	FJ204732	FJ204775
Sphaeralcea obtusiloba G. Don	F. Luebert, T. Böhnert & F. Merklinger 3798 (BONN, FR, ULS)	MN129823	MK728847	MN120532
Sphaeralcea wrightii A.Gray		AY213103	FJ204733	FJ204776
Talipariti elatum (Sw.) Fryxell		AB233276	AB233265	AB233254
Talipariti glabrum (Matsum. ex Nakai) Fryxell		AB181085	AB181053	AB181029
Talipariti hamabo (Siebold & Zucc.) Fryxell		AB181099	EF207292	AB181051
Talipariti macrophyllum (Roxb. ex Hornem.) Fryxell		AB181100	AF384636	AF384589
Talipariti tiliaceum var. *pernambucense* (Arruda) Fryxell		AB233275	AB233264	AB233253
Talipariti tiliaceum var. *tiliaceum* (L.) Fryxell		AB233266		
Tarasa albertii Phil.		AY232631	FJ204734	FJ204777
Tarasa capitata (Cav.) D.M.Bates		AY232632	FJ204740	FJ204788
Tarasa cf. *tenella* (Cav.) Krapov.	F. Luebert, T. Böhnert, F.F. Merklinger & A. Stoll 3604 (BONN, FR, ULS)	MN129838	MK728862	MN120547
Tarasa congestiflora (I.M. Johnst.) Krapov.	F. Luebert & T. Böhnert 3630 (BONN, FR, ULS)	MN129839	MK728863	MN120548
Tarasa nototrichoides (Hochr.) Krapov.	F. Luebert & T. Böhnert 3643 (BONN, FR, ULS)	MN129840	MK728864	MN120549
Tarasa operculata (Cav.) Krapov.	T. Böhnert & F.F. Merklinger 451 (BONN, FR, ULS)	MN129837	MK728861	MN120546

Species	Voucher	*trn*K/*mat*K	*ndh*F	*rpl*16
Tarasa operculata (Cav.) Krapov.		AY213129	FJ204725	FJ204779
Tarasa tenella (Cav.) Krapov.		AY213133	FJ204724	FJ204756
Tarasa thyrsoidea Krapov.		AY232637	FJ204737	FJ204780
Tarasa trisecta (Griseb.) Krapov.		AY213136	FJ204738	FJ204781
Tetrasida weberbaueri (Ulbr.) Fryxell & Fuertes		FJ204712	FJ204749	FJ204782
Thepparatia thailandica Phuph.		KT966989	KT967027	KT967065
Thespesia acutiloba (Baker f.) Exell & Mendonça		KT966978	KT967016	KT967054
Thespesia beatensis (Urb.) Fryxell		KT966972	KT967010	KT967048
Thespesia cubensis (Britton & P.Wilson) J.B.Hutch.		KT966975	KT967013	KT967051
Thespesia danis Oliv.		KT966977	KT967015	KT967053
Thespesia fissicalyx Borss.Waalk.		KT966988	KT967026	KT967064
Thespesia garckeana F.Hoffm.		KT966980	KT967018	KT967056
Thespesia grandiflora DC.		KT966973	KT967011	KT967049
Thespesia gummiflua Capuron		KT966981	KT967019	KT967057
Thespesia patellifera Borss.Waalk.		KT966987	KT967025	KT967063
Thespesia populnea (L.) Sol. ex Corrêa		KT966982	KT967020	KT967058
Thespesia populneoides (Roxb.) Kostel.		KT966986	KT967024	KT967062
Uladendron codesuri Marc.-Berti		AY589067	AY589080	
Urena lobata L.		KT966995	KT967033	KT967071
Urocarpidium albiflorum Ulbr.	F. Luebert, T. Böhnert, F.F. Merklinger, A. Stoll & D. Quandt 3459A (BONN, FR, ULS)	MN129844	MK728868	MN120553
Urocarpidium albiflorum Ulbr.		AY213097	FJ204739	FJ204783
Wercklea tulipiflora (Hook.) Fryxell				KX984275
Wissadula periplocifolia (L.) C.Presl ex Thwaites		FJ204713	FJ204717	FJ204784

Table S2: List of species, voucher information of own collections incl. Herbaria where vouchers are stored and GenBank accession.

Location	Primer	Sequence	Direct.	Reference
*ndh*F	1F	ATGGAGCATACATATCAATATTC	F	Olmstead & Sweere (1994)
*ndh*F	972F	GTCTCAATTGGGTTATATGATG	F	Olmstead & Sweere (1994)
*ndh*F	2110R	CCCCCTAYATATTGATACCTTCTC	R	Olmstead & Sweere (1994)
*ndh*F	1318R	CGAAACATATAAAATGC(AG)GTTAATCC	R	Olmstead & Sweere (1994)
*rpl*16	F71	GGCTATGCTTAGTGTGTGACTCGTT	F	Jordan *et al.* (1996)
*rpl*16	rpl16R	GTAATCCAAGCTGGTTCAAGTGC	R	Olsson *et al.* (2009)
*trn*K	matK2	GGGTTGCTAACTCAATGGTAGAG	F	Wicke & Quandt (2009)
*psb*A	matK13	CGCGTCTCTCTAAAATTGCAGTCAT	R	K. Steele in Johnson & Soltis (1995)
*mat*K	trnK-710F	GTATCGCACTATGT(AT)TCATTTGA	F	Johnson & Soltis (1995)
*mat*K	matK425	ACGATCAACATCTTCTGGAG	F	this study
*mat*K	matK426	GTATCAATGCTCTGGTCAATC	F	this study

Table S3: PCR programm *trn*K-*mat*K.

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

Table S4: PCR programm *ndh*F.

	Temperature [°C]	**Time** [s]	
1	95	300	
2	95	30	
3	46	60	34 x
4	72	60	
5	72	600	
6	8	break	

Table S5: PCR programm *rpl*16.

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

References

Johnson LA & Soltis DE (1995) Phylogenetic Inference in Saxifragaceae Sensu Stricto and *Gilia* (Polemoniaceae) Using *mat*K Sequences. *Annals of the Missouri Botanical Garden* 82 (2): 149. DOI: https://doi.org/10.2307/2399875.

Jordan WC, Courtney MW & Neigel JE (1996) Low Levels of Intraspecific Genetic Variation at a Rapidly Evolving Chloroplast DNA Locus in North American Duckweeds (Lemnaceae). *American Journal of Botany* 83 (4): 430. DOI: https://doi.org/10.2307/2446212.

Olmstead RG & Sweere JA (1994) Combining Data in Phylogenetic Systematics: An Empirical Approach Using Three Molecular Data Sets in the Solanaceae. *Systematic Biology* 43 (4): 467. DOI: https://doi.org/10.2307/2413546.

Olsson S, Buchbender V, Enroth J, Huttunen S, Hedenäs L & Quandt D (2009) Evolution of the Neckeraceae (Bryophyta): Resolving the backbone phylogeny. *Systematics and Biodiversity* 7 (4): 419–432. DOI: https://doi.org/10.1017/S1477200009990132.

Wicke S & Quandt D (2009) Universal primers for the amplification of the plastid *trn*K/*mat*K region in land plants. *Anales del Jardín Botánico de Madrid* 66 (2): 285–288. DOI: https://doi.org/10.3989/ajbm.2231.

Appendix D

— supplementary material of chapter 7 —

Figure S1: Result of the cross-validation criterion.

Figure S2: Dated GBS Maximum Likelihood phylogeny of the genus *Cristaria* using the Penalized Likelihood approach.

Table S1: List of species, voucher information and GBS assembly statistics.

Table S2: List of *Cristaria* vouchers used for distribution maps.

Table S3: BioGeoBEARS species distribution matrix for *Cristaria*.

Python skript 1: Jupyter-Notebook documenting the assembly of *Cristaria* GBS data, assembly statis and phylogenetic analyses.

Python skript 1: Jupyter-Notebook documenting the STRUCTURE analysis of the *C. integerrima*.

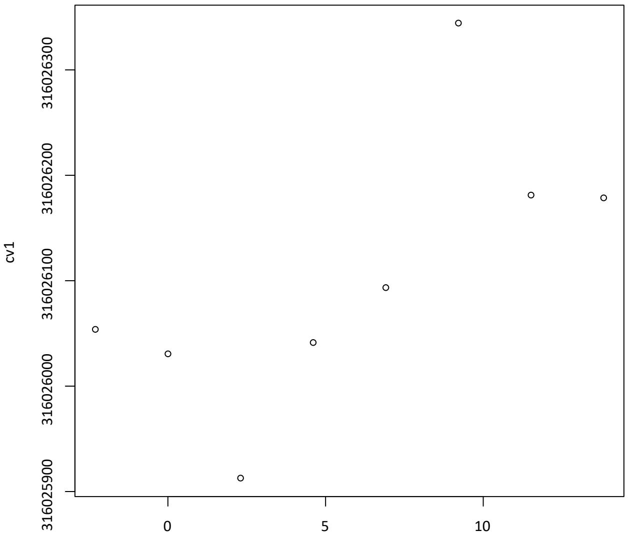


Figure S1: Result of the cross-validation criterion to test for the best fitting smoothing parameter used in the Penalized Likelihood dating analysis.

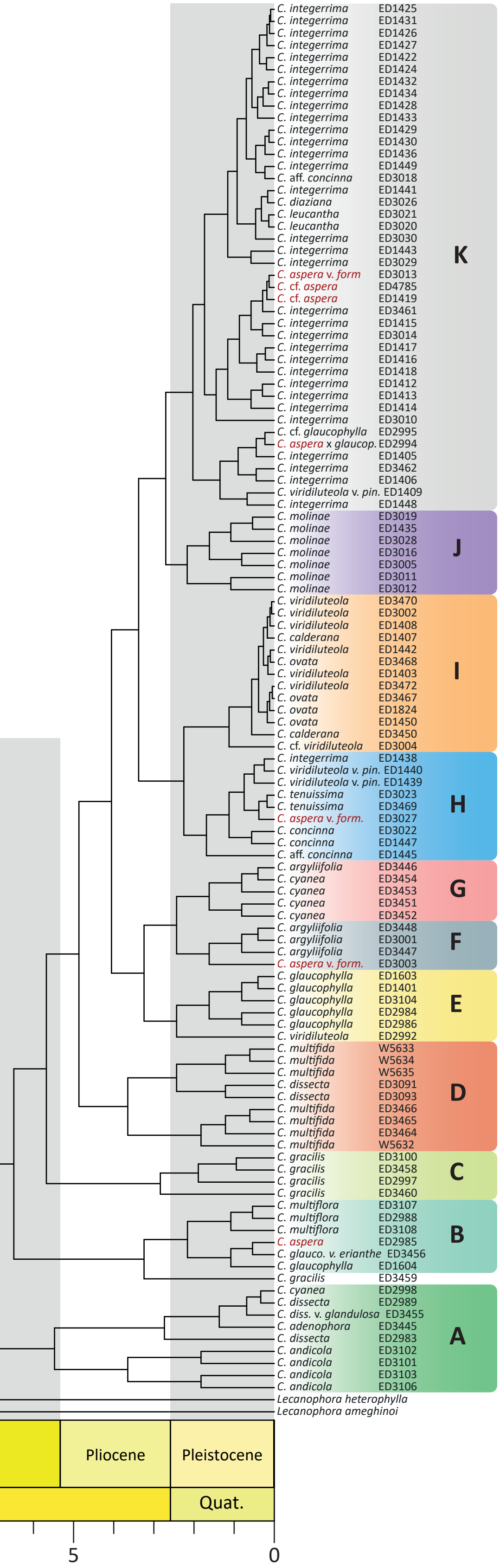


Figure S2: Dated GBS Maximum Likelihood phylogeny of the genus *Cristaria* using the Penalized Likelihood approach after Sanderson (2002). Red dots indicate calibrationed nodes (stem and crown nodes of *Cristaria*; Böhnert *et al.* 2019).

Table S1: List of species, voucher information and GBS assembly statistics. pops30 = all *Cristaria* GBS samples, *C. int* ML = *Cristaria integerrima* assembly for Maximum Likelihood analysis, *C. int* STRU. = *Cristaria integerrima* assembly for STRUCTURE analysis.

Species	Voucher	Lab-ID	Sequencing ID	reads raw	pops30	*C. int* ML	*C. int* STRU.
Cristaria adenophora I.M. Johnst.	J. Schneider & M. Huertas 2914 (FR)	ED3445	C_ade_ED3445	409199	1525		
Cristaria andicola Gay	F. Luebert & T. Böhnert 3667 (Bonn, FR, ULS)	ED3101	C_and_ED3101	639862	1625		
Cristaria andicola Gay	F. Luebert & T. Böhnert 3673 (Bonn, FR, ULS)	ED3102	C_and_ED3102	867107	1307		
Cristaria andicola Gay	F. Luebert & T. Böhnert 3694 (Bonn, FR, ULS)	ED3103	C_and_ED3103	1116270	2097		
Cristaria andicola Gay	F. Luebert & T. Böhnert 3712 (Bonn, FR, ULS)	ED3106	C_and_ED3106	1046333	1762		
Cristaria argyliifolia Phil.	F. Luebert, T. Böhnert & F. Merklinger 3886 (Bonn, FR, ULS)	ED3001	C_arg_ED3001	734115	2552		
Cristaria argyliifolia Phil.	J. Schneider & M. Huertas 2938 (FR)	ED3446	C_arg_ED3446	664604	2416		
Cristaria argyliifolia Phil.	J. Schneider & M. Huertas 2923 (FR)	ED3447	C_arg_ED3447	989316	2437		
Cristaria argyliifolia Phil.	J. Schneider & M. Huertas 2921 (FR)	ED3448	C_arg_ED3448	434304	1685		
Cristaria aspera Gay	F. Luebert, T. Böhnert & F. Merklinger 3784 (Bonn, FR, ULS)	ED2985	C_asp_ED2985	738697	2496		
Cristaria cf. *aspera* Gay	F. Luebert, A. Stoll & T. Böhnert 3318 (Bonn, FR, ULS)	ED1419	C_int_asp_cf_ED1419	1628337	3603	11730	11705
Cristaria cf. *aspera* Gay	F. Luebert, A. Stoll & T. Böhnert 3319 (Bonn, FR, ULS)	ED4785	C_int_asp_cf_ED4785	1599350	1474	5950	5939
Cristaria aspera var. *formosula* (I.M. Johnst.) Muñoz-Schick	F. Luebert, T. Böhnert & F. Merklinger 3891 (Bonn, FR, ULS)	ED3003	C_asp_ED3003	579040	2631		
Cristaria aspera var. *formosula* (I.M. Johnst.) Muñoz-Schick	F. Luebert, T. Böhnert & F. Merklinger 3915 (Bonn, FR, ULS)	ED3013	C_int_asp_form_ED3013	2266892	2500	9066	9046
Cristaria aspera var. *formosula* (I.M. Johnst.) Muñoz-Schick	F. Luebert, T. Böhnert & F. Merklinger 3966 (Bonn, FR, ULS)	ED3027	C_asp_ED3027	526665	2296		
Cristaria aspera x *C. glaucophylla*	F. Luebert, T. Böhnert & F. Merklinger 3850 (Bonn, FR, ULS)	ED2994	C_int_aspXglau_ED2994	503025	2296	7891	7885
Cristaria calderana Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3288 (Bonn, FR, ULS)	ED1407	C_cal_ED1407	2911701	3351		
Cristaria calderana Muñoz-Schick	J. Schneider & M. Huertas 2926 (FR)	ED3450	C_cal_ED3450	231264	1348		
Cristaria aff. *concinna* Phil.	F. Luebert, A. Stoll & T. Böhnert 3409 (Bonn, FR, ULS)	ED1445	C_con_aff_ED1445	531683	2648		
Cristaria aff. *concinna* Phil.	F. Luebert, T. Böhnert & F. Merklinger 3945 (Bonn, FR, ULS)	ED3018	C_int_con_aff_ED3018	729840	2596	8525	8519
Cristaria concinna Phil.	F. Luebert, A. Stoll & T. Böhnert 3387 (Bonn, FR, ULS)	ED1447	C_con_ED1447	501051	2547		
Cristaria concinna Phil.	F. Luebert, T. Böhnert & F. Merklinger 3951 (Bonn, FR, ULS)	ED3022	C_con_ED3022	974644	3057		
Cristaria cyanea Phil. ex Baker f.	F. Luebert, T. Böhnert & F. Merklinger 3879 (Bonn, FR, ULS)	ED2998	C_cya_ED2998	540265	1409		
Cristaria cyanea Phil. ex Baker f.	J. Schneider & M. Huertas 2940 (FR)	ED3451	C_cya_ED3451	774476	2557		
Cristaria cyanea Phil. ex Baker f.	J. Schneider & M. Huertas 2941 (FR)	ED3452	C_cya_ED3452	999930	2548		
Cristaria cyanea Phil. ex Baker f.	J. Schneider & M. Huertas 2942 (FR)	ED3453	C_cya_ED3453	731010	2557		
Cristaria cyanea Phil. ex Baker f.	J. Schneider & M. Huertas 2934 (FR)	ED3454	C_cya_ED3454	841822	2583		
Cristaria diaziana I.M. Johnston	F. Luebert, T. Böhnert & F. Merklinger 3963 (Bonn, FR, ULS)	ED3026	C_int_dia_ED3026	653693	2517	8496	8485
Cristaria dissecta Hook. & Arn.	F. Luebert, T. Böhnert & F. Merklinger 3746 (Bonn, FR, ULS)	ED2983	C_dis_ED2983	635244	1646		
Cristaria dissecta Hook. & Arn.	F. Luebert, T. Böhnert & F. Merklinger 3827 (Bonn, FR, ULS)	ED2989	C_dis_ED2989	848267	2126		
Cristaria dissecta Hook. & Arn.	F. Luebert, T. Böhnert, F.F. Merklinger, A. Stoll & D. Quandt 3517 (Bonn, FR, ULS)	ED3091	C_dis_ED3091	438273	2107		
Cristaria dissecta Hook. & Arn.	F. Luebert, T. Böhnert, F.F. Merklinger & A. Stoll 3614 (Bonn, FR, ULS)	ED3093	C_dis_ED3093	609854	2048		
Cristaria dissecta var. *glandulosa* (Phil.) Muñoz-Schick	J. Schneider & M. Huertas 2935 (FR)	ED3455	C_dis_ED3455	843699	1881		
Cristaria cf. *glaucophylla* Cav.	F. Luebert, T. Böhnert & F. Merklinger 3864 (Bonn, FR, ULS)	ED2995	C_int_gla_cf_ED2995	954199	3044	9361	9347
Cristaria glaucophylla Cav.	F. Luebert, A. Stoll & T. Böhnert 3268 (Bonn, FR, ULS)	ED1401	C_gla_ED1401	1502874	3324		
Cristaria glaucophylla Cav.	F. Luebert & T. Böhnert 3721 (Bonn, FR, ULS)	ED1603	C_gla_ED1603	2625625	3284		
Cristaria glaucophylla Cav.	F. Luebert & T. Böhnert 3724 (Bonn, FR, ULS)	ED1604	C_gla_ED1604	708534	1868		
Cristaria glaucophylla Cav.	F. Luebert, T. Böhnert & F. Merklinger 3755 (Bonn, FR, ULS)	ED2984	C_gla_ED2984	256985	1215		
Cristaria glaucophylla Cav.	F. Luebert, T. Böhnert & F. Merklinger 3793 (Bonn, FR, ULS)	ED2986	C_gla_ED2986	1354922	2770		
Cristaria glaucophylla Cav.	F. Luebert & T. Böhnert 3710 (Bonn, FR, ULS)	ED3104	C_gla_ED3104	326948	1383		

Species	**Voucher**	**Lab-ID**	**Sequencing ID**	**reads raw**	**pops30**	***C. int* ML**	***C. int* STRU.**
Cristaria glaucophylla var. *eriantha* (Hook. & Arn.) Muñoz-Schick	J. Schneider & M. Huertas 2961 (FR)	ED3456	C_gla_ED3456	989472	2072		
Cristaria gracilis Gay	F. Luebert, T. Böhnert & F. Merklinger 3878 (Bonn, FR, ULS)	ED2997	C_gra_ED2997	1070863	2465		
Cristaria gracilis Gay	F. Luebert & T. Böhnert 3659 (Bonn, FR, ULS)	ED3100	C_gra_ED3100	629616	1479		
Cristaria gracilis Gay	J. Schneider & M. Huertas 2861 (FR)	ED3458	C_gra_ED3458	1183425	2554		
Cristaria gracilis Gay	J. Schneider & M. Huertas 2952 (FR)	ED3459	C_gra_ED3459	561116	1953		
Cristaria gracilis Gay	J. Schneider & M. Huertas 2909 (FR)	ED3460	C_gra_ED3460	344981	1927		
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3278 (Bonn, FR, ULS)	ED1405	C_int_ED1405	1385261	3748	11677	11652
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3280 (Bonn, FR, ULS)	ED1406	C_int_ED1406	1561235	3518	11398	11386
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3303 (Bonn, FR, ULS)	ED1412	C_int_ED1412	2038566	3670	11885	11870
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3306 (Bonn, FR, ULS)	ED1413	C_int_ED1413	1154999	3663	11831	11812
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3310 (Bonn, ULS)	ED1414	C_int_ED1414	2039819	3765	12073	12054
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3313 (Bonn, FR, ULS)	ED1415	C_int_ED1415	2094583	3454	11197	11186
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3315 (Bonn, FR, ULS)	ED1416	C_int_ED1416	1345449	3422	11020	11012
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3316 (Bonn, FR, ULS)	ED1417	C_int_ED1417	1928096	3717	12090	12068
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3317 (Bonn, FR, ULS)	ED1418	C_int_ED1418	1373793	3703	11945	11933
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3330 (Bonn, FR, ULS)	ED1422	C_int_ED1422	2301667	3838	12646	12638
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3333 (Bonn, FR, ULS)	ED1424	C_int_ED1424	1565025	3757	12452	12442
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3334 (Bonn, FR, ULS)	ED1425	C_int_ED1425	3228038	3714	12391	12377
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3335 (Bonn, ULS)	ED1426	C_int_ED1426	4039131	1806	7296	7276
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3336 (Bonn, FR, ULS)	ED1427	C_int_ED1427	2468232	3726	12367	12348
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3350 (Bonn, FR, ULS)	ED1428	C_int_ED1428	2107049	2137	8238	8221
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3358 (Bonn, FR, ULS)	ED1429	C_int_ED1429	4193546	3132	10706	10684
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3359 (Bonn, FR, ULS)	ED1430	C_int_ED1430	2579125	3081	10429	10411
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3364 (Bonn, FR, ULS)	ED1431	C_int_ED1431	1541662	2866	9566	9562
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3366 (Bonn, FR, ULS)	ED1432	C_int_ED1432	853614	3128	9992	9977
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3367 (Bonn, FR, ULS)	ED1433	C_int_ED1433	744654	1990	7259	7246
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3368 (Bonn, FR, ULS)	ED1434	C_int_ED1434	2805606	3808	12589	12573
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3375 (Bonn, FR, ULS)	ED1436	C_int_ED1436	3818713	3147	10628	10608
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3388A (Bonn, FR, ULS)	ED1438	C_sp_int_ED1438	2994398	3291		
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3398 (Bonn, FR, ULS)	ED1441	C_int_ED1441	478497	1915	7039	7024
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3403 (Bonn, FR, ULS)	ED1443	C_int_ED1443	889484	2758	9146	9137
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3297 (Bonn, FR, ULS)	ED1448	C_int_ED1448	629203	2403	8143	8130
Cristaria integerrima Phil.	F. Luebert, A. Stoll & T. Böhnert 3388 (Bonn, FR, ULS)	ED1449	C_int_ED1449	1509385	2422	8475	8465
Cristaria integerrima Phil.	F. Luebert, T. Böhnert & F. Merklinger 3908 (Bonn, FR, ULS)	ED3010	C_int_ED3010	1443377	3397	11112	11101
Cristaria integerrima Phil.	F. Luebert, T. Böhnert & F. Merklinger 3982 (Bonn, FR, ULS)	ED3029	C_int_ED3029	1909217	3535	11728	11709
Cristaria integerrima Phil.	F. Luebert, T. Böhnert & F. Merklinger 3990 (Bonn, FR, ULS)	ED3030	C_int_ED3030	3528170	3579	12085	12066
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	F. Luebert, T. Böhnert & F. Merklinger 3917 (Bonn, FR, ULS)	ED3014	C_int_ED3014	901668	3069	9720	9713
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	J. Schneider & M. Huertas 2901 (FR)	ED3461	C_int_ED3461	982060	2815	9237	9219
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	J. Schneider & M. Huertas 2947 (FR)	ED3462	C_int_ED3462	773277	2270	7894	7878
Cristaria leucantha I.M. Johnst.	F. Luebert, T. Böhnert & F. Merklinger 3947 (Bonn, FR, ULS)	ED3020	C_int_leu_ED3020	751100	2552	8675	8658
Cristaria leucantha I.M. Johnst.	F. Luebert, T. Böhnert & F. Merklinger 3948 (Bonn, FR, ULS)	ED3021	C_int_leu_ED3021	466612	2313	7975	7966
Cristaria molinae Gay	F. Luebert, A. Stoll & T. Böhnert 3374 (Bonn, FR, ULS)	ED1435	C_mol_ED1435	1041243	1672	6588	
Cristaria molinae Gay	F. Luebert, T. Böhnert & F. Merklinger 3896 (Bonn, FR, ULS)	ED3005	C_mol_ED3005	442552	2345		
Cristaria molinae Gay	F. Luebert, T. Böhnert & F. Merklinger 3909 (Bonn, FR, ULS)	ED3011	C_mol_ED3011	1141336	2985		
Cristaria molinae Gay	F. Luebert, T. Böhnert & F. Merklinger 3911 (Bonn, FR, ULS)	ED3012	C_mol_ED3012	1247161	3420		
Cristaria molinae Gay	F. Luebert, T. Böhnert & F. Merklinger 3930 (Bonn, FR, ULS)	ED3016	C_mol_ED3016	1090103	3040		
Cristaria molinae Gay	F. Luebert, T. Böhnert & F. Merklinger 3946 (Bonn, FR, ULS)	ED3019	C_mol_ED3019	534575	2306		

Species	**Voucher**	**Lab-ID**	**Sequencing ID**	**reads raw**	**pops30**	***C. int* ML**	***C. int* STRU.**
Cristaria molinae Gay	F. Luebert, T. Böhnert & F. Merklinger 3978 (Bonn, FR, ULS)	ED3028	C_mol_ED3028	543377	2235		
Cristaria multifida Cav.	J. Schneider, M. Chanco & M. Huertas 2798 (FR)	ED3464	C_multifida_ED3464	392983	1948		
Cristaria multifida Cav.	J. Schneider, M. Chanco, M. Huertas & C. Caceres 2790 (FR)	ED3465	C_multifida_ED3465	576885	2229		
Cristaria multifida Cav.	J. Schneider, M. Chanco, M. Huertas & C. Caceres 2776 (FR)	ED3466	C_multifida_ED3466	1009904	2144		
Cristaria multifida Cav.	M. Weigend *et al.* 9866 (USM, HUSA, BONN)	W5632	C_multifida_W5632	2678017	3080		
Cristaria multifida Cav.	M. Weigend *et al.* 9885 (USM, HUSA, BONN)	W5633	C_multifida_W5633	3560550	3441		
Cristaria multifida Cav.	M. Weigend *et al.* 9980 (USM, HUSA, BONN)	W5634	C_multifida_W5634	5130125	2341		
Cristaria multifida Cav.	M. Weigend *et al.* 9978 (USM, HUSA, BONN)	W5635	C_multifida_W5635	3277319	1393		
Cristaria multiflora Gay	F. Luebert, T. Böhnert & F. Merklinger 3817 (Bonn, FR, ULS)	ED2988	C_multiflora_ED2988	590957	2095		
Cristaria multiflora Gay	F. Luebert & T. Böhnert 3718 (Bonn, FR, ULS)	ED3107	C_multiflora_ED3107	722336	2139		
Cristaria multiflora Gay	F. Luebert & T. Böhnert 3719 (Bonn, FR, ULS)	ED3108	C_multiflora_ED3108	601822	2045		
Cristaria ovata Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3300 (Bonn, FR, ULS)	ED1450	C_ova_ED1450	839236	2685		
Cristaria ovata Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3301 (Bonn, FR, ULS)	ED1824	C_ova_CF_ED1824	764302	2574		
Cristaria ovata Muñoz-Schick	J. Schneider & M. Huertas 2905 (FR)	ED3467	C_ova_ED3467	751858	2403		
Cristaria ovata Muñoz-Schick	J. Schneider & M. Huertas 3041 (FR)	ED3468	C_ova_ED3468	602245	2256		
Cristaria tenuissima Muñoz-Schick	F. Luebert, T. Böhnert & F. Merklinger 3958 (Bonn, FR, ULS)	ED3023	C_ten_CF_ED3023	265308	1462		
Cristaria tenuissima Muñoz-Schick	J. Schneider & M. Huertas 2890 (FR)	ED3469	C_ten_ED3469	641663	2081		
Cristaria cf. *viridiluteola* Gay	F. Luebert, T. Böhnert & F. Merklinger 3892 (Bonn, FR, ULS)	ED3004	C_vir_CF_ED3004	727674	2280		
Cristaria viridiluteola Gay	F. Luebert, A. Stoll & T. Böhnert 3273 (Bonn, FR, ULS)	ED1403	C_vir_ED1403	1220692	2786		
Cristaria viridiluteola Gay	F. Luebert, A. Stoll & T. Böhnert 3289 (Bonn, FR, ULS)	ED1408	C_vir_ED1408	1348089	3528		
Cristaria viridiluteola Gay	F. Luebert, T. Böhnert & F. Merklinger 3840 (Bonn, FR, ULS)	ED2992	C_vir_ED2992	439354	1605		
Cristaria viridiluteola Gay	F. Luebert, T. Böhnert & F. Merklinger 3887 (Bonn, FR, ULS)	ED3002	C_vir_ED3002	605072	2301		
Cristaria viridiluteola Gay	J. Schneider & M. Huertas 2922 (FR)	ED3470	C_vir_ED3470	1045665	2523		
Cristaria viridiluteola Gay	J. Schneider & M. Huertas 2906 (FR)	ED3472	C_vir_ED3472	823656	2677		
Cristaria viridiluteola Gay	F. Luebert, A. Stoll & T. Böhnert 3399 (Bonn, FR, ULS)	ED1442	C_vir_ED1442	817648	2416		
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3298 (Bonn, ULS)	ED1409	C_int_vir_pin_ED1409	1823339	3857	12101	12084
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3395 (Bonn, FR, ULS)	ED1439	C_vir_ED1439	1688169	2596		
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	F. Luebert, A. Stoll & T. Böhnert 3396 (Bonn, FR, ULS)	ED1440	C_vir_pin_ED1440	4571564	3039		
Lecanophora ameghinoi (Speg.) Speg.	J. Schneider & Huertas 2967 (FR)	ED1878	Lec_ame_ED1878	639352	1241		
Lecanophora heterophylla (Cav.) Krapov.	J. Schneider & Huertas 2966 (FR)	ED1877	Lec_het_ED1877	433168	976		

Table S2: List of *Cristaria* vouchers used for distribution maps. Source of specimes are either taken from Muñoz-Schick (1995), kindly provided from Julio Schneider (Senkenberg, Frankfurt, Germany) or from own collections. Vocuher used from GBS analyses are indicated in column **phyl** by their Lab-ID (see Table S1).

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria adenophora I.M. Johnst.	Chile	III Region / Atacama	-26.40950	-69.51350	2800	J. Schneider & M. Huertas 2914 (FR, CONC, SGO)	2002	J. Schneider	A	ED3445
Cristaria adenophora I.M. Johnst.	Chile	III Region / Atacama	-26.41041	-69.53038		M. Munoz, Teillier, Meza 2753 (SGO)	1991	Muñoz-Schick 1995	A	
Cristaria adenophora I.M. Johnst.	Chile	III Region / Atacama	-26.39150	-69.46150	2400	J. Schneider & M. Huertas 2915 (FR, CONC, SGO)	2002	J. Schneider	A	
Cristaria adenophora I.M. Johnst.	Chile	III Region / Atacama	-26.39891	-69.40139	2380	F. Luebert, T. Böhnert & F. Merklinger 3900 (Bonn, FR, ULS)	2017	Collection trip 10-2017	A	
Cristaria aff. *concinna* Phil.	Chile	II Region / Antofagasta	-23.54701	-70.37060	200	F. Luebert, A. Stoll & T. Böhnert 3409 (Bonn, FR, ULS)	2016	Collection trip 10-2016	H	ED1445
Cristaria aff. *concinna* Phil.	Chile	II Region / Antofagasta	-24.74954	-70.55518	30	F. Luebert, T. Böhnert & F. Merklinger 3945 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3018
Cristaria andicola Gay	Chile	II Region / Antofagasta	-24.10273	-68.43456	3100	F. Luebert & T. Böhnert 3667 (Bonn, FR, ULS)	2017	Collection trip 03-2017	A	ED3101
Cristaria andicola Gay	Chile	III Redion / Atacama	-25.92073	-69.77171	3600	F. Luebert & T. Böhnert 3673 (Bonn, FR, ULS)	2017	Collection trip 03-2017	A	ED3102
Cristaria andicola Gay	Chile	III Redion / Atacama	-26.87161	-69.22202	3500	F. Luebert & T. Böhnert 3694 (Bonn, FR, ULS)	2017	Collection trip 03-2017	A	ED3103
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-29.90067	-70.04799	2700	F. Luebert & T. Böhnert 3712 (Bonn, FR, ULS)	2017	Collection trip 03-2017	A	ED3106
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-29.84936	-70.14257		Gay 327 (P)		Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-29.84604	-70.14287		Gay 327 (P)	1838	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-27.11667	-69.31667	3280	Marticorena, Matthei, Quezada 535 (CONC)	1973	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-27.08333	-69.25000		Ricardi, Marticorena, Matthei 570 (CONC)	1963	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-27.21667	-69.28333	3700	Marticorena, Matthei, Quezada 573 (CONC)	1973	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-27.05000	-69.36667	3500	Ricardi, Marticorena y Matthei 647 (CONC)	1963	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-29.96667	-70.11667		Ricardi, Marticorena, Manhei 696 (SGO)	1963	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-26.33933	-69.23984	3400	Zollner 853 (CONC)	1966	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-27.81667	-69.38333	3000	Werdermann 938 (CONC)	1926	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-26.28333	-69.53333	2950	Ricardi, Marticorena, Matthei 1571 (CONC)	1966	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-26.36667	-69.25000	3500	Ricardi, Marticorena, Matthei 1592 (CONC)	1966	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-26.96667	-68.91667		Ricardi, Marticorena, Matthei 1663 (CONC)	1966	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	II Region / Antofagasta	-24.27231	-68.80201	3000	Biese 2291 (SGO)	1947	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	II Region / Antofagasta	-24.41590	-68.32962	3500	Biese 2295 (SGO)	1947	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	II Region / Antofagasta	-24.36304	-68.52632	3400	Biese 2312 (SGO)	1947	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	II Region / Antofagasta	-23.83467	-67.86250	4000	J. Schneider & M. Huertas 2868 (FR, CONC, SGO)	2002	J. Schneider	A	
Cristaria andicola Gay	Chile	III Redion / Atacama	-27.07300	-69.38517	3000	J. Schneider & M. Huertas 2917 (FR, CONC, SGO)	2002	J. Schneider	A	
Cristaria andicola Gay	Chile	III Redion / Atacama	-27.04667	-69.31867	3500	J. Schneider & M. Huertas 2918 (FR, CONC, SGO)	2002	J. Schneider	A	
Cristaria andicola Gay	Chile	III Redion / Atacama	-26.86817	-69.43833	3200	J. Schneider & M. Huertas 2919 (FR, CONC, SGO)	2002	J. Schneider	A	
Cristaria andicola Gay	Chile	III Redion / Atacama	-27.72150	-69.70400	2700	J. Schneider & M. Huertas 3061 (FR, SGO)	2003	J. Schneider	A	
Cristaria andicola Gay	Chile	II Region / Antofagasta	-25.51576	-69.58158	3000	Biese 3121 (SGO)	1947	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	II Region / Antofagasta	-24.74625	-69.15708	3920	C. Muñoz 3877 (SGO)	1944	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Redion / Atacama	-26.39891	-69.40139	2380	F. Luebert, T. Böhnert & F. Merklinger 3902 (Bonn, FR, ULS)	2017	Collection trip 10-2017	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-26.98333	-68.95000	4050	Villagran, Kalin 4577 (CONC)		Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-27.05000	-69.68333	1900	Villagran, Kalin 4596 (CONC)	1983	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-30.18333	-70.01667	3400	Kalin 81001 (CONC)	1981	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-30.05000	-70.08333	2600	Kalin 81126 (CONC)	1981	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-28.73333	-69.83333		Kalin 81567 (CONC)	1981	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-28.65000	-69.71667	3900	Marticorena, Kalin y Villagran 83456 (CONC)	1983	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-28.68333	-69.75000	3600	Marticorena, Kalin y Villagran 83562 (CONC)	1983	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-27.78333	-70.00000	3700	Squeo 88054 (CONC)	1988	Muñoz-Schick 1995	A	

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-29.85000	-70.05000	3500	Squeo 88129 (CONC)	1988	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	IV Region / Coquimbo	-29.32756	-69.47108		Perez Moreau 301126 (CONC)	1930	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-26.41667	-69.30000	3300	F. Behn (CONC)	1958	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-26.10000	-69.66667		Gigoux (SGO)	1886	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-26.48333	-69.71667		Niemeyer (CONC)	1973	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-28.00000	-69.48333	3500	Niemeyer (CONC)	1977	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	II Region / Antofagasta	-23.91667	-68.41667		Philippi (SGO)	1854	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-27.91667	-69.40000	3100	Roman (SGO)	1884	Muñoz-Schick 1995	A	
Cristaria andicola Gay	Chile	III Region / Atacama	-28.07940	-69.73445		Villanueva (SGO)	1877	Muñoz-Schick 1995	A	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.10080	-70.67368	340	F. Luebert, T. Böhnert & F. Merklinger 3886 (Bonn, FR, ULS)	2017	Collection trip 10-2017	F	ED3001
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.58333	-70.45000	650	J. Schneider & M. Huertas 2938 (FR)	2002	J. Schneider	G	ED3446
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.29800	-70.75433	300	J. Schneider & M. Huertas 2923 (FR, CONC, SGO)	2002	J. Schneider	F	ED3447
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.34933	-70.68117	250	J. Schneider & M. Huertas 2921 (FR, CONC, SGO)	2002	J. Schneider	F	ED3448
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.85925	-70.59230		Gleisner 44 (CONC)	1965	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.41326	-70.44073		Meza, Barrera 1794 (SGO)	1991	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.55000	-70.46667		Jiles 2163 (CONC)	1952	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.12627	-70.81226		Muñoz, Teillier, Meza 2873 (SGO)	1991	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.34933	-70.68117	300	J. Schneider & M. Huertas 2921 (FR)	2002	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.29800	-70.75722	300	J. Schneider & M. Huertas 2923 (FR)	2002	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.15367	-70.92517	125	J. Schneider & M. Huertas 2932 (FR, CONC, SGO)	2002	J. Schneider	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.58333	-70.45000	650	J. Schneider & M. Huertas 2938 (FR, CONC)	2002	J. Schneider	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.58335	-70.45002	650	J. Schneider & M. Huertas 2943 (FR, CONC, SGO)	2002	J. Schneider	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.37185	-70.33945		Dodt (SGO)	1862	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.86667	-70.36667		Geisse (SGO)		Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.95519	-70.35178		Philippi (SGO)	1885	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.35010	-70.71770		Philippi (SGO)	1885	Muñoz-Schick 1995	F	
Cristaria argyliifolia Phil.	Chile	III Region / Atacama	-27.08306	-70.81449		Philippi (SGO)	1885	Muñoz-Schick 1995	F	
Cristaria aspera Gay	Chile	IV Region / Coquimbo	-30.74102	-71.65348	50	F. Luebert, T. Böhnert & F. Merklinger 3784 (Bonn, FR, ULS)	2017	Collection trip 10-2017	B	ED2985
Cristaria aspera var. *formosula* (I.M. Johnst.) Muñoz-Schick	Chile	III Region / Atacama	-26.96325	-70.74014	210	F. Luebert, T. Böhnert & F. Merklinger 3891 (Bonn, FR, ULS)	2017	Collection trip 10-2017	F	ED3003
Cristaria aspera var. *formosula* (I.M. Johnst.) Muñoz-Schick	Chile	II Region / Antofagasta	-25.89382	-70.64825	220	F. Luebert, T. Böhnert & F. Merklinger 3915 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3013
Cristaria aspera var. *formosula* (I.M. Johnst.) Muñoz-Schick	Chile	II Region / Antofagasta	-24.27102	-70.51755	350	F. Luebert, T. Böhnert & F. Merklinger 3966 (Bonn, FR, ULS)	2017	Collection trip 10-2017	H	ED3027
Cristaria aspera x *C. glaucophylla*	Chile	III Region / Atacama	-28.45746	-71.18279	20	F. Luebert, T. Böhnert & F. Merklinger 3850 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED2994
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.76494	-70.74606		Hannington 19 (SGO)	1987	Muñoz-Schick 1995	I	
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.89356	-70.79672		Ricardi, Marticorena, Matthei 1437 (CONC)	1965	Muñoz-Schick 1995	I	
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.91701	-70.79801		Muñoz, Teillier, Meza 2856 (SGO)	1991	Muñoz-Schick 1995	I	
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.77500	-70.76667	30	J. Schneider & M. Huertas 2907 (FR, CONC, SGO)	2002	J. Schneider	I	
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.77096	-70.76495	50	J. Schneider & M. Huertas 2927 (FR, CONC, SGO)	2002	J. Schneider	I	
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.97067	-70.79150	50	J. Schneider & M. Huertas 2928 (FR, SGO)	2002	J. Schneider	I	
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.97450	-70.77083	40	J. Schneider & M. Huertas 2929 (FR, CONC, SGO)	2002	J. Schneider	I	
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.97280	-70.79279		Kausel 5082 (SGO)	1966	Muñoz-Schick 1995	I	
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-27.09933	-70.67368	350	F. Luebert, A. Stoll & T. Böhnert 3288 (Bonn, FR, ULS)	2016	Collection trip 10-2016	I	ED1407
Cristaria calderana Muñoz-Schick	Chile	III Region / Atacama	-26.77004	-70.76289	50	J. Schneider & M. Huertas 2926 (FR, CONC, SGO)	2002	J. Schneider	I	ED3450

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria cf. *aspera* Gay	Chile	II Region / Antofagasta	-25.89312	-70.64418	250	F. Luebert, A. Stoll & T. Böhnert 3318 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1419
Cristaria cf. *aspera* Gay	Chile	II Region / Antofagasta	-25.89312	-70.64418	250	F. Luebert, A. Stoll & T. Böhnert 3319 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED4785
Cristaria cf. *glaucophylla* Cav.	Chile	III Region / Atacama	-28.09709	-71.16057	10	F. Luebert, T. Böhnert & F. Merklinger 3864 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED2995
Cristaria cf. *viridiluteola* Gay	Chile	III Region / Atacama	-26.96325	-70.74014	210	F. Luebert, T. Böhnert & F. Merklinger 3892 (Bonn, FR, ULS)	2017	Collection trip 10-2017	I	ED3004
Cristaria concinna Phil.	Chile	II Region / Antofagasta	-24.74126	-70.55749	30	F. Luebert, A. Stoll & T. Böhnert 3387 (Bonn, FR, ULS)	2016	Collection trip 10-2016	H	ED1447
Cristaria concinna Phil.	Chile	II Region / Antofagasta	-24.74172	-70.54269	210	F. Luebert, T. Böhnert & F. Merklinger 3951 (Bonn, FR, ULS)	2017	Collection trip 10-2017	H	ED3022
Cristaria concinna Phil.	Chile	II Region / Antofagasta	-24.95517	-70.48050	165	J. Schneider & M. Huertas 3028 (FR)	2003	J. Schneider	H	
Cristaria concinna Phil.	Chile	II Region / Antofagasta	-24.74922	-70.54689	60	J. Schneider & M. Huertas 3040 (FR, SGO)	2003	J. Schneider	H	
Cristaria concinna Phil.	Chile	II Region / Antofagasta	-24.73750	-70.56567		J. Schneider & M. Huertas 3043 (FR, SGO)	2003	J. Schneider	H	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.88015	-70.21933	1550	F. Luebert, T. Böhnert & F. Merklinger 3879 (Bonn, FR, ULS)	2017	Collection trip 10-2017	A	ED2998
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.58333	-70.45000	650	J. Schneider & M. Huertas 2940 (FR)	2002	J. Schneider	G	ED3451
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.58333	-70.45000	650	J. Schneider & M. Huertas 2941 (FR)	2002	J. Schneider	G	ED3452
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.58333	-70.45000	650	J. Schneider & M. Huertas 2942 (FR)	2002	J. Schneider	G	ED3453
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.58083	-70.44583	650	J. Schneider & M. Huertas 2934 (FR)	2002	J. Schneider	G	ED3454
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.98333	-70.56667		Rodriguez, Marticorena 1612 (CONC)	1980	Muñoz-Schick 1995	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-28.20179	-70.66858		Ricardi 2205 (CONC)	1952	Muñoz-Schick 1995	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-28.09210	-70.61112	510	Muñoz, Teillier, Meza 2691 (SGO)	1991	Muñoz-Schick 1995	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-28.27258	-70.72393		Muñoz, Teillier, Meza 2911 (SGO)	1991	Muñoz-Schick 1995	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.57517	-70.44583	650	J. Schneider & M. Huertas 2933 (FR, CONC, SGO)	2002	J. Schneider	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.57519	-70.44585	650	J. Schneider & M. Huertas 2933b (FR, CONC, SGO)	2002	J. Schneider	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.57523	-70.44589	650	J. Schneider & M. Huertas 2934b (FR, SGO)	2002	J. Schneider	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-28.68784	-70.56468	1100	Callejas (SGO)	1985	Muñoz-Schick 1995	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.99451	-70.42543		F. Philippi & Borchers (BM)	1885	Muñoz-Schick 1995	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-27.80626	-70.45504		Geisse (CONC)	1888	Muñoz-Schick 1995	G	
Cristaria cyanea Phil. ex Baker f.	Chile	III Region / Atacama	-28.53023	-70.76178		Rojas (CONC)	1965	Muñoz-Schick 1995	G	
Cristaria diaziana I.M. Johnston	Chile	II Region / Antofagasta	-24.42808	-70.53269	150-200	F. Luebert, T. Böhnert & F. Merklinger 3963 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3026
Cristaria dissecta Hook. & Arn.	Chile	Valparaíso	-32.85180	-70.87507	380	F. Luebert, T. Böhnert & F. Merklinger 3746 (Bonn, FR, ULS)	2017	Collection trip 10-2017	A	ED2983
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.87378	-70.86081	700	F. Luebert, T. Böhnert & F. Merklinger 3827 (Bonn, FR, ULS)	2017	Collection trip 10-2017	A	ED2989
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-20.38819	-69.11255	3000	F. Luebert, T. Böhnert, F.F. Merklinger, A. Stoll & D. Quandt 3517 (Bonn, FR, ULS)	2017	Collection trip 03-2017	D	ED3091
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-20.30425	-69.11324	3200	F. Luebert, T. Böhnert, F.F. Merklinger & A. Stoll 3614 (Bonn, FR, ULS)	2017	Collection trip 03-2017	D	ED3093
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-18.31667	-69.50000	2850	Ricardi, Marticorena, Matthei 97 (CONC)	1961	Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-18.40000	-69.65000	3000	Ricardi. Weldt, Quezada 107 (CONC)	1972	Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	Region Metropolitana	-33.46667	-70.55000	850	Bravo 175 (CONC)	1952	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-19.55551	-69.24616		F. Philippi 232 (SGO)		Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-33.05000	-71.59000		Cunning 245 (GL)	1831	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-19.90000	-69.05000	1120	Ricardi, Maiticorena, Matthei 349 (CONC)	1963	Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	II Region / Antofagasta	-22.78333	-68.36667	3100	Ricardi, Weldt, Ouezada 407 (CONC)	1972	Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.67501	-71.54465		Jiles 683 (CONC)	1948	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	V Region / Valparaiso	-32.83333	-70.60000		Barros 1095 (CONC)	1923	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.66667	-71.18333		Jiles 1296 (CONC)	1949	Muñoz-Schick 1995	A	

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-20.00000	-69.20000	3000	Ricardi, Marticorena, Matthei 1372 (CONC)	1965	Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.90000	-70.93333		Marticorena, Rodríguez, Weldt 1505 (CONC)	1971	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.30000	-71.26667		Jiles 1506 (CONC)	1949	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.65000	-70.66667		Jiles 1552 (CONC)	1949	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	V Region / Valparaiso	-33.08333	-71.26667		Garaventa 1655 (CONC)	1930	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-31.70231	-71.10897	1200	Marticorena, Rodríguez, Weldt 1726 (CONC)	1971	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.18333	-70.71667		Marticorena, Rodríguez, Weldt 1748 (CONC)	1971	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	III Region / Atacama	-27.85000	-70.18333	1600	Meza, Barrera 1765 (SGO)	1991	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	III Region / Atacama	-29.03333	-70.90000	950	Marticorena, Rodríguez, Weldt 1768 (CONC)	1971	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.66667	-70.90000		Jiles 1915 (CONC)	1950	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-31.36106	-71.02615	1275	Biese 2146 (SGO)	1945	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-20.06933	-69.21583	2800	J. Schneider & M. Huertas 2832 (FR, CONC, SGO)	2002	J. Schneider	D	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-20.07933	-69.26600	2700	J. Schneider & M. Huertas 2834 (FR, CONC, SGO)	2002	J. Schneider	D	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-20.23817	-69.63083		J. Schneider & M. Huertas 2835 (FR, CONC, SGO)	2002	J. Schneider	D	
Cristaria dissecta Hook. & Arn.	Chile	II Region / Antofagasta	-22.67783	-68.49600	3100	J. Schneider & M. Huertas 2862 (FR, SGO)	2002	J. Schneider	D	
Cristaria dissecta Hook. & Arn.	Chile	II Region / Antofagasta	-23.24000	-68.00883	2700	J. Schneider & M. Huertas 2866 (FR, CONC, SGO)	2002	J. Schneider	D	
Cristaria dissecta Hook. & Arn.	Chile	II Region / Antofagasta	-23.37150	-67.99767	2700	J. Schneider & M. Huertas 2867 (FR, SGO)	2002	J. Schneider	D	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.43972	-70.80349	1050	Biese 2949 (SGO)	1949	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.98833	-70.53600	1000	J. Schneider & M. Huertas 2955 (FR, CONC, SGO)	2002	J. Schneider	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.73750	-70.55183	1650	J. Schneider & M. Huertas 2963 (FR, CONC, SGO)	2002	J. Schneider	A	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-18.16667	-69.75000	3650	Ricardi 3518 (CONC)	1955	Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-20.30689	-69.13297	3000	F. Luebert, T. Böhnert, F.F. Merklinger & A. Stoll 3622 (Bonn, FR, ULS)	2017	Collection trip 03-2017	D	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-31.71667	-71.20000		Schlegel 3823 (CONC)	1961	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	III Region / Atacama	-28.16667	-70.58333	550	Ricardi, Marticorena 3990 (CONC)	1956	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	III Region / Atacama	-28.79525	-70.39177		Zollner 6096 (CONC)	1972	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-31.58333	-71.53333		Montero 7257 (CONC)	1965	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	III Region / Atacama	-28.96667	-70.18333	1370	Marticorena 9561 (CONC)	1983	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	Region Metropolitana	-33.41667	-70.63333	740	Gunckel 26215 (CONC)	1950	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	V Region / Valparaiso	-32.85000	-70.96667		Ricardi, Marticorena 4193/578 (CONC)	1957	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.21929	-71.00864		Ricardi y Marticorena 4372/757 (CONC)	1957	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	III Region / Atacama	-28.81667	-70.80000		Ricardi, Marticorena 4452/837 (CONC)	1957	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.36667	-71.11667		Ricardi, Marticorena 4903/1288 (CONC)	1958	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	II Region / Antofagasta	-23.04772	-68.04556	3300	Zalensky XVII-970 (SGO)	1968	Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	Region Metropolitana	-33.20000	-70.60000	900	Behn (CONC)	1939	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	V Region / Valparaiso	-33.02366	-71.26354		Behn (CONC)	1916	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.94475	-71.18127		Collantes (CONC)	1948	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.60000	-71.20000		Collantes (CONC)	1948	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.20894	-70.49151	1000	F. Behn (CONC)	1948	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.75000	-71.31667		Garaventa (CONC)	1961	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-30.70000	-71.00000		Gay		Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-31.88333	-70.63333		Germain (SGO)	1894	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	Region Metropolitana	-33.33333	-70.68333		Mahu (CONC)	1969	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	V Region / Valparaiso	-32.84988	-70.57141		Mancilla (CONC)	1952	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	Region Metropolitana	-33.39638	-70.70732		Philippi (SGO)	1854	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	V Region / Valparaiso	-32.76968	-70.95157		Philippi (SGO)	1863	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	V Region / Valparaiso	-33.01062	-71.46507		Philippi (SGO)	1897	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	V Region / Valparaiso	-33.41667	-71.68333		Philippi (SGO)	1974	Muñoz-Schick 1995	A	

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.97393	-70.55614		Reiche (SGO)	1904	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	VI Region / O'Higgins	-34.83333	-70.55000		Ricardi (CONC)	1951	Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	IV Region / Coquimbo	-29.83300	-70.02200				Muñoz-Schick 1995	A	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-19.55551	-69.24616		Rahmer (SGO)	1885	Muñoz-Schick 1995	D	
Cristaria dissecta Hook. & Arn.	Chile	I Region / Tarapaca	-20.17715	-69.78238		Ricardi, Weldt, Quezada (CONC)	1972	Muñoz-Schick 1995	D	
Cristaria dissecta var. *glandulosa* (Phil.) Muñoz-Schick	Chile	III Region / Atacama	-27.06567	-69.33555	3300	J. Schneider & M. Huertas 2935 (FR)	2002	J. Schneider	A	
Cristaria dissecta var. *glandulosa* (Phil.) Muñoz-Schick	Chile	III Region / Atacama	-27.06133	-69.35183	3150	J. Schneider & M. Huertas 2936 (FR)	2002	J. Schneider	A	
Cristaria dissecta var. *glandulosa* (Phil.) Muñoz-Schick	Chile	IV Region / Coquimbo	-29.82384	-70.01441		Volckmann (SGO)		Muñoz-Schick 1995	A	
Cristaria dissecta var. *glandulosa* (Phil.) Muñoz-Schick	Chile	III Region / Atacama	-27.06567	-69.33467	3300	J. Schneider & M. Huertas 2935 (FR, CONC)	2002	J. Schneider	A	ED3455
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.81833	-71.28829	10	F. Luebert, A. Stoll & T. Böhnert 3268 (Bonn, FR, ULS)	2016	Collection trip 10-2016	E	ED1401
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.93453	-71.27660	10	F. Luebert & T. Böhnert 3721 (Bonn, FR, ULS)	2017	Collection trip 03-2017	E	ED1603
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.97322	-71.30319	80	F. Luebert & T. Böhnert 3724 (Bonn, FR, ULS)	2017	Collection trip 03-2017	B	ED1604
Cristaria glaucophylla Cav.	Chile	V Region / Valparaiso	-32.23373	-71.50752	15	F. Luebert, T. Böhnert & F. Merklinger 3755 (Bonn, FR, ULS)	2017	Collection trip 10-2017	E	ED2984
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-30.73724	-71.69991	25	F. Luebert, T. Böhnert & F. Merklinger 3793 (Bonn, FR, ULS)	2017	Collection trip 10-2017	E	ED2986
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.82583	-71.26692	5	F. Luebert & T. Böhnert 3710 (Bonn, FR, ULS)	2017	Collection trip 03-2017	E	ED3104
Cristaria glaucophylla Cav.	Chile	V Region / Valparaiso	-32.33333	-71.30000		Marticorena, Matthei y Rodríguez 136 (CONC)	1974	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	V Region / Valparaiso	-32.33530	-71.42663		Köhler 201 (CONC)	1965	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-30.75006	-71.70223		C. Muñoz y Coronel 1415 (SGO)	1940	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.44953	-71.27197		Barros 1418 (CONC)	1926	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	III Region / Atacama	-28.95000	-71.26667	300	Marticorena, Rodríguez, Weldt 1783 (CONC)	1971	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	III Region / Atacama	-29.10713	-71.45086		C. Muñoz y Johnson 1992 (SGO)	1941	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-31.75307	-71.50718		M. Muñoz 2525 (SGO)	1990	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	III Region / Atacama	-28.36173	-71.15906	5	J. Schneider & M. Huertas 2949 (FR, CONC, SGO)	2002	J. Schneider	E	
Cristaria glaucophylla Cav.	Chile	III Region / Atacama	-28.36171	-71.15904	5	J. Schneider & M. Huertas 2951 (FR, CONC, SGO)	2002	J. Schneider	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-30.30000	-71.50000		Schlegel 4902 (CONC)	1963	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-31.75000	-71.50000		F. Schlegel 5256 (CONC)	1960	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.79844	-71.30089		Zöllner 6914 (CONC)	1973	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.83333	-71.23333		Montero 11054 (CONC)	1978	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.90000	-71.23333		Montero 12039 (CONC)	1981	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	V Region / Valparaiso	-32.76667	-71.53333		Gunckel 23750 (CONC)	1952	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	V Region / Valparaiso	-32.73333	-71.41667		Gunckel 38011 (CONC)	1962	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-30.01667	-71.36667		Kalin 81233 (CONC)	1981	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.83333	-71.26667		Ricardi y Marticorena 4925/1310 (CONC)	1958	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-29.45000	-71.25000		H. Behn (CONC)	1938	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	V Region / Valparaiso	-32.55000	-71.46667		K. Behn. (CONC)	1917	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-30.25000	-71.50000		P. Muñoz (CONC)	1971	Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	III Region / Atacama	-28.42911	-71.16665		Philippi (SGO)		Muñoz-Schick 1995	E	
Cristaria glaucophylla Cav.	Chile	IV Region / Coquimbo	-31.91667	-71.50000		Roesner (CONC)	1946	Muñoz-Schick 1995	E	
Cristaria glaucophylla var. *eriantha* (Hook. & Arn.) Muñoz-Schick	Chile	IV Region / Coquimbo	-30.21500	-71.43112	30	J. Schneider & M. Huertas 2960 (FR)	2002	J. Schneider	E	
Cristaria glaucophylla var. *eriantha* (Hook. & Arn.) Muñoz-Schick	Chile	IV Region / Coquimbo	-30.26167	-71.49112	30	J. Schneider & M. Huertas 2961 (FR)	2002	J. Schneider	B	

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria glaucophylla var. *eriantha* (Hook. & Arn.) Muñoz-Schick	Chile	V Region / Valparaiso	-32.92203	-71.51619		K. Behn. (CONC)	1923	Muñoz-Schick 1995	E	
Cristaria glaucophylla var. *eriantha* (Hook. & Arn.) Muñoz-Schick	Chile	IV Region / Coquimbo	-30.26167	-71.47976	30	J. Schneider & M. Huertas 2962 (FR, CONC, SGO)	2002	J. Schneider	B	ED3456
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.88015	-70.21933	1550	F. Luebert, T. Böhnert & F. Merklinger 3878 (Bonn, FR, ULS)	2017	Collection trip 10-2017	C	ED2997
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-23.78698	-68.16458	2200	F. Luebert & T. Böhnert 3659 (Bonn, FR, ULS)	2017	Collection trip 03-2017	C	ED3100
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-22.67836	-68.49263	3100	J. Schneider & M. Huertas 2861 (FR, SGO)	2002	J. Schneider	C	ED3458
Cristaria gracilis Gay	Chile	IV Region / Coquimbo	-29.14950	-70.82867	1200	J. Schneider & M. Huertas 2952 (FR, CONC, SGO)	2002	J. Schneider	C	ED3459
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.41585	-70.27002	500	J. Schneider & M. Huertas 2909 (FR, CONC, SGO)	2002	J. Schneider	C	ED3460
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-22.29073	-69.08761	3200	Ricardi, Weldt, Quezada 340 (CONC)	1972	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-22.81494	-68.34550	3100	Ricardi, Weldt, Quezada 398 (CONC)	1972	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-26.93204	-69.54992	1200	Ricardi, Marticorena, Matthei 514 (CONC)	1963	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-28.66667	-70.76667	580	Marticorena, Matthei Quezada 612 (CONC)	1973	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.80000	-70.21667	1200	Meza. Barrera 1737 (SGO)	1991	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.28333	-70.21667	800	Meza. Barrera 1777 (SGO)	1991	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-28.28320	-70.71235		M. Muñoz 1840 (SGO)	1983	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-28.14549	-70.84322		C. Muñoz y Johnson 1962 (SGO)	1941	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-28.61088	-70.76777	600	Jiles 2203 (CONC)	1952	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-25.54722	-70.30741		M. Muñoz, Meza 2273 (SGO)	1987	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-26.41449	-70.02480	1800	Muñoz. Teillier. Meza 2738 (SGO)	1991	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-22.67783	-68.49600	3100	J. Schneider & M. Huertas 2861 (FR)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-22.78200	-68.35300	3400	J. Schneider & M. Huertas 2863 (FR, CONC, SGO)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-23.78767	-68.16333	2750	J. Schneider & M. Huertas 2864 (FR, CONC, SGO)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.41583	-70.27050	500	J. Schneider & M. Huertas 2908 (FR, CONC, SGO)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.41583	-70.27305	500	J. Schneider & M. Huertas 2909 (FR)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.12133	-69.27050	1750	J. Schneider & M. Huertas 2910 (FR, CONC, SGO)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-26.88150	-69.92983	2000	J. Schneider & M. Huertas 2911 (FR, CONC, SGO)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.32250	-70.17183	700	J. Schneider & M. Huertas 2937 (FR, CONC)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	IV Region / Coquimbo	-29.14950	-70.82867	1200	J. Schneider & M. Huertas 2952 (FR)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	IV Region / Coquimbo	-29.13333	-70.81667	1200	J. Schneider & M. Huertas 2953 (FR, CONC, SGO)	2002	J. Schneider	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-28.04387	-69.97158	1800	Zollner 4564 (CONC)	1970	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-29.10000	-70.93333	970	Dillon. Teillier 5016 (SGO)	1987	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-25.42376	-70.23579		Dillon, Dillon 5804 (SGO)	1988	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-28.95372	-70.90050		Zöllner 9077 (CONC)		Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-28.15047	-70.91983		Ricardi. Marticorena 4416/801 (CONC)	1957	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.55000	-70.46667		Köhler 534-A (CONC)	1966	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.11667	-69.91667		Hermosilla (SGO)	1963	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-28.25000	-70.51667		Martínez, DC. (CONC)		Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-26.74749	-69.87944	1600	Pfister (CONC)	1950	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-24.96667	-69.56667		Philippi (SGO)		Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.81422	-70.41788		Philippi (SGO)	1885	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	III Region / Atacama	-27.99684	-70.42847		Philippi (SGO)	1885	Muñoz-Schick 1995	C	
Cristaria gracilis Gay	Chile	II Region / Antofagasta	-22.70435	-68.42737		Reiche (SGO)	1901	Muñoz-Schick 1995	C	
Cristaria integerrima Phil.	Chile	III Region / Atacama	-28.11200	-71.11614	45	F. Luebert, A. Stoll & T. Böhnert 3278 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1405
Cristaria integerrima Phil.	Chile	III Region / Atacama	-28.11200	-71.11614	45	F. Luebert, A. Stoll & T. Böhnert 3280 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1406
Cristaria integerrima Phil.	Chile	III Region / Atacama	-26.35380	-70.46948	270	F. Luebert, A. Stoll & T. Böhnert 3303 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1412
Cristaria integerrima Phil.	Chile	III Region / Atacama	-26.03254	-70.43582	470	F. Luebert, A. Stoll & T. Böhnert 3306 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1413

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.89592	-70.58105	500	F. Luebert, A. Stoll & T. Böhnert 3310 (Bonn, ULS)	2016	Collection trip 10-2016	K	ED1414
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.90060	-70.61125	350	F. Luebert, A. Stoll & T. Böhnert 3313 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1415
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.89312	-70.64418	250	F. Luebert, A. Stoll & T. Böhnert 3315 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1416
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.89312	-70.64418	250	F. Luebert, A. Stoll & T. Böhnert 3316 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1417
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.89312	-70.64418	250	F. Luebert, A. Stoll & T. Böhnert 3317 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1418
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.46132	-70.44165	340	F. Luebert, A. Stoll & T. Böhnert 3330 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1422
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.48571	-70.36292	740	F. Luebert, A. Stoll & T. Böhnert 3333 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1424
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.50246	-70.41176	580	F. Luebert, A. Stoll & T. Böhnert 3334 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1425
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.50205	-70.41431	550	F. Luebert, A. Stoll & T. Böhnert 3335 (Bonn, ULS)	2016	Collection trip 10-2016	K	ED1426
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.50211	-70.41336	550	F. Luebert, A. Stoll & T. Böhnert 3336 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1427
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.38526	-70.43665	150	F. Luebert, A. Stoll & T. Böhnert 3350 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1428
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.01240	-70.44647	550	F. Luebert, A. Stoll & T. Böhnert 3358 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1429
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.01240	-70.44647	550	F. Luebert, A. Stoll & T. Böhnert 3359 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1430
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.39356	-70.47090	70	F. Luebert, A. Stoll & T. Böhnert 3364 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1431
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.26618	-70.43738	60	F. Luebert, A. Stoll & T. Böhnert 3366 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1432
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.26618	-70.43738	60	F. Luebert, A. Stoll & T. Böhnert 3367 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1433
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.26618	-70.43738	60	F. Luebert, A. Stoll & T. Böhnert 3368 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1434
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.98810	-70.45839	125	F. Luebert, A. Stoll & T. Böhnert 3375 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1436
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.74126	-70.55749	30	F. Luebert, A. Stoll & T. Böhnert 3388A (Bonn, FR, ULS)	2016	Collection trip 10-2016	H	ED1438
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.51074	-70.55319	100	F. Luebert, A. Stoll & T. Böhnert 3398 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1441
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-23.77639	-70.45578	120	F. Luebert, A. Stoll & T. Böhnert 3403 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1443
Cristaria integerrima Phil.	Chile	III Region / Atacama	-26.75723	-70.73282	20	F. Luebert, A. Stoll & T. Böhnert 3297 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1448
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.74126	-70.55749	30	F. Luebert, A. Stoll & T. Böhnert 3388 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	ED1449
Cristaria integerrima Phil.	Chile	III Region / Atacama	-26.29672	-70.63125	75-600	F. Luebert, T. Böhnert & F. Merklinger 3908 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3010
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-23.87796	-70.49650	180	F. Luebert, T. Böhnert & F. Merklinger 3982 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3029
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-22.42846	-70.21825	680	F. Luebert, T. Böhnert & F. Merklinger 3990 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3030
Cristaria integerrima Phil.	Chile	III Region / Atacama	-28.13333	-71.08333		Ricardi 2298 (CONC)	1952	Muñoz-Schick 1995	K	
Cristaria integerrima Phil.	Chile	III Region / Atacama	-26.43724	-70.23928		Muñoz, Teillier, Meza 2782 (SGO)	1991	Muñoz-Schick 1995	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.25217	-70.52100	100	J. Schneider & M. Huertas 2869 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.01633	-70.44486	700	J. Schneider & M. Huertas 2871 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.38883	-70.46817	15	J. Schneider & M. Huertas 2873 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.38885	-70.46819	15	J. Schneider & M. Huertas 2874 (FR, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.38887	-70.46821	15	J. Schneider & M. Huertas 2877 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.26533	-70.43683	40	J. Schneider & M. Huertas 2879 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.26531	-70.43685	40	J. Schneider & M. Huertas 2881 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.73700	-70.56600	50	J. Schneider & M. Huertas 2884 (FR)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.73742	-70.56513	50	J. Schneider & M. Huertas 2885 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.97446	-70.46942	50	J. Schneider & M. Huertas 2892 (FR, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.01133	-70.46067	150	J. Schneider & M. Huertas 2893 (FR)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.51050	-70.41833	650	J. Schneider & M. Huertas 2897 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	III Region / Atacama	-26.28395	-70.65942	20	J. Schneider & M. Huertas 2899 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.92200	-70.47017	750	J. Schneider & M. Huertas 2900 (FR, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.90302	-70.63819	300	J. Schneider & M. Huertas 2902 (FR, CONC, SGO)	2002	J. Schneider	K	
Cristaria integerrima Phil.	Chile	III Region / Atacama	-26.76606	-70.75465	50	J. Schneider & M. Huertas 2925 (FR, SGO)	2002	J. Schneider	K	

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.01583	-70.43817	550	J. Schneider & M. Huertas 3021 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.02400	-70.45183	350	J. Schneider & M. Huertas 3022 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.00133	-70.46567	10	J. Schneider & M. Huertas 3024 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.95686	-70.47438	300	J. Schneider & M. Huertas 3027 (FR)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.74657	-70.54861	15	J. Schneider & M. Huertas 3035 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.74688	-70.54981	15	J. Schneider & M. Huertas 3036 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.86250	-70.51717		J. Schneider & M. Huertas 3042 (FR)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.73828	-70.56395		J. Schneider & M. Huertas 3044 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.63083	-70.55533		J. Schneider & M. Huertas 3045 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-24.67750	-70.56483		J. Schneider & M. Huertas 3046 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.90000	-70.63333	300	J. Schneider & M. Huertas 3051 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.90005	-70.63335	300	J. Schneider & M. Huertas 3053 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.37117	-70.44700	10	J. Schneider & M. Huertas 3054 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.13150	-70.45700	125	J. Schneider & M. Huertas 3055 (FR, SGO)	2003	J. Schneider	K	
Cristaria integerrima Phil.	Chile	II Region / Antofagasta	-25.50473	-70.40148	615	F. Luebert, A. Stoll & T. Böhnert 3331 (Bonn, FR, ULS)	2016	Collection trip 10-2016	K	
Cristaria integerrima Phil.	Chile	III Region / Atacama	-26.45000	-70.68333		Dillon, Teillier 5053 (SGO)	1987	Muñoz-Schick 1995	K	
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	Chile	II Region / Antofagasta	-24.74719	-70.55101		J. Schneider & M. Huertas 3048 (FR)	2003	J. Schneider	K	
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	Chile	II Region / Antofagasta	-24.74722	-70.55102		J. Schneider & M. Huertas 3049 (FR)	2003	J. Schneider	K	
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	Chile	II Region / Antofagasta	-25.90010	-70.63337	300	J. Schneider & M. Huertas 3052 (FR)	2003	J. Schneider	K	
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	Chile	II Region / Antofagasta	-25.89382	-70.64825	220	F. Luebert, T. Böhnert & F. Merklinger 3917 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3014
Cristaria integerrima var. *lobulata* (Phil.) Muñoz-Schick	Chile	II Region / Antofagasta	-25.90304	-70.63821	300	J. Schneider & M. Huertas 2901 (FR, CONC, SGO)	2002	J. Schneider	K	ED3461
Cristaria leucantha I.M. Johnst.	Chile	II Region / Antofagasta	-24.74172	-70.54269	210	F. Luebert, T. Böhnert & F. Merklinger 3947 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3020
Cristaria leucantha I.M. Johnst.	Chile	II Region / Antofagasta	-24.74172	-70.54269	210	F. Luebert, T. Böhnert & F. Merklinger 3948 (Bonn, FR, ULS)	2017	Collection trip 10-2017	K	ED3021
Cristaria molinae Gay	Chile	II Region / Antofagasta	-24.98810	-70.45839	125	F. Luebert, A. Stoll & T. Böhnert 3374 (Bonn, FR, ULS)	2016	Collection trip 10-2016	J	ED1435
Cristaria molinae Gay	Chile	III Region / Atacama	-26.96325	-70.74014	210	F. Luebert, T. Böhnert & F. Merklinger 3896 (Bonn, FR, ULS)	2017	Collection trip 10-2017	J	ED3005
Cristaria molinae Gay	Chile	III Region / Atacama	-26.29672	-70.63125	75-600	F. Luebert, T. Böhnert & F. Merklinger 3909 (Bonn, FR, ULS)	2017	Collection trip 10-2017	J	ED3011
Cristaria molinae Gay	Chile	III Region / Atacama	-26.29672	-70.63125	75-600	F. Luebert, T. Böhnert & F. Merklinger 3911 (Bonn, FR, ULS)	2017	Collection trip 10-2017	J	ED3012
Cristaria molinae Gay	Chile	II Region / Antofagasta	-25.00660	-70.44997	710	F. Luebert, T. Böhnert & F. Merklinger 3930 (Bonn, FR, ULS)	2017	Collection trip 10-2017	J	ED3016
Cristaria molinae Gay	Chile	II Region / Antofagasta	-24.74954	-70.55518	30	F. Luebert, T. Böhnert & F. Merklinger 3946 (Bonn, FR, ULS)	2017	Collection trip 10-2017	J	ED3019
Cristaria molinae Gay	Chile	II Region / Antofagasta	-23.82627	-70.48677	460	F. Luebert, T. Böhnert & F. Merklinger 3978 (Bonn, FR, ULS)	2017	Collection trip 10-2017	J	ED3028
Cristaria molinae Gay	Chile	I Region / Tarapaca	-20.36400	-70.16127	200	J. Schneider & M. Huertas 2838 (FR, CONC, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	I Region / Tarapaca	-20.81383	-70.17117	700	J. Schneider & M. Huertas 2839 (FR)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	I Region / Tarapaca	-21.03888	-70.15929	300	J. Schneider & M. Huertas 2845 (FR, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	I Region / Tarapaca	-21.27767	-70.07217	190	J. Schneider & M. Huertas 2847 (FR, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-22.37117	-70.22883	225	J. Schneider & M. Huertas 2849 (FR, CONC, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-22.37109	-70.22913	225	J. Schneider & M. Huertas 2850 (FR, CONC, SGO)	2002	J. Schneider	J	

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria molinae Gay	Chile	II Region / Antofagasta	-22.37113	-70.22853	225	J. Schneider & M. Huertas 2854 (FR)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-22.37129	-70.22870	225	J. Schneider & M. Huertas 2855 (FR, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-22.58783	-70.24967	150	J. Schneider & M. Huertas 2856 (FR, CONC, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-22.58759	-70.25030	150	J. Schneider & M. Huertas 2857 (FR, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-25.38889	-70.46823	15	J. Schneider & M. Huertas 2876 (FR, CONC, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-25.26529	-70.43687	40	J. Schneider & M. Huertas 2880 (FR, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-24.55433	-70.56600	75	J. Schneider & M. Huertas 2887 (FR, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-24.55449	-70.56561	75	J. Schneider & M. Huertas 2889 (FR, CONC, SGO)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-25.19167	-70.43550	75	J. Schneider & M. Huertas 2895 (FR)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-25.90306	-70.63823	300	J. Schneider & M. Huertas 2904 (FR)	2002	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-24.95686	-70.47300	165	J. Schneider & M. Huertas 3034 (FR, SGO)	2003	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-24.74725	-70.55103	60	J. Schneider & M. Huertas 3037 (FR, SGO)	2003	J. Schneider	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-23.53647	-70.36055	450	F. Luebert, A. Stoll & T. Böhnert 3407 (Bonn, ULS)	2016	Collection trip 10-2016	J	
Cristaria molinae Gay	Chile	III Region / Atacama	-26.29672	-70.63125	75-600	F. Luebert, T. Böhnert & F. Merklinger 3904 (Bonn, FR, ULS)	2017	Collection trip 10-2017	J	
Cristaria molinae Gay	Chile	II Region / Antofagasta	-24.95550	-70.48050	175	J. Schneider & M. Huertas 3058a (FR)	2003	J. Schneider	J	
Cristaria multifida Cav.	Peru	Arequipa	-17.02900	-71.99844	30	J. Schneider, M. Chanco & M.L. Huertas 2798 (FR, USM)	2002	J. Schneider	D	ED3464
Cristaria multifida Cav.	Peru	Tacna	-17.86450	-71.00833	50	J. Schneider, M. Chanco, C. Cáceres & M.L. Huertas 2790 (FR, USM)	2002	J. Schneider	D	ED3465
Cristaria multifida Cav.	Peru	Tacna	-18.02520	-70.83846	60	J. Schneider, M. Chanco, C. Cáceres & M.L. Huertas 2776 (FR, USM)	2002	J. Schneider	D	ED3466
Cristaria multifida Cav.	Peru	Arequipa	-15.91608	-72.54997	1388	M. Weigend *et al.* 9866 (USM, HUSA, BONN)	2019	M. Weigend *et al.*, 2019	D	W5632
Cristaria multifida Cav.	Peru	Arequipa	-16.45597	-71.61908	2177	M. Weigend *et al.* 9885 (USM, HUSA, BONN)	2019	M. Weigend *et al.*, 2019	D	W5633
Cristaria multifida Cav.	Peru	Moquegua	-16.79986	-70.90539	1663	M. Weigend *et al.* 9980 (USM, HUSA, BONN)	2019	M. Weigend *et al.*, 2019	D	W5634
Cristaria multifida Cav.	Peru	Moquegua	-16.84386	-70.89458	2401	M. Weigend *et al.* 9978 (USM, HUSA, BONN)	2019	M. Weigend *et al.*, 2019	D	W5635
Cristaria multifida Cav.	Peru	Lima	-12.11667	-76.96667	125	J. Schneider & M. Huertas 2738 (USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Lima	-12.11667	-76.96667	150	J. Schneider & M. Huertas 2739 (FR)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Lima	-12.12515	-76.96410	125	J. Schneider & M. Huertas 2740 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Lima	-11.31667	-77.45000	100	J. Schneider & M. Huertas 2743 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Lima	-11.35827	-77.41327	100	J. Schneider & M. Huertas 2749 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Lima	-12.43365	-76.74922	150	J. Schneider & M. Huertas 2757 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Tacna	-18.02683	-70.83400	60	J. Schneider & M. Huertas 2775 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Arequipa	-17.08883	-71.89833	75	J. Schneider, M. Chanco, C. Cáceres & M.L. Huertas 2791 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Arequipa	-17.02900	-72.00050	30	J. Schneider, M. Chanco & M.L. Huertas 2797 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Arequipa	-17.01433	-72.00717	75	J. Schneider, M. Chanco & M.L. Huertas 2801 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Moquegua	-17.70250	-71.27817	100	J. Schneider, M. Chanco & M.L. Huertas 2806 (USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Moquegua	-17.70735	-71.26479	100	J. Schneider, M. Chanco & M.L. Huertas 2815 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Tacna	-17.79183	-70.60015	800	J. Schneider, M. Chanco & M.L. Huertas 2819 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Tacna	-17.85017	-70.95050	150	J. Schneider, M. Chanco & M.L. Huertas 2826 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Tacna	-17.79477	-70.59844	800	J. Schneider, M. Chanco & M.L. Huertas 2831 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Tacna	-17.81367	-70.57567	650	J. Schneider & M. Huertas 2972 (FR, USM)	2002	J. Schneider	D	

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria multifida Cav.	Peru	Arequipa	-15.30318	-74.78882	450	J. Schneider & M. Huertas 2979 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Arequipa	-16.64278	-72.63111	250	J. Schneider & M. Huertas 2982 (FR, USM)	2002	J. Schneider	D	
Cristaria multifida Cav.	Peru	Arequipa	-16.55000	-72.61667	600	J. Schneider & M. Huertas 2991 (FR, USM)	2003	J. Schneider	D	
Cristaria multifida Cav.	Peru	Arequipa	-16.55132	-72.63727	600	J. Schneider & M. Huertas 2996 (FR, USM)	2003	J. Schneider	D	
Cristaria multifida Cav.	Peru	Arequipa	-15.91608	-72.54997	1388	M. Weigend *et al.* 9866 (USM, HUSA, BONN)	2019	M. Weigend *et al.*, 2019	D	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-30.01501	-70.52006	950	F. Luebert, T. Böhnert & F. Merklinger 3817 (Bonn, FR, ULS)	2017	Collection trip 10-2017	B	ED2988
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-30.02440	-70.51820	980	F. Luebert & T. Böhnert 3718 (Bonn, FR, ULS)	2017	Collection trip 03-2017	B	ED3107
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-29.98795	-70.53693	890	F. Luebert & T. Böhnert 3719 (Bonn, FR, ULS)	2017	Collection trip 03-2017	B	ED3108
Cristaria multiflora Gay	Chile	III Region / Atacama	-27.78619	-70.15279		M. Muñoz 3105 (SGO)	1992	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	III Region / Atacama	-28.72164	-70.52388		M. Muñoz 3124 (SGO)	1992	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-30.74076	-70.58119		Jiles 4777 (CONC)	1967	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-30.03993	-70.50641		Montero 11666 (CONC)	1980	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-30.00023	-70.52827		F. Behn (CONC)	1948	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-29.51195	-71.20190		Fonck (SGO)	1886	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-30.86280	-70.77901		Geisse (SGO)	1889	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-30.06147	-70.81325		Mahu (SGO)	1984	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	III Region / Atacama	-28.82369	-70.25861		Niemeyer (CONC)	1965	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	V Region / Valparaiso	-32.73070	-70.68287		Philippi (SGO)	1863	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	V Region / Valparaiso	-32.46955	-70.57107		Philippi (SGO)	1861	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-29.84903	-70.38119		Reiche (SGO)	1904	Muñoz-Schick 1995	B	
Cristaria multiflora Gay	Chile	IV Region / Coquimbo	-29.96206	-70.97008		(SGO)	1897	Muñoz-Schick 1995	B	
Cristaria ovata Muñoz-Schick	Chile	II Region / Antofagasta	-24.85517	-70.52467	30	J. Schneider & M. Huertas 2882 (FR, CONC, SGO)	2002	J. Schneider	I	
Cristaria ovata Muñoz-Schick	Chile	II Region / Antofagasta	-24.83435	-70.53830	50	J. Schneider & M. Huertas 2891 (FR, SGO)	2002	J. Schneider	I	
Cristaria ovata Muñoz-Schick	Chile	III Region / Atacama	-26.75366	-70.73645	3	F. Luebert, A. Stoll & T. Böhnert 3302 (Bonn, FR, ULS)	2016	Collection trip 10-2016	I	
Cristaria ovata Muñoz-Schick	Chile	III Region / Atacama	-26.75366	-70.73645	3	F. Luebert, A. Stoll & T. Böhnert 3300 (Bonn, FR, ULS)	2016	Collection trip 10-2016	I	ED1450
Cristaria ovata Muñoz-Schick	Chile	III Region / Atacama	-26.75366	-70.73645	3	F. Luebert, A. Stoll & T. Böhnert 3301 (Bonn, FR, ULS)	2016	Collection trip 10-2016	I	ED1824
Cristaria ovata Muñoz-Schick	Chile	III Region / Atacama	-26.59367	-70.69200	30	J. Schneider & M. Huertas 2905 (FR, CONC, SGO)	2002	J. Schneider	I	ED3467
Cristaria ovata Muñoz-Schick	Chile	II Region / Antofagasta	-24.83433	-70.53833		J. Schneider & M. Huertas 3041 (FR, SGO)	2003	J. Schneider	I	ED3468
Cristaria tenuissima Muñoz-Schick	Chile	II Region / Antofagasta	-24.51383	-70.55914	300	Hoffmann, Rodriguez 157 (CONC)	1987	Muñoz-Schick 1995	H	
Cristaria tenuissima Muñoz-Schick	Chile	II Region / Antofagasta	-24.43852	-70.53955		Biese 320 (SGO)	1949	Muñoz-Schick 1995	H	
Cristaria tenuissima Muñoz-Schick	Chile	II Region / Antofagasta	-24.55468	-70.56548	225	J. Schneider & M. Huertas 3050 (FR)	2003	J. Schneider	H	
Cristaria tenuissima Muñoz-Schick	Chile	II Region / Antofagasta	-24.56786	-70.55511	225	Dillon, Teillier 5283 (SGO)	1987	Muñoz-Schick 1995	H	
Cristaria tenuissima Muñoz-Schick	Chile	II Region / Antofagasta	-24.55579	-70.55433	60	F. Luebert, T. Böhnert & F. Merklinger 3958 (Bonn, FR, ULS)	2017	Collection trip 10-2017	H	ED3023
Cristaria tenuissima Muñoz-Schick	Chile	II Region / Antofagasta	-24.55445	-70.56590	75	J. Schneider & M. Huertas 2890 (FR, CONC, SGO)	2002	J. Schneider	H	ED3469
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-28.19174	-71.15803	15	F. Luebert, A. Stoll & T. Böhnert 3273 (Bonn, FR, ULS)	2016	Collection trip 10-2016	I	ED1403
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-27.05010	-70.79995	20	F. Luebert, A. Stoll & T. Böhnert 3289 (Bonn, FR, ULS)	2016	Collection trip 10-2016	I	ED1408
Cristaria viridiluteola Gay	Chile	IV Region / Coquimbo	-29.28671	-71.38031	5	F. Luebert, T. Böhnert & F. Merklinger 3840 (Bonn, FR, ULS)	2017	Collection trip 10-2017	E	ED2992
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-27.10080	-70.67368	340	F. Luebert, T. Böhnert & F. Merklinger 3887 (Bonn, FR, ULS)	2017	Collection trip 10-2017	I	ED3002
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-27.34937	-70.68121	250	J. Schneider & M. Huertas 2922 (FR, CONC, SGO)	2002	J. Schneider	I	ED3470
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-26.59365	-70.69205	30	J. Schneider & M. Huertas 2906 (FR, CONC, SGO)	2002	J. Schneider	I	ED3472
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-26.28702	-70.66011	20	J. Schneider & M. Huertas 2898 (FR, CONC, SGO)	2002	J. Schneider	I	
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-27.58343	-70.45010	650	J. Schneider & M. Huertas 2939 (FR, SGO)	2002	J. Schneider	I	
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-28.35662	-71.15827	25	F. Luebert, A. Stoll & T. Böhnert 3270 (Bonn, FR, ULS)	2016	Collection trip 10-2016	I	
Cristaria viridiluteola Gay	Chile	III Region / Atacama	-26.47569	-70.68178		Dillon, Dillon, Poblete 5493 (SGO)	1988	Muñoz-Schick 1995	I	

Scientific name	Country	Region	latitude	long.	elev.	voucher	year	source specimen	clade	phyl.
Cristaria viridiluteola Phil.	Chile	II Region / Antofagasta	-24.40031	-70.54031	110	F. Luebert, A. Stoll & T. Böhnert 3399 (Bonn, FR, ULS)	2016	Collection trip 10-2016	I	ED1442
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	Chile	III Region / Atacama	-27.11150	-70.85050	5	J. Schneider & M. Huertas 2931 (FR, CONC, SGO)	2002	J. Schneider	I	
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	Chile	III Region / Atacama	-28.36177	-71.15910	5	J. Schneider & M. Huertas 2950 (FR, CONC, SGO)	2002	J. Schneider	I	
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	Chile	III Region / Atacama	-26.75723	-70.73282	20	F. Luebert, A. Stoll & T. Böhnert 3298 (Bonn, ULS)	2016	Collection trip 10-2016	K	ED1409
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	Chile	II Region / Antofagasta	-24.54666	-70.57518	50	F. Luebert, A. Stoll & T. Böhnert 3395 (Bonn, FR, ULS)	2016	Collection trip 10-2016	H	ED1439
Cristaria viridiluteola var. *pinnata* (Phil.) Muñoz-Schick	Chile	II Region / Antofagasta	-24.54666	-70.57518	50	F. Luebert, A. Stoll & T. Böhnert 3396 (Bonn, FR, ULS)	2016	Collection trip 10-2016	H	ED1440

Table S3: BioGeoBEARS species distribution matrix for *Cristaria*. Comprising of 11 samples and 6 geographical categories.

Species	A	B	C	D	E	F
Cristaria_andicola	0	0	1	1	1	0
Cristaria_multiflora	0	0	0	1	1	0
Cristaria_gracilis	0	1	1	1	0	0
Cristaria_multifida	1	1	0	0	0	0
Cristaria_glaucophylla	0	0	0	0	1	0
Cristaria_argyliifolia	0	0	0	1	0	1
Cristaria_cyanea	0	0	0	1	0	0
Cristaria_concinna	0	0	0	0	0	1
Cristaria_viridiluteola	0	0	0	1	0	1
Cristaria_molinae	0	0	0	0	0	1
Cristaria_integerrima	0	0	0	1	0	1

References

Böhnert T, Luebert F, Ritter B, Merklinger FF, Stoll A, Schneider JV, Quandt D & Weigend M (2019) Origin and diversification of *Cristaria* (Malvaceae) parallel Andean orogeny and onset of hyperaridity in the Atacama Desert. *Global and Planetary Change* 181: 1–8. DOI: https://doi.org/10.1016/j.gloplacha.2019.102992.

Muñoz-Schick M (1995) Revision del género *Cristaria* (Malvaceae) en Chile. *Boletín del Museo Nacional de Historia Natural Chile* 45: 45–110.

Sanderson MJ (2002) Estimating Absolute Rates of Molecular Evolution and Divergence Times: A Penalized Likelihood Approach. *Molecular Biology and Evolution* 19 (1): 101–109. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a003974.

GBS Assembly & Phylogeny of the genus *Cristaria*

Here a denovo assembly for an empirical Genotype-By-Sequencing (GBS) data set of the genus *Cristaria* Cav. (Malvaceae) using the ipyrad Python API is documented. The assembly subsequent phylogenetic reconstruction was conducted in a Linux environment (Ubuntu 18.04) on a computer with an Intel Core i7 CPU and 32 GB RAM.

For this study we used 117 samples of *Cristaria* incl. two of the sister genus *Lecanophora* Speg. as outgroup. This data set is composed of double digested single-end 100 bp reads from a GBS library prepared with the *Pst*I-*HF* and *Msp*I enzymes. After the Assembly of the data set we make use of the ipyrad analysis tools to run downstream phylogenetic analyses. Unique to this data set is the fact that material of most of the published taxonomic names (accepted names and synonyms mostly from type location) were available in order to understand evolutionary and systematic history of the genus *Cristaria*. A second Notebook provides code and output of a STRUCTURE analysis for the species *Cristaria integerrima*.

Setup (software and parallelization)

[1]:

```
## conda install ipyrad -c ipyrad
## conda install toytree -c eaton-lab
## conda install sra-tools -c bioconda
## conda install entrez-direct -c bioconda
```

[1]:

```
## 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. 0.9.15
toytree v. 0.2.3
```

Parallel processes on independent Python kernels

To start a parallel client you must run the command-line program ‘ipcluster’. This will essentially start a number of independent Python processes (kernels) which we can then send bits of work to do. The cluster can be stopped and restarted independently of this notebook, which is convenient for working on a cluster where connecting to many cores is not always immediately available.

Open a terminal and type the following command to start an ipcluster instance with N engines.

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

```
[2]: ## connect to cluster
     ipyclient = ipp.Client()
     print("{} engines found".format(len(ipyclient)))
```

```
8 engines found
```

Data Assembly

Create an Assembly object and set parameters in *ipyRAD* params file

This object stores the parameters of the assembly and the organization of data files.

```
[4]: ## you must provide a name for the Assembly
     data = ip.Assembly("Cristaria")
```

```
New Assembly: Cristaria
```

```
[5]: ## set parameters
     data.set_params("project_dir", "Cris_Assembly")
     data.set_params("sorted_fastq_path", "../GBS_Cris_Leca/*.fastq.gz")
     data.set_params("clust_threshold", "0.85")
     data.set_params("max_Hs_consens", (0.05))
     data.set_params("restriction_overhang", ('TGCAG', 'GGCC'))
     data.set_params("output_formats", "*")
     data.set_params("datatype", "ddrad")

     ## see / print all parameters
     data.get_params()
```

```
[5]: 0   assembly_name              Cristaria
     1   project_dir                ./Cris_Assembly
     2   raw_fastq_path
     3   barcodes_path
     4   sorted_fastq_path          ~/BioInf/Cristaria_GBS/GBS_Cris_Leca/*.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        6
12  mindepth_majrule            6
13  maxdepth                    10000
14  clust_threshold             0.85
15  max_barcode_mismatch        0
16  filter_adapters             0
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           4
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
```

Assembly GBS data

First steps 1 & 2

Second steps 3-6

Third step 7

[6]:

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

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:01:02 | loading reads        | s1 |
[####################] 100% 0:07:10 | processing reads     | s2 |
```

[8]:

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

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:00:03 | concatenating        | s3 |
[####################] 100% 0:01:09 | dereplicating        | s3 |
[####################] 100% 0:06:45 | clustering/mapping   | s3 |
[####################] 100% 0:00:00 | building clusters    | s3 |
[####################] 100% 0:00:00 | chunking clusters    | s3 |
```

```
[####################] 100% 0:36:38 | aligning clusters     | s3 |
[####################] 100% 0:00:45 | concat clusters       | s3 |
[####################] 100% 0:00:07 | calc cluster stats    | s3 |
[####################] 100% 0:05:59 | inferring [H, E]      | s4 |
[####################] 100% 0:00:07 | calculating depths    | s5 |
[####################] 100% 0:00:10 | chunking clusters     | s5 |
[####################] 100% 0:17:00 | consens calling       | s5 |
[####################] 100% 0:00:19 | indexing alleles      | s5 |
[####################] 100% 0:00:04 | concatenating inputs | s6 |
[####################] 100% 0:00:42 | clustering tier 1     | s6 |
[####################] 100% 0:00:00 | concatenating inputs | s6 |
[####################] 100% 0:00:14 | clustering across     | s6 |
[####################] 100% 0:00:05 | building clusters     | s6 |
[####################] 100% 0:04:07 | aligning clusters     | s6 |
```

[17]:

```
## access the stats of the assembly (so far) from the .stats attribute
data.stats
```

[17]:

```
                  state  reads_raw  reads_passed_filter  clusters_total  \
C_ade_ED3445          6     409199               409179           38396
C_and_ED3101          6     639862               639812           30124
C_and_ED3102          6     867107               867066           42345
C_and_ED3103          6    1116270              1116200           30951
C_and_ED3106          6    1046333              1046263           37917
C_arg_ED3001          6     734115               734059           55094
C_arg_ED3446          6     664604               664553           57254
C_arg_ED3447          6     989316               989249           70553
C_arg_ED3448          6     434304               434276           42185
C_asp_ED2985          6     738697               738633           36964
C_asp_ED3003          6     579040               578996           34823
C_asp_ED3027          6     526665               526628           39281
C_cal_ED1407          6    2911701              2911661          220621
C_cal_ED3450          6     231264               231246           29714
C_con_ED1447          6     501051               501013           32113
C_con_ED3022          6     974644               974584           39702
C_con_aff_ED1445      6     531683               531655           66947
C_cya_ED2998          6     540265               540220           25787
C_cya_ED3451          6     774476               774410           73164
C_cya_ED3452          6     999930               999857          145257
C_cya_ED3453          6     731010               730960           58043
C_cya_ED3454          6     841822               841754           66978
C_dis_ED2983          6     635244               635201           25660
C_dis_ED2989          6     848267               848220           41083
C_dis_ED3091          6     438273               438252           30619
C_dis_ED3093          6     609854               609806           30904
C_dis_ED3455          6     843699               843646           67752
C_gla_ED1401          6    1502874              1502845          112865
```

C_gla_ED1603	6	2625625	2624751	54329
C_gla_ED1604	6	708534	708491	90263
C_gla_ED2984	6	256985	256971	47199
C_gla_ED2986	6	1354922	1354832	73577
C_gla_ED3104	6	326948	326925	23188
C_gla_ED3456	6	989472	989400	87024
C_gra_ED2997	6	1070863	1070789	33872
C_gra_ED3100	6	629616	629569	22558
C_gra_ED3458	6	1183425	1183351	107057
C_gra_ED3459	6	561116	561067	63615
C_gra_ED3460	6	344981	344962	46817
C_int_ED1405	6	1385261	1385234	84830
C_int_ED1406	6	1561235	1561211	69011
C_int_ED1412	6	2038566	2038528	96195
C_int_ED1413	6	1154999	1154982	55507
C_int_ED1414	6	2039819	2039793	113980
C_int_ED1415	6	2094583	2094541	71421
C_int_ED1416	6	1345449	1345429	73842
C_int_ED1417	6	1928096	1928067	71241
C_int_ED1418	6	1373793	1373772	60182
C_int_ED1422	6	2301667	2300849	286918
C_int_ED1424	6	1565025	1564468	55758
C_int_ED1425	6	3228038	3226997	56152
C_int_ED1426	6	4039131	4038026	68152
C_int_ED1427	6	2468232	2467406	46191
C_int_ED1428	6	2107049	2106397	35929
C_int_ED1429	6	4193546	4192232	95262
C_int_ED1430	6	2579125	2578317	144467
C_int_ED1431	6	1541662	1541556	83220
C_int_ED1432	6	853614	853550	50914
C_int_ED1433	6	744654	744592	42441
C_int_ED1434	6	2805606	2804600	80765
C_int_ED1436	6	3818713	3817495	64890
C_int_ED1441	6	478497	478469	27582
C_int_ED1443	6	889484	889439	35057
C_int_ED1448	6	629203	629171	35792
C_int_ED1449	6	1509385	1509286	32838
C_int_ED3010	6	1443377	1442790	41011
C_int_ED3014	6	901668	901605	44369
C_int_ED3029	6	1909217	1908588	40232
C_int_ED3030	6	3528170	3526968	54166
C_int_ED3461	6	982060	981994	86785
C_int_ED3462	6	773277	773205	64484
C_int_aspXglau_ED2994	6	503025	502988	28833
C_int_asp_cf_ED1419	6	1628337	1628304	75759
C_int_asp_cf_ED4785	6	1599350	1598942	63369
C_int_asp_form_ED3013	6	2266892	2266110	43433

```
C_int_con_aff_ED3018       6     729840               729795           31291
C_int_dia_ED3026           6     653693               653648           32856
C_int_gla_cf_ED2995        6     954199               954116           46535
C_int_leu_ED3020           6     751100               751040           33085
C_int_leu_ED3021           6     466612               466576           42291
C_int_vir_pin_ED1409       6    1823339              1823301          120360
C_mol_ED1435               6    1041243              1041166           52069
C_mol_ED3005               6     442552               442525           29210
C_mol_ED3011               6    1141336              1140897           47431
C_mol_ED3012               6    1247161              1246657           43426
C_mol_ED3016               6    1090103              1089641           42541
C_mol_ED3019               6     534575               534542           26095
C_mol_ED3028               6     543377               543335           29383
C_multifida_ED3464         6     392983               392956           43473
C_multifida_ED3465         6     576885               576839           39966
C_multifida_ED3466         6    1009904              1009834          142047
C_multifida_W5632          6    2678017              2677057           85112
C_multifida_W5633          6    3560550              3559293           84670
C_multifida_W5634          6    5130125              5128467           69842
C_multifida_W5635          6    3277319              3276481           44150
C_multiflora_ED2988        6     590957               590914           35940
C_multiflora_ED3107        6     722336               722290           39514
C_multiflora_ED3108        6     601822               601778           37587
C_ova_CF_ED1824            6     764302               764250           72724
C_ova_ED1450               6     839236               839180           44028
C_ova_ED3467               6     751858               751807           70993
C_ova_ED3468               6     602245               602201           52050
C_sp_int_ED1438            6    2994398              2993373           51419
C_ten_CF_ED3023            6     265308               265287           24361
C_ten_ED3469               6     641663               641621           58201
C_vir_CF_ED3004            6     727674               727628           29867
C_vir_ED1403               6    1220692              1220614           66414
C_vir_ED1408               6    1348089              1348060           88697
C_vir_ED1439               6    1688169              1688078           61472
C_vir_ED1442               6     817648               817605           39907
C_vir_ED2992               6     439354               439325           41769
C_vir_ED3002               6     605072               605038           34122
C_vir_ED3470               6    1045665              1045604           77460
C_vir_ED3472               6     823656               823601           78750
C_vir_pin_ED1440           6    4571564              4570273           53107
Lec_ame_ED1878             6     639352               639316           74422
Lec_het_ED1877             6     433168               433131           71616

                       clusters_hidepth  hetero_est  error_est  reads_consens
C_ade_ED3445                      10385    0.005386   0.001061           9975
C_and_ED3101                      12529    0.019319   0.000965          11386
C_and_ED3102                      12330    0.021684   0.001460          10876
```

C_and_ED3103	13445	0.009891	0.000769	12851
C_and_ED3106	12077	0.009358	0.000817	11514
C_arg_ED3001	14798	0.013219	0.000885	13824
C_arg_ED3446	13701	0.016240	0.001566	12551
C_arg_ED3447	14516	0.013296	0.001133	13524
C_arg_ED3448	10280	0.013898	0.001469	9447
C_asp_ED2985	15143	0.011058	0.000765	14369
C_asp_ED3003	13533	0.012754	0.000879	12711
C_asp_ED3027	11941	0.010662	0.000859	11274
C_cal_ED1407	40023	0.022699	0.001084	33568
C_cal_ED3450	8228	0.012027	0.001393	7661
C_con_ED1447	12610	0.015478	0.000949	11763
C_con_ED3022	14940	0.009960	0.000761	14246
C_con_aff_ED1445	13952	0.012817	0.001278	13000
C_cya_ED2998	9440	0.003394	0.000742	9176
C_cya_ED3451	14610	0.014488	0.001076	13554
C_cya_ED3452	17981	0.023725	0.001579	15341
C_cya_ED3453	14426	0.013708	0.000995	13505
C_cya_ED3454	14379	0.013556	0.000974	13429
C_dis_ED2983	10971	0.004497	0.000900	10602
C_dis_ED2989	13269	0.004522	0.000802	12828
C_dis_ED3091	13048	0.017399	0.000964	11972
C_dis_ED3093	13089	0.017103	0.000927	12076
C_dis_ED3455	13484	0.013620	0.001081	12504
C_gla_ED1401	24938	0.010312	0.000937	23484
C_gla_ED1603	21471	0.009019	0.001236	20498
C_gla_ED1604	12503	0.011840	0.000908	11728
C_gla_ED2984	8542	0.008065	0.000953	8096
C_gla_ED2986	14958	0.009409	0.000775	14242
C_gla_ED3104	8683	0.009062	0.000913	8187
C_gla_ED3456	14097	0.012934	0.001305	13089
C_gra_ED2997	14254	0.014532	0.000796	13440
C_gra_ED3100	9486	0.008142	0.000710	9078
C_gra_ED3458	16534	0.013429	0.001045	15253
C_gra_ED3459	12630	0.013883	0.001121	11777
C_gra_ED3460	12141	0.019616	0.001405	10928
C_int_ED1405	20579	0.014079	0.000875	19254
C_int_ED1406	18910	0.010090	0.000904	17928
C_int_ED1412	21023	0.017208	0.000979	19293
C_int_ED1413	20144	0.012645	0.000877	18978
C_int_ED1414	21990	0.014775	0.000915	20259
C_int_ED1415	18054	0.015919	0.000888	16738
C_int_ED1416	22219	0.011606	0.000856	20883
C_int_ED1417	20403	0.014553	0.000835	19091
C_int_ED1418	19060	0.013247	0.000780	18006
C_int_ED1422	24602	0.022812	0.002048	21468
C_int_ED1424	21746	0.010723	0.001226	20668

C_int_ED1425	20824	0.011981	0.001226	19719
C_int_ED1426	11215	0.011961	0.000783	10587
C_int_ED1427	20980	0.012072	0.001257	19827
C_int_ED1428	12301	0.011140	0.001133	11698
C_int_ED1429	18060	0.012335	0.000914	16769
C_int_ED1430	33849	0.012842	0.001401	30889
C_int_ED1431	23190	0.018254	0.001215	20964
C_int_ED1432	14956	0.011946	0.000889	14094
C_int_ED1433	10956	0.011952	0.000874	10346
C_int_ED1434	21862	0.012282	0.001242	20501
C_int_ED1436	15990	0.010537	0.000968	15146
C_int_ED1441	10241	0.010352	0.000840	9716
C_int_ED1443	13624	0.011990	0.000870	12884
C_int_ED1448	12246	0.012662	0.000820	11550
C_int_ED1449	12201	0.009546	0.000727	11672
C_int_ED3010	19954	0.008714	0.001351	18991
C_int_ED3014	15590	0.012892	0.000830	14657
C_int_ED3029	19399	0.010234	0.001186	18450
C_int_ED3030	19792	0.009252	0.001053	18915
C_int_ED3461	16078	0.019802	0.001872	14254
C_int_ED3462	12353	0.010232	0.001242	11644
C_int_aspXglau_ED2994	12223	0.012635	0.000830	11538
C_int_asp_cf_ED1419	21424	0.015756	0.001183	19649
C_int_asp_cf_ED4785	10450	0.013581	0.001087	9675
C_int_asp_form_ED3013	13846	0.012102	0.001102	13149
C_int_con_aff_ED3018	12641	0.011421	0.000764	11982
C_int_dia_ED3026	12507	0.010191	0.000848	11910
C_int_gla_cf_ED2995	15767	0.014046	0.000921	14739
C_int_leu_ED3020	12882	0.009832	0.000797	12265
C_int_leu_ED3021	11633	0.009593	0.000810	11086
C_int_vir_pin_ED1409	27071	0.017284	0.001043	24430
C_mol_ED1435	9997	0.005264	0.000723	9639
C_mol_ED3005	12218	0.005426	0.000903	11762
C_mol_ED3011	19343	0.010050	0.001320	18295
C_mol_ED3012	20855	0.010491	0.001207	19766
C_mol_ED3016	16323	0.004634	0.001207	15729
C_mol_ED3019	11844	0.004087	0.000772	11495
C_mol_ED3028	11733	0.004250	0.000790	11374
C_multifida_ED3464	11057	0.004828	0.001047	10630
C_multifida_ED3465	11978	0.004579	0.000904	11543
C_multifida_ED3466	15669	0.013223	0.001393	14100
C_multifida_W5632	17314	0.008545	0.001210	16454
C_multifida_W5633	22839	0.017763	0.001194	20957
C_multifida_W5634	17200	0.016374	0.000858	15701
C_multifida_W5635	10875	0.017357	0.000891	9937
C_multiflora_ED2988	12927	0.013343	0.000956	12097
C_multiflora_ED3107	13526	0.012789	0.000876	12697

```
C_multiflora_ED3108                 12856    0.013217   0.000849          12096
C_ova_CF_ED1824                     14471    0.010744   0.000938          13627
C_ova_ED1450                        15891    0.010415   0.001032          15098
C_ova_ED3467                        13401    0.010814   0.001093          12615
C_ova_ED3468                        12337    0.010309   0.000916          11671
C_sp_int_ED1438                     17236    0.009468   0.001139          16419
C_ten_CF_ED3023                      8636    0.009295   0.000901           8171
C_ten_ED3469                        11766    0.010463   0.001003          11101
C_vir_CF_ED3004                     12461    0.009955   0.000765          11906
C_vir_ED1403                        19013    0.011901   0.001099          17886
C_vir_ED1408                        21072    0.013180   0.000874          19341
C_vir_ED1439                        14190    0.009301   0.000834          13502
C_vir_ED1442                        12712    0.007686   0.000791          12199
C_vir_ED2992                        10228    0.013322   0.001081           9472
C_vir_ED3002                        12255    0.010535   0.000894          11604
C_vir_ED3470                        14919    0.015517   0.001160          13649
C_vir_ED3472                        15725    0.011322   0.001061          14724
C_vir_pin_ED1440                    17487    0.008955   0.000844          16623
Lec_ame_ED1878                      16190    0.020358   0.001086          13973
Lec_het_ED1877                      12225    0.019297   0.001319          11026
```

Load assembly object (until step 6) **to avoid rerunning the assembly until here when comming back later!**

[3]:
```
## load assembly object from previous assemblies
data = ip.load_json("Cris_Assembly/Cristaria.json")
```

```
loading Assembly: Cristaria
from saved path: ~/BioInf/Cristaria_GBS/Cris_Anal/Cris_Assembly/Cristaria.json
```

Step 7: Population assembly for *Cristaria* phylogeny with outgroup Create a branch with 70 % missing data for ingroup, but allow missing data in the outgroup by setting population assignments. The population min-samples values overrule the min-samples-locus in the parameter file defined above.

[19]:
```
## 70% missing data in ingroup
Cris_pops30 = data.branch("Cris_pops30")
Cris_pops30.populations = {
    "ingroup": (34, [i for i in Cris_pops30.samples if "C_" in i]),
    "outgourp": (0, [i for i in Cris_pops30.samples if "Lec_" in i]),
    }
#print(pops30.populations)
Cris_pops30.run("7", force = True)
```

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:00:06 | applying filters     | s7 |
[####################] 100% 0:00:21 | building arrays      | s7 |
[####################] 100% 0:00:07 | writing conversions  | s7 |
```

```
[####################] 100% 0:00:35 | indexing vcf depths  | s7 |
[####################] 100% 0:01:08 | writing vcf output   | s7 |
```

Phylogenetic reconstruction whole dataset

[7]:

```
## following programs are required
# conda install toytree -c eaton-lab
# conda install tetrad -c eaton-lab -c conda-forge
# conda install raxml -c bioconda
```

Load assemblies

[20]:

```
## reload assemblies from their JSON files
Cris_pops30 = ip.load_json("Cris_Assembly/Cris_pops30.json")
```

```
loading Assembly: Cris_pops30
from saved path: ~/BioInf/Cristaria_GBS/Cris_Anal/Cris_Assembly/Cris_pops30.json
```

RAxML — concatenation tree inference

[23]:

```
## create a raxml analysis object for the Backbone tree
rax = ipa.raxml(
    name = Cris_pops30.name,
    data = Cris_pops30.outfiles.phy,
    workdir = "./Cris_RAxML/New",
    T = 8,
    N = 200,
    o = ["Lec_ame_ED1878", "Lec_het_ED1877"],
    )
```

[24]:

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

```
/home/nees/anaconda3/envs/ipyrad_09/bin/raxmlHPC-PTHREADS-AVX2 -f a -T 8 -m
GTRGAMMA -n Cris_pops30 -w
/home/nees/BioInf/Cristaria_GBS/Cris_Anal/Cris_RAxML/New -s /home/nees/BioInf/Cr
istaria_GBS/Cris_Anal/Cris_Assembly/Cris_pops30_outfiles/Cris_pops30.phy -p
54321 -N 200 -x 12345
```

[25]:

```
## run RAxML analysis
rax.run(force = True)
```

```
job Cris_pops30 finished successfully
```

Plotting results

```
[ ]: ## load tree
     Cris_pops30 = toytree.tree("./Cris_RAxML/RAxML_bipartitions.Cris_pops30")

     ## root tree with Halimione portulacoides
     rAtriAll = AtriAll.root(wildcard = "OUT_A-port_ED3985")

     ## define aesthetics for tree plotting
     canvas, axes = rAtriAll.ladderize(1).draw(
         height = 1300, width = 800,
         tip_labels_align = True,
         node_labels = rAtriAll.get_node_values("support"),
         node_sizes = 0,
         node_labels_style = {"font-size": "9px",
                              "baseline-shift": "7px",
                              "-toyplot-anchor-shift": "-11px"},
     );

     ## export trees
     import toyplot.pdf
     import toyplot.svg
     toyplot.pdf.render(canvas, "Atri_RAxML/Atri_ML_Fig/Atri_All_pops30_GBS_RAxML.
      ↪pdf")
     toyplot.pdf.render(canvas, "Atri_RAxML/Atri_ML_Fig/Atri_All_pops30_GBS_RAxML.
      ↪svg")
```

Assembly and phylogenetic analyses of *C. integerrima*

Assembly for *Cristaria integerrima* clade

First assembly includes all memeber of the *C. integerrima* clade and one sample of *C. molinae* as outgroup for RAxML. **Second** assembly is identical to the fist but without outgroup to use be for STRUCTURE analysis (see second Notebook for the STRUCUTRE analyses).

```
[9]: ## create a subset with only C. integerrima samples & 1 outgroup for RAxML␣
      ↪analysis
     ## allowing 70 % missing data
     C_int_ML2 = [i for i in data.samples.keys() if "C_int" in i]
     C_int_ML2.append('C_mol_ED1435')                        ## adds sample to list
     C_int_ML2 = data.branch('C_int_ML2', subsamples = C_int_ML2)
     C_int_ML2.set_params("min_samples_locus", 12)
     C_int_ML2.run("7", force = True)
```

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:00:08 | applying filters     | s7 |
```

```
[####################] 100% 0:00:10 | building arrays      | s7 |
[####################] 100% 0:00:05 | writing conversions  | s7 |
[####################] 100% 0:00:25 | indexing vcf depths  | s7 |
[####################] 100% 0:00:28 | writing vcf output   | s7 |
```

[10]:
```
## create a subset with only C. integerrima for population analysis
## allowing 70 % missing data
C_int_POP2 = [i for i in data.samples.keys() if "C_int" in i]
C_int_POP2 = data.branch('C_int_POP2', subsamples = C_int_POP2)
C_int_POP2.set_params("min_samples_locus", 12)
C_int_POP2.run("7", force = True)
```

```
Parallel connection | Eulychnia: 8 cores
[####################] 100% 0:00:04 | applying filters     | s7 |
[####################] 100% 0:00:10 | building arrays      | s7 |
[####################] 100% 0:00:05 | writing conversions  | s7 |
[####################] 100% 0:00:23 | indexing vcf depths  | s7 |
[####################] 100% 0:00:27 | writing vcf output   | s7 |
```

RAxML analyses

[16]:
```
## reload assemblies from their JSON files
C_int_ML2 = ip.load_json("Cris_Assembly/C_int_ML2.json")
```

```
loading Assembly: C_int_ML2
from saved path: ~/BioInf/Cristaria_GBS/Cris_Anal/Cris_Assembly/C_int_ML2.json
```

[19]:
```
## create a raxml analysis object for the Backbone tree
rax = ipa.raxml(
    name = C_int_ML2.name,
    data = C_int_ML2.outfiles.phy,
    workdir = "./Cris_RAxML/New",
    T = 8,
    N = 200,
    o = ["C_mol_ED1435"],
    )

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

```
/home/nees/anaconda3/envs/ipyrad_09/bin/raxmlHPC-PTHREADS-AVX2 -f a -T 8 -m
GTRGAMMA -n C_int_ML2 -w
/home/nees/BioInf/Cristaria_GBS/Cris_Anal/Cris_RAxML/New -s /home/nees/BioInf/Cr
istaria_GBS/Cris_Anal/Cris_Assembly/C_int_ML2_outfiles/C_int_ML2.phy -p 54321 -N
200 -x 12345
```

[20]:
```
## Run the RAxML analyses
rax.run(force = True)
```

```
job C_int_ML2 finished successfully
```

Cristaria GBS: structure analyses

This notebook provides the code for a STRUCTURE analyses of the coastal species *Cristaria integerrima*

Required software

[ ]:

```
## conda install ipyrad -c ipyrad
## conda install toytree -c eaton-lab
## conda install structure -c ipyrad
## conda install clumpp -c ipyrad
```

Imports

[1]:

```
## 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. 0.9.15
toytree v. 0.2.3
```

Connect to cluster

[2]:

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

```
8 engines found
```

Population structure of *C. integerrima*

[10]:
```
## the structure formatted file
strfile = "./Cris_Assembly/C_int_POP2_outfiles/C_int_POP.str"

## an optional mapfile, to sample unlinked SNPs
mapfile = "./Cris_Assembly/C_int_POP2_outfiles/C_int_POP.snps.map"

## the directory where outfiles should be written
workdir = "./Cris_STRUCTURE/"
```

[11]:
```
## create a STRUCTURE object
struct = ipa.structure(name = 'CrisInt_STRUCT2',
                        data = strfile,
                        mapfile = mapfile,
                        workdir = workdir)
```

Set parameter options for this object

[12]:
```
## set mainparams for object
struct2.mainparams.burnin = 50000
struct2.mainparams.numreps = 100000

## see all mainparams
print struct.mainparams

#see or ser extraparams
print struct.extraparams
```

```
burnin             50000
extracols          0
label              1
locdata            0
mapdistances       0
markernames        0
markovphase        0
missing            -9
notambiguous       -999
numreps            100000
onerowperind       0
phased             0
phaseinfo          0
phenotype          0
ploidy             2
popdata            0
popflag            0
recessivealleles   0
```

```
admburnin           500
alpha               1.0
alphamax            10.0
alphapriora         1.0
alphapriorb         2.0
alphapropsd         0.025
ancestdist          0
ancestpint          0.9
computeprob         1
echodata            0
fpriormean          0.01
fpriorsd            0.05
freqscorr           1
gensback            2
inferalpha          1
inferlambda         0
intermedsave        0
lambda_             1.0
linkage             0
locispop            0
locprior            0
locpriorinit        1.0
log10rmax           1.0
log10rmin           -4.0
log10rpropsd        0.1
log10rstart         -2.0
maxlocprior         20.0
metrofreq           10
migrprior           0.01
noadmix             0
numboxes            1000
onefst              0
pfrompopflagonly    0
popalphas           0
popspecificlambda   0
printlambda         1
printlikes          0
printnet            1
printqhat           0
printqsum           1
randomize           0
reporthitrate       0
seed                12345
sitebysite          0
startatpopinfo      0
unifprioralpha      1
updatefreq          10000
```

```
usepopinfo          0
```

[13]:
```
## set a range of k-values to test
kvalues = [2, 3, 4, 5, 6]
```

[14]:
```
## submit batches of 10 replicates jobs for each value of k
for kpop in kvalues:
    struct.run(kpop = kpop, nreps = 10, seed = 12345, ipyclient=ipyclient)#,␣
 ↪force = True)
```

```
submitted 10 structure jobs [CrisInt_STRUCT-K-2]
submitted 10 structure jobs [CrisInt_STRUCT-K-3]
submitted 10 structure jobs [CrisInt_STRUCT-K-4]
submitted 10 structure jobs [CrisInt_STRUCT-K-5]
submitted 10 structure jobs [CrisInt_STRUCT-K-6]
```

[16]:
```
## block/wait until all jobs finished
ipyclient.wait()
```

[16]:
```
True
```

Check results in evanno table

[17]:
```
## return the evanno table (deltaK) for best K
etable = struct.get_evanno_table(kvalues) #(kvalues)
etable
```

[17]:
```
   Nreps  deltaK  estLnProbMean  estLnProbStdev       lnPK      lnPPK
2     10   0.000      -98553.29        2620.755       0.00       0.00
3     10   1.882      -96621.57        4660.383    1931.72    8770.04
4     10   7.162     -103459.89       14008.322   -6838.32  100332.32
5     10   0.502     -210630.53      159877.846 -107170.64   80210.14
6     10   0.000     -237591.03      285541.048  -26960.50       0.00
```

[19]:
```
struct.get_evanno_table(kvalues, max_var_multiple=0, quiet=True)
```

[19]:
```
   Nreps  deltaK  estLnProbMean  estLnProbStdev       lnPK      lnPPK
2     10   0.000      -98553.29        2620.755       0.00       0.00
3     10   1.882      -96621.57        4660.383    1931.72    8770.04
4     10   7.162     -103459.89       14008.322   -6838.32  100332.32
5     10   0.502     -210630.53      159877.846 -107170.64   80210.14
6     10   0.000     -237591.03      285541.048  -26960.50       0.00
```

[20]:
```
struct.get_evanno_table(kvalues, max_var_multiple=10, quiet=True)
```

```
[20]:    Nreps  deltaK  estLnProbMean  estLnProbStdev       lnPK      lnPPK
      2     10   0.000     -98553.290        2620.755      0.000      0.000
      3     10   1.882     -96621.570        4660.383   1931.720   8770.040
      4     10   0.951    -103459.890       14008.322  -6838.320  13326.119
      5      7   0.696    -123624.329       37471.761 -20164.439  26081.980
      6      8   0.000    -117706.788       34019.574   5917.541      0.000
```

```
[22]: struct.get_evanno_table(kvalues, max_var_multiple=50, quiet=True)
```

```
[22]:    Nreps  deltaK  estLnProbMean  estLnProbStdev       lnPK      lnPPK
      2     10   0.000      -98553.29        2620.755       0.00       0.00
      3     10   1.882      -96621.57        4660.383    1931.72    8770.04
      4     10   7.162     -103459.89       14008.322   -6838.32  100332.32
      5     10   1.026     -210630.53      159877.846 -107170.64  164003.37
      6      9   0.000     -153797.80      112852.628   56832.73       0.00
```

```
[21]: struct.get_evanno_table(kvalues, max_var_multiple=100, quiet=True)
```

```
[21]:    Nreps  deltaK  estLnProbMean  estLnProbStdev       lnPK      lnPPK
      2     10   0.000      -98553.29        2620.755       0.00       0.00
      3     10   1.882      -96621.57        4660.383    1931.72    8770.04
      4     10   7.162     -103459.89       14008.322   -6838.32  100332.32
      5     10   0.502     -210630.53      159877.846 -107170.64   80210.14
      6     10   0.000     -237591.03      285541.048  -26960.50       0.00
```

Get permuted reps with CLUMPP

Calculate a permuted table of results across replicate runs for each value of K while excluding reps based on the max_var_multiple parameter (see above for description).

```
[31]: ## summarize results
      struct.clumppparams.m = 3                ## use largegreedy algorithm
      struct.clumppparams.greedy_option = 2    ## test nrepeat possible orders
      struct.clumppparams.repeats = 100000     ## number of repeats
```

```
[32]: qtable = struct.get_clumpp_table(kvalues, max_var_multiple=100.)
```

```
[K2] 10/10 results permuted across replicates (max_var=100.0).
[K3] 10/10 results permuted across replicates (max_var=100.0).
[K4] 10/10 results permuted across replicates (max_var=100.0).
[K5] 10/10 results permuted across replicates (max_var=100.0).
[K6] 10/10 results permuted across replicates (max_var=100.0).
```

Custom order of STRUCTURE bar plots

First load a tree the STRUCTURE bar plots should be plotted against.

[195]:

```
## load a tree topology for ordering tips on barplot
tre = toytree.tree("./Cris_RAxML/New/RAxML_bipartitions.C_int_ML2")
tre.ladderize(1).draw()
#rtre = tre.root(names="C_mol_ED1435")
#dtre = rtre.drop_tips(names="C_mol_ED1435")
#dtre.ladderize(1).draw();

## root on outgroups and then drop outgroups from the tree
#outg = ["C_mol_ED1435"]
#tre = tre.drop_tips(wildcard="C_mol")
```

[195]:

```
(<toyplot.canvas.Canvas at 0x7f62bf264e50>,
 <toyplot.coordinates.Cartesian at 0x7f62987d4790>)
```

Make a list of samples and sort them in the desired order

[86]:

```
## custom sorting order
myorder = ["C_int_ED1448", "C_int_vir_pin_ED1409",
           "C_int_ED1406", "C_int_ED3462", "C_int_ED1405",
           "C_int_gla_cf_ED2995", "C_int_aspXglau_ED2994", "C_int_ED3010",
           "C_int_ED1413", "C_int_ED1414","C_int_ED1412",
           "C_int_ED1415", "C_int_ED1418", "C_int_ED1416",
           "C_int_ED1417", "C_int_ED3014", "C_int_ED3461",
           "C_int_asp_cf_ED1419", "C_int_asp_form_ED3013",␣
 ↪"C_int_asp_cf_ED4785",
           "C_int_ED3030", "C_int_ED1424", "C_int_ED1426",
           "C_int_ED1427", "C_int_ED1422", "C_int_ED1431",
           "C_int_ED1425", "C_int_ED1428", "C_int_ED1432",
           "C_int_ED1434", "C_int_ED1433", "C_int_ED1429",
           "C_int_ED1430", "C_int_ED1436", "C_int_con_aff_ED3018",
           "C_int_ED1449", "C_int_ED1443", "C_int_ED3029", "C_int_ED1441",
           "C_int_dia_ED3026", "C_int_leu_ED3021", "C_int_leu_ED3020",]
print "custom ordering"
print qtable[4].loc[myorder]
```

```
custom ordering
                               0          1          2      3
C_int_ED1448           8.440e-02  5.174e-01  2.210e-02  0.376
C_int_vir_pin_ED1409   2.082e-01  4.167e-01  5.190e-02  0.323
C_int_ED1406           2.700e-03  6.896e-01  8.000e-04  0.307
C_int_ED3462           8.000e-04  7.471e-01  6.000e-04  0.252
C_int_ED1405           7.650e-02  7.182e-01  1.800e-03  0.203
C_int_gla_cf_ED2995    8.120e-02  7.733e-01  9.000e-04  0.145
C_int_aspXglau_ED2994  5.270e-02  7.715e-01  2.300e-03  0.173
C_int_ED3010           1.031e-01  2.220e-02  4.979e-01  0.377
C_int_ED1413           1.193e-01  1.000e-03  6.141e-01  0.266
C_int_ED1414           1.167e-01  9.000e-04  5.624e-01  0.320
C_int_ED1412           1.351e-01  4.670e-02  5.029e-01  0.315
```

```
C_int_ED1415          5.490e-02  9.000e-04  6.253e-01  0.319
C_int_ED1418          5.190e-02  2.000e-04  6.416e-01  0.306
C_int_ED1416          7.620e-02  5.000e-04  5.990e-01  0.324
C_int_ED1417          4.660e-02  2.000e-04  6.151e-01  0.338
C_int_ED3014          4.720e-02  6.000e-04  6.671e-01  0.285
C_int_ED3461          9.320e-02  1.200e-03  6.451e-01  0.261
C_int_asp_cf_ED1419   9.220e-02  3.000e-04  6.237e-01  0.284
C_int_asp_form_ED3013 2.870e-02  8.000e-04  7.835e-01  0.187
C_int_asp_cf_ED4785   1.640e-02  1.000e-03  7.474e-01  0.235
C_int_ED3030          2.424e-01  1.000e-04  4.000e-04  0.757
C_int_ED1424          1.681e-01  2.000e-04  2.510e-02  0.807
C_int_ED1426          1.300e-01  1.200e-03  8.000e-03  0.861
C_int_ED1427          1.795e-01  2.700e-03  3.930e-02  0.778
C_int_ED1422          1.277e-01  5.000e-04  2.150e-02  0.850
C_int_ED1431          8.790e-02  7.000e-04  1.900e-03  0.909
C_int_ED1425          1.598e-01  2.000e-04  2.960e-02  0.810
C_int_ED1428          8.280e-02  5.000e-04  9.000e-04  0.916
C_int_ED1432          7.190e-02  6.000e-04  9.000e-04  0.927
C_int_ED1434          7.750e-02  3.000e-04  5.000e-04  0.922
C_int_ED1433          7.030e-02  4.000e-04  4.000e-04  0.929
C_int_ED1429          6.190e-02  0.000e+00  2.000e-04  0.938
C_int_ED1430          7.190e-02  0.000e+00  1.000e-04  0.928
C_int_ED1436          7.590e-02  1.000e-04  3.000e-04  0.924
C_int_con_aff_ED3018  1.690e-01  1.200e-03  5.000e-04  0.829
C_int_ED1449          6.630e-02  7.000e-04  3.000e-04  0.933
C_int_ED1443          3.361e-01  9.500e-03  7.460e-02  0.580
C_int_ED3029          3.477e-01  1.130e-02  2.530e-02  0.616
C_int_ED1441          2.899e-01  1.200e-03  1.500e-03  0.707
C_int_dia_ED3026      2.931e-01  5.000e-04  1.000e-03  0.705
C_int_leu_ED3021      3.438e-01  3.000e-04  2.000e-04  0.656
C_int_leu_ED3020      3.225e-01  1.000e-04  0.000e+00  0.677
```

Plot all STRUCTURE results against Phylogeny

[189]:

```
## get tree from RAxML results
stre = toytree.tree("./Cris_RAxML/RAxML_bipartitions.C_int_ML2")

## further styling of plot with css
style = {"stroke":toyplot.color.near_black,
         "stroke-width": 0.5}

## Define the leucantha clade to be rotated in the tree
leu = ["C_int_leu_ED3020", "C_int_leu_ED3021", "C_int_dia_ED3026",
       "C_int_ED1441", "C_int_ED3029", "C_int_ED1443", ]

## built & dissect canvas into multiple cartesian areas (x1, x2, y1, y2)
```

```
c = toyplot.Canvas()
a1 = c.cartesian(bounds=('1%', '40%', '5%', '95%'))       # The tree
a2 = c.cartesian(bounds=('45.5%', '55%', '5%', '92.5%'))  # K=2
a3 = c.cartesian(bounds=('55.5%', '65%', '5%', '92.5%'))  # K=3
a4 = c.cartesian(bounds=('65.5%', '75%', '5%', '92.5%'))  # K=4
a5 = c.cartesian(bounds=('75.5%', '85%', '5%', '92.5%'))  # K=5
a6 = c.cartesian(bounds=('85.5%', '95%', '5%', '92.5%'))  # K=6
a1.show = False
a2.show = False
a3.show = False
a4.show = False
a5.show = False
a6.show = False

## draw the tree
stre.ladderize(1).rotate_node(leu).draw(
    axes = a1,
    use_edge_lengths = True,
    tip_labels_align = True,
    node_labels = "support",
    node_sizes = 0,
    node_labels_style={"font-size": "9px",
                       "baseline-shift": "7px",
                       "-toyplot-anchor-shift": "-13px"});

## draw the STRUCTURE bar plots
## 'along' defines plot orientation; x = vertical; y = horizontal
a2.bars(qtable[2].loc[myorder], style = style, along = 'y');
a3.bars(qtable[3].loc[myorder], style = style, along = 'y');
a4.bars(qtable[4].loc[myorder], style = style, along = 'y');
a5.bars(qtable[5].loc[myorder], style = style, along = 'y');
a6.bars(qtable[6].loc[myorder], style = style, along = 'y');

## add header for the bar plots
c.text(300, 20, 'K = 2', style={"font-size": "13px"})
c.text(360, 20, 'K = 3', style={"font-size": "13px"})
c.text(420, 20, 'K = 4', style={"font-size": "13px"})
c.text(480, 20, 'K = 5', style={"font-size": "13px"})
c.text(540, 20, 'K = 6', style={"font-size": "13px"})

## add deltaK values below the bar plots
c.text(305, 565, '0.0', style={"font-size": "10px"})
c.text(365, 565, '1.9', style={"font-size": "10px"})
c.text(425, 565, '7.2', style={"font-size": "10px"})
c.text(485, 565, '0.5', style={"font-size": "10px"})
c.text(545, 565, '0.0', style={"font-size": "10px"})
c.text(425, 580, 'delta <b>K</b>', style={"font-size": "10px"})
```

[190]:

```
## safe the plot
import toyplot.pdf
import toyplot.svg
toyplot.pdf.render(c, "Cris_STRUCTURE/figures/C_int_Phyl_Str_K_all.pdf")
toyplot.svg.render(c, "Cris_STRUCTURE/figures/C_int_Phyl_Str_K_all.svg")
```