RESEARCH ARTICLE

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An integrative approach to understanding the evolution and diversity of *Copiapoa* (Cactaceae), a threatened endemic Chilean genus from the Atacama Desert[1]

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PREMISE OF THE STUDY: Species of the endemic Chilean cactus genus *Copiapoa* have cylindrical or (sub)globose stems that are solitary or form (large) clusters and typically yellow flowers. Many species are threatened with extinction. Despite being icons of the Atacama Desert and well loved by cactus enthusiasts, the evolution and diversity of *Copiapoa* has not yet been studied using a molecular approach.

METHODS: Sequence data of three plastid DNA markers (*rpl32-trnL*, *trnH-psbA*, *ycf1*) of 39 *Copiapoa* taxa were analyzed using maximum likelihood and Bayesian inference approaches. Species distributions were modeled based on geo-referenced localities and climatic data. Evolution of character states of four characters (root morphology, stem branching, stem shape, and stem diameter) as well as ancestral areas were reconstructed using a Bayesian and maximum likelihood framework, respectively.

KEY RESULTS: Clades of species are revealed. Though 32 morphologically defined species can be recognized, genetic diversity between some species and infraspecific taxa is too low to delimit their boundaries using plastid DNA markers. Recovered relationships are often supported by morphological and biogeographical patterns. The origin of *Copiapoa* likely lies between southern Peru and the extreme north of Chile. The Copiapó Valley limited colonization between two biogeographical areas.

CONCLUSIONS: *Copiapoa* is here defined to include 32 species and five heterotypic subspecies. Thirty species are classified into four sections and two subsections, while two species remain unplaced. A better understanding of evolution and diversity of *Copiapoa* will allow allocating conservation resources to the most threatened lineages and focusing conservation action on real biodiversity.

KEY WORDS ancestral state reconstruction; Cactaceae; Cactoideae; *Copiapoa*; cpDNA; evolutionary biogeography; infrageneric classification; morphology; phylogeny; systematics

More than 20% of the world's plants are threatened with extinction (IUCN, 2010). Among angiosperms, Cactaceae is one of the plant families whose species are most prone to extinction, with almost one third of the ca. 1500 species assessed as threatened (Hernández and Bárcenas, 1995, 1996; Mourelle and Ezcurra, 1997; Ortega-Baes and Godínez-Álvarez, 2006; Walter, 2011a; IUCN, 2014). Small species distributions correlate with elevated extinction risks (Gaston, 2003), and Cactaceae follow this pattern with many species restricted in distribution.

The Atacama Desert (Guerrero et al., 2013) and mediterranean central Chile harbor a high diversity of cacti, most of which represent endemic lineages (genera and species) (Guerrero et al., 2011a, b; Walter, 2011a; Duarte et al., 2014). Threats to their continued survival include increasing aridity due to climate change, (very)

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restricted distributions (extent of occurrence <100 to <1000 km^2, area of occupation <10 to <2000 km^2 [IUCN, 2014; Hunt et al., 2006]), leaving them vulnerable to habitat destruction, human impact (illegal collecting, mining, agriculture, road construction, etc.), and herbivory (Walter, 2011a; Larridon et al., 2014), as well as genetic erosion (e.g., Kramer and Havens, 2009). Threatened plant species are, in general, poorly studied (e.g., Samain and Cires, 2012; Larridon et al., 2014), although the lack of basic taxonomic knowledge is a limiting factor when aiming toward better understanding and the conservation of their biodiversity (e.g., Leadley and Jury, 2006; Walter, 2011a; Bornholdt et al., 2013). Patterns of lineage divergence within Cactaceae indicate priority sites within the Atacama Desert and mediterranean central Chile as important reservoirs of biodiversity (Guerrero et al., 2011b; Walter, 2011a; Duarte et al., 2014). However, phylogenetic relationships and species delimitations in some of the genera are still poorly known, challenging our comprehension of the evolutionary processes at the origin of the observed diversity, and hindering efforts and resource allocation for conservation.

The genus *Copiapoa* Britton & Rose has been considered a well-defined cohesive genus by the vast majority of authors since Britton and Rose (1922) erected it with *Copiapoa marginata* (Salm-Dyck) Britton & Rose (basionym: *Echinocactus marginatus* Salm-Dyck) (Fig. 1A) as its type and defined it as globose or short cylindrical, spiny cacti with diurnal, yellow flowers. Their new genus comprised only six species, five of which had formerly been placed in the genus *Echinocactus* Link & Otto. In the following years, more taxa from *Echinocactus* were combined into *Copiapoa*, i.e., *Echinocactus humilis* Phil., *Echinocactus conglomeratus* Phil., and *Echinocactus fiedlerianus* K.Schum. From the 1930s to the 1980s, authors such as Backeberg (1966) and especially Ritter (1980) described many new species and accepted up to 46 species in total. Since the mid-1980s, many authors proposed to greatly reduce the number of species by lumping taxa into broadly circumscribed species (e.g., Hoffmann, 1989: 17 spp.; Hunt et al., 2006: 21 spp.), followed by a flood of new combinations (mainly lowering taxonomic rank). More recently, several new species have been described (e.g., Schaub and Keim, 2006; Walter and Mächler, 2006).

All taxonomic proposals and species delimitations published to date (e.g., Backeberg, 1966; Ritter, 1980; Hoffmann, 1989; Charles, 1998; Anderson, 2001; Doweld, 2002; Hoffmann and Walter, 2004; Schulz, 2006; Hunt et al., 2006) were based on morphological affinities. This basis is problematic due to significant homoplasy documented in Cactaceae (Hernández-Hernández et al., 2011; Guerrero et al., 2011a; Schlumpberger and Renner, 2012). Ritter (1980) was the first to suggest an infrageneric classification of *Copiapoa*. Previously in 1961, Ritter published the monotypic genus *Pilocopiapoa* F.Ritter, only including *Pilocopiapoa solaris* F.Ritter [*Copiapoa solaris* (F.Ritter) F.Ritter; Fig. 1B]. However, in 1980, he recognized two subgenera in *Copiapoa*, i.e., subgenus *Pilocopiapoa* and subgenus *Copiapoa*, with the latter subgenus divided into five nameless sections and four nameless series based on general plant morphology. More recently, Doweld (2002) published an infrageneric classification with three sections (*Pilocopiapoa*, *Echinopoa*, and *Copiapoa*) and five series (*Feroces*, *Echinoidei*, *Cinerei*, *Humiles*, and *Copiapoa*) based on morphological and anatomical seed characters. Hoffmann and Walter, 2004) adopted Ritter's concept of two subgenera. Anderson (2001) and Hunt et al. (2006, 2014) did not propose an infrageneric classification of *Copiapoa*.

Different suprageneric relationships have been suggested for *Copiapoa*, e.g., Barthlott and Hunt (1993), Anderson (2001), and Hunt et al. (2006) included the genus in tribe Notocacteae. However, in molecular phylogenetic studies (e.g., Nyffeler, 2002; Arakaki et al., 2011; Bárcenas et al., 2011; Hernández-Hernández et al., 2011, 2014), *Copiapoa* appears isolated on its own branch. Nyffeler and Eggli (2010) treated *Copiapoa* as incertae sedis in their suprageneric classification of Cactaceae, while Korotkova et al. (2010) suggested a close relationship between *Copiapoa*, *Calymmanthium* F.Ritter—a monotypic cereoid genus—and *Lymanbensonia* Kimnach, but hesitated to include *Copiapoa* in their tribe Lymanbensonieae due to its different morphology, ecology, and distribution. Hunt et al. (2014) adopted Doweld's (2002) proposal of a monotypic tribe Copiapoeae. According to Hernández-Hernández et al. (2014), *Copiapoa* evolved within what they define as the Andean region of Chile and Argentina during the Pliocene (stem group age: 12.34 (8.3–18.15) million years ago (Ma); crown group age: 3.38 (1.40–5.84) Ma).

The stems of *Copiapoa* are solitary, globular to elongate cylindrical, or form clusters containing up to hundreds of simple subcolumnar cylindrical stems. Some species are partially geophytic, e.g., *Copiapoa esmeraldana* F.Ritter (Fig. 1C) and *Copiapoa hypogaea* F.Ritter subsp. *hypogaea* (Fig. 1D). Plant size ranges between ca. 2 cm in single-bodied and 2 m in diameter in mound-forming species, and their stem color varies widely. Roots are fibrous or greatly enlarged taproots. The plant apex is often covered in dense soft wool. Spines are very variable in number and color, and their form can be needle- or awl-shaped, straight to strongly bent, but never hooked. One or more central spines are usually more developed than the marginal spines. The apically born campanulate to short-funnelform diurnal flowers are usually yellow, with the outer perianth segments sometimes reddish or purplish. In a few taxa, the entire flower is red, e.g., in *Copiapoa taltalensis* subsp. *desertorum* (F.Ritter) G.J.Charles (Fig. 1E) and *Copiapoa rubriflora* F.Ritter [*C. taltalensis* (Werderm.) Looser subsp. *taltalensis*]. The floral tube is longer than the pericarpel. The well-developed nectar chamber is short and tubular, except in *Copiapoa angustiflora* Helmut Walter, G.J.Charles & Mächler where it is remarkably long. Bract scales (rudimentary leaves) are usually scarce and mainly present near the hypanthium rim (only in *C. solaris*, the scales are numerous and arranged over the entire surface of the pericarpel and hypanthium). In *C. solaris*, the axils of the bract scales are woolly, while in *C. angustiflora*, *C. hypogaea* subsp. *hypogaea*, and *C. hypogaea* subsp. *laui* (Diers) G.J.Charles, scant, long, fine hairs sometimes arise from the scale axils (Walter and Mächler, 2006). In all the other *Copiapoa* taxa they are naked. The stamens and pistil are typically pale yellow. Pollination of *Copiapoa* flowers is performed by insects (Hoffmann and Walter, 2004). Hernández-Hernández et al. (2014) describe the *Copiapoa* species as mellitophilic (bee-pollinated) with this condition having originated secondarily from species with other pollination syndromes. The fruits are small and smooth, dehiscing by circumscissile, apical splitting (Fig. 1F). In Cactaceae, this type of fruit is unique to *Copiapoa*. The seeds are shiny and black (Fig. 1F) and are ant-dispersed (H. E. Walter, P. C. Guerrero, personal observations), and the fruit structure is specifically adapted to this (Ritter, 1980).

Copiapoa is restricted to a narrow latitudinal belt in Chile, exclusively occurring between Tocopilla (22°S) and the coastal hills north of the Choapa Valley (31°20°S), from sea level to 1300 m elevation. Its distribution range is located in the coastal area of the

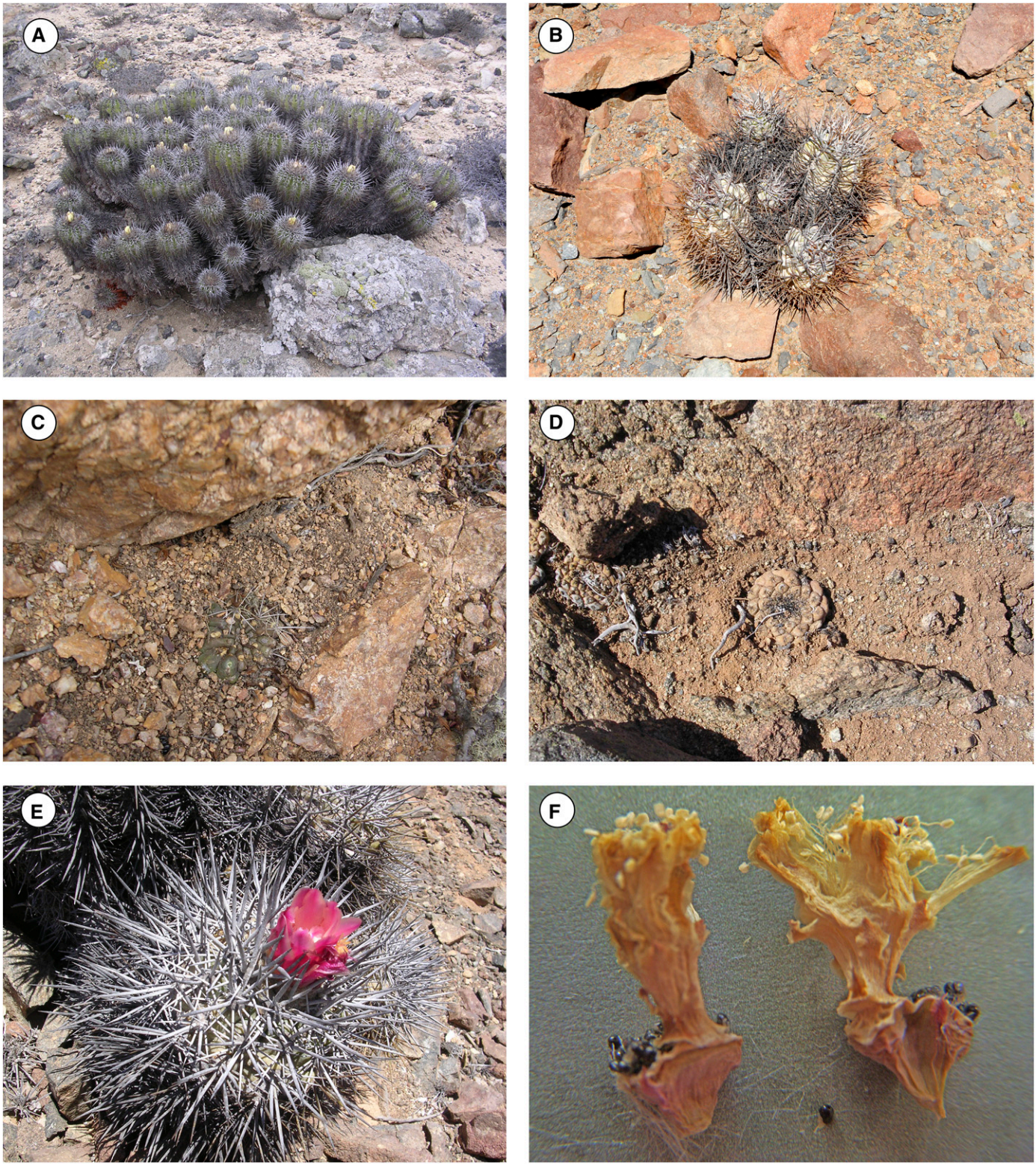


FIGURE 1 Diversity and morphology of *Copiapoa*. (A) Habit of *Copiapoa marginata* at Morro Copiapó, (B) habit of *C. solaris* at El Cobre (800 m a.s.l.), (C) habit of *C. esmeraldana* at Las Lomitas in Pan de Azúcar National Park (850 m a.s.l.), (D) habit of *C. hypogaea*, at Barquito (400 m a.s.l.), (E) *C. desertorum*, stem with flower, (F) mature *Copiapoa* fruit with seeds, fruit dehiscence by a small round apical lid opening at the top of the fruit with the perianth remnant detaching is visible.

Atacama Desert within the northern part of one of the world's biodiversity hotspots, i.e., central Chile, also identified as the Chilean Winter Rainfall-Valdivian Forests (Myers et al., 2000; Arroyo et al., 2005). This biodiversity hotspot encompasses many different vegetation types along an aridity gradient: to the south, different temperate forest types can be observed; in central Chile, a sclerophyllous vegetation type occurs; and in the north, two fragile and unique ecosystems are found, the coastal fog oasis or "lomas formation" and the blooming desert (e.g., Arroyo et al., 2008; Larridon et al., 2014). Most of the *Copiapoa* species are endemic to these two ecosystems, with some species having an extremely limited distribution range (Walter and Mächler, 2006; Guerrero et al., 2011a, b; Walter, 2011a; IUCN, 2014; Larridon et al., 2014).

Copiapoa representatives are common in botanic gardens and private cactus collections around the world and are particularly popular in countries such as the United States, Germany, and the United Kingdom (Larridon et al., 2014). Although germination of seeds in culture is fairly easy, germination and, specifically, the development of the hypocotyl under habitat conditions are often difficult. Consequently, seedlings can be observed in few wild populations. Low recruitment may be a factor contributing to rarity and thus to threat. Furthermore, the process of desertification and erosion as a consequence of global climate change may affect existing *Copiapoa* populations by diminishing their capacity to regenerate in the medium and long term (e.g., Walter, 2011a; IUCN, 2014).

Despite their ecological and horticultural relevance, and the fact that half of the species are vulnerable, endangered, or critically endangered in the wild (IUCN, 2014; Larridon et al., 2014), species delimitation, evolutionary relationships, and diversity in *Copiapoa* have not yet been studied in depth using molecular data. In this study, we aim to (1) infer a species-level phylogenetic hypothesis, (2) reconstruct ancestral states of taxonomically important characters, (3) model species distributions, and (4) reconstruct ancestral areas to obtain insights on the evolution of *Copiapoa* species and to test existing infrageneric classifications for the genus.

MATERIALS AND METHODS

***Taxon selection*—**The current study included 109 individuals, of which 16 belong to the outgroup and 93 represent 39 *Copiapoa* taxa. Appendix S1 (see Supplemental Data with the online version of this article) contains a list of taxa with associated collection information and GenBank accession numbers. The outgroup includes the genus *Pereskia* Mill. (subfamily Pereskioideae) and six genera from the subfamily Cactoideae that were suspected to be closely related to *Copiapoa* based on literature (e.g., Anderson, 2001; Nyffeler, 2002; Bárcenas et al., 2011; Hernández-Hernández et al., 2014). Multiple individuals were included of ca. 50% of the *Copiapoa* taxa, often encompassing both wild populations and botanic garden accessions. As a starting point for taxon selection, we largely followed Hunt et al. (2006) with the addition of four taxa (i.e., *C. coquimbana* var. *armata* F.Ritter, *C. longispina* F.Ritter, *C. mollicula* F.Ritter, and *C. rupestris* F.Ritter) described by Ritter (1980), and five recently published taxa—one of which is included in the IUCN Red List of Threatened Species (IUCN, 2014).

***Molecular methods*—**In Cactaceae, DNA extraction is complicated compared with most other plants since they usually do not have leaves. In previous studies, DNA has been extracted from different plant parts including the stem cortex, cladodes, and flowers (e.g., Korotkova et al., 2010; Guerrero et al., 2011a; Majure et al., 2012). Recently, a protocol to extract DNA from cactus spines was published, presenting an alternative to sampling cortical tissue from cactus stems, which can result in damage to the plants and exposure to pathogens (Fehlberg et al., 2013). We adapted and improved the protocol of Fehlberg et al. (2013) to conform to our laboratory conditions and needs (online Appendix S2: Cactus DNA extraction protocol). The protocol developed in our laboratory also allows extracting DNA from cortical tissue without formation of mucilage, which is often problematic in Cactaceae (e.g., Korotkova et al., 2010; Guerrero et al., 2011a). DNA was extracted from spines in most botanic garden samples used in this study. For samples collected from wild populations, either cortical stem tissue or young spines were used.

Three plastid DNA loci, two noncoding introns (*rpl32-trnL* and *trnH-psbA*) and one gene (*ycf1*), were selected based on their usefulness in previous studies of Cactaceae (e.g., Nyffeler, 2002; Butterworth and Wallace, 2004; Korotkova et al., 2010; Calvente et al., 2011; Guerrero et al., 2011a; Hernández-Hernández et al., 2011; Yesson et al., 2011; Majure et al., 2012; Schlumpberger and Renner, 2012; Franck et al., 2012, 2013) and a wide range of other plant families (e.g., Cires et al., 2012; Rakotoarivelo et al., 2012; Granados-Mendoza et al., 2013; Bauters et al., 2014). Nine chloroplast (*rpl16* intron, *petL-psbE*, *psbJ-petA*, *trnS-trnfM*, *psbD-trnT*, *trnS-trnG*, *matK-trnK*, *psbA-3′trnK*, *ndhF-rpl32*) and two nuclear (ITS, PHYC) markers were tested by C. Peña Hernández (Universidad de Concepción, unpublished data); however, the phylogenetic informativeness and/or the amplification rate of these markers proved insufficient for further use in the phylogenetic analyses.

Amplification and sequencing was carried out using the following primer pairs: $trnH^{(GUG)}$ (Tate and Simpson, 2003) and *psbA* (Sang et al., 1997), *rpl32F* and $trnL^{(UAG)}$ (Shaw et al., 2007), and *Ycf1*-4182F and *Ycf1*-5248R (Franck et al., 2012). PCR reactions were carried out in a Biometra thermocycler (Westburg, Leusden, Netherlands) in a 26 µL total volume using 15 µL of H_2O, 2.5 µL 10× polymerase reaction buffer, 2.5 µL dNTP (1.25 µM each), 1 µL of each primer (5 µM), 1 µL of BSA (10 mg/ml), 1 µL of ampliTaq DNA polymerase (Life Technologies, Ghent, Belgium), and 1 µL DNA (~10 ng). PCR amplifications had an initial denaturation of 96°C for 3 min; 35 cycles of 95°C for 45 s, annealing at 52°C for 30 s, and extension at 72°C for 90 s; and a final extension at 72°C for 6 min. PCR products were analyzed on agarose gels stained with ethidium bromide. The cleaned PCR products were then sent to Macrogen Europe (Amsterdam, Netherlands) for sequencing on ABI3730XL machines.

Sequences were assembled and edited in the program Geneious R8 (Biomatters Ltd, Auckland, New Zealand), and manually aligned in the program PhyDE v0.9971 (Müller et al., 2010). Individual markers did not show discordant relationships (>0.7 Bayesian posterior probability [PP], or >70% maximum likelihood bootstrap [BS]) and were combined and analyzed simultaneously. Two versions of the sequence alignment were prepared, (1) the full sampling alignment including all 109 sequences and (2) a reduced sampling alignment including one individual per *Copiapoa* taxon and a reduced number of outgroup species. The latter was used for both the character state and ancestral area reconstructions. The alignments used to produce the phylogenies were submitted to Dryad (http://datadryad.org; doi:10.5061/dryad.hj20g).

***Phylogenetic analysis*—**The program PartitionFinder v.1.1.1 (Lanfear et al., 2012) was used to determine an appropriate data-partitioning scheme from potential partitions that were defined a priori (in this case, each codon position of the *ycf1* gene and the noncoding markers), as well as the best-fitting model of molecular evolution for each partition, using the Bayesian information criterion. The GTRGAMMA model of sequence evolution was determined to be the best-fitting model for each nucleotide partition in the concatenated data set.

Maximum likelihood (ML) analyses of the optimally partitioned data were performed using the program RAxML v8.1.11 (Stamatakis, 2014). The search for an optimal ML tree was combined with a rapid bootstrap analysis of 1000 replicates. Partitioned analyses were conducted using Bayesian inference in the program MrBayes v3.2.3 (Ronquist et al., 2012). The parameters of each of the partitions were the same as in the ML analysis. Rate heterogeneity, base frequencies, and substitution rates across partitions were unlinked. The analysis was allowed to run for 100 million generations across four independent runs with four chains each, sampling every 10 000 generations. Convergence, associated likelihood values, effective sample size (ESS) values, and burn-in values of the different runs were verified with the program Tracer v1.6 (Rambaut et al., 2014. The first 25% of the trees from all runs were excluded as burn-in before making a majority-rule consensus of the 7500 posterior distribution trees using the "sumt" function. All phylogenetic analyses were run using the CIPRES portal (http://www.phylo.org/; Miller et al., 2010) and were executed for both full and reduced sampling alignments. Trees were drawn using the programs TreeGraph 2 (Stöver and Müller, 2010) and Adobe Photoshop CS3.

***Character state reconstruction*—**Plants of all taxa included in the molecular study were studied by the authors both in situ during field expeditions and in a large number of ex situ living collections in Europe and North and South America. The existing morphological knowledge is here used to discuss the morphological trends in the obtained phylogenetic hypothesis. Binary character states were assigned for four morphological characters: root morphology (fascicular vs. long or short taproot), stem branching (much branching vs. solitary or little branching [2–3]), stem shape (cylindrical vs. [sub]globose), and stem diameter (≤7.5 cm vs. > 7.5 cm). The selection of these characters was partly based on recent literature, e.g., Hernández-Hernández et al. (2011) and Schlumpberger and Renner (2012) studied growth form as a potentially informative morphological character, while Ritz et al. (2012) reconstructed character states for seven morphological traits, i.e., life form, root and embryo, plus four characters specific to *Opuntia* Mill. Initially, other characters were tested, e.g., flower shape, seed morphology, stem firmness, epidermis color, presence of spines); however, they did not prove very informative at infrageneric levels, although they are often useful at specific and infraspecific levels. The program BayesTraits v1.0 (Pagel et al., 2004; Pagel and Meade, 2006) was used to perform ancestral state reconstructions. The sampled trees from independent runs (.t-files) of the Bayesian analysis on the concatenated matrix (see above) were loaded into the program Mesquite v 2.75 (Maddison and Maddison, 2011). For all four .t-files the first 25% of trees were discarded as burn-in, and 250 trees were sampled randomly out of the remaining trees and merged in a separate file, resulting in 1000 sampled trees. The outgroup was used to root the trees. Next, 12 well-supported nodes were chosen for ancestral state reconstruction. The command lines for these 12 nodes were generated in the program BayesTrees v1.3 (Meade and Pagel, 2011). The 1000 sampled trees were used for analyzing each character separately using the Multistate module as implemented in BayesTraits. Initially, a maximum likelihood analysis was run to derive empirical priors. After setting these priors, a Bayesian analysis was performed using a MCMC approach, 50 000 000 generations, sampling every 1000th generation, discarding the first 25% as burn-in. Acceptance rates were checked manually, and RateDev parameters were varied to reach acceptance rate values between 20–40% to ensure adequate mixing. Ancestral states were plotted on the Bayesian consensus tree using pie charts in TreeGraph 2 (Stöver and Müller, 2010).

***Species distribution modeling*—**Predictive distribution modeling was used to infer species range extent associated with spatial distribution of environmental suitability. Climatic variables determine species distribution at broad evolutionary and biogeographic scales (Soberón, 2007; Colwell and Rangel, 2009); therefore, methods that use these variables to produce occurrence probability maps are appropriate to estimate the distribution of species. Information on locality data was obtained from different sources: field excursions, literature (e.g., Eggli et al., 1995; Schulz and Kapitany, 1996; Schulz, 2006; Guerrero et al., 2011b), and the Chilean herbaria CONC and SGO. These locality data are not included here, as it concerns CITES listed species under significant threat due to illegal collecting. However, more information may be obtained from us. The current climatic variables were obtained from Pliscoff et al. (2014) who modified and corrected biases caused by heterogeneous distribution of data records in northern Chile that were detected for 19 of the original bioclimatic variables of Hijmans et al. (2005). Also, we used the month surface radiation value of the year 2000 (Ohmura et al., 1998 and posterior updates), and the Global Potential Evapo-Transpiration and Global Aridity Index (Zomer et al., 2007, 2008). To select the variables, a Pearson correlation analysis in the program ENMTools (Warren et al., 2008) was performed, discarding those variables with a correlation over 0.9. After filtering, 12 variables were retained: mean diurnal range, isothermality, maximum temperature of warmest month, temperature annual range, mean temperature of driest quarter, precipitation of driest month, precipitation of warmest quarter, precipitation of coldest quarter, precipitation seasonality (coefficient of variation), global potential evapotranspiration, and two month radiation (January and October). The resolution of all climatic layers was 1 km^2. Climatic layers were managed with the program ArcGIS v9.3 (ESRI, Redlands, California, USA).

To model species distributions, we generated species distribution models (SDMs, Elith et al., 2011) based on a maximum entropy algorithm implemented in the program MaxEnt v3.3.3 (Phillips et al., 2006). First, we selected a background as the rectangle between −8.98 to 55.97 latitude, and −78.91 to −62.01 longitude. Because MaxEnt only uses occurrence data; absence data (pseudoabsence) are defined randomly within the background. We made 50 replicates (with bootstrap adjustment based on 500 iterations) for each species and used the average models as predicted distributions. The random test percentage of 25% was selected for evaluating the accuracy of each model, and area under the curves (AUCs) were calculated using MaxEnt, which allows evaluating the sensitivity and specificity of the model. Since 13 *Copiapoa* species have less than five recorded occurrences, distribution models were not run for these species, and their distributions are shown relying on occurrence data.

***Ancestral area reconstruction*—**To trace the biogeographic history of the *Copiapoa* species, we inferred ancestral areas using S-DIVA analysis implemented in the program RASP (Reconstruct Ancestral State in Phylogenies) v2.1 (Yu et al., 2015). The distribution range of *Copiapoa* was divided into seven areas, based on the presence of one or more endemic species as shown in Figs. 3A–H: A (Peru), B (south of Antofagasta), C (north of Taltal), D (south of Taltal), E (north of Copiapó Valley), F (south of Copiapó Valley), and G (central Chile). The S-DIVA analysis was run on the .t output files of the MrBayes reduced data set analysis. The number of maximum areas was kept as 2. The possible ancestral ranges at each node on a selected tree were obtained (see online Appendix S3).

RESULTS

***Characteristics of the plastid data set*—**The final concatenated full sampling alignment included 3047 bp of aligned sequence data for 109 accessions, and the final concatenated reduced sampling alignment included 2869 bp of aligned sequence data for 42 accessions. Table 1 contains the summary statistics for the individual markers and for the coverage of each marker in the two data sets.

***Phylogenetic results*—**The results of the phylogenetic analyses of the three single-marker alignments were largely congruent. None of the relationships that differed between trees were well supported (i.e., PP > 0.7 or BS > 70), and differences below these values were largely restricted to closely related taxa. In online Appendix S4 (full sampling alignment analysis), the outgroup is represented by species of the genera *Blossfeldia* Werderm., *Calymmanthium*, *Eriosyce* Phil., *Eulychnia* Phil., *Parodia* Speg., *Pereskia*, and *Rhipsalis* Gaertn. In Fig. 2 (see also online Appendix S5, the reduced sampling alignment analysis depicting branch lengths), the outgroup is reduced to *Eriosyce subgibbosa* (Haw.) Katt. subsp. *subgibbosa*, *Eulychnia iquiquensis* (K.Schum.) Britton & Rose, and *Calymmanthium substerile* F.Ritter. In all analyses, the genus *Copiapoa* forms a well-supported (PP = 1, BS = 100) monophyletic clade. The first branch in *Copiapoa* encompasses *Copiapoa solaris* and *Copiapoa humilis* subsp. *australis* P.Hoxey (further as '*C. australis*'). The sister relationship between these taxa is weak, and in all ML analyses performed, the latter taxon occurred on a separate branch between *C. solaris* and *C. hypogaea* subsp. *laui* (further as *C. laui* Diers). The clade (or grade in ML results) formed by *C. solaris* and '*C. australis*' is sister to the rest of the genus *Copiapoa*. First branching after this clade is *C. laui*, a well-supported taxon (PP = 1, BS = 93). Subsequently branching is a well-supported clade including accessions of several infraspecific taxa recognized under the species *C. humilis* (Phil.) Hutchison, i.e., *C. humilis* subsp. *humilis*, *C. humilis* subsp. *tenuissima* (F.Ritter ex D.R.Hunt) D.R.Hunt, *C. humilis* subsp. *tocopillana* (F.Ritter) D.R.Hunt, and *C. humilis* subsp. *variispinata* (F.Ritter) D.R.Hunt (PP = 1, BS = 100). The relationships between most infraspecific taxa of *C. humilis* in this clade (see II in Fig. 2) are not resolved (polytomy). However, in Appendix S4, the two included samples of *C. humilis* subsp. *tenuissima* are well supported as a clade (PP = 1, BS = 83). Sister to clade II is a clade (PP = 1, BS = 100) encompassing all other *Copiapoa* species, which is divided in two well-supported clades (see III and IV in Fig. 2).

Clade III can be split into two clades (see clade IIIa and clade IIIb in Fig. 2). Clade IIIa includes two species accepted by Hunt et al. (2006), i.e., *C. cinerea* (Phil.) Britton & Rose and *C. krainziana* F.Ritter. However, our data suggests that *C. cinerea* subsp. *haseltoniana* is a separate species (PP = 0.96) (further referred to as *C. gigantea* Backeb.), while *C. krainziana* is nested in *C. cinerea* (further referred to as *C. cinerea* subsp. *krainziana* (F.Ritter) Slaba). As is shown by the polytomy in Appendix S4, the included samples of *C. cinerea* subsp. *cinerea* (3), *C. cinerea* subsp. *columna-alba* (F.Ritter) D.R.Hunt (4) and *C. cinerea* subsp. *krainziana* (8) cannot be differentiated from each other based on our data (no sequence variation). Though no inference about hybridity can be made studying only chloroplast markers, two samples which were identified in the field as of presumed hybrid origin (intermediate morphology and locality at edge of distribution area of *C. cinerea* and *C. gigantea*) here form a weakly supported clade between both species (Appendix S4).

Clade IIIb can be split up into several clades and grades. Branching off first are *C. longispina*, *C. megarhiza* Britton & Rose subsp. *megarhiza*, and *C. conglomerata* (F.Phil.) Lembcke (Fig. 2). The three branches are well supported (PP > 0.95, BS > 70). Sister to *C. conglomerata* is a well-supported clade (PP = 0.9), which includes a polytomy and several recognizable species groups. Within clade IIIb, a first clade encompasses the species *C. longistaminea* F.Ritter, *C. aphanes* Mächler & Helmut Walter, *C. desertorum* F.Ritter, and *C. rupestris* and is sister to a clade comprising a polytomy (of *C. serpentisulcata* F.Ritter, *C. taltalensis* and *C. decorticans* N.P.Taylor & G.J.Charles) and another supported clade (PP ≥ 0.75) including *C. cinerascens* (Salm-Dyck) Britton & Rose and 11 other taxa. First branching after *C. cinerascens* is a well-supported clade (PP = 0.89) including *C. angustiflora*, *C. esmeraldana*, and *C. mollicula* F.Ritter, in which *C. angustiflora* and *C. esmeraldana* are strongly supported as sister taxa (PP = 1, BS = 95). This clade is sister to *C. grandiflora* plus the clade indicated by node 10 in Fig. 2. The latter clade (PP = 0.93, BS = 71) encompasses a polytomy of *C. montana* F.Ritter, *C. calderana* F.Ritter subsp. *calderana* (further as *C. calderana*), and *C. marginata*, and a well-supported clade (PP = 0.91). The latter includes *C. hypogaea* subsp. *hypogaea*, *C. calderana* subsp. *atacamensis* (Middled.) D.R.Hunt (further as *C. atacamensis* Middled.), *C. leonensis* I.Schaub & Keim, and *C. megarhiza* subsp. *parvula* Mächler & Helmut Walter (further as '*C. parvula*'). The relationships among these species are not well resolved or well supported by PP or BS values, reflecting the limited genetic distances between the taxa in this group.

Clade IV includes two sister clades, the first of which includes an accession of *C. coquimbana* var. *armata* (further as '*C. armata*'), and *C. fiedleriana* (K.Schum.) Backeb. (in Appendix S4, a polytomy of five accessions plus one accession of *C. megarhiza* subsp. *echinata*

TABLE 1. Summary statistics of the full sampling alignment including all 109 sequences and of the reduced sampling alignment including one individual per *Copiapoa* taxon and a reduced number of outgroup species.

Locus	Total length (bp)	Variable characters (*N*)	PI characters (*N*)	Coverage (%)
Full sampling				
rpl32-trnL	1318	310	150	86
trnH-psbA	421	81	41	99
ycf1	1308	560	378	100
Reduced sampling				
rpl32-trnL	1306	124	35	76
trnH-psbA	419	79	13	98
ycf1	1144	361	101	100

Notes: PI = parsimony informative.

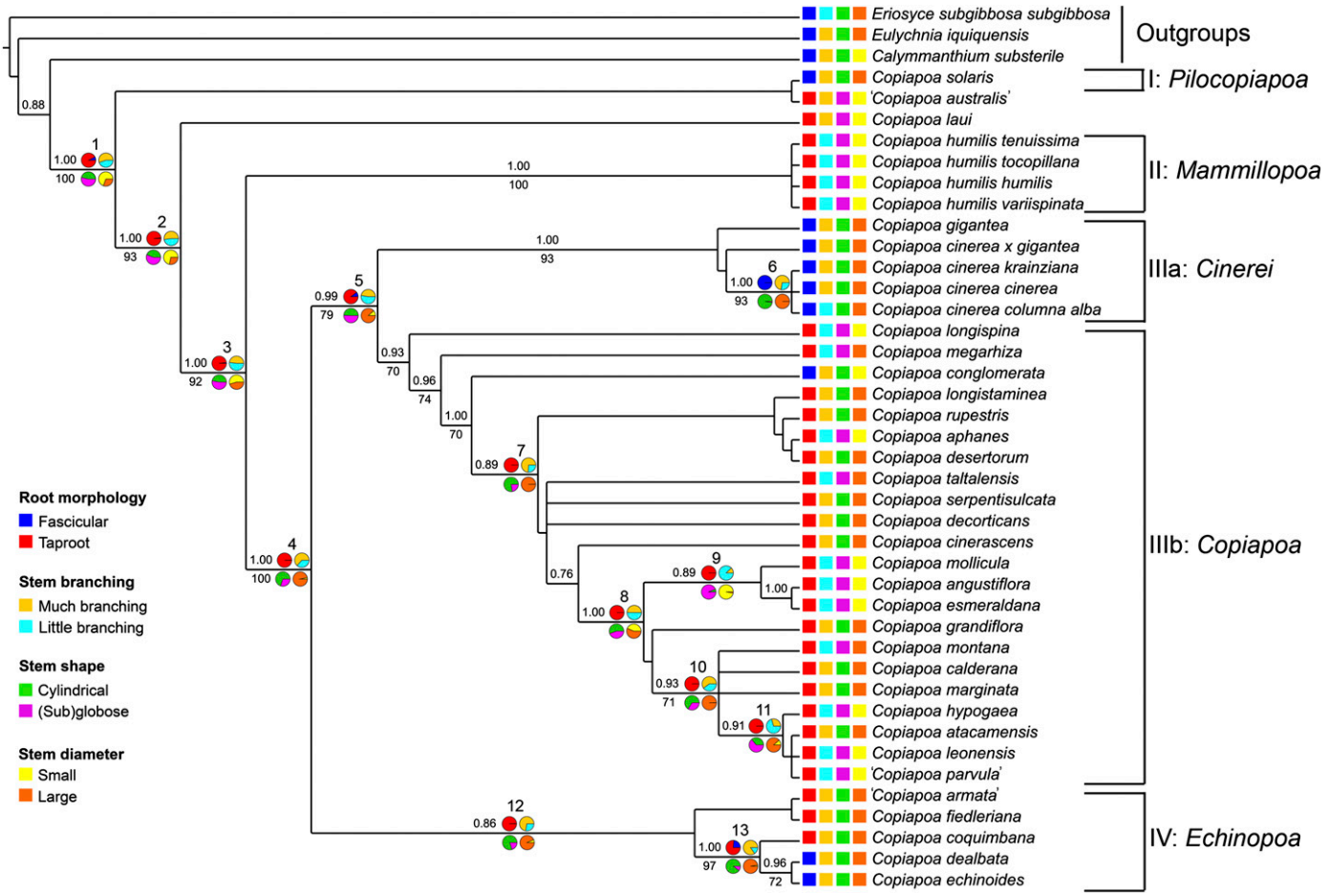


FIGURE 2 Three-locus plastid phylogenetic hypothesis of *Copiapoa* based on a reduced sampling alignment of 42 accessions. Bayesian posterior probabilities (PP) are shown above branches. Maximum-likelihood bootstrap support values (BS) are shown below branches; PP > 0.7 and BS > 70 are given, and branches with support PP < 0.5 or BS < 50 are shown as polytomies. See also Appendix S4 for full sampling (109 accessions) phylogram and Appendix S5 for reduced sampling (42 accessions) phylogenetic hypotheses depicting branch lengths. The ancestral state reconstruction executed in BayesTraits is shown for 13 relevant nodes, as are the separate character states for each of the four characters per taxon.

(F.Ritter) Doweld, which is nested in *C. fiedleriana*). The second clade includes three species, i.e., *C. coquimbana* (Karw. ex Rümpler) Britton & Rose, *C. dealbata* F.Ritter, and *C. echinoides* (Lem. ex Salm-Dyck) Britton & Rose, the latter are well supported as sister taxa (PP = 0.93).

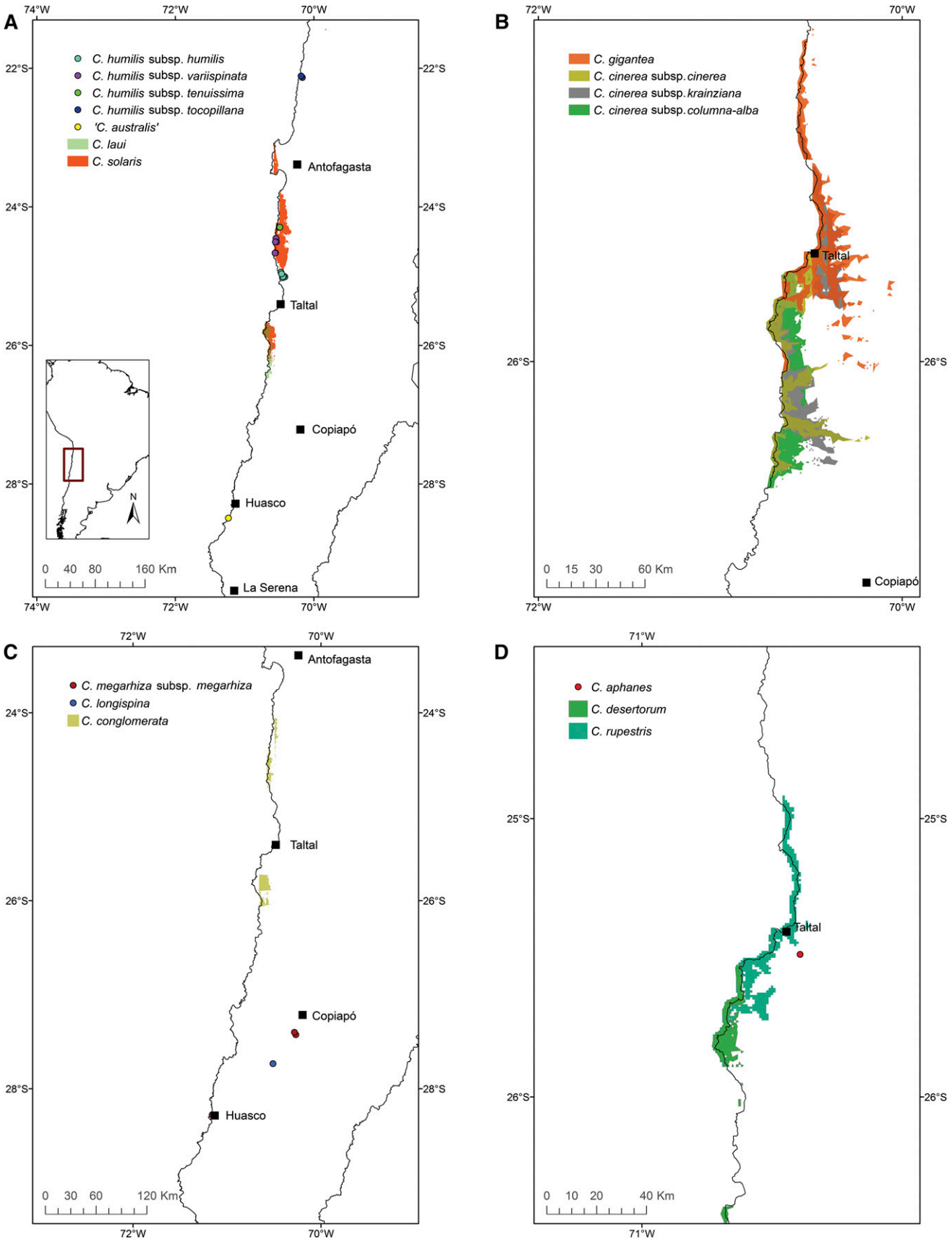
***Ancestral state reconstruction*—**The reconstructed character states for 13 nodes are shown in Fig. 2, with the likelihood values given in online Appendix S6. Character state reconstruction indicates that taproots are likely to be ancestral in *Copiapoa* with a much higher probability than a fascicular root state (node 1, PP = 0.93 vs. PP = 0.07). Taproots are the most common state in *Copiapoa*, and fascicular roots originate at least four times in *C. solaris*, clade IIIa, *C. conglomerata*, and the *C. dealbata*–*C. echinoides* sister pair (Fig. 2). Interestingly, all the outgroup taxa included in our analysis have fascicular roots.

A much-branching habit is more likely to be ancestral than the little-branching habit (node 1, PP = 0.55 vs. PP = 0.45). Two clades (*C. humilis* s.l. and the clade indicated by node 9) are characterized by a little-branching habit. In total, this character state originated at least seven times from the more common much-branching state.

Concerning stem shape, character state reconstruction is less certain as to which is ancestral, although the (sub)globose state has a slightly higher likelihood than the cylindrical state (node 1, PP = 0.54 vs. PP = 0.46). Except *C. solaris*, the other early-branching species of *Copiapoa* (see II in Fig. 2) have (sub)globose stems. The most likely ancestral state for clades III and IV (Fig. 2) is cylindrical instead of (sub)globose (node 4, PP = 0.70 vs. PP = 0.30). All clade IIIa and clade IV taxa have cylindrical stems, in clade IIIb the state reverted at least six times to the (sub)globose state.

The ancestral state for stem diameter is more likely to be small than large (node 1, PP = 0.69 vs. PP = 0.31). Except for *C. solaris*, all other early-branching *Copiapoa* species have a small stem diameter (see II in Fig. 2). However, for clades III and IV, the most likely ancestral state is a large stem diameter (node 4, PP = 0.97 vs. PP = 0.03), and a small stem diameter in these clades is only found in five separate species or clades.

***Species distribution modeling*—**Figure 3 shows the modeled species distributions of the *Copiapoa* species. Figure 3A gives the modeled distribution of *C. solaris*, '*C. australis*', *C. laui*, and *C. humilis* s.l. The distribution ranges of *C. solaris* and *C. laui* overlap


A
74°W
72°W
70°W
22°S
24°S
26°S
28°S
C. humilis subsp. humilis
C. humilis subsp. variispinata
C. humilis subsp. tenuissima
C. humilis subsp. tocopillana
'C. australis'
C. laui
C. solaris
Antofagasta
Taltal
Copiapó
Huasco
La Serena
N
0 40 80 160 Km
B
72°W
70°W
26°S
C. gigantea
C. cinerea subsp. cinerea
C. cinerea subsp. krainziana
C. cinerea subsp. columna-alba
Taltal
Copiapó
0 15 30 60 Km
C
72°W
70°W
24°S
26°S
28°S
C. megarhiza subsp. megarhiza
C. longispina
C. conglomerata
Antofagasta
Taltal
Copiapó
Huasco
0 30 60 120 Km
D
71°W
25°S
26°S
C. aphanes
C. desertorum
C. rupestris
Taltal
0 10 20 40 Km

(Continued)

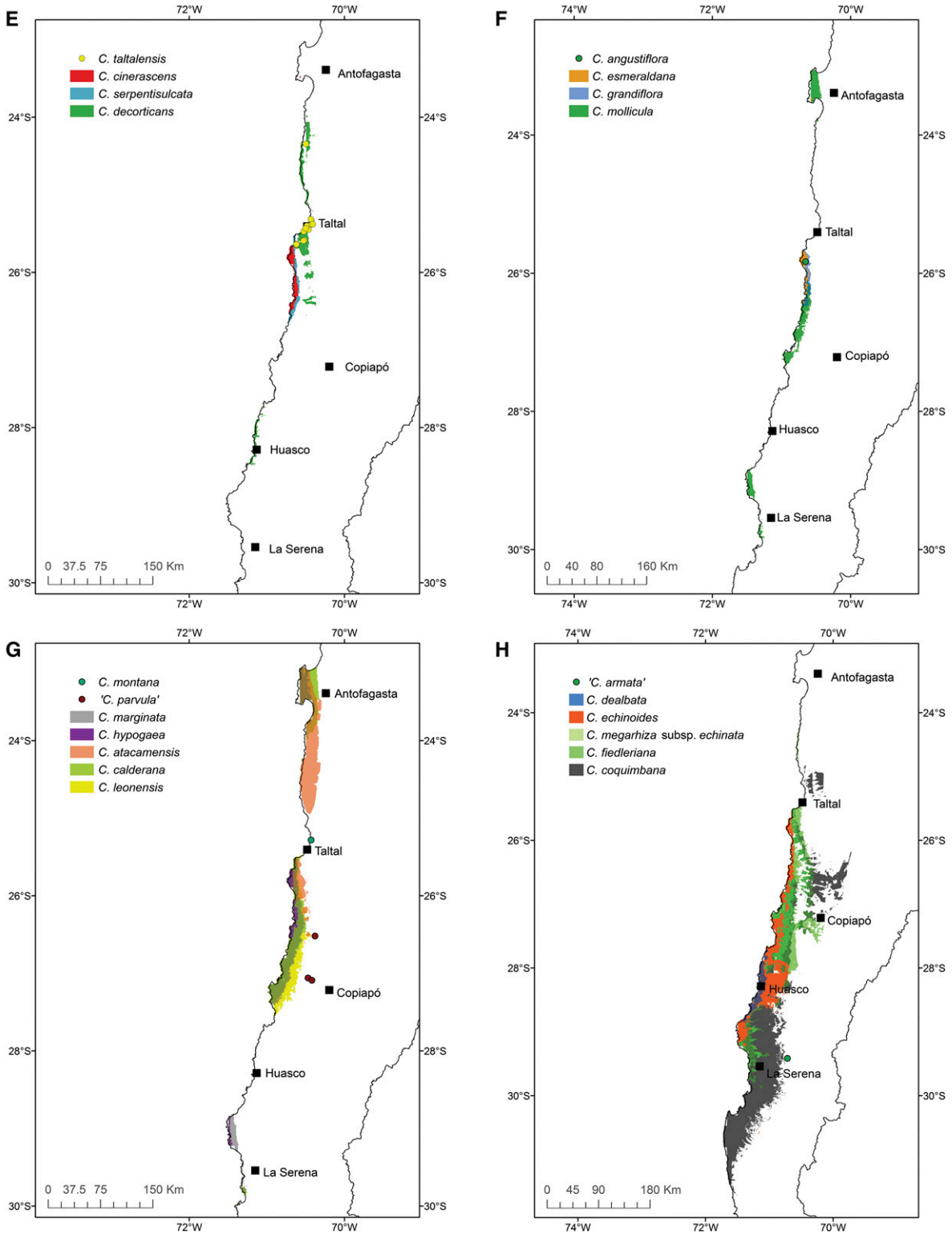


FIGURE 3 Modeled species distributions of the *Copiapoa* species grouped according to their phylogenetic relationships as shown in Figure 2. (A) *C. solaris*, '*C. australis*' (*C. humilis* subp. *australis*), *C. humilis* subsp. *humilis*, *C. humilis* subsp. *tenuissima*, *C. humilis* subsp. *tocopillana*, and *C. humilis* subsp. *variispinata*. (B) *C. cinerea* subsp. *cinerea*, *C. cinerea* subsp. *columna-alba*, *C. cinerea* subsp. *krainziana*, and *C. gigantea*. (C) *C. conglomerata*, *C. longispina*, and *C. megarhiza*. (D) *C. aphanes*, *C. desertorum*, *C. longistaminea*, and *C. rupestris*. (E) *C. cinerascens*, *C. decorticans*, *C. serpentisulcata*, *C. taltalensis*. (F) *C. angustiflora*, *C. esmeraldana*, *C. grandiflora*, and *C. mollicula*. (G) *C. atacamensis*, *C. calderana*, *C. hypogaea*, *C. leonensis*, *C. marginata*, *C. montana*, and '*C. parvula*' (*C. megarhiza* subsp. *parvula*). (H) '*C. armata*' (*C. coquimbana* var. *armata*), *C. coquimbana*, *C. dealbata*, *C. echinoides*, and *C. fiedleriana* (including *C. echinata*).

at ca. 26°S, while '*C. australis*' occurs much farther south than the species it is related to. Figure 3B shows the modeled distribution of *C. cinerea* s.l. and *C. gigantea*. *Copiapoa gigantea* is the most northerly distributed, while *C. cinerea* subsp. *columna-alba* is the most southerly distributed taxon of clade IIIa (Fig. 2). *Copiapoa cinerea* subsp. *cinerea* and *C. cinerea* subsp. *krainziana* only occur in the immediate vicinity of Taltal. *Copiapoa conglomerata* is distributed more northerly from around 24°S to the area around 26°S, while *C. longispina* and *C. megarhiza* occur farther to the south (27–28°S) (Fig. 3C). *Copiapoa aphanes*, *C. desertorum*, *C. longistaminea*, and *C. rupestris* are mainly distributed just south and east of Taltal (Fig. 3D), while *C. cinerascens*, *C. serpentisulcata*, and *C. taltalensis* are found around 26°S (Fig. 3E). *Copiapoa angustiflora*, *C. esmeraldana*, *C. grandiflora*, and *C. mollicula* largely occur in and around Pan de Azúcar National Park (Fig. 3F). Figure 3G shows the modeled distribution of *C. atacamensis*, *C. calderana*, *C. hypogaea*, *C. leonensis*, *C. marginata*, and *C. montana*. The species of this clade are distributed over nearly all areas where the genus occurs (Appendix S3). Figure 3H gives the modeled distribution of '*C. armata*', *C. coquimbana*, *C. dealbata*, *C. echinoides*, and *C. fiedleriana* (see IV in Fig. 2). The species of this clade are largely distributed between 26°S and 30°S. *Copiapoa dealbata*, *C. echinoides*, and *C. fiedleriana*, as well as *C. coquimbana* and *C. fiedleriana* sometimes grow sympatrically, while '*C. armata*' occurs further inland.

***Ancestral area reconstruction*—**The S-DIVA analysis shown in Appendix S3 suggests that *Copiapoa* has a complex biogeographical history in which dispersal, vicariance, and extinction have all been important. The results of the analysis indicate that the ancestors of the genus *Copiapoa* originated in southern Peru or the extreme north of Chile (see area reconstruction at basal node 81, with the frequency of occurrence of this range being 100%). Nodes 80 and 43 suggest an early dispersal to the most southern area of dispersal by the ancestor of '*C. australis*'. At nodes 78, 77, 72, and 67, the possible ancestral area switches twice between ranges BC (ca. between 22° and 25°S) and F (south of the Copiapó Valley). In clade III, after node 65, the ancestral and current ranges in general gradually pass from north to south (Fig. 3D–G; Appendix S3).

DISCUSSION

***General patterns of evolution and diversity*—**The relationships shown among the genera representing the outgroup in Appendix S4 conform to those recovered in recent molecular phylogenetic studies of Cactaceae (e.g., Nyffeler, 2002; Bárcenas et al., 2011; Hernández-Hernández et al., 2014). Despite the comparatively low sequence variation in the markers used in previous phylogenetic studies of Cactaceae (Korotkova et al., 2011), as also confirmed by our results, relationships between genera, clades of related species, and many previously recognized *Copiapoa* taxa were resolved.

First-branching species—Copiapoa solaris is a clearly defined species, very different in morphology to '*C. australis*', *C. laui*, and *C. humilis* (Fig. 2; Appendix S4). *Copiapoa laui* is well supported on a branch in between the *C. solaris*+'*C. australis*' clade and the *C. humilis* clade. *Copiapoa humilis* forms a monophyletic clade (see II in Fig. 2) including *C. humilis* subsp. *humilis*, *C. humilis* subsp. *tenuissima*, *C. humilis* subsp. *tocopillana*, and *C. humilis* subsp. *variispinata*. *Copiapoa solaris*, *C. laui*, and *C. humilis* occur in the extreme north of the distribution range of the genus, while '*C. australis*' is distributed farther south (Fig. 3A) as is indicated by its epithet, which was chosen when it was considered to be the southernmost infraspecific taxon of *C. humilis*.

Clades III and IV—Interestingly, virtually all species of clade III (except *C. longispina* and southerly populations of *C. megarhiza*) occur north of the Copiapó Valley (27°10′S to 23°30′S, Antofagasta) (Fig. 3B–G), while all species of clade IV are distributed in the area to the south of the Copiapó Valley, between Totoral (27°50′S) and the Choapa Valley (31°S) (Fig. 3H). The Copiapó Valley also marks the northernmost distribution of *Eriosyce* subgenus *Neoporteria* (Walter, 2008), and in *Nolana* (Solanaceae) a genus occurring in a similar habitat, the Copiapó Valley, also marks one of the strongest barriers to gene flux (Ossa et al., 2013). These results suggest that the Copiapó Valley acts as a barrier in distinct Cactaceae and other plant lineages. In *Copiapoa* it particularly separates the evolutionary histories of clades III and IV. Apart from forming a geographic barrier, the precipitation regime changes from arid (to the south) to hyper-arid (to the north) and the bioclimate from desertic-oceanic to hyperdesertic in this zone (Luebert and Pliscoff, 2006). Another reason the Copiapó Valley acts as a distribution barrier may be because the valley widens to >60 km in the coastal zone where unstable sandy soil could constrain growth of most *Copiapoa* species and significantly hinder the ant-mediated seed dispersal. However, several scattered populations of *C. marginata* occur within the coastal zone of the Copiapó Valley (Schulz, 2006; H. E. Walter, personal observations). Additionally, destructive water floods run down the Copiapó Valley after unusually heavy rain, as it did in March 2015, with devastating consequences on infrastructure and wildlife (Dirección General de Aguas de Chile, 2015). These phenomena are thought to occur once each century, making the presence of long-lived populations of cacti in riverbeds of the Copiapó Valley unlikely. Based on the historical biogeographic reconstructions, few dispersal events occurred between these two areas suggesting that climatic conservatism might constrain colonization, since arid environments are relatively harsh habitats that may require novel physiological adaptations to allow organisms to invade them (Guerrero et al., 2013). An ancient dispersal event occurred from north to south crossing the Copiapó Valley (at node 80) and another from south to north (at node 66) in the diversification history of the genus (Appendix S3). Complementary to the dispersal history of the group, morphological evolution in *Copiapoa* showed substantial homoplasy and lability, which may be caused by similar microclimates in both areas, and also parallel evolutionary response of species to adapt to extremely dry conditions and use new hydric niches such as fog oasis.

***Taxonomic implications*—**The poor fit of the clades shown in Fig. 2 with previously published classifications, indicates the need for a revised infrageneric classification of *Copiapoa* (see also online Appendix S7).

***Copiapoa* section *Pilocopiapoa* (F.Ritter) Doweld—**Ritter (1961) described the monotypic genus *Pilocopiapoa* for *C. solaris* because of the abundant wool covering the pericarpel, the floral tube, and the fruits of this species. While Ritter (1980) and Hoffmann and Walter (2004) placed *Pilocopiapoa* at the subgeneric level in *Copiapoa*, Doweld (2002) recognized it at the sectional level.

***Unplaced taxa*—**Two species are left unplaced in the suggested infrageneric classification: *C. australis* and *C. laui*.

Copiapoa australis (Hoxey) Helmut Walter & Larridon, comb. et stat. nov. Basionym: *Copiapoa humilis* subsp. *australis* Hoxey, Brit. Cact. Succ. J. 22 (1): 39. 2004.

Copiapoa australis warrants recognition at the species level (Fig. 2; Appendix S4), separate from *C. humilis* of which it was previously considered an infraspecific taxon (Hoxey, 2004). *Copiapoa australis* has rather low conical tubercles, while *C. laui* and the various *C. humilis* subspecies have pronounced conical tubercles.

Many authors (e.g., Charles, 1998; Hunt et al., 2006) considered *C. laui* to be a variety or subspecies of *C. hypogaea* (see clade IIIf). However, this relationship is not supported by our analyses (Fig. 2; Appendix S4). Although *C. laui* and *C. hypogaea* share some morphological characters (e.g., taproot, very short to geophytic [sub] globose stems, gray-green epidermis mimicking the surrounding terrain in color and texture, no or few very small spines, fine hairs from the axils of the hypanthium bract-scales), these similarities most likely result from convergent evolution in similar habitats.

Copiapoa australis, *C. laui*, and the taxa of the *C. humilis* clade do not form a monophyletic clade, but do share a number of morphological characters besides the presence of conical tubercles, e.g., taproots, (sub)globose stems, and small stem diameter (Fig. 2). The observed morphological similarities may result from homoplasies as morphology is often convergent in cacti (Hernández-Hernández et al., 2011; Schlumpberger and Renner, 2012). This hypothesis is supported by the fact that *C. australis* shows much more morphological similarity to the *C. humilis* clade compared with *C. laui*. Alternatively, they may represent the ancestral character states. Following this hypothesis, ancestors of *C. australis* showing this morphology dispersed from the more northerly ancestral distribution range of *Copiapoa* (Appendix S3) as far south as Huasco (28°S), and today's *C. australis* could be considered a relict species, with a population that currently consists of less than 2000 individuals within an extremely small extent of occurrence (<10 km^2) near Huasco.

Based on our results, several hypotheses concerning the early-branching lineages in *Copiapoa* can be put forward. A number of these lineages may have gone extinct, which might have been the case for closely related species of *C. solaris*, *C. australis*, and *C. laui*. If representing relicts of old lineages, *C. solaris*, *C. australis*, and *C. laui* may each deserve their own section. Another hypothesis is that these species may represent lineages that did not further diversify. A similar situation where several monotypic lineages could not be placed into a classification was recently described in *Peperomia* (Frenzke et al., 2015). Pending further study, we opt not to publish additional sectional names for *C. australis* and *C. laui*, and these species therefore remain unplaced in the proposed classification.

Copiapoa section *Mammillopoa* Helmut Walter & Larridon, sect. nov. Type: *Copiapoa humilis* (Phil.) Hutchison.

Solitary or little branching (sub)globose cacti, with a small stem diameter (usually ≤7.5 cm) and having taproots. The ribs in mature plants are dissolved into ± conical tubercles. Section *Mammillopoa* is here circumscribed as monotypic, only including *C. humilis*.

Copiapoa australis, *C. laui*, and the taxa of section *Mammillopoa* can all be recognized by the presence of conical tubercles, and more or less conform to Ritter's (1980) section 3 and to *C.* series *Humiles* Doweld (2002: p. 49). However, Ritter's (1980) section 3 not only comprised all the *C. humilis* taxa but also *C. longispina*, *C. esmeraldana*, and *C. taltalensis*, taxa that are placed within different clades in Fig. 2. Doweld (2002) also included additional species in his series *Humiles* and chose the species *C. hypogaea* as the type of this series. As *C. hypogaea* is not related to *C. humilis* according to our results, Doweld's name cannot be used for this group. Therefore, the new name *Copiapoa* section *Mammillopoa* is proposed based on *C. humulis* subsp. *humilis* as the type and including only the four *C. humilis* subspecies to conform to the monophyly criterium (Fig. 2).

***Copiapoa Britton & Rose section Copiapoa*—**Based on our results, section *Copiapoa* includes two sister clades, here recognized at the subsectional level (see clades IIIa and IIIb in Fig. 2).

Copiapoa section *Copiapoa* subsection *Cinerei* (Doweld) Helmut Walter & Larridon, comb. et stat. nov. Basionym: *Copiapoa* ser. *Cinerei* Doweld, Sukkulenty 4 (1–2): 48. 2002.

In his section 5, Ritter (1980) placed all the taxa here included in subsection *Cinerei*, but also included *C. dealbata*, *C. longistaminea*, and *C. serpentisulcata*, taxa that are placed in different clades in Fig. 2. Doweld (2002) largely followed Ritter's (1980) circumscription when formally describing this taxon, though he excluded *C. longistaminea*. The species of subsection *Cinerei* (Fig. 2) have a large number of ribs (up to 40), and their stem tissue is very hard compared with other *Copiapoa* species. The two species supported by our data, i.e., *C. cinerea* and *C. gigantea*, can also be distinguished from each other morphologically. While *C. cinerea* plants form loose groups of stems or have solitary stems with the apex covered in gray wool, *C. gigantea* forms large dense mounds with (orange-) brown apical wool. Although no sequence variation was found between the three subspecies of *C. cinerea* (*C. cinerea* subsp. *cinerea*, *C. cinerea* subsp. *columna-alba* and *C. cinerea* subsp. *krainziana*), these taxa are usually very easy to distinguish from each other based on their morphology and distribution. Nevertheless, plants with intermediate morphologies are known from the areas where the distribution ranges of the subspecies meet. Also, putative hybrid plants between *C. cinerea* and *C. gigantea* have been documented and were here included in the analysis (Fig. 2; Appendix S4). To obtain a clearer picture of the relationships in subsection *Cinerei* and to investigate the genetic diversity, gene flow, and population structure of these taxa, we have already started a microsatellite study of these taxa. The four taxa are mainly distributed around Paposo and Taltal (Fig. 3B), an area well known for its high richness in cactus species and the presence of many endemic taxa (e.g., Guerrero et al., 2011b; Walter, 2011a; Larridon et al., 2014).

***Copiapoa section Copiapoa subsection Copiapoa*—**The first-branching species in clade IIIb (Fig. 2), i.e., *Copiapoa longispina*, *C. megarhiza*, and *C. conglomerata*, form a grade. *Copiapoa longispina* was considered to be related to *C. humilis* by various authors (Doweld, 2002, as a subspecies; Hunt et al., 2006, as a synonym of *C. humilis* subsp. *humilis*). Based on their morphology, *C. australis*, *C. laui*, and *C. humilis*, plus *C. longispina* are indeed recognizable as a group based on shared morphological traits (ribs dissolved into ± conical tubercles, root morphology, stem shape, and stem diameter) (Fig. 2). However, according to our results, *C. longispina* is not closely related to the other taxa that share this morphology. The similarities between *C. longispina* and the species *C. australis*, *C. humilis*, and *C. laui* may be due to convergent evolution. Alternatively, they could represent the ancestral state, with *C. solaris* and the clade IIIa taxa having developed a different morphology. The latter hypothesis is better supported by the character state

reconstruction (Fig. 2). Previously, two or more infrageneric taxa were treated as subspecies of *C. megarhiza* (i.e., subspecies *megarhiza*, *echinata*, and *parvula*) (Mächler and Walter, 2005; Hunt et al., 2006). However, in our results subsp. *echinata* is placed among the *C. fiedleriana* accessions included in the analyses (see above), while subsp. *parvula* is placed in the clade branching at node 11 in Fig. 2 (see below). *Copiapoa conglomerata* was listed by Hunt et al. (2006) and IUCN (2014) under the name *C. ahremephiana*, but the earliest published and thus correct name for this taxon is *C. conglomerata* (Walter, 2011b). *Copiapoa conglomerata*, *C. longispina* and *C. megarhiza* are quite dissimilar in morphology. Concerning their distribution, *C. longispina* and *C. megarhiza* occur more inland in the vicinity of Copiapó (27–28°S), while *C. conglomerata* is only known from the vicinity of Quebrada Botija north of Paposo (24°S) (Fig. 3C). Hunt et al. (2006) considered this species to be the northernmost member of the *C. cinerea* group, a view not supported by our results.

Copiapoa aphanes, *C. decorticans*, *C. desertorum*, *C. longistaminea*, *C. rupestris*, *C. serpentisulcata*, and *C. taltalensis* occur in the broad area around and to the south of the coastal town of Taltal (Fig. 3D, E). *Copiapoa aphanes*, *C. desertorum*, and *C. rupestris* can be distinguished from *C. longistaminea* and other related species by the presence of red mid-stripes of different widths on the otherwise yellowish interior perianth segments, and by large taproots. *Copiapoa serpentisulcata*, *C. taltalensis*, and *C. decorticans* form a polytomy, with the next clade including *C. cinerascens* and 11 species with a more southern distribution (Figs. 2, 3F, 3G; Appendix S3). Our results do not corroborate the broadly circumscribed concept of *C. taltalensis* sensu Hunt et al. (2006) including the taxa *C. aphanes*, *C. rupestris*, and *C. rubriflora*, as *C. taltalensis* is placed in a different clade than *C. aphanes*, *C. desertorum*, and *C. rupestris*. *Copiapoa longistaminea* and *C. serpentisulcata* have been considered as related to *C. cinerea* since Ritter (1980) put them within his section 5 or '*C. cinerea* group' (comprising 11 species, among them the southerly distributed *C. dealbata*). Our data do not support this view as *C. dealbata* is placed within clade IV. Though the exact nature of their relationship is not clear from our results, in both *C. cinerascens* and *C. decorticans*, a process of exposing the vascular cylinder through destruction of the soft tissue by effects of heat and/or water stress has been observed (Taylor and Charles, 2002).

The clade indicated by node 9 (Fig. 2) encompasses *C. angustiflora*, *C. esmeraldana*, and *C. mollicula*. The latter species was previously placed in synonymy of *C. montana* (Hunt et al., 2006). *Copiapoa angustiflora* is one of the more recently described *Copiapoa* species (Walter and Mächler, 2006). On the basis of the molecular data, the close relationship between the sister-pair *C. angustiflora* and *C. esmeraldana* needs further study. However, the two taxa can easily be distinguished based on morphology. *Copiapoa esmeraldana* has large, broadly campanulate flowers whose nectar chambers are short and broadly cup-shaped, stems up to 7 cm in diameter, and a green, not pruinose epidermis. In contrast, *C. angustiflora* bears small and narrowly funnelform flowers with a long and narrowly tubular nectar chamber, and has stems up to 4 cm in diameter, with a gray-brown, somewhat pruinose epidermis. Additionally, *C. esmeraldana* is only known from the steep cliffs (fog oasis, 980 m a.s.l.) around Las Lomitas within Pan de Azúcar National Park, while *C. angustiflora* exclusively occurs in and around the Guanillos Valley (some 20 km further northeast in a very dry inland area at 350 m a.s.l.) (Fig. 3F).

Copiapoa montana, *C. calderana*, and *C. marginata* form a polytomy with a well-supported clade encompassing *C. hypogaea* and a polytomy of *C. atacamensis*, *C. leonensis*, and '*C. parvula*'. *Copiapoa montana* can be distinguished from *C. calderana* and *C. marginata* by having little branching and globose stems. The morphological differences between *C. calderana* and *C. marginata* are more subtle. *Copiapoa atacamensis* can easily be distinguished from *C. leonensis* and '*C. parvula*', as it is a much-branching species with large cylindrically shaped stems, and it has a much more northerly distribution (Fig. 3G). In contrast, the morphology of *C. leonensis* and '*C. parvula*' (*C. megarhiza* subsp. *parvula*) is rather similar (stem diameter, globose stems, little branching to solitary, taproot), and their distribution overlaps north of Caldera (Fig. 3G). Therefore, we here opt to synonymize these taxa instead of raising '*C. parvula*' to species level.

In general, the current data set was unable to clearly resolve the close relationships between the species of subsection *Copiapoa* due to the limited genetic diversity. Further study using other techniques is required.

***Copiapoa* section *Echinopoa* Doweld—**Ritter (1980) placed the five species here included in section *Echinopoa* in three of his five nameless sections. Doweld's (2002) section *Echinopoa* originally only comprised taxa with fascicular roots (except for *C. serpentisulcata*), and his section *Copiapoa* only included taxa with tuberous roots. The Doweld (2002) circumscriptions of these sections are not corroborated here. The species of clade IV radiated locally after an ancestral dispersal event to the area south of the Copiapó Valley (Fig. 3H; Appendix S3).

Copiapoa armata (F.Ritter) Helmut Walter & Larridon, comb. et stat. nov. Basionym: *Copiapoa coquimbana* var. *armata* F.Ritter, Kakteen in Südamerika 3: 1075. 1980.

Copiapoa armata was described as a variety of *C. coquimbana* by Ritter (1980). However, our data suggest it to be a separate species more closely related to *C. fiedleriana* than to the typical *C. coquimbana* (Fig. 2; Appendix S4).

Copiapoa echinata F.Ritter, which was considered a subspecies of *C. megarhiza* by Hunt et al. (2006), is nested in *C. fiedleriana* (Appendix S4). Because it is genetically very similar and morphologically not very distinct from *C. fiedleriana*, it seems appropriate to consider it as a synonym of *C. fiedleriana* (see also Appendix S7).

Copiapoa coquimbana, *C. dealbata*, and *C. echinoides* are three well-defined species generally forming large multistemmed mounds. *Copiapoa fiedleriana* also forms dense clusters of stems, but the stems are much smaller and noticeably gray-brown.

***Significance for conservation*—**Our results indicate that the conservation status (IUCN, 2014) for 21 species or >60% of the genus (see Appendix S7, indicated with †) will need to be reassessed, as their previously accepted circumscription does not conform to the molecular phylogenetic findings. Because the species boundaries used in IUCN (2014) were too broad in several cases, the conservation status of these *Copiapoa* species will likely be assessed at a higher level of threat, as their extent of occurrence and area of occupancy will be smaller than was assumed thus far.

CONCLUSIONS

In general, *Copiapoa* clades and species clustering in the molecular phylogenetic hypothesis are often supported by geographical patterns

as well as by shared diagnostic morphological characters. The origin of *Copiapoa* likely lies between southern Peru and the extreme north of Chile, and the Copiapó Valley barrier clearly limited colonization between biogeographical areas. Although some groups share some diagnostic characters, repeated occurrence of homoplasies are detected for characters like root and stem morphology. A new infrageneric classification of *Copiapoa* is established. As defined here, the genus includes 32 species plus five heterotypic subspecies. Thirty species are classified into four sections and two subsections, while two species remain unplaced. Our study provides a phylogenetic baseline for future research (e.g., population genetics, ecology) focusing on selected *Copiapoa* taxa. It also shows that further efforts are needed to urgently reassess the conservation status of 21 *Copiapoa* species.

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