

Systematic placement and biogeographical relationships of the monotypic genera *Gypothamnium* and *Oxyphyllum* (Asteraceae: Mutisioideae) from the Atacama Desert

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Gypothamnium and *Oxyphyllum* (Asteraceae) are two monotypic genera endemic to the Atacama Desert of northern Chile. We performed a phylogenetic analysis using published sequences of the plastid *rbcL* and *ndhF* genes, the *trnL-trnF* region and the nuclear ribosomal internal transcribed spacer (ITS) to assess the systematic placement of the two genera within Mutisioideae. On the basis of the phylogenetic results, we constructed area cladograms to explore the biogeographical relationships and origin of the genera. The phylogenetic analysis showed that *Gypothamnium* is closely related to *Aphyllocladus*, *Plazia*, *Urmenetia*, *Lycoseris* and *Onoseris*, whereas *Oxyphyllum* is closely related to *Leucheria*, *Moscharia*, *Polyachyrus* and, with low support, *Jungia*. These results do not differ substantially from those proposed in previous treatments based on morphological characters. The biogeographical analysis suggests that *Gypothamnium* in the coastal Atacama Desert is related to taxa that are currently distributed in eastern subtropical South America and in the Puna. *Oxyphyllum* may have originated from central Chile and other areas in southern South America, but its sister group (*Leucheria* + *Polyachyrus*) also reaches the Puna and the coastal Atacama Desert. Both groups show ancestral affinities with elements currently distributed in north-western South America and Mesoamerica. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, 159, 32–51.

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INTRODUCTION

The systematics of the tribe Mutisieae *s.l.* (Asteraceae) has been the subject of several recent studies. Such studies have shown Barnadesiinae, formerly included in Mutisieae (Benthams, 1873; Cronquist, 1955; Cabrera, 1977; Bremer, 1987; Bremer *et al.*,

1992), to be sister to the rest of Asteraceae (Jansen & Palmer, 1987, 1988; Jansen *et al.*, 1990; Hansen, 1991a; Jansen, Michaels & Palmer, 1991; Bremer *et al.*, 1992; Kim & Jansen, 1995; Bayer & Starr, 1998; Kim, Choi & Jansen, 2005), which subsequently has been segregated as subfamily Barnadesioideae (Bremer & Jansen, 1992; Bremer, 1994; Gustafsson *et al.*, 2001; Panero & Funk, 2002; Funk *et al.*, 2005). Since then, the tribal, subtribal and

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generic relationships concerning Mutisieae have remained controversial (Kim, Loockerman & Jansen, 2002). It has been suggested that Mutisieae *s.l.* is polyphyletic (Kim *et al.*, 2002; Panero & Funk, 2002; Funk *et al.*, 2005), the subtribe Nassauviinae is monophyletic (Jansen & Palmer, 1988; Bremer, 1994; Kim *et al.*, 2002; Katinas *et al.*, 2008; Panero & Funk, 2008), whereas each of subtribes Gochnatiinae and Mutisiinae seems to be non-monophyletic (Jansen & Palmer, 1988; Kim *et al.*, 2002). At the generic level, Funk *et al.* (2005) synthesized results from all major phylogenetic studies on Asteraceae (see references therein) to build a supertree of the family. These authors considered 34 genera in Mutisieae, which represent 40% of the generic diversity of the group. Recently, Panero & Funk (2008) presented a phylogenetic hypothesis of the entire Asteraceae based on ten plastid loci and with a sampling of 108 taxa, among which 53 genera belonged to Mutisieae *s.l.* (Cabrera, 1977). These authors provided support for the main clades of the family and circumscribed subfamily Mutisioideae to be composed of the monophyletic tribes Onoserideae, Nassauvieae and Mutisieae (Panero & Funk, 2002, 2007, 2008). Several taxa traditionally included in Mutisieae were proposed to be segregated to subfamilies Sitfftioideae, Wunderlichioideae, Gochnatioideae, Hecastocleidoideae and Carduoideae (Panero & Funk, 2008). The genera *Gypothamnium* Phil. and *Oxyphyllum* Phil., traditionally treated as members of Mutisieae *s.l.* (Hoffmann, 1897; Reiche, 1905; Crisci, 1974a; Cabrera, 1977; Bremer, 1994; Hind, 2007; Panero & Funk, 2007), have not been included in any of the previous molecular studies. The systematic position of *Gypothamnium* and *Oxyphyllum* needs to be assessed with molecular evidence.

Gypothamnium and *Oxyphyllum* (Fig. 1) were described by Philippi (1860) after his travel to the Atacama Desert in the 19th century. They are both monotypic and endemic to the coastal Atacama Desert of northern Chile (Johnston, 1929; Moreira-Muñoz & Muñoz-Schick, 2007).

Gypothamnium has been associated to *Plazia* Ruiz & Pav. (Johnston, 1929; Cabrera, 1951; Grau, 1987; Bremer, 1994; Panero & Funk, 2007). Hoffmann (1897) and Reiche (1905) actually included it in *Plazia*. *Plazia* may be related to *Onoseris* Willd., *Lycoseris* Cass., *Aphyllclados* Wedd. and *Urmenetia* Phil., according to morphological and molecular evidence (Hoffmann, 1897; Cabrera, 1951; Hansen, 1991a; Karis, Kallersjö & Bremer, 1992; Bremer, 1994; Kim *et al.*, 2002; Katinas *et al.*, 2008; Panero & Funk, 2008), and together they constitute tribe Onoserideae (Panero & Funk, 2007, 2008). Bremer (1994) included *Gypothamnium* in the '*Plazia* group', but did not assign the group to any subtribe within

Mutisieae. The *Plazia* group is characterized by the foliage of closely set and narrow leaves, sessile capitula and, especially, the rather stout, truncate, coloured anther appendages, that are shared by all genera in the group, i.e. *Aphyllclados*, *Gypothamnium* and *Plazia* (Bremer, 1994). Hind (2007) included these genera in subtribe Mutisiinae. Panero & Funk (2008) showed phylogenetic relationships between *Onoseris* [associated by Bremer (1994) to *Urmenetia*] and *Lycoseris*, and argued for the monophyly of Onoserideae (Panero & Funk, 2007, 2008). Morphology generally confirms these relationships (Hansen, 1991b; Tellería, Urtubey & Katinas, 2003; Sancho, 2004; Tellería & Katinas, 2004). On the basis of karyology, Grau (1987) considered *Gypothamnium* ($2n = 72$) as a palaeopolyploid in relation to the allied genera *Plazia* ($2n = 54$) and *Onoseris* ($2n = 36$), with lower chromosome numbers (Covas & Schnack, 1946; Cabrera, 1977; Grau, 1987). To our knowledge, no chromosome counts have been reported for *Aphyllclados*, *Lycoseris* and *Urmenetia*.

Oxyphyllum was included by Hoffmann (1897) and Reiche (1905) in Mutisieae-Nassauviinae, as a close relative of *Leucheria* Lag., from which it differs by its shrubby habit and glabrous leaves. Johnston (1929) and Crisci (1980) also suggested its position as a close relative of *Leucheria* within Nassauviinae. Crisci (1974a) suggested that *Oxyphyllum* is an 'advanced stage of development in the subtribe, the product of colonization by the Nassauviinae of a very special environment.' Bremer (1994) included *Oxyphyllum* in the '*Leucheria* group', which belongs to Nassauviinae, together with *Nassauvia* Comm. ex Juss., *Leucheria*, *Perezia* Lag., *Holocheilus* Cass., *Pamphalea* Lag., *Macrachaenium* Hook. f., *Moscharia* Ruiz & Pav., *Polyachyrus* Lag. and *Triptilion* Ruiz & Pav., and related *Oxyphyllum* to *Nassauvia*, but specified that the intergeneric relationships are unclear. In agreement with Vuilleumier (1969), Crisci (1974a) and Hansen (1991a), Bremer (1994) pointed out that two subgroups can be recognized within the *Leucheria* group: one composed of the two large genera *Leucheria* and *Perezia* and the other composed of the rest of the genera, with the exception of *Oxyphyllum*, the position of which remains uncertain. Chromosome numbers have not been reported for *Oxyphyllum* but, for the rest of the putative allied genera of the *Leucheria* group, the numbers vary from $2n = 8$ (*Perezia*), $2n = 16$ (*Perezia*), $2n = 22$ (*Holocheilus*, *Nassauvia*) and $2n = 24$ (*Perezia*) to $2n = 40$ (*Leucheria*, *Moscharia*) and $2n = 42$ (*Nassauvia*, *Polyachyrus*) (Crisci, 1974a; Cabrera, 1977; Grau, 1987), although the monophyly of the relatively large and karyologically variable genera *Nassauvia* (c. 40 species) and *Perezia* (c. 30 species) has not been assessed. Pollen data (Hansen,



Figure 1. *Gypothamnium pinifolium* (A–C) and *Oxyphyllum ulicinum* (D, E) in their natural habitats. Photographs by Michael O. Dillon.

1991b; Tellería *et al.*, 2003) also confirm the close relationships of *Oxyphyllum* with *Leucheria*, *Moscharia*, *Nassauvia*, *Perezia*, *Polyachyrus* and *Triptilion*. Recent phylogenetic studies (Katinas *et al.*, 2008; Panero & Funk, 2008) have confirmed the monophyly of Nassauvieae. These studies, although based on different taxon samplings and different molecular markers, provided largely congruent hypotheses of the phylogenetic relationships within Nassauvieae, which differ from previously published studies (Bremer, 1994; Kim *et al.*, 2002; Funk *et al.*, 2005).

Chemical studies, largely focused on di- and sesquiterpenes, have been carried out on the species under study (Zdero, Bohlmann & Niemeyer, 1988a, b) and allied taxa (Bittner *et al.*, 1994). They seem to be of little systematic value in Asteraceae, at least at the subtribal and lower taxonomic levels (Zdero *et al.*, 1988a, b; Alvarenga *et al.*, 2005).

Biogeographical analyses of *Gypothamnium* and *Oxyphyllum* have not been published. Moreira-Muñoz & Muñoz-Schick (2007) placed the genera in the category of the 'endemic' element of the flora of Chile, showing a distribution map of each genus. In the context of the biogeography of the Atacama Desert, Katinas & Crisci (2000) and Katinas *et al.* (2008) suggested that the close relatives of the genus *Polyachyrus* are distributed in central Chile and that some species of *Polyachyrus* have reached the arid environments of the coastal Atacama Desert from central Chilean ancestors through range expansion in response to wetter phases during the Pleistocene. For *Malesherbia* Ruiz & Pav. section *Malesherbia* (Malesherbiaceae), Gengler-Nowak (2002) has postulated a Pleistocene colonization of the coastal environments of the Atacama Desert from Andean ancestors currently distributed in the valleys and canyons of the Andean foothills in southern Peru and northern Chile; the isolation of the coastal representatives may have been the result of increasing aridity in the Quaternary. *Heliotropium* L. section *Cochranea* (Miers) Kuntze has been suggested to have originated in the middle Miocene as a consequence of the uplift of the Andes, following a Pliocene radiation in the coastal Atacama (Luebert & Wen, 2008). For *Tropaeolum* L. section *Chilensia* Sparre, Hershkovitz, Hernández-Pellicer & Arroyo (2006b) suggested that the desert taxa are closely related to species from central Chile.

We examine the systematic relationships of *Gypothamnium* and *Oxyphyllum* using sequences of the plastid *rbcL* and *ndhF* genes, the *trnL-trnF* region and the nuclear ribosomal internal transcribed spacer (ITS), and assess their systematic placement in Mutisioideae. The phylogenetic results are employed to analyse the geographical diversification of the allied

genera within the framework of the Atacama Desert biogeography.

MATERIAL AND METHODS

PLANT MATERIAL SELECTION AND NOMENCLATURE

Two samples of each of the two species, *Gypothamnium pinifolium* and *Oxyphyllum ulicinum*, were sequenced for the plastid *rbcL* gene, the *ndhF* gene, the *trnL-trnF* region and the nuclear ribosomal ITS. These molecular markers have been extensively sampled in Mutisioideae (Kim *et al.*, 2002; Katinas *et al.*, 2008; Panero & Funk, 2008). Sequences were deposited in GenBank. We obtained 38 additional *ndhF* sequences of Mutisioideae (*sensu* Panero & Funk, 2008) and three sequences of Barnadesioideae from GenBank. These sequences were reported previously by Kim & Jansen (1995) and Kim *et al.* (2002). We also obtained 78 sequences of Mutisioideae and six of Barnadesioideae of *trnL-trnF* and ITS from GenBank. These sequences correspond to 42 species and were reported previously by Funk, Chan & Keeley (2004) and Katinas *et al.* (2008). In addition, the *rbcL*, *ndhF* and *trnL-trnF* sequences reported by Panero & Funk (2008), corresponding to 22 species of Mutisioideae and two of Barnadesioideae, were aligned together with our sequences of *Gypothamnium* and *Oxyphyllum*. The GenBank accession numbers of these sequences are listed in Appendix 1.

To assess the systematic placement of *Gypothamnium* and *Oxyphyllum*, we built three different matrices, one with the *ndhF* gene, one with the combined *trnL-trnF* and ITS, and one with *rbcL*, *ndhF* and *trnL-trnF*, mainly relying on the mentioned GenBank accessions. Nomenclature at the generic level follows Hind (2007).

Gypothamnium Philippi, Fl. Atacam. 27. 1860.
Gypothamnium pinifolium Philippi, Fl. Atacam. 27, pl. 3. 1860. Johnston, Contr. Gray Herb. 85: 131. 1929. Cabrera, Darwiniana 9: 373. 1951. Grau, Bot. Jahrb. Syst. 108: 230. 1987. = *Plazia pinifolia* (Phil.) Hoffmann ex Reiche, Anal. Univ. Chile 114: 736. 1904 & Fl. Chile 4: 301. 1905. Hoffmann, Nat. Pflanzenfam. IV,5: 338. 1897.

DNA samples: Luebert & García 2747/1141 (*ndhF*, EU729345; *trnL-trnF*, EU729337; ITS, EU729341), Luebert & García 2762/1156 (*rbcL*, EU736105; *ndhF*, EU729346; *trnL-trnF*, EU729338; ITS, EU729342).

Oxyphyllum Philippi, Fl. Atacam. 28. 1860.
Oxyphyllum ulicinum Philippi, Fl. Atacam. 28, pl. 4. 1860. Hoffmann, Nat. Pflanzenfam. IV,5: 349. 1897. Reiche, Anal. Univ. Chile 116: 417. 1905 & Fl. Chile 4: 435. 1905. Johnston, Contr. Gray Herb. 85: 136. 1929.

DNA samples: Luebert & García 2722/1116 (*rbcL*, EU736103; *ndhF*, EU729347; *trnL-trnF*, EU729339; ITS, EU729343), Luebert & García 2829/1223 (*rbcL*, EU736104; *ndhF*, EU729348; *trnL-trnF*, EU729340; ITS, EU729344).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA was extracted from leaf tissue samples dried with silica gel using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987). Polymerase chain reaction (PCR) amplifications were performed in a Peltier PTC-255 thermal cycler (MJ Research Inc., Watertown, MA, USA) in a 20- μ L volume containing 1 U of *Taq* polymerase, 2.5 mM MgCl₂, 125 μ M of each deoxynucleoside triphosphate (dNTP), 0.5 μ M of each primer and about 25 ng of template. Amplification primers for *ndhF* were the same as those in Olmstead & Sweere (1994) and Oxelman, Backlund & Bremer (1999), and the cycling conditions followed Kim & Jansen (1995). Amplification primers for *trnL-trnF* and ITS were those of Taberlet *et al.* (1991) and White *et al.* (1990), respectively, and cycling conditions followed Katinas *et al.* (2008). The *rbcL* gene was amplified in two segments with the primer pairs 1F-724R and 636F-1460R (Olmstead *et al.*, 1992; Fay, Swensen & Chase, 1997) in 35 cycles of 45 s at 94 °C, 45 s at 55 °C and 1 min at 72 °C, with a final extension of 4 min at 72 °C. The PCR products were purified with the polyethylene glycol (PEG) precipitation procedure (Rosenthal, Coutelle & Craxton, 1993) and cycle sequencing was performed with BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Each sample was sequenced in both the forward and reverse directions. The resulting sequences were assembled using Sequencher 4.1.10 (Gene Codes Corporation, Ann Arbor, MI, USA), and then aligned manually using Se-Al 2.0a11 (Rambaut, 1996).

PHYLOGENETIC ANALYSIS

Maximum parsimony (MP; Farris, Kluge & Eckardt, 1970), maximum likelihood (ML; Felsenstein, 1981) and Bayesian (BA; Mau, Newton & Larget, 1999) analyses were carried out for each data matrix. MP and ML analyses were conducted using PAUP* 4.0 (Swofford, 2003), and BA was performed using BEAST v.1.4.7 (Drummond & Rambaut, 2007). Modeltest v.3.06 (Posada & Crandall, 1998) was used prior to ML and BA to determine the best-fit nucleotide substitution model for the dataset. MP analysis was performed with the heuristic search setting MaxTrees to 50 000, random taxon-addition replicates to 100, tree bisection–reconnection branch swapping, multi-

trees in effect, collapsing branches of zero length, characters as equally weighted and treating gaps as both missing data and coded as recommended by Simmons & Ochoterena (2000). Branch support of the maximally parsimonious trees (MPTs) was assessed with bootstrap analysis, using a heuristic search with 1000 replicates and the same settings as used in the MP search. ML analysis was performed with the heuristic search under the selected substitution model and setting random taxon-addition replicates to 10, tree bisection–reconnection branch swapping, multitrees in effect and collapsing branches of zero length. BA was carried out under the selected model for 10 000 000 generations with a sampling frequency every 1000 generations. The monophyly of Mutisioideae was enforced using the program BEAUTi v.1.4.7 (Drummond & Rambaut, 2007). The first 1000 trees were discarded as 'burnin'.

Both samples of *O. ulicinum* present incomplete *ndhF* sequences with 65% missing data at the end of the *ndhF* aligned matrix. It is certainly desirable to have the complete sequences for these samples. Given the total number of characters taken into account, the amount of missing data should not affect dramatically the phylogenetic accuracy of the analyses (Wiens, 2003). To assess the accuracy of the resolved position of *Oxyphyllum*, given the amount of real missing data in the species, we bootstrapped our original data matrix 1000 times, excluding *Oxyphyllum*, using the program Mesquite v.1.12 (Maddison & Maddison, 2006). For each bootstrapped matrix, an additional matrix with a randomly selected taxon with 65% missing data at the end, was generated. For each matrix, an MP analysis was carried out with PAUP, saving 1000 trees (Maxtrees) in each search, to ensure a reasonable processing time, and, for each search, a strict consensus tree was computed. For each tree containing one taxon with missing data, the position in the tree of this taxon was compared with its position in the tree generated from the corresponding complete bootstrapped matrix. The same position was considered when the three closest related taxa were the same and formed the same topology. The percentage of times in which the position of the taxon with missing data did not change in relation to the complete dataset was computed. This value was used as a measure of the accuracy of the position of *Oxyphyllum*.

BIOGEOGRAPHICAL ANALYSIS

The geographical distribution of *Oxyphyllum* and *Gypothamnium* was determined by examining material from the herbaria SGO, EIF, F, GH, CONC and ULS. The distribution of their related groups was accounted for by checking into the pertaining mono-

graphic and floristic studies (for example, Bacigalupi, 1931; Cabrera, 1936, 1951, 1953a, b, 1968, 1977, 1982; Ferreyra, 1944, 1995; Fabris, 1968; Vuilleumier, 1969; Anderson, 1972; Crisci, 1974a, b, 1976; Ricardi & Weldt, 1974; Egeröd & Ståhl, 1991; Harling, 1995; Dillon, 2005). The resulting distribution areas were visually analysed by tracing them onto the respective cladograms obtained from the phylogenetic analysis. Area cladograms (Page, 1988) were then constructed for each dataset using the software Component v.2.0 (Page, 1993), available at <http://taxonomy.zoology.gla.ac.uk/rod/cpw/index.html>, in order to examine possible relationships among the distribution areas of the related taxa at the generic level. For this purpose, areas of endemism were defined on the basis of Morrone (2006). Heuristic searches with nearest-neighbor interchange branch swapping, minimizing the number of leaves added, were performed and the strict consensus trees were computed (Page, 1988, 1993). Missing areas were not included because they do not provide information (Page, 1988). The last procedure corresponds to treating widespread taxa under 'Assumption 0' (Nelson & Ladiges, 1991). 'Assumption 1' was explored in Component using the option of not mapping widespread taxa (Page, 1993). 'Assumption 2' was not explored.

As different phylogenetic hypotheses have been put forward for Nassauvieae at the generic level, and the placement of *Urmenetia* within Onoserideae still remains uncertain, we reconstructed and evaluated different possible scenarios according to published phylogenetic results (Kim *et al.*, 2002; Funk *et al.*, 2005; Katinas *et al.*, 2008; Panero & Funk, 2008). For Nassauvieae, three topologies were taken into account, which correspond to the three phylogenetic hypotheses available in the literature (Funk *et al.*, 2005; Katinas *et al.*, 2008; Panero & Funk, 2008). We re-analysed the data in the present study with the inclusion of *Oxyphyllum*. For Onoserideae, the position of *Urmenetia* was considered to be: (1) sister to the *Plazia* group (*sensu* Bremer, 1994), (2) sister to the *Onoseris-Lycoseris* clade (Panero & Funk, 2008), and (3) sister to all other Onoserideae, as the three plausible hypotheses given the available molecular and morphological information (Bremer, 1994; Sancho, 2004; Tellería & Katinas, 2004; Katinas *et al.*, 2008; Panero & Funk, 2008).

RESULTS

PHYLOGENETIC ANALYSIS

Analysis with the ndhF gene

The data matrix of *ndhF* consisted of 972 aligned positions, with a total of 281 variable sites, 171 of which were parsimony informative. The 3285 MPTs

had 503 steps, a consistency index (CI) of 0.730 and a retention index (RI) of 0.803. No gaps were coded. The selected substitution model was GTR + I + Γ .

All three analyses (MP, ML and BA) produced the same topology concerning the close relatives of *Gypothamnium* and *Oxyphyllum*. The resulting tree is shown in Fig. 2A. These analyses suggest that *Gypothamnium* is closely related to *Aphyloclados* (96% bootstrap support and 100% Bayesian posterior probability), which form a well-supported monophyletic group with *Onoseris* and *Plazia* (87% bootstrap support and 100% Bayesian posterior probability). None of the trees obtained shows this group as directly related to any other taxa (Fig. 2A). The analyses suggest that *Oxyphyllum* is closely related to *Leucheria* (97% bootstrap support and 100% Bayesian posterior probability), which may be sister to *Jungia* L.f., but the latter relationship did not have high bootstrap support and only had low Bayesian posterior probability (69%). The ML and BA analyses support *Leucheria*, *Oxyphyllum* and *Jungia* as sister to the rest of Nassauviinae (represented in this analysis by *Adenocaulon* Hook., *Perezia*, *Nassauvia*, *Tripilium*, *Acourtia* D. Don, *Proustia* Lag. and *Trixis* P. Browne), with strong support (99% Bayesian posterior probability, tree not shown), but this relationship is not suggested by the MP strict consensus tree (Fig. 2A). The result of the simulation analysis indicates that the resolved position of *O. ulicinum*, given the amount of missing data, has an accuracy of 81.6%.

Analysis with trnL-trnF and ITS

The data matrix of *trnL-trnF* and ITS consisted of 1824 aligned positions, with a total of 606 variable sites, 451 of which were parsimony informative. One MPT was recovered and had 2031 steps, CI of 0.47 and RI of 0.72. Sixteen parsimony-informative indels were coded. The MPT (gap codes included) had 2049 steps, CI of 0.48 and RI of 0.72. Concerning the closely related taxa of *Gypothamnium* and *Oxyphyllum*, the topologies of the MP analysis were identical between the gap-coded dataset and the non-gap-coded matrix. The selected substitution model was GTR + I + Γ .

All analyses (MP, ML and BA) suggested the same close relationships of *Gypothamnium* and *Oxyphyllum*. The results (Fig. 2B) suggest that *Gypothamnium* is sister to *Plazia* (99% bootstrap support and 100% Bayesian posterior probability) and both are related to *Urmenetia*, although with weak branch support (54% bootstrap support and 83% Bayesian posterior probability). *Aphyloclados*, *Onoseris* and *Lycoseris* were not included in this analysis. The results also suggest that *Oxyphyllum*, *Moscharia*, *Leucheria* and *Polyachyrus* form a well-supported monophyletic group (100% bootstrap support and 100% Bayesian posterior

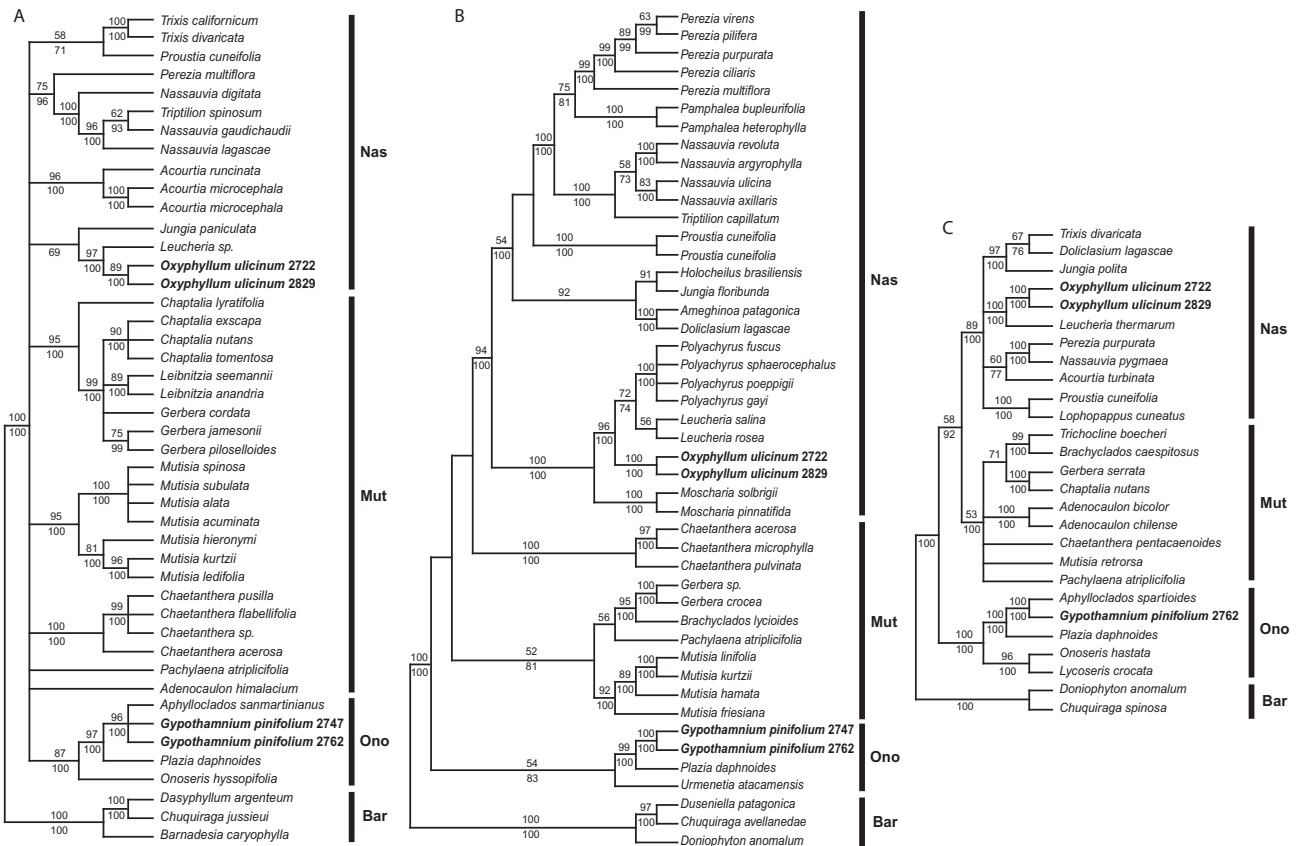


Figure 2. Maximum parsimony strict consensus cladograms obtained from the analysis of three molecular datasets: A, *ndhF*; B, *trnL-trnF* + ITS; C, *rbcL* + *ndhF* + *trnL-trnF*. The topologies obtained from maximum parsimony (MP), maximum likelihood (ML) and Bayesian (BA) analyses are the same concerning the close relatives of *Gypothamnium* and *Oxyphyllum* in each dataset. Parsimony bootstrap support values of > 50% are indicated above the branches and Bayesian posterior probabilities of > 50% are shown below the branches. The main groups are indicated as follows: Bar, Barnadesioideae; Mut, Mutisieae; Nas, Nassauvieae; Ono, Onoserideae.

probability) in which *Moschardia* is sister to the clade consisting of all other genera (96% bootstrap support and 100% Bayesian posterior probability). The analyses resolved *Oxyphyllum* as sister to the *Leucheria*–*Polyachyrus* clade, but this relationship was only moderately supported (Fig. 2B).

Analysis with *rbcL*, *ndhF* and *trnL-trnF*

The data matrix of *rbcL*, *ndhF* and *trnL-trnF* consisted of 4538 aligned positions, with a total of 649 variable sites, 298 of which were parsimony informative. Twenty-eight MPTs were recovered and had 986 steps, CI of 0.76 and RI of 0.67. Eight parsimony-informative gaps were coded, and all were in the *trnL-trnF* region. The MPT (gap codes included) had 993 steps, CI of 0.76 and RI of 0.67. The topologies of the MP analysis, concerning close relatives of *Gypothamnium* and *Oxyphyllum*, were identical between the gap-coded dataset and the non-gap-coded matrix. The selected substitution model was GTR + I + Γ .

The three analyses (MP, ML and BA) suggested the same close relationships of *Gypothamnium* and *Oxyphyllum*. The results (Fig. 2C) suggest that *Gypothamnium* is related to *Aphyllclados* (100% bootstrap support and 100% Bayesian posterior probability) and both are related to *Plazia* (100% bootstrap support and 100% Bayesian posterior probability). These three genera form a group sister to the clade of *Onoseris* and *Lycoseris* (99% bootstrap support and 100% Bayesian posterior probability). The results resolve *Oxyphyllum* as sister to *Leucheria* (100% bootstrap support and 100% Bayesian posterior probability) (Fig. 2C).

BIOGEOGRAPHICAL ANALYSIS

The geographical distribution of *Gypothamnium* and *Oxyphyllum*, resulting from the spatialization of the examined herbarium material (Appendix 2), is shown in Fig. 3. The geographical distribution of the related

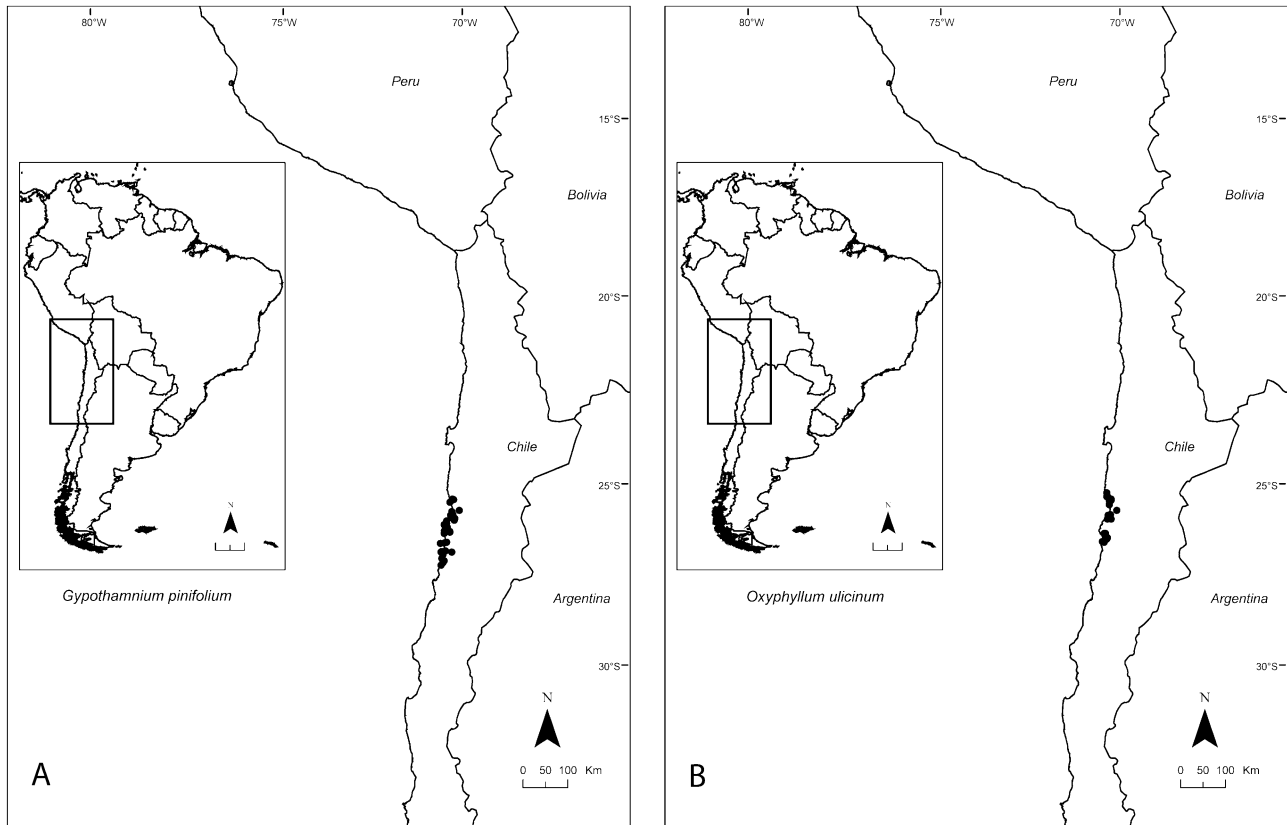


Figure 3. Map of the geographical distribution of *Gybothamnium* (A) and *Oxyphyllum* (B).

genera is detailed in Table 1. The genera related to *Gybothamnium* are distributed in the tropical to subtropical areas of Central and South America, including Mesoamerica, north-western South America, Yungas, Prepuna, Monte, Chaco, coastal Peruvian Desert and Andean Atacama Desert, but none reaches the coastal Atacama Desert, where *Gybothamnium* is endemic. The genera related to *Oxyphyllum* are also distributed in tropical to subtropical areas of Central and South America, but extend southwards to the central Chilean, sub-Antarctic and Patagonian subregions, and northwards up to the south of the Nearctic region in the southern USA, and the genera *Leucheria*, *Polyachyrus* and *Proustia* reach the coastal Atacama Desert.

The resulting strict consensus area cladograms generated under 'Assumption 0' are shown in Fig. 4. In all cases, the strict consensus area cladograms under 'Assumptions 0 and 1' were fully congruent. In the case of the relatives of *Gybothamnium*, the area cladograms generated under 'Assumption 0' resolve the coastal Atacama Desert as a sister area to the Andean Atacama Desert, Chaco, Prepuna and Monte (Fig. 4D–F), whereas the area cladograms generated under 'Assumption 1' (not shown) regard all these

areas in a single polytomy. The strict consensus area cladograms of the relative genera of *Oxyphyllum* (Fig. 4J–L), generated under both assumptions, were identical.

With regard to *Gybothamnium* and its relatives (Fig. 4D–F), the coastal Atacama Desert forms a clade with and is sister to the rest of the areas of tropical and subtropical southern South America (Prepuna, Monte, Chaco, Andean Atacama Desert); the tropical areas of north-western South America (Yungas, Paramo and coastal Peruvian Desert provinces and the north-western South America dominion) and Mesoamerica form a basal polytomy. These relationships resulted after the analysis of all phylogenetic hypotheses, i.e. regardless of the phylogenetic position of *Urmenetia* within Onoserideae. Only the first phylogenetic hypothesis analysed (Fig. 4A) shows a different topology regarding the position of the Puna province being sister to the coastal Atacama Desert, Andean Atacama Desert, Prepuna, Monte and Chaco (Fig. 4D), whereas the other two hypotheses (Fig. 4B, C) resulted in the Puna province being included in the basal polytomy (Fig. 4E, F).

The area cladograms of *Oxyphyllum* and its allied genera (Fig. 4J–L) show the coastal Atacama Desert

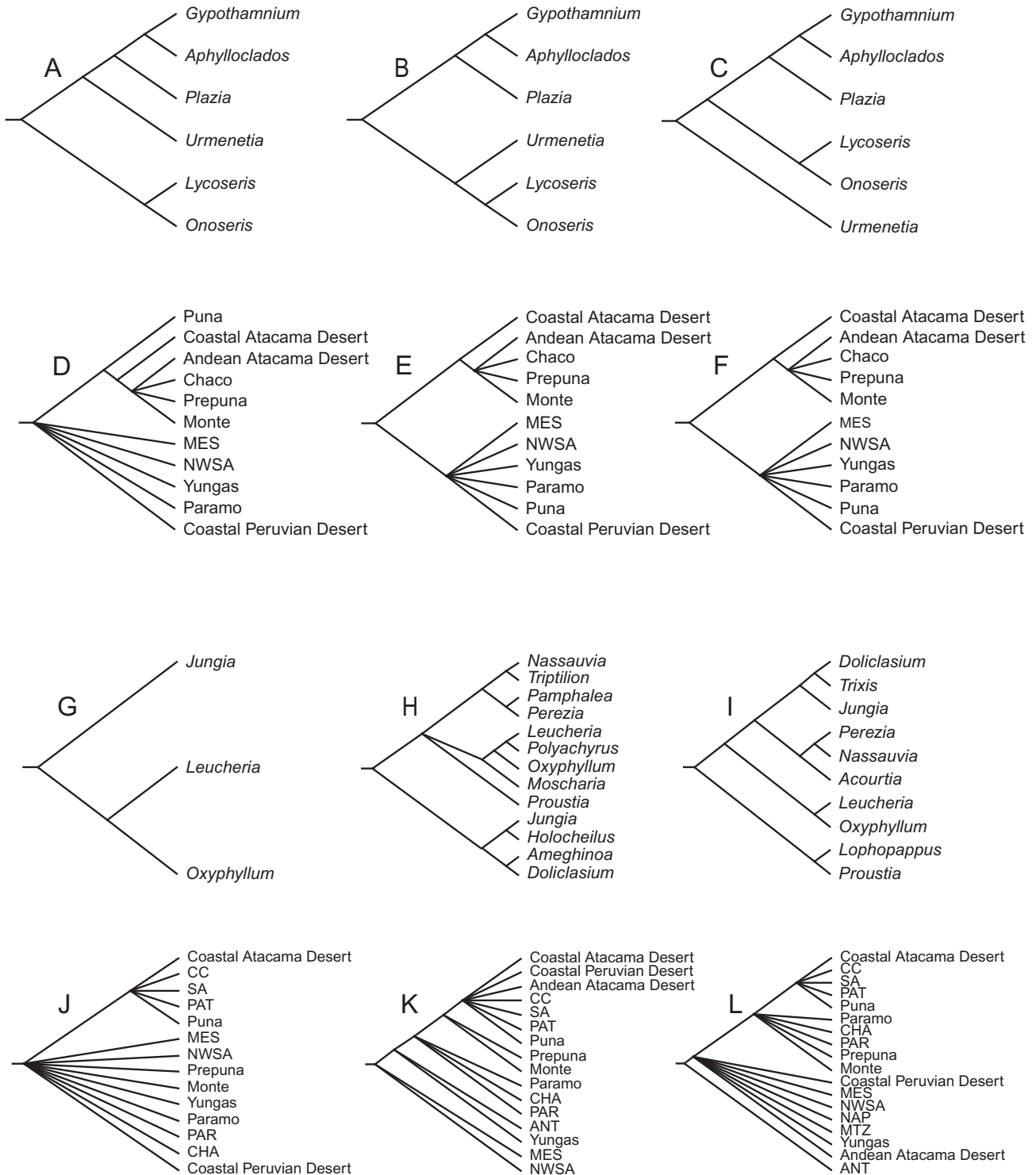


Figure 4. Simplified cladograms and resulting strict consensus area cladograms (generated under 'Assumption 0') of the genera closely related to *Gybothamnium* (A–F) and *Oxyphyllum* (G–L). The taxon cladograms of *Gybothamnium* (A–C) and *Oxyphyllum* (G–I) correspond to the generic relationships shown in Fig. 2A–C, respectively, and are placed above the corresponding consensus area cladograms (*Gybothamnium*, D–F; *Oxyphyllum*, J–L). The abbreviations of the biogeographical units are given in Table 1.

in a clade in a polytomy together with the sub-Antarctic, central Chilean and Patagonian subregions and the Puna province; the Mesoamerican dominion, the tropical areas of north-western South America, the Yungas, Monte, Prepuna and Paramo provinces, and the tropical and subtropical areas of eastern South America (Chacoan and Parana subregions), form a polytomy (Fig. 4J) in the first phylogenetic hypothesis (Fig. 4G), where only *Jungia* and *Leucheria* are considered as close relatives of *Oxyphyllum* (see Fig. 2A). However, when more taxa are taken into account (Fig. 4H, I), in accordance with Katinas *et al.* (2008) and Panero & Funk (2008), respectively, the polytomy becomes more differentiated (Fig. 4K, L), whereas the closely related areas to the coastal Atacama Desert remain the same (Fig. 4L) or include also the coastal Peruvian Desert and the Andean Atacama Desert (Fig. 4K), which seems to be the direct effect of the inclusion of *Polyachyrus* in the analysis (Fig. 4H; see Fig. 2B and Table 1).

DISCUSSION

SYSTEMATIC PLACEMENT

The systematic position of the monotypic genera *Gypothamnium* and *Oxyphyllum*, based on our molecular phylogenetic analysis, largely supports previous assignments using morphology. The close relationship of *Gypothamnium* to the *Plazia* group of Bremer (1994), previously suggested by Cabrera (1951), indicates the phylogenetic relevance of the morphological synapomorphies that unite this group, i.e. foliage of closely set and narrow leaves, sessile capitula, rather stout, truncate, coloured anther appendages (Bremer, 1994), pollen ectosexine slightly thicker than the endosexine, a diffuse mesoaperture and an apertural membrane densely microgranulate at the equator (Telleria & Katinas, 2004). Cabrera (1951) included in this group the genus *Hyalis* D. Don ex Hook. & Arn., which Bremer (1994) assigned to the relatives of *Gochnatia* Kunth. Hoffmann (1897) reunited *Aphyllocladus*, *Hyalis* and *Gypothamnium* under the name *Plazia*, whereas Cabrera (1977) and Bremer (1994) treated them as separate genera. The results of Panero & Funk (2008) resolved the position of *Hyalis* as unrelated to the *Plazia* group and outside Mutisioideae, although not directly related to *Gochnatia*. The sister relationship of *Onoseris* with the *Plazia* group (Kim *et al.*, 2002; Funk *et al.*, 2005) seems to be well supported (Fig. 2), as already suggested by Hoffmann (1897), although some authors have considered it differently. Jansen & Palmer (1988) suggested that *Onoseris* was closely related to *Stiffitia* Mikan, but with low branch support and a much

smaller sampling scheme, whereas Kim & Jansen (1995) did not report well-resolved relationships of *Onoseris*. Karis *et al.* (1992) showed *Onoseris* as sister to Nassauviinae, whereas Hansen (1991a) and Bremer (1994) considered it as a separate group related to *Urmenetia*, grouping together the *Onoseris* group. Sancho (2004) used *Urmenetia* as the only outgroup in her phylogenetic analysis of *Onoseris*. The pollen study of Telleria & Katinas (2004) suggested, however, that *Urmenetia* is a close relative of *Pachylaena* Don, occupying an intermediate position between the *Plazia* and *Mutisia* groups, and that not all studied species of *Onoseris* (*On. odorata* (D. Don) Hook. & Arn. and *On. alata* Rusby) share the same pollen characters. Sancho (2004) resolved distant positions for these species within *Onoseris*. The results of Katinas *et al.* (2008) and those shown here support the inclusion of *Urmenetia* in Onoserideae, which is also in agreement with Panero & Funk (2007). Furthermore, Panero & Funk (2008) suggested the monophyly of Onoserideae as composed of *Onoseris*, *Lycoseris*, *Plazia* and *Aphyllocladus*. Our analysis shows that *Gypothamnium* clearly belongs to this group. Unfortunately, the sampling of *Onoseris* and *Lycoseris* in our analysis is limited to one species each, reported by Panero & Funk (2008). The inclusion of more species of the genera *Aphyllocladus*, *Onoseris*, *Lycoseris* and *Plazia* should help to resolve generic limits within Onoserideae. Bremer (1994) placed these genera in separate groups (the *Plazia* and *Onoseris* groups) and considered *Lycoseris* as an isolated genus. Traditionally, genera belonging to the *Plazia* and *Onoseris* groups have been assigned to Gochnatiinae (Hoffmann, 1897; Cabrera, 1977). According to Panero & Funk (2008), Onoserideae is well resolved as sister to all other Mutisioideae (*sensu* Panero & Funk, 2002, 2007), whereas Gochnatiinae occupies a more derived phylogenetic position outside Mutisioideae.

Concerning the relationships of *Oxyphyllum*, our study supports the previous results of Crisci (1980), who suggested its closest relative to be *Leucheria*. Sister to the clade of these genera is *Jungia* (Kim *et al.*, 2002; Funk *et al.*, 2005), but this clade has low bootstrap support and only a moderate Bayesian posterior probability (Fig. 2A). The phylogenetic relationships of *Leucheria* and *Oxyphyllum* within the *Leucheria* group are unclear (Bremer, 1994); the *Leucheria* group does not possess obvious morphological synapomorphies. *Jungia* was formerly associated with the monotypic genus *Pleocarphus* D. Don endemic to north-central Chile (for example, Hoffmann, 1897; Reiche, 1905; Crisci, 1974a, 1980; Hansen, 1991a; Bremer, 1994). However, Harling (1995) argued that it is more likely to be closely

related to *Marticoenia* Crisci, also monotypic and endemic to central Chile, and to *Holocheilus* Cass., with c. six species from Brazil, Paraguay, Uruguay and Argentina (Cabrera, 1968, 1977). Bremer (1994) included *Holocheilus*, *Leucheria* and *Oxyphyllum* in the *Leucheria* group, and treated *Jungia*, *Marticoenia* and *Pleocarphus* as 'other Nassauviinae genera', indicating that, in agreement with Crisci (1974a, 1980), they are related to *Trixis* and *Ameghinoa* Speg. This last scenario seems to be supported by recent phylogenetic molecular studies (for example, Katinas *et al.*, 2008; Panero & Funk, 2008), but none of these genera appears to be closely related to *Leucheria* or *Oxyphyllum*, but to *Dolichlasium* Lag. and *Holocheilus* (Katinas *et al.*, 2008; Panero & Funk, 2008; Fig. 2B, C), although the molecular analyses to date have not sampled *Pleocarphus* or *Marticoenia*. Bremer (1994) suggested that the intergeneric relationships within Nassauviinae are still unresolved. Kim *et al.* (2002: 603) were also unable to resolve well the relationships in Nassauvieae. As suggested by Kim *et al.* (2002: 604), the monophyly of this subtribe has not been confirmed by *ndhF* data with MP analysis (Kim *et al.*, 2002: 603–604). Our results from ML analysis and BA provide good support for its monophyly (99% Bayesian posterior probability, not shown; cf. Kim *et al.*, 2002: 601). Two groups can be recognized within the Nassauvieae in the Bayesian *ndhF* phylogeny (not shown), one composed of the above-mentioned *Jungia*, *Leucheria* and *Oxyphyllum*, and the other consisting of the rest of the analysed genera, including *Adenocaulon*, (sub-)tribal placement of which remains controversial (Katinas, 2000; Kim *et al.*, 2002; Hind, 2007), although Panero & Funk (2008) resolved *Adenocaulon* as nested within Mutisieae. The monophyly of Nassauvieae has also been suggested by morphological characters, penicillate style branches (Crisci, 1974a; Cabrera, 1977; Bremer, 1994) and exine sublayers with similar structure (Crisci, 1974a; Tellería *et al.*, 2003), but the latter seems to be symplesiomorphic (cf. Urtubey & Tellería, 1998; Tellería *et al.*, 2003). The monophyly of Nassauvieae was well supported in the analyses of Katinas *et al.* (2008) and Panero & Funk (2008), within which *Oxyphyllum* is deeply nested and related to *Leucheria*, but not to *Jungia* (Fig. 2B, C). Including *Oxyphyllum* in the dataset of Katinas *et al.* (2008) shows that the genus forms a well-supported clade, which also includes *Leucheria*, *Moscharia* and *Polyachyrus* (Fig. 2B). The sister position of *Moscharia* with respect to the three other genera is well resolved (Katinas *et al.*, 2008; Fig. 2B), but the relationships among *Leucheria*, *Oxyphyllum* and *Polyachyrus* remain only moderately supported (Fig. 2B). Although the monophyly of the genera *Moscharia* and *Polyachyrus* has been suggested to be well established (Katinas & Crisci, 2000;

Katinas *et al.*, 2008; Fig. 2B), a broader sampling of *Leucheria* may be necessary to clarify the relationships among these genera, and the interpretation of the evolution of the secondary heads in Nassauvieae, developed by Katinas *et al.* (2008), may need to be re-evaluated in the light of the phylogenetic placement of *Oxyphyllum*. Indeed, the results of Katinas *et al.* (2008) are in agreement with the position of Hellwig (1985), who argued that any comparison between *Moscharia* and *Polyachyrus* must also include *Leucheria*. Our results show that such a comparison should also include *Oxyphyllum*.

The systematic position of *Gypothamnium* and *Oxyphyllum* among their closest relatives seems to be confirmed by different molecular datasets and by morphology. However, several aspects of the systematics of Mutisioideae remain largely unresolved. Some of these aspects have been discussed in previous studies (for example, Cabrera, 1977; Bremer, 1994; Kim *et al.*, 2002; Hind, 2007; Katinas *et al.*, 2008; Panero & Funk, 2008) and a deeper discussion on the subject is beyond the scope of this contribution. Nevertheless, the re-analyses performed here on published datasets have yielded slightly different results. Such differences may be a result of different alignments and/or sampling schemes.

BIOGEOGRAPHY

The biogeographical analyses performed for the genera *Gypothamnium* and *Oxyphyllum* (Figs 3, 4; Table 1) suggest two biogeographical patterns. One is represented by *Gypothamnium* and its allies, in which the origin of the species in the coastal Atacama Desert was directly linked to groups inhabiting the subtropical areas of eastern South America and the Andean Atacama Desert (*sensu* Gajardo, 1994; Fig. 4D–F). This pattern is similar to that observed in *Heliotropium* section *Cochranea*, endemic to the Atacama Desert (Luebert & Wen, 2008), in which the close relatives largely occupy areas of eastern South America (Johnston, 1928; Förther, 1998). It is worthwhile noting that one of the primitive lineages within *Heliotropium* section *Cochranea* (*H. glutinosum* Phil.) is an inhabitant of the Andean Atacama Desert, whereas most of the derived taxa are restricted to the coastal zone of Atacama. *Chuquiraga* section *Acanthophyllae* (Ezcurra, Ruggiero & Crisci, 1997), *Malesherbia* section *Malesherbia* (Gengler-Nowak, 2002) and *Nolana* (Dillon *et al.*, 2007) also show ancestral taxa in the Andean range and a later diversification in the coast. This distribution pattern can be explained by mass landslides (Ochsenius, 1999) and streamflow (Nester *et al.*, 2007) from high to low altitudes that have taken place in the Quaternary and possibly earlier, which may have promoted gene

flow from the Andes to the coast, followed by isolation of the established populations in the coastal Atacama. Assuming such a scenario, it is unlikely that *G. pinifolium* is a palaeopolyploid, as suggested by Grau (1987); instead, it is more likely to be a neopolyploid.

In the case of *Oxyphyllum*, the closely related *Leucheria*, *Moscharia* and *Polyachyrus* are largely distributed in central Chile, with representatives in southern Chile and in Patagonia, as well as in the Puna and the Atacama Desert (Table 1; Fig. 4K). Although a broader sampling of *Leucheria* is needed to fully understand the biogeographical origin of *Oxyphyllum*, it has been suggested that *Oxyphyllum* represents a later stage in the geographical diversification of *Leucheria* by colonizing the arid environments of the Atacama from central Chile (Crisci, 1974a). *Polyachyrus*, mainly diversified in the coastal Atacama Desert (Johnston, 1929; Ricardi & Weldt, 1974), could also represent a later colonization of arid environments of the Atacama and Peruvian Deserts from central Chilean ancestors (Katinas & Crisci, 2000; Katinas *et al.*, 2008). The phylogenetic position of *Moscharia*, almost endemic to central Chile (Crisci, 1974b), in relation to *Oxyphyllum* and *Polyachyrus* (Fig. 2B), and the concentration of the specific diversity of *Leucheria* in central Chile (Crisci, 1976), seem to support these hypotheses. A similar scenario has been proposed for *Tropaeolum* section *Chilensia* (Hershkovitz *et al.*, 2006b). This clearly represents a different pattern of biogeographical relationships of the Atacama endemic taxa in comparison with *Gypothamnium*. A preliminary chromosome count of *Oxyphyllum* based on material of Dillon & Teillier 5106 (F, see Appendix 2) shows $n = 33$, as reported here. This species should also be treated as a neopolyploid.

Furthermore, deep in the phylogenetic relationships, the ancestral group related to *Gypothamnium*, represented by *Onoseris* and *Lycoseris*, is distributed in the tropical areas of north-western South America and Mesoamerica (Table 1; Fig. 4D–F). The distribution of the sister groups of the group formed by *Leucheria*, *Moscharia*, *Oxyphyllum* and *Polyachyrus* (Table 1; Fig. 4J–L) suggests, as for *Gypothamnium*, an ancestral origin of the southern South American taxa in the tropical areas of north-western South America and Mesoamerica, but with the taxa more broadly distributed reaching into south-eastern Brazil, Uruguay and north-eastern Argentina, as well as the southern part of the USA. A similar ancestral biogeographical pattern has been detected in *Heliotropium*, in which the sister clade of *Heliotropium* section *Cochranea* reaches north-western South America and Mesoamerica (Luebert & Wen, 2008). *Croton chilensis* Müll.Arg., endemic to nearly the same area as *Gypothamnium* and *Oxyphyllum*, seems

to be related to a species group of tropical Andean origin (Berry *et al.*, 2005).

Table 1 shows that the Puna province has the largest number of analysed genera present, both within Onoserideae and Nassauvieae. This is in agreement with the suggestion of Katinas, Morrone & Crisci (1999) that the Puna province (where the authors included the area of the Atacama Desert) is an area of confluence of neotropical and austral elements.

Kim *et al.* (2005), using an internal fossil-based calibration of an outgroup and a non-parametric method, have proposed that Asteraceae originated in the mid-Eocene (45–49 Ma). Later, Hershkovitz *et al.* (2006a), using a secondary calibration for the asterids (Bremer, Friis & Bremer, 2004) and a semi-parametric method, suggested a more recent origin (36 Ma). This discrepancy cannot be attributed to the different methods employed when using the same dataset and the same fossil calibrations, as has been suggested by Anderson, Bremer & Friis (2005), who showed that excluding fossils gives younger ages for the clade to which the fossils belong. Unfortunately, the scarcity of reliable fossil records for Asteraceae makes it difficult to elaborate and test hypotheses on the time of origin of the family. The minimum age of Asteraceae may be regarded as the mid-Eocene (c. 38 Ma), because the oldest reliable fossil record of the family comes from that time (Graham, 1996; Zavada & De Villiers, 2000; Scott, Cadman & McMillan, 2006). Recent studies have shown that the area of the current location of the Atacama Desert has long been arid, even in pre-Tertiary times (Hartley *et al.*, 2005; Clarke, 2006). We argue that it is not possible to trace so far back for the origin of the present Atacama Desert flora, for the following reasons: (1) the evolutionary replacement rate of plant taxa in arid environments has been suggested to be rapid (Stebbins, 1952); (2) there has been a history of climatic (for example, Zachos *et al.*, 2001) and geological (for example, Gregory-Wodzicki, 2000; Garzzone *et al.*, 2008) events since then that may have played an important role, leading to extinction and diversification of biotic groups; and (3) hyperaridity did not develop until the Pliocene (Hartley, 2003). Luebert & Wen (2008) proposed the middle Miocene as the minimum age for the origin of *Heliotropium* section *Cochranea*, facilitated by the vicariant effect of the Andean uplift, with a Pliocene diversification in the arid environments of Atacama. A similar timing or even more recent times have been proposed for the diversification of other groups in the Atacama Desert, such as *Polyachyrus* (Katinas & Crisci, 2000), *Malesherbia* (Gengler-Nowak, 2002), *Chaetanthera* Ruiz & Pav. (Hershkovitz *et al.*, 2006a) and *Tropaeolum* (Hershkovitz *et al.*, 2006b). The vicariant effect

of the Andes has also been suggested for the fauna of arthropods (Roig-Juñent *et al.*, 2006). Based on the data presented here and those from the published literature, we propose that the Andes have played an important role in the origin and diversification of the Atacama Desert flora in three different ways. First, they have acted as a corridor and allowed the north-south geographical range expansion of taxa. Second, they have been a vicariant barrier and separated groups on the eastern and western sides of the Andes. Third, they have served as a new niche for speciation in high-elevation environments. The first may explain the pattern of some related groups having a distribution range along the Andes and adjacent zones; the second may account for the close relationships among taxa on both sides of the Andes; and the third may explain the fact that there are phylogenetically related taxa in the basal areas of both sides of the Andes and also at high elevations. These three kinds of process and the development of aridity in the Atacama may have led to the complex origin and diversification of *Gypothamnium* and *Oxyphyllum*.

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APPENDIX 1. GENBANK ACCESSIONS USED IN THIS STUDY

ndhF

Most sequences were from Kim *et al.* (2002), where information on all voucher specimens is given. Those indicated with asterisks were published by Kim & Jansen (1995).

Acourtia microcephala DC. (L39407*, L39408), *Acourtia runcinata* (D. Don) B.L. Turner (AF233807), *Adenocaulon himalaicum* Edgew. (L39401), *Aphyllclados sanmartinianus* Molino (AF233804), *Barnadesia caryophylla* S.F. Blake (L39394), *Chaetanthera acerosa* (J. Rémy) Benth. & Hook. f. (AF233831), *Chaetanthera flabellifolia* Cabrera (AF233822), *Chaetanthera* sp. (AF233830), *Chaetanthera pusilla* Hook. & Arn. (AF233821), *Chaptalia exscapa* (Pers.) Baker (AF233811), *Chaptalia lyratifolia* Burkart (AF233810), *Chaptalia nutans* (L.) Polak (AF233812), *Chaptalia tomentosa* Vent. (AF233837), *Chuquiraga jussieui* J.F. Gmel. (L39393), *Dasyphyllum argenteum* Kunth (L39392), *Gerbera cordata* (Thunb.) Less. (AF233820), *Gerbera jamesonii* Adlam (L39403), *Gerbera piloselloides* (L.) Cass. (L39404*), *Jungia paniculata* A. Gray (AF233816), *Leibnitzia anandria* (L.) Turcz. (L39402*), *Leibnitzia seemannii* (Schultz-Bip.) G.L. Nesom (AF233815), *Leucheria* sp. (AF233829), *Mutisia acuminata* Ruiz & Pav. (L39400*), *Mutisia alata* Hieron. (AF233833), *Mutisia hieronymi* Sodiro ex Cabrera (AF233834), *Mutisia kurtzii* R.E. Fr. (AF233835), *Mutisia ledifolia* Decne. ex Wedd. (AF233836), *Mutisia spinosa* Ruiz & Pav. (AF233818), *Mutisia subulata* Ruiz & Pav. (AF233819), *Nassauvia digitata* Wedd. (AF233824), *Nassauvia gaudichaudii* Cass. (L39405), *Nassauvia lagascae* F. Meigen (AF233826), *Onoseris hyssopifolia* Kunth (L39398), *Pachylaena atriplicifolia* D. Don ex Hook. & Arn. (AF233827), *Perezia multiflora* Less. (AF233814), *Plazia daphnoides* Wedd. (AF233840), *Proustia cuneifolia* D. Don (AF233817), *Triptilion spinosum* Ruiz & Pav. (AF233825), *Trixis divaricata* (Kunth) Spreng. (AF233841), *Trixis californicum* Kellogg (AF233803, L39406*).

ITS AND *trnL-trnF* (ITS GENBANK ACCESSION NUMBER FIRST)

Most sequences were published by Katinas *et al.* (2008), where information on all voucher specimens is provided. Sequences indicated with asterisks were published by Funk *et al.* (2004).

Ameghinoa patagonica Speg. (EF530223, EF530269), *Brachyclados lycioides* D. Don (EF530224, EF530270), *Chaetanthera acerosa* (J. Rémy) Benth. & Hook. (EF530229, EF530275), *Chaetanthera microphylla* (Cass.) Hook. & Arn. (EF530240, EF530286), *Chaetanthera pulvinata* (Phil.) Hauman (EF530258, EF530304), *Chuquiraga avellanadae* Lorenz (EF530230, EF530276), *Dolichlasium lagascae* D. Don (EF530259, EF530305), *Doniophyton anomalum* (D. Don) Kurtz (EF530256, EF530302), *Dusenilla patagonica* (O. Hoffm.) K. Schum. (EF530227, EF530273), *Gerbera crocea* Kuntze (AY504687*, AY504769*), *Gerbera* sp. (AY504688*, AY504770*), *Holocheilus brasiliensis* (L.) Cabrera (EF530247, EF530293), *Jungia floribunda* Less. (EF530233, EF530279), *Leucheria rosea* Less. (EF530254, EF530300), *Leucheria salina* (J. Rémy) Hieron. (EF530236, EF530282), *Moschardia pinnatifida* Ruiz & Pav. (EF530218, EF530264), *Moschardia solbrigii* Crisci (EF530219, EF530265), *Mutisia friesiana* Cabrera (EF530241, EF530287), *Mutisia hamata* Reiche (EF530242, EF530288), *Mutisia kurtzii* R. E. Fr. (EF530235, EF530281), *Mutisia linifolia* Hook. (EF530252, EF530298), *Nassauvia argyrophylla* Cabrera (EF530246, EF530292), *Nassauvia axillaris* (Lag.) D. Don (EF530232, EF530278), *Nassauvia revoluta* D. Don (EF530253, EF530299), *Nassauvia ulicina* (Hook. f.) Macloskie (EF530245, EF530291), *Pachylaena atriplicifolia* Hook. & Arn. (EF530250, EF530296), *Pamphalea bupleurifolia* Less. (EF530257, EF530303), *Pamphalea heterophylla* Less. (EF530248, EF530294), *Perezia ciliaris* Hook. & Arn. (EF530238, EF530284), *Perezia multiflora* (Humb. & Bonpl.) Less. ssp. *sonchifolia* (Baker) Vuilleum. (EF530249, EF530295), *Perezia pilifera* (D. Don) Hook. & Arn. (EF530225, EF530271), *Perezia purpurata* Wedd. (EF530239, EF530285), *Perezia virens* (D. Don) Hook. & Arn. (EF530243, EF530289), *Plazia daphnoides* Wedd. (EF530226, EF530272), *Polyachyrus fuscus* (Meyen) Walpers (EF530263, EF530309), *Polyachyrus gayi* J. Rémy (EF530220, EF530266), *Polyachyrus poeppigii* (Less.) Less. (EF530234, EF530280), *Polyachyrus sphaerocephalus* D. Don (EF530221, EF530267), *Proustia cuneifolia* D. Don var. *mendocina* (Phil.) Ariza (1: EF530244, EF530290; 2: EF530251, EF530297), *Triptilion capillatum* (D. Don) Hook. & Arn. (EF530222, EF530268), *Urmenetia atacamensis* Phil. (EF530231, EF530277).

rbcL, *ndhF* AND *trnL-trnF* (*rbcL* GENBANK ACCESSION NUMBER FIRST AND *trnL-trnF* LAST)

Sequences were published by Panero & Funk (2008), where information on all voucher specimens is given.

Acourtia turbinata (La Llave & Lex.) Reveal & R. M. King (EU384940, EU385126, EU385032), *Adenocaulon chilense* Less. (EU384942, EU385128, EU385034), *Adenocaulon bicolor* Hook. (EU384943, EU385129, EU385035), *Aphyllocladus spartioides* Wedd. (EU384946, EU385132, EU385038), *Brachyclados caespitosus* Speg. (EU384951, EU385137, EU385044), *Chaetanthera pentacaenoides* Hauman (EU384956, EU385142, EU385049), *Chaptalia nutans* (L.) Polak (EU384957, EU385143, EU385050), *Chuquiraga spinosa* D. Don (EU384960, EU385146, EU385053), *Dolichlasium lagascae* Gill. ex D. Don (EU384968, EU385155, EU385062), *Doniophyton anomalum* (D. Don) Kurtz (EU384969, EU385156, EU385063), *Gerbera serrata* (Thunb.) Druce (EU384976, EU385164, EU385069), *Jungia polita* Griseb. (EU384991, EU385178, EU385084), *Leucheria thermarum* Reiche (EU384992, EU385179, EU385085), *Lophopappus cuneatus* R. E. Fr. (EU384995, EU385182, EU385088), *Lycoseris crocata* S. F. Blake (EU384996, EU385183, EU385089), *Mutisia retrorsa* Cav. (EU384998, EU385185, EU385091), *Nassauvia pygmaea* Hook. f. (EU384999, EU385186, EU385092), *Onoseris hastata* Wedd. (EU385003, EU385190, EU385096), *Pachylaena atriplicifolia* D. Don ex Hook. & Arn. (EU385005, EU385192, EU385098), *Perezia purpurata* Wedd. (EU385007, EU385194, EU385100), *Plazia daphnoides* Wedd. (EU385010, EU385197, EU385103), *Proustia cuneifolia* D. Don (EU385012, EU385199, EU385105), *Trichocline boecheri* Cabrera (EU385024, EU385213, EU385119), *Trixis divaricata* (Kunth) Spreng. (EU385025, EU385214, EU385120).

APPENDIX 2. EXAMINED MATERIAL

GYPOTHAMNIUM PHIL.

Representative material examined (total specimens examined: 81)

Chile. **II Región.** Prov. Antofagasta. Quebrada Yumbe, 24°58'S, 70°26'W, 1150 m, 14.ii.1998, G. Arancio & F. Squeo 11344 (ULS); Mina Liverpool, al W de Paposo 24°59'S, 70°24'W, 1035 m, 1.xii.2001, C. Aedo 7031 (CONC); Quebrada Paposo, c. 12 km E of Caleta Paposo, 950 m, 7.xii.1987, M. O. Dillon & J. T. S. Teillier 5120 (F); Mina Abundancia, Posada (Quebrada de Paposo), 25.ix.1953, M. Ricardi 2632 (CONC); Punta Grande, Vicinity of Paposo, c. 25°02'S, 9.xii.1925, I. M. Johnston 5234 (GH); Quebrada de Paposo, 800 m, 7.x.1966, C. Jiles 4954 (CONC); vicinity of Paposo, Quebrada de Guanillo, c. 25°02'S, 750 m, 8.xii.1925, I. M. Johnston 5597 (GH);

Alrededores de Santa Catalina, 1300 m, 21.ii.1969, M. Ricardi & O. Parra 78 (CONC); Cascabeles, x.1940, C. Grandjot 4576 (CONC); Quebrada Cascabel, 3.ii.1947, W. Biese 2247 (SGO); Camino Taltal-Paposo 25°18'S, 70°27'W, 13.xi.1996, R. Rodríguez 3111 (CONC); Quebrada San Ramón, c. 6 km NE of Taltal, 50–80 m, 9.xii.1987, M.O. Dillon & J.T.S. Teillier 5199 (F); Quebrada San Ramón, 25°23'S, 70°25'W, 300 m, 9.xi.1985, F. Schlegel 7980 (CONC); 6 km. al N de Taltal, 25°24'S, 70°29'W, 50 m, 6.x.1988, G. Arancio 88258 (ULS); Hueso Parado, c. 5 km N of Taltal, 60–70 m, 6.x.1988, M.O. Dillon & D. Dillon 5647 (F); Taltal, 100 m, ii.1926, E. Werdermann 1044 (F, GH, CONC), Taltal, 20.x.1964, M Ricardi, C. Marticorena & O. Matthei 1061 (CONC); on Hwy 1 E of Taltal, 12.ix.2001, M. McMahon & L. Hufford 569 (F); Hills south of Taltal, 25.ii.1939; A.A. Beetle 26170 (GH); Cerro Perales, 25°25'S, 70°25'W, 1065 m, 7.x.1988, G. Arancio 88257 (ULS), 5 km south-east of Taltal, Quebrada de Taltal, 200 m, 14.i.1939, J.L. Morrison 17093 (GH); Quebrada de Taltal, 17.ix.1968, O. Zöllner 2778 (CONC); Quebrada de Taltal, 150 m, 10.ix.1936, G. Montero 2914 (CONC); Quebrada Taltal, vicinity of Taltal, c. 25°25'S, 25.xi.1925, I.M. Johnston 5113 (GH); Quebrada Taltal, 570 m, 14.ix.1992, S. Teillier, P. Rundel, & P. García 2660 (F); Taltal, Quebrada Changos, 21.ix.1953, M. Ricardi 2583 (CONC); Quebrada Breas, 25°29'S, 70°22'W, 640 m, 10.x.2005, F. Luebert & N. García 2747/1141 (SGO, EIF); Breas, 1888, A. Larrañaga (SGO 61849); PanAmericana, 8 km S Las Breas, 950 m, 15.ix.1992, S. Teillier, P. Rundel, & P. García 2722 (F); Trayecto Agua Verde-Chañaral, 25°30'S, 70°00'W, 1300 m, 26.ii.2001, C. Villagrán, C. Latorre & A. Maldonado 264 (CONC); Quebrada del Gritón on route to Pta.Tórtolas, c. 15 km SSW of Taltal, 350 m, 25.xi.1987, M.O. Dillon 5401 (F); Las Tórtolas, Sierra de San Pedro, 25°33'S, 70°35'W, 170 m, 12.x.2005, F. Luebert & N. García 2762/1156 (SGO, EIF); Sierra Vetada, km. 18.316, 25°37'S, 70°35'W, 340 m, 10.ii.1998, G. Arancio & F. Squeo 11022 (ULS); Cifuncho, 17.ix.1954, M. Ricardi 3071 (CONC); Caleta Cifuncho, 1 km. al sur, 25°39'S, 70°39'W, 100 m, 10.ii.1998, G. Arancio & F. Squeo 10984 (ULS); Quebrada Tigrillo, 25°43'S, 70°34'W, 370 m, 9.ii.1998, G. Arancio & F. Squeo 10967 (ULS); Mineral Esmeralda, 700–800 m, 3.ii.1947, W. Biese 2269 (SGO); Quebrada La Cachina, 25°50'S, 70°30'W, 780 m, 9.ii.1998, G. Arancio & F. Squeo 10913 (ULS); Camino entre Guanillo y Quebrada Cachina, 300 m, x.1941, E. Pisano & R. Bravo 648 (CONC); **III Región**, Prov. Chañaral. Parque Nacional Pan de Azúcar, frente a cerro Soldado, 28.x.1991, R. Rodríguez 2633 (CONC); Entre Chañaral y Pan de Azúcar, km. 10, 26°08'S, 70°38'W, 50 m, 19.i.2004, F. Luebert & R. Torres 1963 (EIF); Chañaral, 13.ix.1965, A. Kohler 111 (CONC);

vicinity of Puerto de Chañaral, hill back of El Barquito, c. 26°23'S, 28–29.x.1925, I.M. Johnston 4798 (GH); 15 km al norte de Salado, 300 m, 14.ix.1958, M. Ricardi & C. Marticorena 4619/1004 (CONC); Las Ánimas, ix.1909, K. Reiche (SGO 73904); Quebrada Las Ánimas, 26°23'S, 70°44'W, 100 m, 17.x.1993, P. Castillo, 17 (ULS); near Playa Hippie, c. 13 km S of Chañaral along PanAmerican Hwy 5, 20 m, 3.x.1987, M.O. Dillon & J.T.S. Teillier 5052 (F); Camino de Chañaral a Caldera, km. 13, 25.x.1964, M. Ricardi, C. Marticorena & O. Matthei 1099 (CONC); Chañaral-Caldera, 14.3 km, 9.ii.1987, C. Marticorena, T. Stuessy & M. Baeza 9904 (CONC), Interior Caleta Flamenco, 26°37'S, 70°39'W, 262 m, 17.ix.2003, F. Luebert & L. Kritznner 1845 (EIF); Caleta Obispito, 26°44'S, 70°44'W, 5 m, 15.iii.1987, O. Matthei & R. Rodríguez 192 (CONC).

OXYPHYLLUM PHIL.

Representative material examined (total specimens examined: 38)

Chile. **II Región**. Prov. Antofagasta. Paposo, Quebrada El Médano, iv.1985, H. Niemeyer (SGO 104085); Sector La Rinconada, Quebrada El Resbalón, 24°55'S, 70°29'W, 120 m, 14.xi.1996, R. Rodríguez 3122 (CONC); El Rincón, just north of Paposo, along trail to old Parañas Mine, c. 24°59'S, 7.xii.1925, I.M. Johnston 5537 (GH); 8 km E (above) Paposo on road connecting to the Panamericana, 25°00'S, 70°27'W, 760 m, 15.xii.1994, U. Eggl & B.E. Leuenberger 2649 (CONC); Al W de Paposo, 25°00'S, 70°24'W, 830 m, 1.xii.2001, C. Aedo 7036 (CONC); Cuesta de Paposo en camino a Taltal (57 km al norte), 600–800 m, 25.iv.1955, R. Wagenknecht, 640 (CONC); Paposo, Mina abundancia, 25.ix.1953, M. Ricardi 2542 (CONC); Quebrada Portezuelo, 25°00'S, 70°27'W, 550 m, 26.i.1997, G. Arancio 10712 (CONC, ULS); Paposo, 17.ix.1958, M Ricardi & C. Marticorena 4634/1019 (CONC); Quebrada Matancilla, 25°06'S, 70°27'W, 170 m, 8.x.2005, F. Luebert & N. García 2722/1116 (SGO, EIF); Quebrada Matancilla, c. 5 km S of Punta Plata, 170–350 m, 27.x.1988, M.O. Dillon, D. Dillon, V. Asencio & M. Villarroel 5751 (F); Quebrada Matancilla, 25°07'S, 70°27'W, 200 m, 6.xi.1985, F. Schlegel 7914 (CONC); Taltal, Quebrada Cascabeles, 4.x.1954; M. Ricardi 3096 (CONC); Quebrada de San Ramón, north of Taltal, c. 25°24'S, 26.xi.1925, I.M. Johnston 5153 (GH); Taltal, 20.x.1964, M Ricardi, C. Marticorena & O. Matthei 1060 (CONC); Taltal, 16.ix.1968, O. Zöllner 2681 (CONC); 5 km south-east of Taltal, Quebrada de Taltal, 200 m, 14.i.1939, J.L. Morrison 17096 (GH); 9–12 km into canyon road which goes to Taltal from Hwy. 5, 25°30'S, 70°30'W, 9.xi.1990, O.F. Clarke 17-12 (CONC); Breas, 1888, A. Larrañaga (SGO 61854);

Quebrada del Gritón on route to Pta. Tórtolas, c. 15 km SSW of Taltal, c. 350 m, 25.xii.1987, M.O. Dillon 5403 (F); Posada Hidalgo, 13.xii.1925, I.M. Johnston 5668 (GH); Quebrada Esmeralda, 11.xii.1983, X. Rodríguez (SGO); c. 60 km S of Taltal, near mouth of Quebrada de la Cachina, 50–100 m, 28.xi.1988, M. Dillon & D. Dillon 6036 (F); region about Aguada Cachina (Waterhole in Quebrada Cachina c. 6 km inland from Caleta Esmeralda), c. 25°53'S, 14–15.xii.1925, I.M. Johnston 5725 (GH); Quebrada las Quiscuda, entre Pan de Azúcar y Caleta Esmeralda, 400 m, 29.x.1941, E. Pisano & R. Bravo 551 (CONC); **III Región**. Prov. Chañaral. Vicinity of

Aguada Grande ['Cachinal de la Costa' of Philippi], near Antofagasta–Atacama provincial boundary, c. 26°02'S, 16–18.xii.1925, I.M. Johnston 5794 (GH); Parque Nacional Pan de Azúcar, Quebrada Coquimbo, 140–190 m, 30.ix.1998, M.O. Dillon, D. Dillon, & V. Poblete 5597 (F); Camino al mirador de Pan de Azúcar, 26°06'S, 70°38'W, 270 m, 14.x.2005, F. Luebert & N. García 2829/1223 (SGO, EIF); Parque Nacional Pan de Azúcar, 26°07'S, 70°35'W, 200 m, 1.xii.1997, M.O. Dillon 8147 (F, CONC); Parque Nacional Pan de Azúcar, 160–200 m, 5.xii.1987, M.O. Dillon & J.T.S. Teillier 5106 (F).