

EVOLUTION OF SECONDARY HEADS IN NASSAUVIINAE (ASTERACEAE, MUTISIEAE)¹

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The evolution of the inflorescence head in Asteraceae is important in the diversification of this largest angiosperm family. The aggregation of heads into higher-order capitulescences (secondary heads or syncephalia) is considered evolutionarily advanced. The genera *Moscharia*, *Nassauvia*, *Polyachyrus*, and *Triptilion* of the subtribe Nassauviinae (Mutisieae) have syncephalia with differing degrees of capitula condensation. ITS and plastid *trnL-trnF* regions were analyzed separately and together using maximum parsimony and maximum likelihood to examine the evolution of syncephalia in the Nassauviinae. The four genera displaying syncephalia do not form a clade minus taxa without syncephalia, indicating that secondary heads in Nassauviinae have either convergently evolved twice in the subtribe (or, very unlikely) once with multiple reversions. Strong support was obtained for a sister relationship between *Leucheria* (without syncephalium) and *Polyachyrus*, and both sister to *Moscharia*. *Nassauvia* and *Triptilion* form a distinct clade but are sister to other genera, *Perezia* and *Panphalea*, without syncephalium. Previous hypotheses postulated the evolution from simple to more complex secondary heads. We show that the ancestor of *Moscharia*, *Polyachyrus*, and *Leucheria*, in a more arid habitat, had a complex type of secondary head, and loss of complexity occurred in response to a shift from arid to mesic conditions.

Key words: ITS; *Leucheria*; *Moscharia*; Mutisieae; Nassauviinae; *Polyachyrus*; syncephalia; *trnL-trnF*.

The capitulum or head, the typical inflorescence of members of the plant family Asteraceae, traditionally has been regarded as an advanced feature in comparison with other inflorescences found in asterids. Along with secondary metabolites, the inflorescence head has been argued to be a key ecological feature behind the diversification of this largest family of flowering plants (Cronquist, 1981; John, 1996; Stevens, 2001). Putative advantages to species possessing a capitulum include more attraction to insect pollinators, better protection for the seeds, and a potential capacity for producing more recombinants (Stebbins, 1967). Aggregation of two or more capitula into inflorescences of a second order is termed secondary heads, syncephalia, or pseudocephalia (Troll, 1928; Leppik, 1960; Weberling, 1992; Harris, 1995; Claßen-Bockhoff, 1996). These aggregations of capitula to higher-order capitulescences are considered an advanced evolutionary feature in the family. Stebbins (1967) suggested that the union of few-flowered heads into a secondary head provides a more efficient mechanism to increase the size of the functional inflorescence relative to the acquisition of new flowers into a reduced capitulum. Stebbins (1967) hypothesized a scenario for the formation of secondary heads involving three steps: (1) the capitula aggregate closely without losing their individual identities; (2) the capitula aggregate into a functional head, the individual capitula being still recognizable, but

without the formation of a pseudoinvolucre (the series of bracts that surrounds the secondary head); and (3) the complete loss of identity of individual capitula and the acquisition of a pseudoinvolucre.

Secondary heads occur in approximately 70 genera (4.4% of all genera in Asteraceae) and in 11 tribes (Good, 1931; Crisci, 1974b; Stuessy, 1978; Harris, 1999). One of these tribes is Mutisieae, an early-diverging lineage in the family and a key tribe for understanding the systematics and evolution of Asteraceae (Jansen and Palmer, 1987; Bremer, 1994). The tribe Mutisieae in its traditional sense (subfamily Mutisioideae for some authors; e.g., Jeffrey, 2007; Katinas et al., in press) is constituted by two subtribes, the monophyletic Nassauviinae and the nonmonophyletic Mutisiinae (Crisci, 1974a; Cabrera, 1977; Bremer, 1994). The focus of our investigation is the subtribe Nassauviinae, which includes 25 genera and about 300 species. It is a neotropical group, with the greatest concentration of genera and species in the southern Andes, where they are an important component of the flora.

Four genera of Nassauviinae, *Moscharia* (two species), *Nassauvia* (38 species), *Polyachyrus* (seven species), and *Triptilion* (seven species), have secondary heads with differing degrees of capitula condensation (Fig. 1). In *Nassauvia* and *Triptilion*, all stages are found between a cymose, lax capitulescence, and the aggregation of few-flowered capitula into condensed, glomerulose capitulescences still containing the identities of the individual capitula. Each capitulum has 1–5 flowers surrounded by two series of bracts or phyllaries (Fig. 2). *Polyachyrus* and *Moscharia*, on the other hand, have more condensed heads bearing and not bearing a pseudoinvolucre, respectively. In *Polyachyrus*, each capitulum is two-flowered with an involucre of four phyllaries, the outermost being keeled and more or less surrounding the whole capitulum; both flowers are separated by a palea (Fig. 3). *Moscharia* has a central one- or two-flowered capitulum with an involucre of 4–7 phyllaries, which is surrounded by marginal, two-flowered capitula. As in *Polyachyrus*, each marginal capitulum is enclosed by a keeled bract, and both flowers are separated

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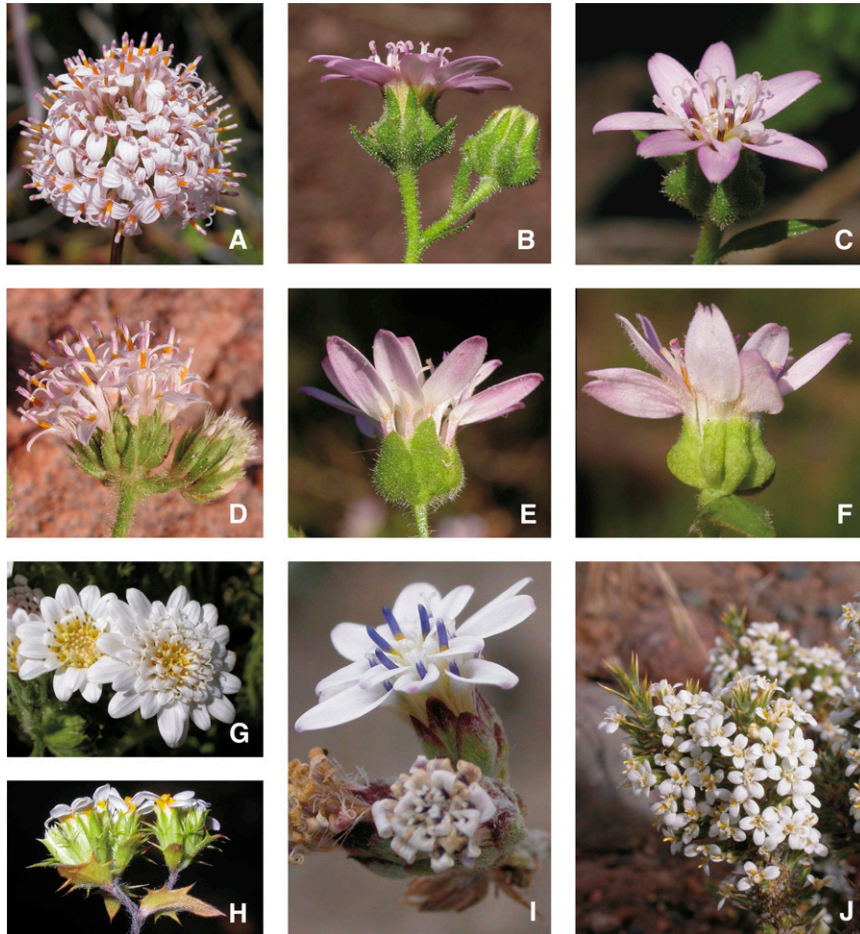
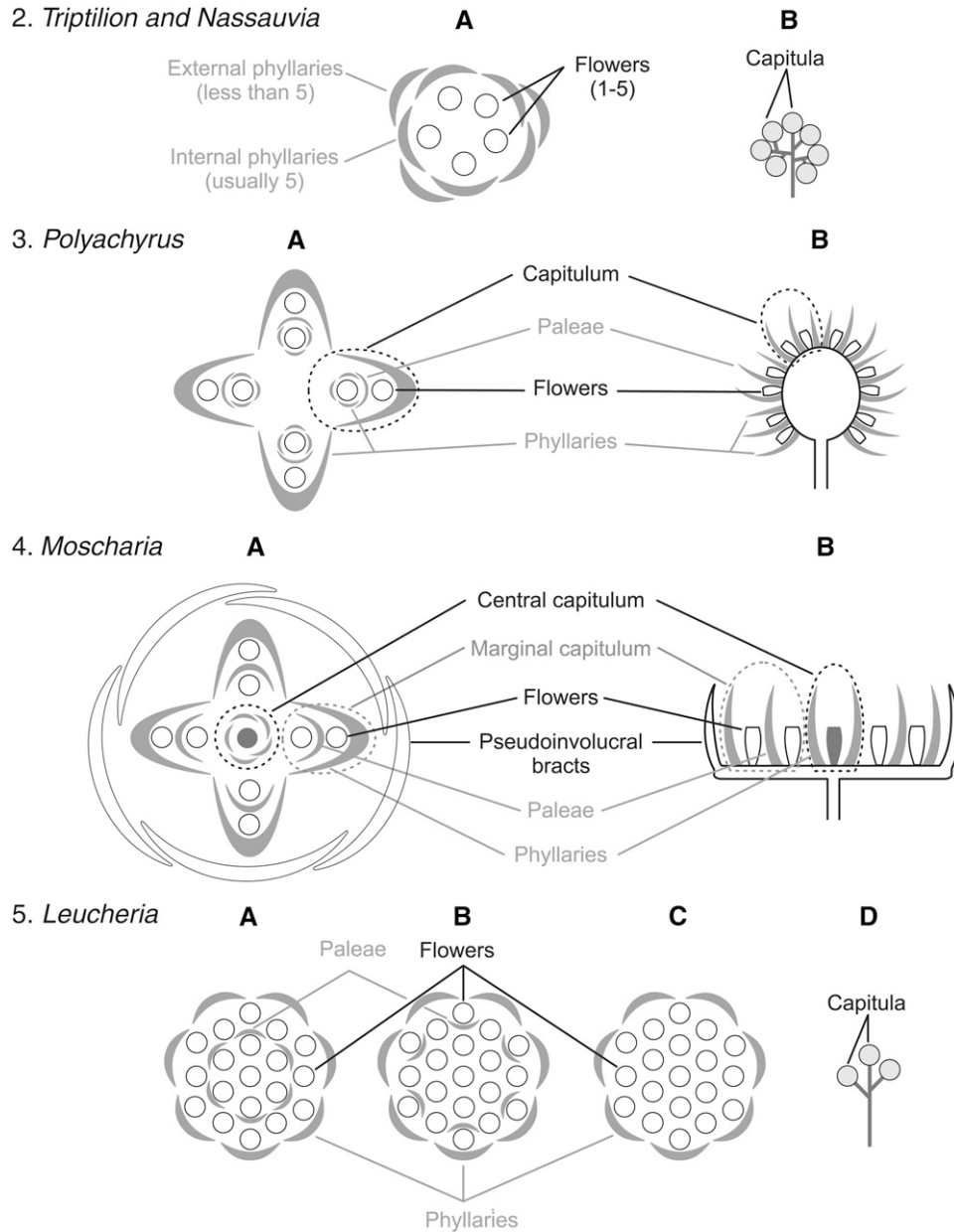


Fig. 1. Representatives of Nassauviinae (Asteraceae), some of them with synccephalia. (A) *Polyachyrus poeppigii* (lateral view of the synccephalium). (B) *Moscharia solbrigii* (lateral view of the synccephalium; note keeled phyllaries and absence of pseudoinvolucre). (C) *M. solbrigii* (superior view of the synccephalium). (D) *P. sphaerocephalus* (lateral view of the synccephalium). (E) *M. pinnatifida* (lateral view of synccephalium; note presence of pseudoinvolucre). (F) *M. pinnatifida* (pseudoinvolucral bracts removed; note keeled phyllaries). (G) *Leucheria salina* (superior view of two capitula). (H) *Triptilion capillatum* (lateral view of condensed capitula). (I) *L. rosea* (lateral view of one capitulum and two fruiting capitula). (J) *Nassauvia glomerulosa* (lateral view of condensed capitula).

by a palea, but the total number of phyllaries are reduced to only the keeled phyllary (Fig. 4). The lack of secondary heads in other genera of the subtribe Nassauviinae is represented by *Leucheria* with true capitula each with their own phyllaries, although the capitula can be somewhat aggregated (Figs. 1, 5).

The presence of secondary heads in *Nassauvia*, *Polyachyrus*, and *Triptilion* was never doubted by past researchers (Ricardi and Weldt, 1974; Burtt, 1977; Cabrera, 1982; Hellwig, 1985; Katinas et al., 1992; Tortosa et al., 2004), but there was controversy regarding the occurrence of this feature in *Moscharia*. The floral head of *Moscharia* traditionally had been considered to be a true, individual capitulum until a numerical taxonomic study of the subtribe Nassauviinae indicated that *Nassauvia*, *Triptilion*, and *Polyachyrus* were closely related to *Moscharia* (Crisci, 1974a). Because of the close relationship between *Polyachyrus* and *Moscharia*, the floral head of *Moscharia* was reinterpreted as a secondary head when comparing it with that possessed by *Polyachyrus*. This reinterpretation of the floral head of *Moscharia* is supported by more recent studies (Freire et al., 1993; Katinas and Crisci, 2000). There are many similarities between the reproductive structures in both genera (Katinas and Crisci, 2000), such

as the presence of few flowers tightly surrounded by one keeled bract, reduction of the pappus, and synaptospermy (i.e., when the heads are enclosed by a keeled bract and are dropped off as a whole and dispersed by gravity; Murbeck, 1920). These features constitute a syndrome typical of genera with secondary heads (Claßen-Bockhoff, 1996). A series of changes from less morphologically complex synccephalia in *Polyachyrus* to more complex ones in *Moscharia* was established (Crisci, 1974b): each group of two flowers and their surrounding bract in the head of *Moscharia* is equivalent to a capitulum in the head of *Polyachyrus*. The central group of one to two flowers and their surrounding bracts in *Moscharia* correspond to a much reduced first-order capitulum. *Moscharia pinnatifida* would represent the most advanced stage in this series because of the presence of pseudoinvolucre, a structure lacking in the other species, *M. solbrigii*. In morphology-based cladistic analyses involving *Moscharia*, *Polyachyrus*, *Nassauvia*, and *Triptilion* (Freire et al., 1993; Katinas and Crisci, 2000), *Moscharia* and *Polyachyrus* resolved as sister taxa by the presence of seven synapomorphies, one of them the presence of secondary heads. The capitula arranged in secondary heads in *Moscharia* and *Polyachyrus* were interpreted



Figs. 2–5. Inflorescence structure in Nassauviinae. **2.** Capitula in *Triptilion* and *Nassauvia*. (A) Cross section of capitula. (B) Lateral view of capitulescence. Capitula very densely set, in some species syncephalia present (e.g., *Triptilion benaventii* and *Nassauvia argentea*) but not as specialized as in *Moscharia* and *Polyachyrus*. **3.** Syncephalium in *Polyachyrus*. (A) Cross section (only four capitula shown). (B) Longitudinal section. Dotted lines indicate individual capitula. **4.** Syncephalium in *Moscharia*. (A) Cross section (only five capitula shown). (B) Longitudinal section (diagram based on *M. pinnatifida*; in *M. solbrigii* pseudoinvolucral bracts are lacking). Dotted lines indicate individual capitula. **5.** Arrangement of capitula in *Leucheria* in corymbs, racemes, etc., sometimes somewhat condensed. (A) Paleae with the open part facing the center of the capitulum, e.g., cross section in *L. landbeckii*. (B) Paleae face the outer part of the capitula, e.g., cross section in *L. rosea*. (C) Capitula without paleae, e.g., cross section in *L. achillaeifolia*. (D) Lateral view of capitulescence.

as a parallel development to the secondary heads in species of *Nassauvia* (and in one species of *Triptilion*).

Some authors have interpreted *Moscharia* as having a simple capitulum instead of a secondary head. Burt (1977) stated that *Moscharia* has a much greater resemblance to some species of *Leucheria*, a genus of Nassauviinae with simple capitula, in which the inner involucre bracts may be decidedly concave on the inner surface and embrace an outer achene. According to Burt, *Moscharia* might well have developed from such a species

of *Leucheria*. Hellwig (1985) compared the morphology of *Moscharia*, *Polyachyrus*, and *Leucheria* and rejected the idea of secondary heads in *Moscharia*, emphasizing the great similarity between the capitula in *Moscharia* and *Leucheria*. Hellwig found that (1) similarities in flowers and capitula are few between *Moscharia* and *Polyachyrus*, (2) the embryological development of the capitula in *Moscharia* is not very different from that in *Leucheria*, and (3) *Moscharia* and *Leucheria* are $2n = 40$, whereas *Polyachyrus* are $2n = 42$.

Recent molecular phylogenetic analyses (Kim et al., 2002; Funk et al., 2005) show *Leucheria* as sister to *Jungia*, another member of Nassauviinae, with *Nassauvia* and *Triptilion* also sister taxa but independent of the pair *Leucheria*–*Jungia*. The genera *Moscharia* and *Polyachyrus* were not sampled in these studies.

For testing these controversial ideas, a molecular phylogenetic analysis should sample more widely within Nassauviinae and include species of *Moscharia* and *Polyachyrus* with other representative genera of Nassauviinae, such as *Leucheria*, *Nassauvia*, and *Triptilion*. Analysis of the results of the molecular phylogenetic analysis could now use new advances in the knowledge of the structure and ontogeny of secondary heads (e.g., Harris, 1995, 1999; Claßen-Bockhoff, 1996); this combined approach may help researchers interpret the reproductive structures in Nassauviinae. The goal of this study is to use molecular data to answer the following questions: Does the molecular approach support the sister relationship between *Moscharia* and *Polyachyrus*? What are the phylogenetic positions of *Leucheria*, *Nassauvia*, and *Triptilion* with respect to *Moscharia* and *Polyachyrus*? How can the evolution of secondary heads in Nassauviinae be interpreted with new molecular evidence in context of structure and ontogeny?

MATERIALS AND METHODS

Taxa sampling—A total of 46 taxa, representing 20 genera of Asteraceae from the two subtribes Mutisieae, Nassauviinae, and Mutisiinae, were sampled in this study (Appendix). Three outgroups were selected from the monophyletic subfamily Barnadesioideae: *Chuquiraga*, *Doniophyton*, and *Dusenilla*. Specimens were collected in Chile, Argentina, and Uruguay by M. Bonifacio and determined by the authors. Classification of genera into subfamilies, tribes, and subtribes follows that of Bremer (1994).

DNA extraction, amplification, and sequencing—Total genomic DNA was extracted from silica-dried leaves following the manufacturers protocol for the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). Both the nuclear ITS region and chloroplast *trnL-trnF* region were amplified by polymerase chain reaction (PCR) using *trnL-trnF* primers following Taberlet (1991) and ITS primers ITS leu1 and ITS 4 (White et al., 1990). PCR reaction mixes for ITS and *trnL-trnF* were 13.75 μ L ddH₂O, 1 μ L DNA, 3 μ L MgCl₂, 3.5 μ L New England Biolabs (Ipswich, Massachusetts, USA) buffer, 0.5 μ L BSA, 1.25 μ L dimethyl sulfoxide (DMSO), 0.5 μ L Q-Soln (Qiagen), 3.5 μ L dNTP, 0.25 μ L Tween, 0.5 μ L of each primer, and 0.25 μ L of New England Biolabs *Taq* polymerase. A second protocol was used for some ITS amplifications of difficult taxa, with PCR mixes consisting of 0.3 μ L Mango (Bioline, Boston Massachusetts) *Taq* polymerase, 8.55 μ L ddH₂O, 0.8 μ L DNA, 6 μ L MgCl₂, 3 μ L buffer, 0.3 μ L BSA, 0.8 μ L DMSO, 0.5 μ L Q-solution (Qiagen), 3.5 μ L dNTP, 0.25 μ L Tween, and 0.5 μ L of each primer.

All 25- μ L PCR reactions were performed in an MJ Research PTC-200 thermal cycler (Waltham, Massachusetts) using an initial 94°C denaturation for 5 min; followed by 32 cycles of 94°C denaturation for 30 s, 60 s annealing at 52°C, and 90 s extension at 72°C; followed by a 7-min final extension at 72°C. PCR products were then purified and cleaned using the AMPure PCR purification protocol (Agencourt, Beverly, Massachusetts, USA). Cycle sequencing reactions used the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA).

Sequences were manually edited in Sequencer 3.0 (Gene Codes, Ann Arbor, Michigan, USA) and the resulting sequences were manually aligned in Se-Al v2.0a7b (Rambaut, 2003). Indels for *trnL-trnF* were treated as single events and were included in the analysis and coded as present or absent. Unalignable regions of ITS were excluded from the analyses. Aligned sequences have been submitted to NCBI GenBank (Appendix).

Phylogenetic analyses—The nuclear ITS and plastid *trnL-trnF* regions were analyzed separately and together using maximum parsimony and maximum likelihood. Congruence between the two data sets under parsimony was assessed with the incongruence length difference (ILD) test (Farris et al., 1994)

using a heuristic search and simple taxon addition for 100 random partitions of data in PAUP* version 4.0b10 (Swofford, 2002). PAUP* 4.0b10 was used to perform heuristic searches using 1000 stepwise random addition sequences replicates, holding one tree at each step, with tree-bisection-reconnection (TBR) swapping and MULTREES and steepest descent in effect. All characters were unweighted and treated as unordered. Bootstrap replicates (Felsenstein, 1985) were performed for each analysis to assess character support. One thousand bootstrap replicates were performed with stepwise random addition sequences, holding three trees at each step, with MulTrees and steepest descent in effect. Consistency indices (CIs) and retention indices (RIs) (Farris, 1989) were calculated to evaluate the amount of homoplasy in the data. Bremer support (Bremer, 1988) for all clades was calculated using (1) saved trees, relaxing parsimony one step at a time, or (2) topological constraints for clades and evaluating the length of shortest trees necessary to break each clade.

Parameters for maximum likelihood (ML) analysis of the combined data sets excluding gaps was obtained with ModelTest version 3.6 (Posada and Crandall, 1998). The ML analysis was run with 100 replicates, TBR branch swapping, and random taxon addition.

Biogeographical and habitat analyses—The shifts in biogeographical province and habitat type among the sampled Mutisieae were assessed in MacClade version 4.0 (Maddison and Maddison, 2000). Each species was scored for its presence in one or more of 13 recognized biogeographical provinces in South America (Cabrera, 1971; Cabrera and Willink, 1980; Marticorena and Rodríguez, 1995; Squeo et al., 2004): 1, Desert (northern Chile and Peru); 2, Central Chile; 3, Altoandean; 4, Puna; 5, Prepuna; 6, Patagonian; 7, Subantarctic; 8, Paranaense; 9, Pampean; 10, Espinal; 11, Monte; 12, Cerrado; 13, Amazonian. Additionally, each species was scored based on its adaptation to specific habitat–moisture regimes. These included desert-dry (regions 1–2), montane-dry (regions 3–5), south-wetter (regions 6–7), mesic (regions 8–12), and Amazonian (region 13). Character-state reconstructions for these two characters were obtained using phylogenetic trees from both maximum parsimony (MP) and maximum likelihood (ML) analyses. We used the two characters with unordered character states but explored the effects of ordering (with different permutations) the habitat–moisture regime character. Additionally, we explored whether resolving hard polytomies affected the character reconstructions.

RESULTS

The aligned length of *trnL-trnF* was 977 bp, with 11 indel events coded. A total of 105 characters were parsimony informative. MP analysis of *trnL-trnF* and indels found 2932 most parsimonious trees of 178 steps and a CI of 0.775 and RI of 0.913. The full ITS data set was 893 bp long, with two unalignable regions of 69 and 40 bp excluded, of which 140 characters were potentially parsimony informative. MP analysis of the ITS data yielded 284 trees, 1284 bp long with a CI of 0.431 and RI of 0.744. The ITS and *trnL-trnF* data sets were highly congruent ($P = 0.9$). Combining the ITS and *trnL-trnF* data sets yielded 24 trees of length 1650 with a CI of 0.471 and RI of 0.795.

The strict consensus of the 24 MP trees from the combined data analysis is well resolved (Fig. 6). Lack of resolution is seen at the base of a moderately supported (73%) subtribe Nassauviinae with no resolution among each of the four strongly supported (100%) subclades. The subtribe Mutisiinae is not supported as monophyletic. Most bootstrap support values for resolved clades in all three analyses were high (>70%). Importantly, the bootstrap support of two groups is 100%: first, *Leucheria* embedded in the *Polyachyrus* and *Moscharia* clade (and 99% for the clade of *Leucheria* + *Polyachyrus*); and second, the two other genera with syncephalia, *Nassauvia* and *Triptilion*, sister to *Perezia* and *Panphalea*. Also, bootstrap and Bremer decay analyses supported the monophyly of most genera with two or more species sampled such as *Moscharia* (100%, 25 decay), *Nassauvia* (100%, 23 decay), *Perezia* (99%, 9 decay), and *Polyachyrus* (100%, 13 decay) from Nassauviinae, and *Chaetanthera* (100%, 19 decay) and *Mutisia* (97%, 8 decay)

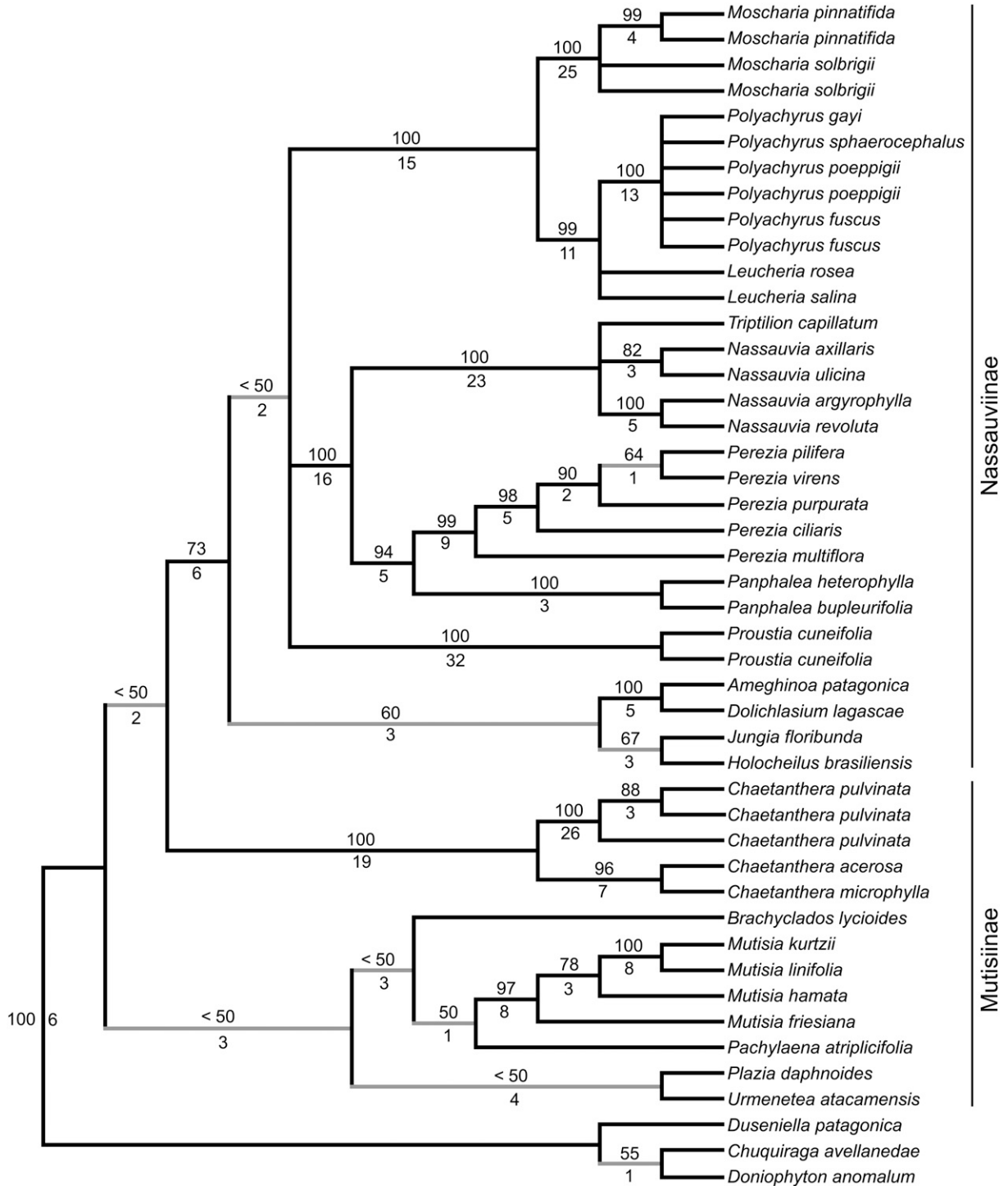


Fig. 6. Strict consensus of 24 most parsimonious trees from combined ITS and *trnL-trnF* data for subtribes Nassauviinae and Mutisiinae of the tribe Mutisieae. Tree is rooted with a monophyletic Barnadesioideae. Bootstrap support values are above each branch and decay values are below. Branches with less than 70% support are gray. The subtribe Nassauviinae is monophyletic, although not strongly so, and the subtribe Mutisiinae is paraphyletic.

from Mutisiinae. *Nassauvia* and *Leucheria* are the only genera not clearly monophyletic with MP analyses.

ML analysis of the combined data sets excluding gaps using the TIM + I + G model selected by ModelTest generates a tree (Fig. 7) very similar to those obtained with MP. The ML tree provides evidence for monophyly of two groups not seen in the

MP analysis. The subtribe Mutisiinae, as well as the subtribe Nassauviinae, is monophyletic. *Nassauvia* with four species sampled is monophyletic relative to *Triptilion*. Only the genus *Leucheria* still remains nonmonophyletic and appears paraphyletic in the ML tree, although the branch length placing *L. salina* as sister to *L. rosea* + *Polyachyrus* is extremely small.

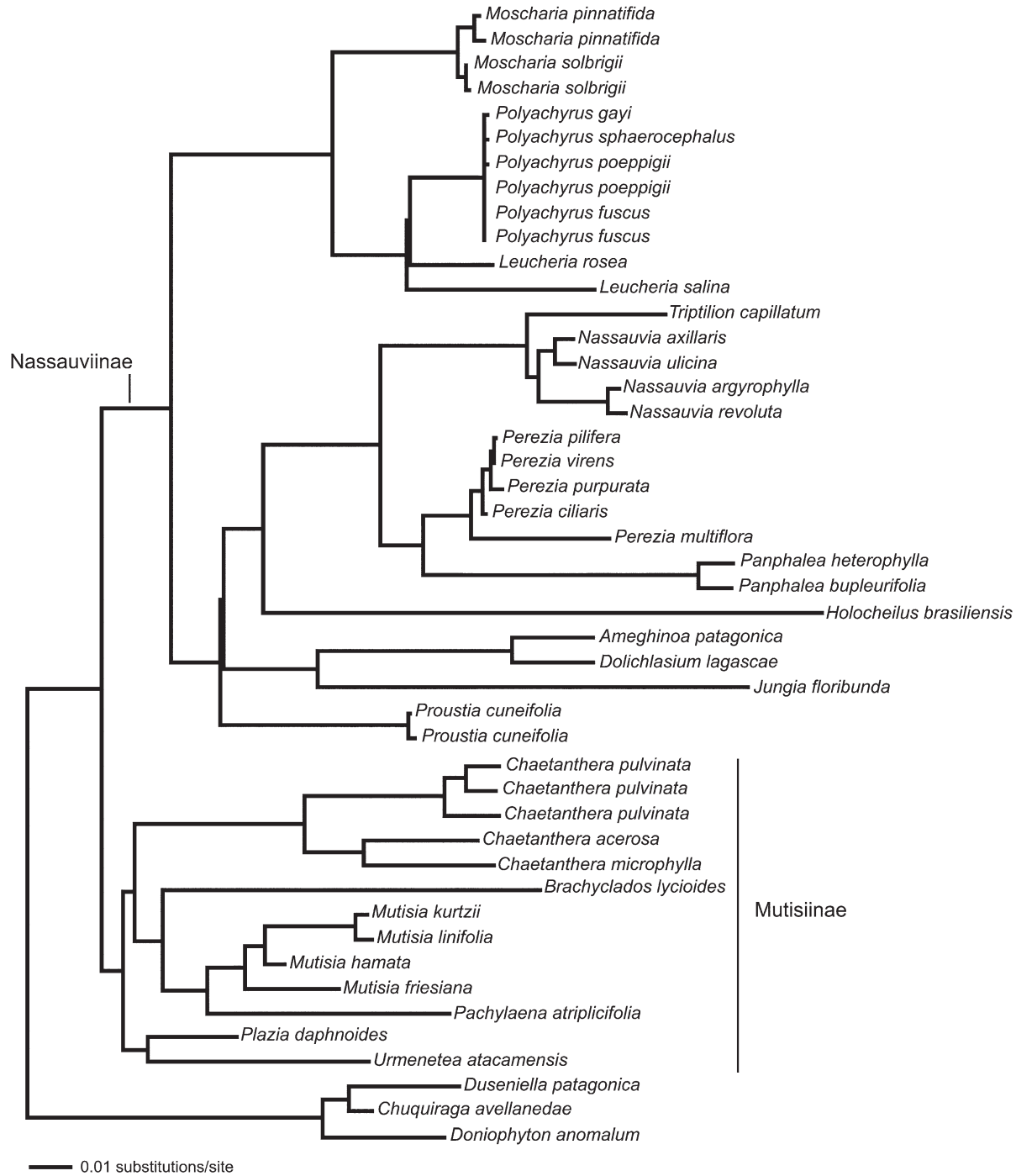


Fig. 7. Maximum likelihood tree from combined ITS and *trnL-trnF* data for subtribes Nassauviinae and Mutisiinae of the tribe Mutisieae. Tree is rooted with a monophyletic Barnadesioideae. Both subtribes Nassauviinae and Mutisiinae are monophyletic.

There is also a strong congruence in the results of the MP and ML trees regarding the generic relationships of Nassauviinae. The only difference is the sister relationship of *Holocheilus* with *Ameghinoa*, *Dolichlasium*, and *Jungia* in the MP tree. In the ML tree, *Holocheilus* is the sister of the monophyletic group constituted by *Triptilion*, *Nassauvia*, *Perezia*, and *Panphalea*. Both trees support generic relationships previously postulated with other types of evidence, such as *Nassauvia-Triptilion*

(Crisci, 1974a; Cabrera, 1982; Freire et al., 1993) and *Ameghinoa-Dolichlasium* (Hansen, 1991; Katinas, 1994). The problematic *Proustia*, previously placed either in Nassauviinae or in Mutisiinae, appears clearly placed in Nassauviinae. The most striking finding of the MP and ML analyses is the sister relationship of *Polyachyrus* with *Leucheria*, both constituting a monophyletic group sister to *Moscharia*. This clade is independent of the pair *Nassauvia-Triptilion*, the other group with secondary heads.

Biogeographical and habitat–moisture analyses in the context of the phylogenetic relationships within the tribe Mutisieae (as sampled here) indicate that the ancestral biogeographic province is likely the Altoandean and its associated montane-dry conditions (Fig. 8). This finding is robust under all 24 MP trees and the ML tree; for a conservative view, we depict in Fig. 8 the reconstructions onto the consensus MP tree (see Fig. 6). The shift solely to desert-dry habitats (deserts of northern Chile and Peru or dry, mediterranean central Chile) is seen in lineages of the subtribe Nassauviinae that exhibit syncephalia. Because of the lack of resolution within the *Moscharia-Polyachyrus-Leucheria* clade in all phylogenetic trees examined, the shift from montane-dry to desert-dry conditions could have occurred once (with a subsequent reversal to montane-dry in *Leucheria*) or independently twice to *Moscharia* and to *Polyachyrus* (Fig. 8).

DISCUSSION

This study includes many more taxa of Nassauviinae (12 genera, 30 species) than previous molecular systematic studies (Kim et al., 2002; Funk et al., 2005), leading us to establish, with a higher degree of certainty, some generic relationships in the subtribe. This is the first time that all species of *Moscharia* and four of the seven species of *Polyachyrus* were included in a molecular phylogenetic analysis of the subtribe. This sampling, together with the inclusion of many other genera and species of Nassauviinae, allows us to suggest hypotheses on the evolution of secondary heads in the subtribe with some confidence.

Generic relationships in Nassauviinae—*Nassauvia* (38 species) and *Triptilion* (seven species) are two southern South American genera. *Nassauvia* species occur in the Andean zone, from the Falkland (Malvinas) Islands to southern Bolivia, and in Patagonia. One species of *Triptilion* inhabits Patagonia, with the rest in central Chile. Both genera were treated as synonyms by David Don in 1832 and by de Candolle in 1838. Other authors (Philippi, 1864; Crisci, 1974a; Cabrera, 1982; Katinas et al., 1992; Freire et al., 1993) treated them as independent genera, although Cabrera (1982) suggested the possibility of considering *Triptilion* as a section of *Nassauvia*. Both genera share many characteristics such as capitulum with 3–6 flowers, similar flower and involucre bract morphology, secondary heads in some of their species, a similar type of twin hairs on fruits, pollen morphology, and a pappus of 3–6 caducous paleae. The main character for their differentiation is the palea of the pappus plicate along the median line, with the upper part expanded and laciniate in *Triptilion* and the narrow pappus (with sometimes plumose bristles), which is not laciniate, in *Nassauvia*. Crisci (1974a, 1980) considered the relationship between *Nassauvia* and *Triptilion* one of the closest within Nassauviinae. Our results support this relationship but maintain them as independent genera in the ML tree (Fig. 7), although the strict consensus parsimony analysis is equivocal on the monophyly of *Nassauvia* (Fig. 6).

Ameghinoa (one species) of Patagonia and *Dolichlasium* (one species) of the central Andes in Argentina were determined previously to be related to the genus *Trixis* (Cabrera, 1936; Simpson Vuilleumier, 1969), *Dolichlasium* as a species of *Trixis* and *Ameghinoa* as very close to this genus. They are shrubby genera with biseriate involucre, yellow flowers, and glabrous receptacle. *Ameghinoa* can be distinguished from *Dolichlasium* by its branches with brachyblasts and entire leaves. In previous analyses, they were considered to be either

sister genera (Hansen, 1991; Katinas, 1994) or closely related but not sister taxa (Crisci, 1974a, 1980). Our analysis agrees with their sister relationship.

Proustia (three species) from southern Peru, Bolivia, central Chile, and Argentina have sometimes been placed in the subtribe Mutisiinae (Cabrera, 1961; Fabris, 1968) because the rounded, dorsally papillose style branches differ from the typical penicillate style branches of Nassauviinae. Crisci (1974a) and Tellería et al. (2003), on the basis of palynological features, emphasized the position of this genus within Nassauviinae because the pollen of Nassauviinae can be clearly distinguished from the pollen of Mutisiinae. The recent molecular phylogenetic analysis of Asteraceae of Funk et al. (2005) positioned *Proustia* in Nassauviinae, and this placement is corroborated in the current study.

Leucheria (46 species), *Moscharia* (two species), and *Polyachyrus* (seven species) were first postulated by Crisci (1980) to be an evolutionarily close group in one of his phylogenetic trees of Nassauviinae that used the genus *Trixis* as outgroup. In Crisci (1980) and all subsequent analyses, except that of Hellwig (1985), *Moscharia* and *Polyachyrus* formed a sister group relationship. As earlier mentioned, Hellwig (1985) related *Leucheria* to *Moscharia* by stating that they both have capitula and not secondary heads as in *Polyachyrus*. Our molecular analysis strongly supports *Leucheria* as sister to *Polyachyrus*, and both as sister to *Moscharia*. As observed in some previous analyses, this clade of three genera is not sister to a clade of *Nassauvia* and *Triptilion*, indicating that the secondary heads have independently evolved twice in the subtribe in Nassauviinae—a phenomenon also seen in unrelated genera of other tribes of Asteraceae (Harris, 1999). On the basis of the homology assessment for these syncephalia, as detailed in the next section, it is highly unlikely that secondary heads evolved only once within Nassauviinae followed by subsequent and repeated reversals (up to five times under ML, Fig. 7).

Morphological evolution of secondary heads—It is widely accepted that *Polyachyrus* have secondary heads and that *Leucheria* have single capitula. However, whether the two species of *Moscharia* have capitula or secondary heads is still debatable with good arguments in favor of both views. In the context of this debate, close relationships have been postulated between *Moscharia* and *Polyachyrus* (e.g., Crisci, 1980; Freire et al., 1993; Katinas and Crisci, 2000) and between *Leucheria* and *Moscharia* (e.g., Burt, 1977; Hellwig, 1985). The results presented here differ fundamentally from these traditionally held views. The analysis supports, with 99% of bootstrap replicates, a sister relationship between *Leucheria* and *Polyachyrus*.

In light of these new DNA-based results, the question still remains: how did secondary heads evolve in Nassauviinae and specifically within the clade *Moscharia-Leucheria-Polyachyrus*? Probably, the simplest solution would be that *Moscharia* and *Leucheria* have capitula and that secondary heads later evolved in *Polyachyrus*. We, however, reject this simple scenario on the basis that it is incorrect to interpret the reproductive structure of *Moscharia* as a simple capitulum. Why view *Moscharia* as possessing syncephalia? As we indicate in some detail, there are very strong morphological similarities between *Moscharia* and *Polyachyrus* in their flowers, keeled bracts, capitulum organization, paleae, pappus, and mode of dispersal (Figs. 3, 4). All these features differ in the simple capitulum of *Leucheria*, which lacks the complex of keeled bracts surrounding two flowers, has a developed pappus, and does not have synaptospermy

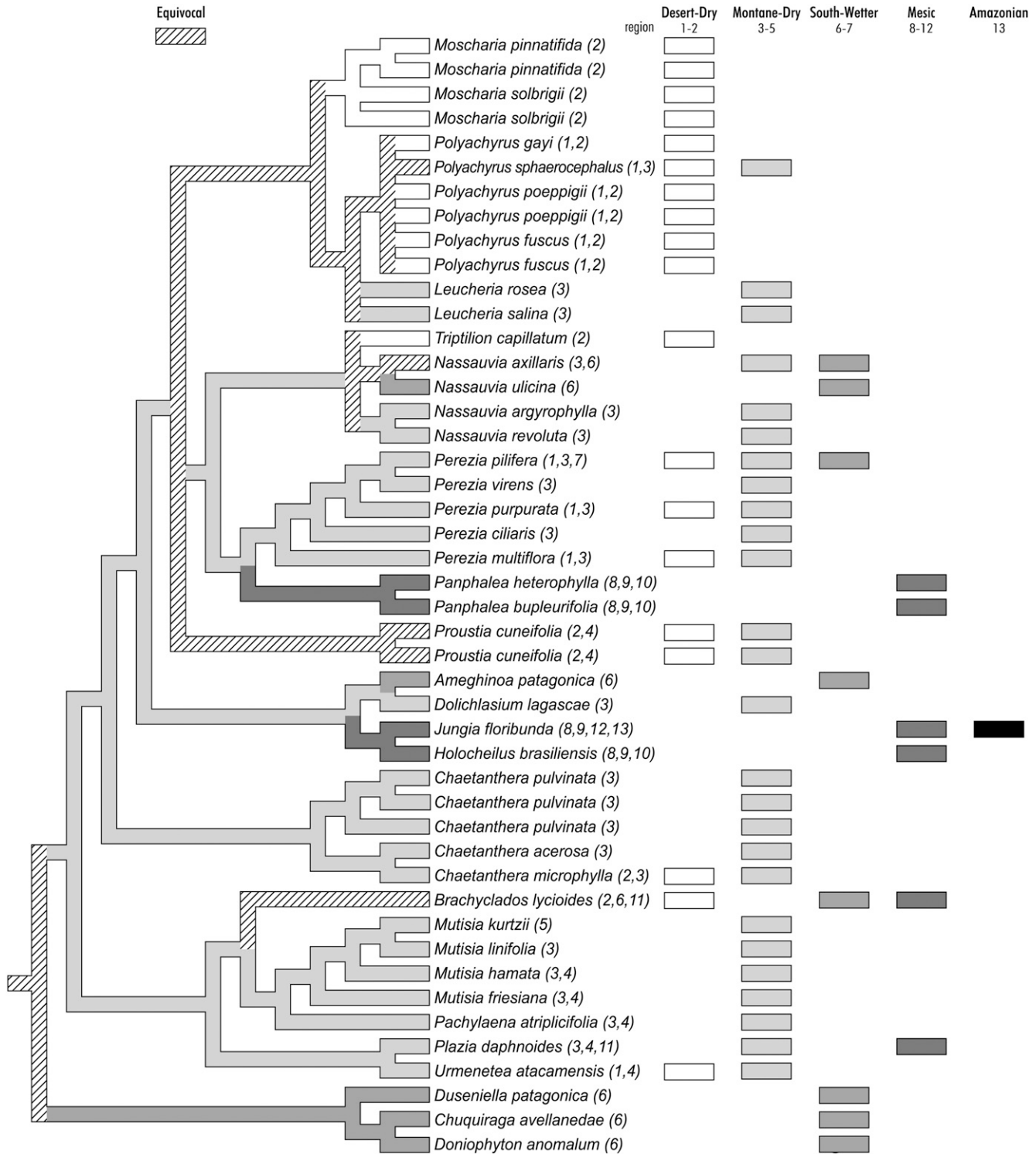


Fig. 8. Overlay of geographical and habitat data onto the consensus most parsimonious tree (see Fig. 6). The mean temperature and annual precipitations are provided for each designated biogeographic province (Cabrera, 1971; Cabrera and Willink, 1980; Marticorena and Rodríguez, 1995; Squeo et al., 2004). Region 1: desert (northern Chile and Peru), less than 20°C, 0–110 mm; region 2: central Chile, 13–15°C, less than 50 mm (except relict area of Fray Jorge Forest: 4–24°C, 85 mm); region 3: Altoandean, –8°C, less than 300 mm, principally as snow; region 4: Puna, 8.5–9.5°C, 50–700 mm; region 5: Prepuna, 10°C, 170–200 mm; region 6: Patagonian, 5.0–13.5°C, 100–500 mm; region 7: Subantarctic, 5.4–9.5°C, 700–5000 mm; region 8: Paranense, 16–22°C, 1500–2000 mm; region 9: Pampean, 13–17°C, 600–1200 mm; region 10: Espinal, 15–20°C, 340–1170 mm; region 11: Monte, 13.0–15.5°C, 80–250 mm; region 12: Cerrado, 21–25°C, 1200–2000 mm; region 13: Amazonian, 26°C, 2000–2600 mm.

(Fig. 5). The clear homology of the reproductive structures of *Moscharia* to *Polyachyrus* strongly supports the argument that both genera, not just *Polyachyrus*, possess syncephalia.

Hellwig (1985), however, argued that in a true secondary head the sequence of floral maturation should be centrifugal or basipetal, as typically happens in the capitulescences (inflorescences of capitula) in some other Asteraceae. He then pointed out that *Moscharia* instead has a racemose or centripetal order of flower maturation—like that in the simple capitula of *Leucheria*—and thus argued that *Moscharia* should possess simple capitula. We analyzed this feature in *Moscharia*, *Leucheria*, and *Polyachyrus* and confirmed the centripetal sequence in *Moscharia* and *Leucheria*, as first noted by Hellwig (1985). However, the centripetal sequence is also present in syncephalia of *Polyachyrus*. Hence, if *Moscharia* is argued to possess a true capitulum instead of a secondary head based on centripetal maturation of flowers, *Polyachyrus* should be also considered as having capitula not clustered into secondary heads or syncephalia—an argument, however, no one would be willing to make. Furthermore, it is clear that a centripetal sequence of floral maturation is found in other genera and species of Asteraceae with syncephalia (besides *Moscharia* and *Polyachyrus*) such as *Flaveria repanda* (Kunze, 1969) and *Myriocephalus gracilis* (Claßen-Bockhoff, 1996). In *Myriocephalus gracilis*, the terminal capitulum is missing and the flowering sequence is centripetal. The capitula are thus arranged in a racemose branching pattern like the flowers in a single capitulum. Loss of the terminal capitulum (truncation), equalization of the branches (homogenization), and change of the flowering sequence (race-mization) have modified the secondary head in such a way that it acts like a single capitulum (Claßen-Bockhoff, 1996).

The centripetal sequence in the flowering of *Moscharia*, *Leucheria*, and *Polyachyrus* would indicate common ancestry of the three genera. A genus with single capitula like *Leucheria*, nested between two genera with secondary heads, is not implausible if we analyze a very unusual feature in the capitula of species of *Leucheria*. The genus has species with and without phyllaries placed among the marginal flowers (Fig. 5). These phyllaries, interpreted sometimes as paleae, change their

spatial orientation in the different species (Crisci, 1976). Some species (e.g., *Leucheria gilliesii*, *L. glandulosa*, *L. purpurea*, *L. senecioides*) have the paleae enclosing florets with the open part facing the center of the capitulum (Fig. 5A). In other species (*L. amoena*, *L. congesta*, *L. hieracioides*, *L. multiflora*, *L. paniculata*, *L. rosea*, *L. tenuis*), the paleae enclosing florets face the outer part of the capitula (Fig. 5B).

A new hypothesis of secondary head evolution should now be considered for these three genera. This hypothesis is based on the strong molecular phylogenetic relationships presented here, on the clear homology in the order of floral maturation of all three genera and between the reproductive structures in the syncephalia of *Moscharia* and *Polyachyrus*, and on the presence and absence of paleae and the change of the palea orientation in *Leucheria*. It is most parsimonious to view the ancestor of the group as possessing morphologically complex secondary heads with subsequent descendants having rearrangements and reductions. A plausible reconstruction of the evolution in floral and head arrangement for this group would involve the following events (Fig. 9): (A) a common ancestor that has syncephalia of many central capitula and many marginal capitula with keeled outer phyllaries and has a pseudoinvolucre; (B) *Polyachyrus*-like ancestor with central capitula but with rearrangement of their phyllaries to the marginal capitula; (C) loss of central capitula (truncation) and pseudoinvolucre in *Polyachyrus*; (D) loss of marginal capitula in *Leucheria* (the remnants of the phyllaries rearrangement in the ancestor is evidenced in the phyllaries position seen among different extant species); and (E) planation to a common receptacle in *Moscharia*, which is very similar to the hypothetical ancestor except that the former has only one central capitulum. This hypothesis of syncephalia evolution thus explains two interesting features of this group: the different palea orientation in *Leucheria* and the centripetal sequence in the flowering of *Moscharia* and *Polyachyrus*. A similar argument of inflorescence reduction has been recently suggested for the genus *Nassauvia* (Tortosa et al., 2004), for which secondary heads are now considered to be the basal type of capitulescence from which the other, lax types were derived.

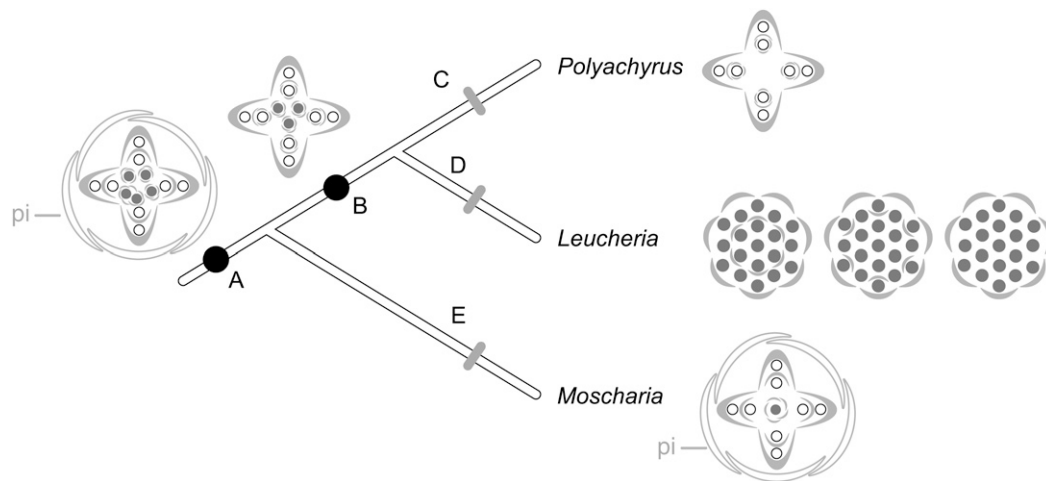


Fig. 9. Syncephalia evolution. (A) Hypothetical common ancestor of *Moscharia*, *Polyachyrus*, and *Leucheria* with several central and marginal capitula and a pseudoinvolucre (only a few central flowers shown). (B) Hypothetical common ancestor of *Polyachyrus* and *Leucheria* with rearrangement of central phyllaries to marginal capitula and loss of pseudoinvolucre. (C) Loss of central capitula (truncation) in *Polyachyrus*. (D) Loss of all marginal capitula, rearrangement of phyllaries in the different species of *Leucheria*. (E) Planation of common receptacle and loss of all but one central capitula in *Moscharia*. Filled circles, flowers from central capitula; empty circles, flowers from marginal capitula; pi, pseudoinvolucre.

Ecological evolution of secondary heads—A plausible explanation for this simplification in the morphology of the reproductive structures can be invoked through habitat pressure and selection. It was postulated, for example, that synaptospermy—and thus secondary heads—had evolved several times within the tribe Arctotideae (Asteraceae) (Roessler, 1959) and that the evolution of synaptospermy is a characteristic and adaptive feature of arid areas (Murbeck, 1920; Zohary, 1950; Schmidt, 1991). Evidence indicates that seeds enclosed in synaptospermous disseminules are better protected against drought. When these seeds germinate, they have more water at their disposal than do single seeds because more cell walls are present, which can absorb and store water (Freire and Katinas, 1995; Claßen-Bockhoff, 1996).

In the case of the genera under discussion, *Moscharia* and *Polyachyrus* occupy well-defined geographical areas in southwestern Peru and north and central Chile (Fig. 8). Most of the species grow in the Mediterranean central valley of Chile, the Atacama Desert, and the dry littoral in Chile and Peru, all areas characterized by their extreme summer drought. *Moscharia solbrigii* grows in the Fray Jorge forest, and it was also found north of La Serena, both areas of central Chile. The area of Fray Jorge is a relict of the Subantarctic forest but is 250 km north of any other relict Subantarctic forest and 500 km north of the northern border of that type of vegetation (Crisci, 1974b). Field observations indicate that *M. solbrigii* appears in the more arid margins and slopes of the Fray Jorge forest and not in the wetter conditions prevailing inside the forest—this distribution is consistent with the more arid specializations of related *M. pinnatifida* and *Polyachyrus*. *Leucheria* and *Nassauvia*, on the other hand, constitute characteristic Altoandean and Patagonian elements, with some species reaching the subantarctic forests (Fig. 8). These areas are dry and cold, with scarce precipitation, but more humid than the areas where *Moscharia* and *Polyachyrus* occur. Species of *Triptilion* grow in both dry and more mesic areas (species sampled only in dry, Fig. 8). Morphological changes in genera of Mutisieae as adaptations to different habitats and pollinators have previously been postulated by Tellería and Katinas (2004). They document palynological and other differences between taxa inhabiting central Chile as opposed to more humid regions of the Altoandean or Patagonia. Our reconstructions suggest that the ancestor of *Moscharia*, *Leucheria*, and *Polyachyrus* with secondary heads probably originally evolved in a very arid region such as the Andes or in the central Chilean areas. These areas have been subjected to Andean uplift in the Tertiary and to Pleistocene glaciations in southern South America.

An ancestral area analysis (Katinas and Crisci, 2000) revealed that the probable ancestral geographic area for *Moscharia* and *Polyachyrus* is the area in central Chile between 30° and 35° S. This hypothesis, however, did not consider *Leucheria* as more closely related to *Polyachyrus*. Although our reconstructions of biogeographical and habitat–moisture changes in this group are equivocal (Fig. 8), the reinterpretation of the inflorescence structure in *Leucheria* strongly argues for a single origin of syncephalia in this subclade under desert-dry conditions. The origin in dry areas would be then followed by a shift of some descendants to more mesic conditions (Altoandean, Patagonian, Subantarctic) where extant species of *Leucheria* are found today (see Fig. 8). The shift from xeric to more mesic habitats would be correlated with a corresponding decompression (i.e., loss of marginal capitula) of the reproductive structures.

In conclusion, this assessment of inflorescence structure in a molecular phylogenetic framework of the Nassauviinae permits

exploration of the role of head evolution in species and habitat diversification within the largest family of angiosperms. The molecular phylogenetic trees obtained led us to review previous hypotheses concerning the relationships and the reproductive structures in *Moscharia*, *Polyachyrus*, and *Leucheria*. *Moscharia* and *Polyachyrus* are not sister taxa as previously thought, and *Leucheria* occupies a novel position as sister to *Polyachyrus*. Consequently, a reconsideration of the evolution of the secondary heads was necessary. Based on morphological assessment of homologous structures, we provide evidence that *Moscharia* species possess secondary heads and not capitula as suggested by others. Previous hypotheses postulated the evolution from simple to more complex secondary heads within this group. The emerging hypothesis established here suggests the opposite, that the ancestor of *Moscharia*, *Polyachyrus*, and *Leucheria* had a more complex type of secondary head than do the extant genera. The reduction in complexity of the syncephalia within the group principally involved the loss of capitula and rearrangement of phyllaries and flowers, perhaps in response to a shift from arid to more mesic conditions in the late Tertiary and Pleistocene.

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APPENDIX. Taxa, vouchers, localities, and ITS and *trnL-trnF* GenBank accession numbers for sampled Mutisieae and outgroups.

- Ameghinoa patagonica* Speg.; *M. Bonifacino* 448; Argentina. Prov.: Chubut, Dept.: Sarmiento. Ruta N° 24, 91 km N of Sarmiento, slopes W of Laguna Seca, 44°53'17"S, 69°12'53"W, 15 Jan 2002; EF530223, EF530269.
- Brachyclados lycioides* D. Don; *M. Bonifacino* 800; Argentina. Prov.: Chubut, Dept.: Gaiman. Ruta nacional N° 25, 43°35'45"S, 66°29'17"W, 5 Dec 2002; EF530224, EF530270.
- Chaetanthera acerosa* (J. Rémy) Benth. et Hook. var. *acerosa*; *M. Bonifacino* 1323; Chile. Reg.: Metropolitana, Prov. Cordillera. RN Río Clarillo, aprox. 3 km N of the entrance to the Reserva, 33°43'47"S, 70°28'9"W, 6 Dec 2004; EF530229, EF530275. *Chaetanthera microphylla* (Cass.) Hook. et Arn. var. *microphylla*; *M. Bonifacino* 1328; Chile. Reg.: Metropolitana, Prov. Cordillera, RN Río Clarillo, aprox. 3 km N of the entrance to the Reserva, 33°43'47"S, 70°28'9"W, 6 Dec 2004; EF530240, EF530286. *Chaetanthera pulvinata* (Phil.) Hauman; *M. Bonifacino* 1505; Argentina. Prov.: Salta, Dept.: Los Andes. Base of the hill Llullaillaco, 24°41'48"S, 68°28'16"W, 10 Jan 2005; EF530228, EF530274. *Chaetanthera pulvinata* (Phil.) Hauman; *M. Bonifacino* 1597; Argentina. Prov.: Mendoza, Dept.: Las Heras. Cordillera del Tigre, Ea. El Yalguaraz, 32°12'12"S, 69°29'53"W, 19 Jan 2005; EF530255, EF530301. *Chaetanthera pulvinata* (Phil.) Hauman; *M. Bonifacino* 1644; Argentina. Prov.: Mendoza, Dept.: Las Heras. Cordillera del Tigre, Ea. San Alberto, 32°27'49"S, 69°25'47"W, 21 Jan 2005; EF530258, EF530304. *Chuiraga avellanadae* Lorenz.; *M. Bonifacino* 1616; Argentina. Prov.: Mendoza, Dept.: Las Heras. Cordillera del Tigre, Ea. El Yalguaraz, 32°12'12"S, 69°29'53"W, 19 Jan 2005; EF530230, EF530276.
- Dolichlasium lagascae* D. Don; *M. Bonifacino* 1635; Argentina. Prov.: Mendoza, Dept.: Las Heras. Cordillera del Tigre, Ea. San Alberto, 32°27'49"S, 69°25'47"W, 21 Jan 2005; EF530259, EF530305. *Doniophyton anomalum* (D. Don) Kurtz; *M. Bonifacino* 773; Argentina. Prov.: Santa Cruz, Dept.: Deseado. Ruta N° 12, 46°51'55"S, 68°3'11"W, 3 Dec 2002; EF530256, EF530302. *Dusenilla patagonica* (O. Hoffm.) K. Schum.; *M. Bonifacino* 437; Argentina. Prov.: Chubut, Dept.: Sarmiento. Ruta N° 20, 20 km E of Sarmiento, 45°38'33"S, 68°57'1"W, 14 Jan 2002; EF530227, EF530273.
- Holocheilus brasiliensis* (L.) Cabrera; *M. Bonifacino* 1203; Uruguay. Dept.: Maldonado. Cerro de las Animas, 34°45'16"S, 55°19'43"W, 28 Oct 2004; EF530247, EF530293.
- Jungia floribunda* Less.; *M. Bonifacino* 1306; Uruguay. Dept.: Cerro Largo. Sierra de Aceguá, 31°52'34"S, 54°23'52"W, 2 Nov 2004; EF530233, EF530279.
- Leucheria rosea* LESS.; *M. Bonifacino* 1322; Chile. Reg.: Metropolitana, Prov.: Cordillera. RN Río Clarillo, aprox. 3 km N of the entrance to the Reserva, 33°43'47"S, 70°28'9"W, 6 Dec 2004; EF530254, EF530300. *Leucheria salina* (J. Rémy) Hieron. subsp. *salina*; *M. Bonifacino* 1557; Argentina. Prov.: San Juan, Dept.: Iglesia. Cordillera de Olivares, Ojos de Agua, 30°21'24"S, 69°41'28"W, 16 Jan 2005; EF530236, EF530282.
- Moscharia pinnatifida* Ruiz et Pav.; *M. Bonifacino* 1321; Chile. Reg.: Metropolitana, Prov.: Cordillera. Reserva Nacional Río Clarillo, 33°43'24"S, 70°29'32"W, 6 Dec 2004; EF530218, EF530264. *Moscharia pinnatifida* Ruiz et Pav.; *M. Bonifacino* 1364; Chile. Reg.: Coquimbo, Prov.: Limarí. PN Bosque Fray Jorge, 30°38'57"S, 71°37'31"W, 8 Dec 2004; EF530237, EF530283. *Moscharia solbrigii* Crisci; *M. Bonifacino* 1428; Chile. Reg.: Coquimbo, Prov.: Limarí. PN Bosque Fray Jorge, 30°39'52"S, 71°40'60"W, 7 Dec 2004; EF530219, EF530265. *Moscharia solbrigii* Crisci; *M. Bonifacino* 1368; Chile. Reg.: Coquimbo, Prov.: Elqui. Quebrada in Ruta 5 and Puente Juan Soldado, 29°39'29"S, 71°18'6"W, 8 Dec 2004; EF530260, EF530306. *Mutisia friesiana* Cabrera; *M. Bonifacino* 1477; Argentina. Prov.: Jujuy, Dept.: Susques. Between Sey and San Antonio de los Cobres, 23°59'56"S, 66°31'38"W, 6 Jan 2005; EF530241, EF530287. *Mutisia hamata* Reiche; *M. Bonifacino* 1428; Argentina. Prov.: Jujuy, Dept.: Rinconada. E of Coyahuasi, 22°47'46"S, 66°25'22"W, 5 Jan 2005; EF530242, EF530288. *Mutisia kurtzii* R. E. Fr. var. *kurtzii*; *M. Bonifacino* 1535; Argentina. Prov.: Catamarca, Dept.: Belén. Cuesta de Randolph, 26°51'57"S, 66°44'16"W, 14 Jan 2005; EF530235, EF530281. *Mutisia linifolia* Hook.; *M. Bonifacino* 1643; Argentina. Prov.: Mendoza, Dept.: Las Heras. Cordillera del Tigre, Ea. San Alberto, 32°27'49"S, 69°25'47"W, 21 Jan 2005; EF530252, EF530298.
- Nassauvia argyrophylla* Cabrera; *M. Bonifacino* 384; Argentina. Prov.: Chubut, Dept.: Cushamen. Ruta N° 15, 2 km W of intersection with Ruta N° 40, 42°23'37"S, 71°09'54"W, 11 Jan 2002; EF530246, EF530292. *Nassauvia axillaris* (Lag.) D. Don; *M. Bonifacino* 1610; Argentina. Prov.: Mendoza, Dept.: Las Heras. Cordillera del Tigre, Ea. El Yalguaraz, 32°12'12"S, 69°29'53"W, 19 Jan 2005; EF530232, EF530278. *Nassauvia revoluta* D. Don.; *M. Bonifacino* 527; Argentina. Prov.: Santa Cruz, Dept.: Lago Argentino. Chaltén, 49°19'18"S, 72°53'42"W, 21 Jan 2002; EF530253, EF530299. *Nassauvia ulicina* (Hook. f.) Macloskie; *M. Bonifacino* 802; Argentina. Prov.: Chubut, Dept.: Gaiman. Ruta nacional N° 25, 43°35'45"S, 66°29'17"W, 5 Dec 2002; EF530245, EF530291.
- Pachylaena atriplicifolia* Hook. et Arn.; *M. Bonifacino* 1602; Argentina. Prov.: Mendoza, Dept.: Las Heras. Cordillera del Tigre, Ea. El Yalguaraz, 32°12'12"S, 69°29'53"W, 19 Jan 2005; EF530250, EF530296. *Panphalea bupleurifolia* Less.; *M. Bonifacino* 1257; Uruguay. Dept.: Cerro Largo. Sierra de Aceguá, 31°49'22"S, 54°19'47"W, 20 Nov 2004; EF530257, EF530303. *Panphalea heterophylla* Less.; *M. Bonifacino* 1222; Uruguay. Dept.: Maldonado. Cerro de las Animas, 34°45'04"S, 55°19'22"W, 28 Oct 2004; EF530248, EF530294. *Perezia ciliaris* Hook. et Arn.; *M. Bonifacino* 1647; Argentina. Prov.: Mendoza, Dept.: Las Heras. Cordillera del Tigre, Ea. San Alberto, 32°27'49"S, 69°25'47"W, 21 Jan 2005; EF530238, EF530284. *Perezia multiflora* (Humb. et Bonpl.) Less. subsp. *sonchifolia* (Baker) Vuilleum.; *M. Bonifacino* 1219; Uruguay. Dept.: Maldonado. Cerro de las Animas, 34°45'04"S, 55°19'22"W, 28 Oct 2004; EF530249, EF530295. *Perezia pilifera* (D. Don) Hook. et Arn.; *M. Bonifacino* 1442; Argentina. Prov.: Jujuy, Dept.: Susques. S of Coranzulí, 23°1'41"S, 66°24'11"W, 6 Jan 2005; EF530225, EF530271. *Perezia purpurata* Wedd.; *M. Bonifacino* 1501; Argentina. Prov.: Salta, Dept.: Los Andes. Base of hill Llullaillaco, 24°41'48"S, 68°28'16"W, 10 Jan 2005; EF530239, EF530285. *Perezia virens* (D. Don) Hook. et Arn.; *M. Bonifacino* 1493; Argentina. Prov.: Salta, Dept.: Los Andes. Base of Abra del Gallo, 24°19'35"S, 66°28'45"W, 8 Jan 2005; EF530243, EF530289. *Plazia daphnoides* Wedd.; *M. Bonifacino* 1470; Argentina. Prov.: Jujuy, Dept.: Susques. Between Susques and Sey, 23°28'22"S, 66°24'16"W, 6 Jan 2005; EF530226, EF530272. *Polyachyrus fuscus* (Meyen) Walpers; *M. Bonifacino* 1380; Chile. Reg.: Antofagasta. Prov.: Antofagasta. Between Ruta 5 and Tal Tal, 25°28'13"S, 70°26'23"W, 9 Dec 2004; EF530263, EF530309. *Polyachyrus fuscus* (Meyen) Walpers; *M. Bonifacino* 1377; Chile. Reg.: Atacama, Prov.: Chañaral. PN Pan de Azúcar, Quebrada Castillo and Quebrada los Locos, 26°12'14"S, 70°37'11"W, 9 Dec 2004; EF530262, EF530308. *Polyachyrus gayi* J. Rémy; *M. Bonifacino* 1367; Chile. Reg.: Coquimbo, Prov.: Elqui. Quebrada in Ruta 5 and Puente Juan Soldado 29°39'29"S, 71°18'6"W, 8 Dec 2004; EF530220, EF530266. *Polyachyrus poeppigii* (Less.) Less. subsp. *poeppigii*; *M. Bonifacino* 1356; Chile. Reg.: Coquimbo, Prov.: Choapa. Ruta 5, 32°4'58"S, 71°31'29"W, 7 Dec 2004; EF530234, EF530280. *Polyachyrus poeppigii* (Less.) Less. subsp. *poeppigi*; *M. Bonifacino* 1366; Chile. Reg.: Coquimbo, Prov.: Elqui. Quebrada in Ruta 5 and Puente Juan Soldado, 29°39'29"S, 71°18'6"W, 8 Dec 2004; EF530261, EF530307. *Polyachyrus sphaerocephalus* D. Don; *M. Bonifacino* 1397; Chile. Reg.: Antofagasta, Prov.: El Loa. Quebrada de Guatín, 22°45'25"S, 68°4'23"W, 11 Dec 2004; EF530221, EF530267. *Proustia cuneifolia* D. Don var. *mendocina* (Phil.) Ariza; *M. Bonifacino* 1547; Argentina. Prov.: La Rioja, Dept.: Famatina. Cuesta de Miranda, 29°23'56"S, 67°48'6"W, 15 Jan 2005; EF530244, EF530290. *Proustia cuneifolia* D. Don var. *mendocina* (Phil.) Ariza; *M. Bonifacino* 1593; Argentina. Prov.: San Juan, Dept.: Ullún. Ruta 436 between Portillo del Colorado and Ciénaga Gualilán, 30°42'29"S, 68°58'59"W, 17 Jan 2005; EF530251, EF530297.
- Triptilion capillatum* (D. Don) Hook. et Arn.; *M. Bonifacino* 1336; Chile. Reg.: Metropolitana, Prov.: Cordillera. RN Río Clarillo, approx., 3 km N of entrance to the Reserva, 33°43'47"S, 70°28'9"W, 6 Dec 2004; EF530222, EF530268.
- Urmenetea atacamensis* Phil.; *M. Bonifacino* 1500; Argentina. Prov.: Salta, Dept.: Los Andes. Estación km 1506, 24°39'10"S, 67°18'28"W, 9 Jan 2005; EF530231, EF530277.