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A Molecular Phylogeny of the Feathery Mistletoe *Misodendrum*

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ABSTRACT. *Misodendrum* comprises eight species of aerial hemiparasites endemic to temperate forests of Chile and Argentina that parasitize *Nothofagus*. This mistletoe is unique in that it has feathery staminodes on its wind dispersed achenes. Previous classifications included two subgenera, *Misodendrum* (two sections) and *Angelopogon* (three sections). The present study tested this classification using two chloroplast genes (*trnL-F* and *matK*) and 31 morphological characters. Maximum parsimony, likelihood and Bayesian analyses were performed for individual and combined partitions. Results from analyses of the separate partitions differed only in the positions of *M. linearifolium* and *M. quadriflorum*; however, the 2-gene tree gave higher support for *M. quadriflorum* as sister to all other species. *Misodendrum brachystachyum* and *M. oblongifolium* form a well supported clade that is sister to one composed of *M. punctulatum, M. gayanum,* and *M. angulatum*. These phylogenetic relationships generally agree with previous taxonomic classifications. Subgenus *Misodendrum*, characterized by warty stems and two stamens, here resolves as a polytomy: *M. punctulatum, M. gayanum,* and *M. angulatum*. Subgenus *Angelopogon*, characterized by the plesiomorphies three stamens and foliacious bracts, is paraphyletic given our rooting. *Misodendrum brachystachyum* and *M. oblongifolium* (section *Archiphyllum*) differ morphologically only by the length of their fruiting staminodes.

KEYWORDS: *matK*, parasitic plant, Santalales, South America, *trnL-F*.

Misodendrum Banks ex DC, the sole genus of Misodendraceae, comprises eight species of aerial hemiparasitic shrubs endemic to temperate forests from 36° 30′ S in central Chile to 55° S on Tierra del Fuego Island, Argentina. These mistletoes are host specific, naturally parasitizing various species of *Nothofagus* (rarely parasitic on other hosts; Skottsberg 1914). Although the range of *Nothofagus* extends to other Gondwanan landmasses such as Australia and New Zealand, *Misodendrum* is restricted to the New World.

Misodendrum is characterized by sympodial branches with alternate leaves. Plants are dioecious; however, monoecious individuals are rarely found, including some with bisexual flowers. The inflorescence is basically a raceme or spike with multiple flowers, although sometimes reduced to one or two flowers. The inflorescence bracts are similar to the leaves. Flowers are very small and dull in color. Staminate flowers lack a perianth and bear two or three stamens that surround a central nectariferous disk. In the carpellate flowers the perianth and the bases of the staminodes are fused to the ovary. From the grooves formed by the edges of the perianth members, three staminodes emerge approximately midway along the ovary. After fertilization, these staminodes will develop into the characteristic feathery appendages of the fruit. In some species, perianth lobes are recognizable at the apex of the ovary. The nature of these lobes has not been adequately investigated, but we favor the concept that they are petals (as in Takhtajan 1997), where fusion and reduction have progressed to such a degree that no evidence of a calyx (or calyculus) exists. Because the perianth is fused to the ovary for nearly its entire length, we interpret it as epigynous, not hypogynous as stated in Orfila (1978). Because the staminodes (or stamens in bisexual flowers) are fused to the ovary only at the base, they could be considered epihypogynous. A nectariferous disk occurs inside the petal lobes and surrounds a short style ending in three stigmas. The unilocular ovary is tricarpellate at the base and bears three ovules, pendulous from a free-central placenta; each has an undifferentiated nucellus and integument. Following ovular abortion, only one seed remains in the mature fruit. The presence of nectary disks on flowers of both sexes, in conjunction with characteristics of the pollen exine, suggest insect pollination (Orfila 1978), although empirical data are lacking. In the Lake Vintter area of Argentina, Orfila (1978) observed many adult Cantharidae (Coleoptera) on flowering *Misodendrum*. These beetles are known to eat petals and stamens and Orfila (1978) states that they could be involved in pollinating *Misodendrum*.

The fruit is a wind dispersed achene with three feathery appendages of varying length depending upon the species. The appendages not only keep the fruit aloft but also aid in attachment to the host branch. Upon germination, the green hypocotyl with a sticky apical holdfast adheres to the host branch. The radicle develops an haustorium that first attaches to the host epidermis and then enters the cortex. The haustorium branches out and

penetrates into the host phloem and then xylem in a manner analagous to the cortical strands and sinkers of Viscaceae (Johnson 1889; Tercero-Bucardo and Kitzberger 2004). In species such as *M. punctulatum*, the parasite can live endophytically for up to two years prior to the emergence of the first shoot from the host branch (Tercero-Bucardo and Kitzberger 2004).

Misodendrum was first described by Candolle (1830) who placed the genus within the large mistletoe family Loranthaceae. In 1858, J. G. Agardh named the new family, Misodendraceae, placing it between Loranthaceae and Santalaceae. Possibly unaware of this work, Bentham and Hooker (1880) moved the genus from Loranthaceae to Santalaceae based on the similarities of the carpel, placentation and ovules. In 1889, Hieronymus classified Misodendraceae as a separate family (between Loranthaceae and Santalaceae) not knowing that Agardh had named it 30 years earlier.

The first infrageneric classification was the monograph by Skottsberg (1913). Orfila (1978) added two new species to Skottsberg's classification, resulting in 12 species in two subgenera (*Misodendrum* and *Gymnophyton*). Some years later, Rossow (1982) published a revision of the family where he proposed several synonyms that reduced the number of species to eight. His classification, used in this study, recognizes subgenus *Misodendrum*, which is characterized by warty stems and two stamens. This subgenus contains sections *Misodendrum* (*M. punctulatum* Banks ex DC and *M. gayanum* Tiegh.) and *Heterophyllum* Skottsb. (*M. angulatum* Phil. and *M. macrolepis* Phil.). Subgenus *Angelopogon* (Tiegh.) Rossow, characterized by three stamens and foliacious bracts, contains three sections: *Angelopogon* (Tiegh.) Skottsb. (*M. linearifolium* DC), *Archiphyllum* (Tiegh.) Skottsb. (*M. brachystachyum* DC and *M. oblongifolium* DC), and *Telophyllum* (Tiegh.) Skotsb. (*M. quadriflorum* DC). Zavaro et al. (1997) published a cladistic analysis based on 18 external morphological and anatomical characters that supported Rossow's classification.

To date, there has been little molecular phylogenetic work on Misodendraceae. Placeholders for the family have been included in broad-scale phylogenetic studies aimed at placing all families within Santalales (Nickrent and Duff 1996; Nickrent et al. 1998; Nickrent and Malécot 2001). That work indicated that Misodendraceae is sister to *Schoepfia* (Schoepfiaceae) and this clade sister to Loranthaceae. Both *Schoepfia* and basal Loranthaceae (e.g. *Nuytsia*) are root parasites, thus aerial parasitism evolved twice independently, once in the ancestor of *Misodendrum* and again in more derived Loranthaceae. Using a calibrated tree for the order Santalales (Malécot 2002), aerial parasitism must have arisen first in *Misodendrum* ca. 75 mybp. This date is not incompatible with the fossil history of *Nothofagus* which had already differentiated into four subgenera (Knapp et al. 2005). The calibrated Santalales tree also indicates that aerial parasitism in Loranthaceae evolved later, ca. 40 mybp.

This study had two objectives: 1) to test the existing traditional classification by reconstructing the phylogeny of the species of *Misodendrum* using chloroplast markers and morphology and 2) to examine the evolution of morphological characters in this mistletoe genus using the phylogeny inferred from the molecular data.

Materials and Methods

Sampling. The ingroup for molecular analyses consisted of all species of Misodendraceae except the rare *Misodendrum macrolepis*. This species is restricted to a small area in Chile and only a few collections of female individuals exist. All other species were collected near Bariloche, Argentina (the northern part of the distribution of the genus). The outgroup comprises two species of *Schoepfia* (Schoepfiaceae) and the three root parasitic and relictual species of Loranthaceae, *Nuytsia floribunda* from western Australia, *Atkinsonia ligustrina* from eastern Australia and the neotropical *Gaiadendron punctatum*. Accession and voucher information, as well as GenBank numbers for all taxa used, are given in Appendix 1.

DNA Extraction and Sequencing. DNA was obtained using a standard CTAB method from silica dried tissue (Nickrent 1994). The nuclear ribosomal DNA internal transcribed spacer (ITS-1 and ITS-2) and 5.8S rDNA were amplified and sequenced. Excessive length variation in the ITS-1 region precluded unambiguous alignment. Analysis of the remaining regions yielded no resolution, thus this data partition was not used in this study (sequences were deposited with GenBank). Primers and methodology used are available from RVR upon request. The chloroplast spacer *trnL-F*, including the intron between the *trnL* exons, was amplified and sequenced using the primers described in Taberlet et al. (1991). The chloroplast gene *matK* was amplified and sequenced with primer 78f (5′-CAG GAG TAT ATT TAT GCA CT) and 1420r (5′-TCG AAG TAT ATA CTT TAT TCG).

Polymerase chain reactions (PCR) were performed in a total volume of 25 µl using 1× PCR buffer (50 mM KCl, 10mM tris HCl, pH 8.3), 1.5 mM $MgCl_2$, 50 µM of dNTPs, 0.4 µM of each primer, ca. 1 unit *Taq* DNA polymerase and 1µl of genomic DNA diluted 1:9. Amplifications were carried out in a GeneAmp system 9600 thermocycler (Applied Biosystems). For *trnL-F* reactions, a touch down profile was used: 5 min at 95°C, 5 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min, followed by 33 cycles of 94°C for 30 sec, 48°C for 30 sec, and 72°C for 1 min, with a final extension of 72°C for 10 min. For the *matK* reactions, a step up PCR profile was used: 5 min at 95°C, 5 cycles of 94°C for 1 min, 46°C for 1 min, and 72°C for 1 min, followed by 35 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min, with a final extension of 72°C for 10 min.

PCR products were purified using either the QIAquick gel extraction kit (Qiagen Inc., Valencia, California) or the EZNA

TABLE 1. Summary of tree statistics from parsimony analyses of molecular and morphological data sets.

	trnL-F	*matK*	*matK* + *trnL-F*	Morphology	*matK* + *trnL-F* + morphology
Alignment length	1365	1151	2516	31	2547
Missing data %	0	10.25	4.69	3.20	4.72
Variable characters	275	432	707	31	738
Informative characters	147	260	407	29	436
Number of trees	1	2	2	2	1
Tree length	367	632	1015	56	1074
CI	0.910	0.847	0.856	0.714	0.846
RI	0.869	0.836	0.827	0.709	0.817

purification kit (Omega Biotek, Inc. Doraville, Georgia). Cycle sequencing reactions were performed directly on the purified PCR products following standard protocols accompanying the BigDye terminator Cycle Sequencing Ready Reaction Kit, with AmpliTaq DNA Polymerase (Applied Biosystems, Foster City, California) and Better Buffer (The Gel Company, San Francisco, California). Sequencing reactions were purified with ethanol precipitation or with Centri-Sep 100 spin columns with Sephadex (Princeton Separations, Inc. Adelphia, New Jersey). Sequences were generated using an ABI 377 automated sequencer (Applied Biosystems).

Alignment and Phylogenetic Analysis. Sequences were aligned by eye in Se-Al (Rambaut 2004). Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were performed for individual and combined partitions. Parsimony and bootstrap analyses (BS, 1000 replications) were conducted in PAUP* (Swofford 2003) with branch and bound searches. Gaps were considered homologous if they shared identical boundaries and length. They were coded as a substitution only for the *trnL-F* region if they were shared by more than one taxon. For *matK*, all gaps were coded as missing. The final data matrices are available from TreeBASE (study number S1728).

Maximum likelihood analyses were conducted in PAUP* (Swofford 2003), with a model of sequence evolution selected using ModelTest (Posada and Crandall 1998) for each gene partition as well as for all combined partitions, implementing the AIC criteria. Tree heuristic searches were performed using Tree Bisection Reconnection (TBR) branch swapping with a Neighbor Joining (NJ) starting tree. Bootstrap nodal support (MLBS) was determined by analyzing 100 replicates, starting each time with a NJ tree and heuristic search and the TBR branch swapping algorithm.

Bayesian inference was performed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) with a model selected by MrModeltest (Nylander 2004). The model of molecular evolution selected for *matK, trnL-F* and both partitions combined was the general time reversible plus a gamma distribution to account for variation among sites (GTR+ Γ). Two independent analyses with four chains each were performed for five millions generations. Trees and parameters were saved every 100 generations, producing 50,000 trees each run. Model parameters were estimated as part of the analysis; uniform prior probabilities were assigned to all parameters except the state frequencies for which a Dirichlet prior distribution was assigned. When more than one partition was analyzed, parameter estimations were unlinked and the rate of the priors was set to vary, thus allowing partitions to evolve at different rates. The burn-in was determined by identifying stationarity using the –ln likelihood score. The variance between runs in all cases was below 0.001, thus runs were combined thereby increasing the number of trees in the posterior probability (PP) distribution.

Morphological Analysis. Morphological data were collected from the available literature for Misodendraceae (Johnson 1889; Skottsberg 1913; Orfila 1978; Rossow 1982; Carlquist 1985; Zavaro et al. 1997). *Schoepfia schreberi* and *Nuytsia floribunda* were used as outgroup by assembling information from the literature (Herbert 1919; Metcalfe and Chalk 1950; Reed 1955; Narayana 1958; Barlow 1966; Werth et al. 1979; Robertson 1982). A matrix with 31 discrete characters was constructed (Appendix 2 and 3) and analyzed with unweighted parsimony, treating all characters as unordered. Tree search and nodal support (1000 bootstrap replicates) were performed with a Branch and Bound search in PAUP* (Swofford 2003). This matrix was concatenated with the molecular data set and was analyzed using the same parsimony settings used for the molecular data set alone. Morphological character state changes were optimized on the molecular topology using MacClade (Maddison and Maddison 2000) under both DELTRAN and ACCTRAN options.

RESULTS

For the *trnL-F* region, the ingroup varied in length from 620 to 772 base pairs. There were 48 gaps ranging from 1 to 146 base pairs; of these gaps, 10 were coded for parsimony analysis. Most of the larger size differences were deletions, whereas the largest insertion was found in *M. linearifolium*, consisting of 35 bp. Among both ingroup and outgroup taxa, tandem duplications in at least 17 positions were seen that averaged six base pairs in length. Ambiguous alignment positions were present in the *trnL-F* matrix but were generally confined to ingroup and outgroup comparisons. When the outgroup was removed, the alignment was largely unambiguous. Inclusion and exclusion of ambiguous sites did not change relationships among the taxa. Mutations in the upstream and downstream duplicate segments involved small length mutations and substitutional changes. The model of molecular evolution selected for *trnL-F* was a transitional model with rate variation among sites estimated through a gamma distribution with four categories (TIM+Γ).

The final *trnL-F* data matrix (with outgroups included) consisted of 1365 aligned positions, of which 147 were parsimony informative (Table 1). One most parsimonious tree was found (367 steps), however, when gaps were coded three equally

parsimonious trees were found (383 steps). The difference in these trees was the position of *M. quadriflorum*. The *trnL-F* consensus tree strongly supported the sister relationship of *Misodendrum* and *Schoepfia*. When ingroup nodes unsupported by at least one method of analysis were collapsed, four clades emerged from a polytomy: 1) *M. linearifolium*, 2) *M. quadriflorum*, 3) *M. brachystachyum and M. oblongifolium* (BS = 100, MLBS = 100, PP = 1.00), and 4) *M. angulatum, M. gayanum*, and *M. punctulatum* (BS = 99, MLBS = 97, PP = 1.00). *Misodendrum linearifolium* is weakly supported (BS = 79, MLBS = 50, PP = 0.52) as sister to all other species in the ingroup.

The *matK* gene does not vary greatly in length among species of *Misodendrum*. Due to amplifications problems *M. punctulatum* is missing 656 bp, and *S. schreberi* is missing 878 bp of this gene. There is one in-frame deletion of 6 bp in *M. quadriflorum*. *Misodendrum linearifolium* shows a frame shift mutation caused by one base deletion and then the reading frame is recovered by an insertion 17 bases downstream. This frameshift mutation causes a change in six amino acids. A tranversional model with rate variation among sites estimated through a gamma distribution with four categories (TVM+Γ) was selected for *matK*.

The *matK* data set consisted of 1151 aligned positions, of which 260 were parsimony informative (Table 1). Two most parsimonious tree were found (632 steps) that differed in the relationship among the species of the monophyletic subg. *Misodendrum*. The same four clades found with the *trnL-F* region were found analyzing *matK*. Subgenus *Angelopogon* was paraphyletic, and *M. quadriflorum* was highly supported as sister to all species (BS = 93, MLBS = 88, PP = 0.97).

The topology of the *matK* tree was more resolved than the one from *trnL-F*, but the two trees were not incongruent. For this reason, the two datasets were concatenated. This analysis yielded one most parsimonious tree of 1028 steps (Table 1; Fig. 1A) which placed *M. quadriflorum* as sister to all other taxa but with low support (BS = 63, MLBS = 55, PP = 0.73). The signal for this relationship derives from the *matK* gene tree. All analyses recovered section *Archiphyllum* (subg. *Angelopogon*) as monophyletic. This section is sister to subg. *Misodendrum*, however, this relationship is weakly supported (BS = 63, MLBS = 54, PP = 0.89). Subgenus *Misodendrum* is monophyletic with high support from all three analytical methods (BS = 100, MLBS = 100, PP = 1.00).

The two most parsimonous *matK* trees differed from the *trnL-F* tree in the relative positions of *M. punctulatum*, *M. gayanum* and *M. angulatum*. In the combined analysis, parsimony places *M. punctulatum* as sister to *M. angulatum* (as with *matK*) with 63% bootstrap support, however, this relationship collapses to a polytomy with likelihood and Bayesian inference. These taxa are best viewed as emerging from a polytomy, especially given that *M. macrolepis*, included in this group by other authors, was not sampled in this study; its inclusion might help to resolve the relationships among species of subg. *Misodendrum*.

The morphological character analysis yielded two trees, each of 56 steps (Table 1). The topologies of the two trees captured some of the same well-supported relationships obtained from the molecular data (Fig. 1B). The clades supported by both methodologies were subg. *Misodendrum* and section *Archiphyllum* (subg. *Angelopogon*). *Misodendrum quadriflorum* is sister to section *Archiphyllum* with relatively high support (BS = 86). This topology is also found with the *trnL-F* data set but with low support (BS = 52). *Misodendrum linearifolium* changes position in the two morphology trees. It appears either as sister to subg. *Misodendrum* or as part of subg. *Angelopogon*.

Analysis of the concatenated molecular plus morphology data set, as with both data sets analyzed separately, did not resolve a monophyletic subg. *Angelopogon*, thus the two subgenera are not reciprocally monophyletic. In this analysis, *Misodendrum linearifolium* (subg. *Angelopogon*) is sister to the remaining species, but with no support. As in the other analyses, subg. *Misodendrum* is highly supported as monophyletic. Moderate support (BS = 72) is found for the sister relationship between *M. angulatum* and *M. macrolepis* (this latter species was included only in the morphological data analysis); however, there were no morphological synapomorphies for this clade.

When the character state changes are optimized on the molecular tree (with *M. macrolepis* added in its most likely position based on the morphological analysis), five morphological synapomorphies support the monophyly of subgenus *Misodendrum* (Fig. 2A). Additional synapomorphies are found for this clade when one explores the different reconstructions of ancestral states for the six characters for which optimization was equivocal. If character 4 (leaf shape) is reconstructed under the DELTRAN option (Fig. 2B), then linear leaves characterize the subgenus *Misodendrum* clade. In this case, *M. linearifolium* acquired the same leaf shape independently. With ACCTRAN reconstruction, three more characters (absence of sclereids, absence of rays, and lignified parenchyma) characterize this clade (Fig. 2B). Section *Archiphyllum* shares with subg. *Misodendrum* the alternate

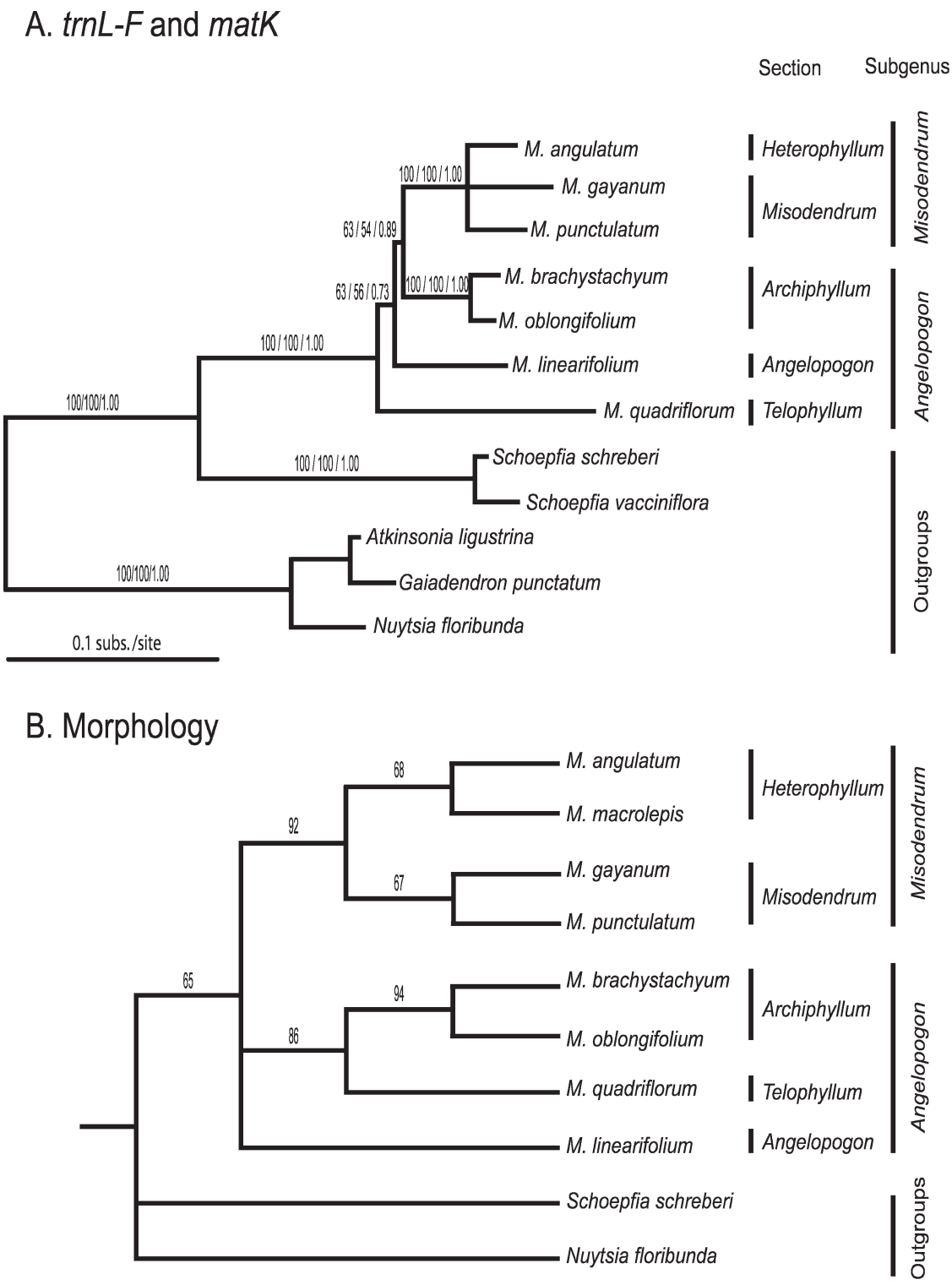

FIG. 1. A. Majority rule consensus tree of 75,000 trees from Bayesian analysis of the combined *matK* and *trnL-F* sequences. Branch lengths are represented by mean values of those trees. Nodal support is given above the branches as bootstrap values for parsimony, likelihood, and posterior probabilities, respectively. B. Strict consensus of two most parsimonious trees resulting from the analysis of 31 morphological characters with bootstrap support above each branch (1000 replicates).

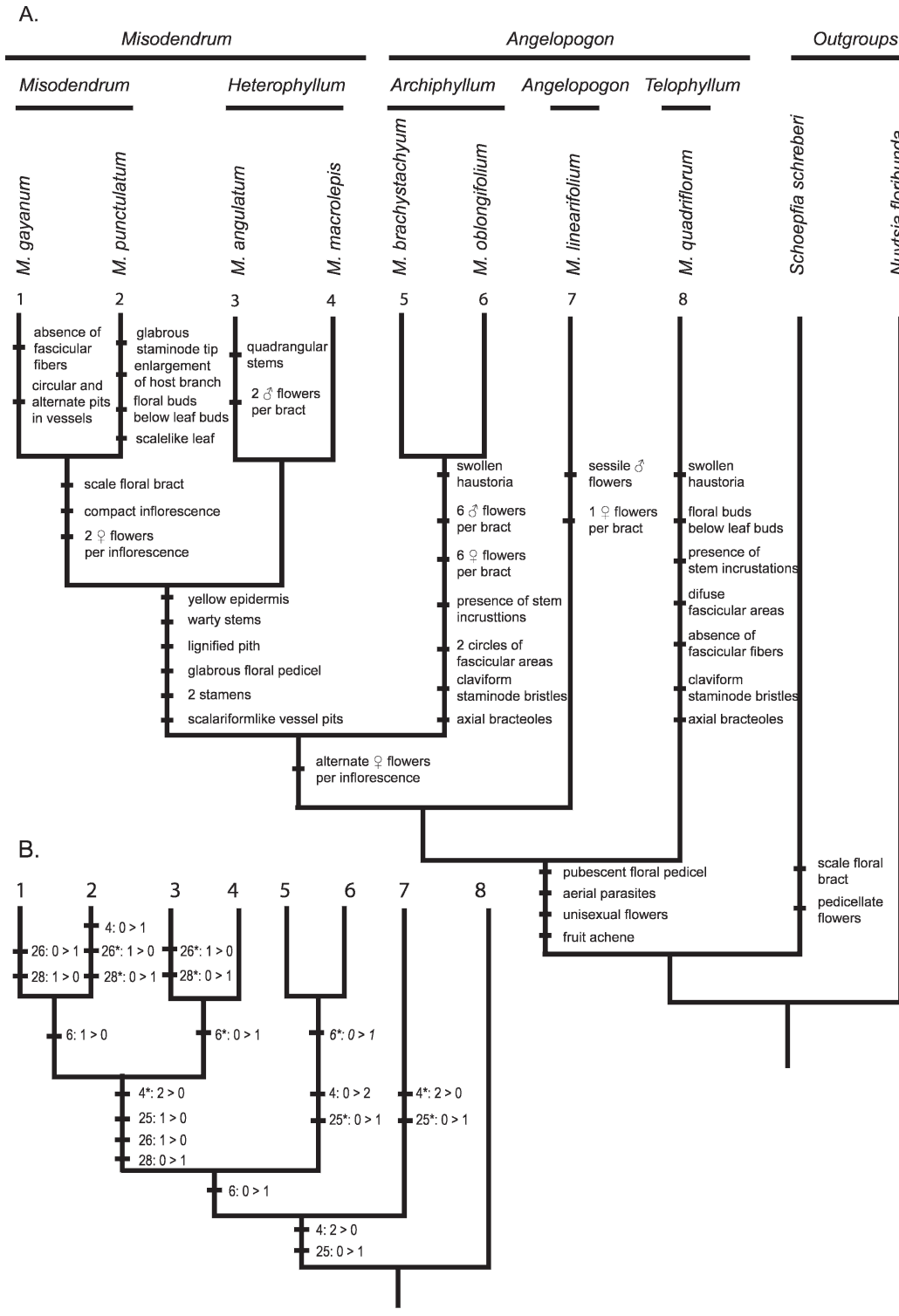

FIG. 2. Character optimization. A. Character state changes optimized on the molecular tree. B. Different resolution for the characters where equivocal reconstructions were found. Numbers refer to characters and states in Appendix 2. A star next to the character number refers to DELTRAN optimization, the others to ACCTRAN.

position of the females flowers on the inflorescence. In addition, when character 6 (female flowers) is optimized under ACCTRAN (Fig. 2B), these two clades have pedicellate female flowers with a reversal to sessile flowers in section *Misodendrum* (subg. *Misodendrum*). When optimized under DELTRAN, pedicellate flowers in section *Heterophyllum* (subg. *Misodendrum*) are homoplasious with the ones found in section *Archiphyllum* (subg. *Angelopogon*).

Many character states are shared among subg. *Angelopogon* taxa, but these become pleisomorphic given that subg. *Angelopogon* is paraphyletic. Especially, similarities are seen between section *Archiphyllum* and *Telophyllum*, that share four character states: 1) swollen haustoria, 2) axial bracteoles, 3) claviform staminode bristles, and 4) stem incrustations.

Discussion

All species of *Misodendrum* are very similar morphologically and unique among aerial parasites with their wind dispersed fruits. Molecular data strongly support the monophyly of Misodendraceae (BS = 100, MLBS = 100, PP = 1.00). The phylogenetic relationships found among species in this study generally support previous classifications of the family. Subgenus *Misodendrum* is a distinct clade characterized by warty yellow stems and two stamens (Fig. 2A), but relationships among its component species are unresolved. The rare species *M. macrolepis* was not included in this molecular analysis, but was placed as sister to *M. angulatum* in the morphological analysis reported here and in the one conducted by Zavaro et al. (1997). In the latter study, *M. punctulatum* and *M. gayanum* appear as sister to that clade, but with low support (BS = 50), whereas our morphological cladistic analysis yielded high support (BS = 92) for this clade. The 13 additional characters added in our study, specifically those relating to stem anatomy, may have resulted in higher clade support. *Misodendrum gayanum* is one of the most distinctive species within the subgenus based on its stem and wood anatomy (Fig. 2). Specifically, it lacks fibers in fascicular areas, its axial parenchyma is not lignified, and the vessel pits are rounded whereas in other species they are elliptical and scalariform (Carlquist 1985). *Misodendrum punctulatum* appears to be a more specialized parasite in that its leaves have become reduced to scales. In addition, its leaves and stems are more yellow than other species, probably indicating a loss of chlorophyll and concomitant relaxation of photosynthetic activity. A greater dependence on carbohydrates from the host can also be inferred from the fact that this species can exist completely endophytically for the first two years and sometimes up to six years after infection (Tercero-Bucardo and Kitzberger 2004). The morphological and physiological similarity of *M. punctulatum* to the dwarf mistletoes (*Arceuthobium*, Viscaceae) represents an example of convergent evolution.

Misodendrum quadriflorum is weakly supported as sister to all other species in the genus in the combined analysis, but strongly so with the *matK* data set. With the *trnL-F* data, *Misodendrum linearifolium* is weakly supported as the first diverging taxon. Any of these topologies results in a paraphyletic subgenus *Angelopogon*. Compared with subgenus *Misodendrum*, members of subgenus *Angelopogon* have larger and more succulent stems with swollen haustorial bases (an exception is *M. linearifolium*). In contrast to subgenus *Misodendrum*, species within *Angelopogon* differ greatly in their wood anatomy (Carlquist 1985). Specifically, the vascular bundles in *M. quadriflorum* are irregularly oriented, whereas section *Archiphyllum* (*M. brachystachyum* and *M. oblongifolium*) has two fascicular circles. *Misodendrum linearifolium* (section *Angelopogon*) appears to be the least specialized species having only one circle of fascicles. Based on the optimization of character states, species previously classified in subgenus *Angelopogon,* share only ancestral characteristics such as gray and smooth stems, unlignified pith, pubescent floral shoots and three stamens in the male flowers. The phylogeny inferred by the chloroplast genome, here represented by *matK* and *trnL-F*, shows a paraphyletic subg. *Angelopogon*. Because of the low support and the examination of only one genome, this paraphyly should be tested with genes from the nucleus prior to proposing a change in the classification.

Section *Archiphyllum* (*M. brachystachyum* and *M. oblongifolium*) was strongly supported as monophyletic in every analysis conducted in this study and with all examined partitions. These two taxa share the same states for all morphological characters examined in this study. They differ in two continuous characters, with *M. oblongifolium* having longer staminodes and a higher degree of leaf pubescence (Orfila 1978; Rossow 1982). *Misodendrum oblongifolium* is more geographically restricted than *M. brachystachyum* and parasitizes hosts that are generally found at higher elevations. Only two hosts are known for *M. oblongifolium* (*Nothofagus pumilio* and *N. dombeyi*), while *M. brachystachyum* parasitize these plus three more species (*N. antarctica*, *N. betuloides*, *N. nitida*). Genetic distances (HKY85 in PAUP*) between these two species were the lowest among all

species of *Misodendrum*. For the gene *matK*, the average distance among species was 0.1 whereas the value between *M. brachystachym* and *M. oblongifolium* (section *Archiphyllum*) was 0.02. A similar difference was seen for the *trnL-F* region where the mean distance among *Misodendrum* species was 0.1 as opposed to 0.03 between species in section *Archiphyllum*. More individuals of these two taxa should be sampled along their entire geographic range to further examine their distinctiveness.

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APPENDIX 1. Taxa included in phylogenetic analyses with voucher information (in parentheses: herbarium acronym and DNA accession number from collection maintained by D.

L. Nickrent at SIUC) and GenBank accession numbers for *matK*, *trnL-F*, and ITS, respectively.

***Misodendrum angulatum* Phil.** Bariloche, RN, Argentina *G. Amico 131* (BCRU; DLN4587), DQ787437, DQ788707, DQ788697. ***Misodendrum brachystachyum* DC** Bariloche, RN, Argentina *G. Amico 132* (BCRU; DLN4588), DQ787440, DQ788708, DQ788698. ***Misodendrum gayanum* Tiegh.** Villa La Angostura NQN, Argentina *G. Amico 134* (BCRU; DLN4590), DQ787439, DQ788709, DQ788699. ***Misodendrum linearifolium* DC** Chillan, Chile *G. Amico 136* (BCRU; DLN4591), DQ787438, DQ788712, DQ788700. ***Misodendrum oblongifolium* DC** Bariloche, RN, Argentina *G. Amico 137* (BCRU; DLN4592), DQ787442, DQ788710, DQ788701. ***Misodendrum punctulatum* Banks ex DC** Bariloche, RN, Argentina *G. Amico 139* (BCRU; DLN4593), DQ787443, DQ788711, DQ788702. ***Misodendrum quadriflorum* DC** Bariloche, RN, Argentina *N. Tercero & G. Amico 140* (BCRU; DLN4594), DQ787441, DQ788713, DQ788703. ***Atkinsonia ligustrina* (Lindl.) F. Muell.** Blue Mountains, NSW, Australia *Dave Watson* (DLN4343), DQ787444, DQ788714. ***Gaiadendron punctatum* G. Don.** Monteverde, Costa Rica *Sara Sargent* (DLN2729), DQ787445, DQ788715, DQ788704. ***Nuytsia floribunda* (Labill.) R. Br.** WA, Australia *Adrienne Markey* (DLN2747), DQ787446, DQ788716, DQ788705. ***Schoepfia schreberi* J.F. Gmel.** Palo verde, Costa Rica. *R. Vidal-Russell* 21 (INB; DLN4915), DQ787447, DQ788717, DQ788706. ***Schoepfia fragrans* Wall.** S. Yunnan, Mengla Xian, China *Tsi zhanhuo 91–417* (MO 4252063; DLN5009), DQ788718. ***Schoepfia vacciniflora* Planch. ex Triana & Planch.**. Chiriqui, Panama *G. McPherson and P. M. Richardson 15981* (DLN3069), DQ787448. ***Schoepfia jasminodora* Sieb. & Zucc.** previously published sequence AF534681.

APPENDIX 2. List of characters and character states used in the morphological analysis.

***General Characters*. 1. Bark color:** 0 = yellow, 1 = gray. **2. Stem texture:** 0 = warty, 1 = smooth. **3. Floral bract:** 0 = scale, 1 = leafy. **4. Leaf shape:** 0 = linear, 1 = scalelike, 2 = lanceolate. **5. Male flowers:** 0 = sessile, 1 = pedicellate. **6. Female flowers:** 0 = sessile, 1 = pedicellate. **7. Tip of staminode:** 0 = pubescent, 1 = glabrous. **8. Swollen haustorium base:** 0 = absent, 1 = present. **9. Enlargement of host branch:** 0 = absent, 1 = present. **10. Position of floral buds:** 0 = above leaf buds, 1 = below leaf buds. **11. Lignified pith:** 0 = absent, 1 = present. **12. Leaves:** 0 = sessile, 1 = petiolate. **13. Stem section:** 0 = circular, 1 = quadrangular. **14. Pubescence of floral pedicel:** 0 = absent, 1 = present. **15. Number of male flower per bract:** 0 = one, 1 = two, 2 = six. **16. Number of stamens:** 0 = two, 1 = three, 2 = four, 3 = six. **17. Inflorescence shape:** 0 = compact, 1 = lax. **18. Position of bracteoles:** 0 = at base of flowers, 1 = on the floral axis. **19. Number of female flowers per bract:** 0 = one, 1 = two, 2 = six. **20. Position of female flowers on the inflorescence:** 0 = alternate, 1 = opposite. **21. Staminode bristles:** 0 = straight, 1= incurved or claviform. **22. Stem incrustations:** 0 = absent, 1 = present. **23. Fascicular areas:** 0 = diffuse, 1 = one circle, 2 = two circles. **24. Fibers in fascicular areas:** 0 = absent, 1 = present. **25. Sclereids in outer cortex:** 0 = absent, 1 = present. **26. Rays:** 0 = absent, 1 = present. **27. Vessel pits:** 0 = laterally extended, in helical bands, 1 = elliptical, scalariformlike, 2 = circular, alternate. **28. Axial parenchyma:** 0 = abundant, nonlignified, 1 = sparse, lignified. **29. Flowers:** 0 = bisexual, 1 = unisexual. **30. Fruit:** 0 = achene, 1 = samara, 2 = drupe. **31. Parasitism**: 0 = root, 1 = aerial.

APPENDIX 3. Data matrix used for the morphological analysis for each taxon, characters are described in Appendix 2. Parenthesis indicate a polymorphic character state; ? indicates unknown character state.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
M. angulatum	(0,1)	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	2	0	0	0	1	1	0	0	1	1	1	0	1
M. brachystachyum	1	1	1	2	1	1	0	1	0	0	0	1	0	1	2	1	1	1	3	0	1	1	2	1	1	1	0	0	1	0	1
M. gayanum	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	2	0	1	0	1
M. linearifolium	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	1	1	1	1	0	0	1	0	1
M. macrolepis	0	0	1	0	?	1	?	0	0	0	1	0	0	0	0	0	1	0	2	0	?	0	?	?	?	?	?	?	1	0	1
M. oblongifolium	1	1	1	2	1	1	0	1	0	0	0	1	0	0	2	1	1	1	3	0	1	1	2	1	1	1	0	0	1	0	1
M. punctulatum	0	0	0	1	1	0	1	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	0	1
M. quadriflorum	1	1	1	2	1	0	1	1	0	1	0	1	0	1	1	1	1	1	2	1	1	1	0	0	0	1	0	0	1	0	1
Schoepfia schreberi	1	1	0	2	1	1	n/a	0	0	0	0	1	0	0	n/a	2	1	0	n/a	n/a	n/a	0	1	1	0	1	0	0	0	2	0
Nuytsia floribunda	1	1	1	2	0	0	n/a	0	0	0	0	0	0	0	n/a	3	1	1	n/a	n/a	n/a	0	3	1	0	1	?	0	0	1	0