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Abstract—The genus *Lilaeopsis* (Apiaceae subfamily Apioideae) comprises 15 species and exhibits both American amphitropic and amphiantarctic patterns of disjunction. The group is difficult taxonomically because of its simplified habit, phenotypic plasticity of vegetative characters, and extensive variation in fruit characters. Sequence data from the nrDNA ITS and cpDNA *rps16* intron and *rps16-trnK* intergenic spacer regions were obtained for 60 accessions, representing 13 species of *Lilaeopsis* and five closely related outgroup genera from the North American Endemics clade of tribe Oenantheae. These molecular data were subjected to maximum parsimony, Bayesian inference, and dispersal-vicariance analyses in an effort to reconstruct evolutionary relationships and infer biogeographic scenarios. The results suggest that: (1) *L. macloviana*, *L. masonii*, and *L. occidentalis*, distributed in western South America and western North America, collectively represent a single, polymorphic species of amphitropic distribution; (2) The Australasian species *L. brisbanica, L. novae-zelandiae, L. polyantha*, and *L. ruthiana* comprise a well-supported clade. However, *L. novae-zelandiae* is not monophyletic, but may be rendered so by the inclusion of all Australasian taxa into one polymorphic species; (3) *L. mauritiana* from Mauritius is closely related to *L. brasiliensis* from South America and may even be subsumed under the latter pending further investigation; and (4) *Lilaeopsis* probably originated in South America following a dispersal of its ancestor from North America. A minimum of seven dispersal events is necessary to explain its present-day distribution, including one dispersal from South America to Australia or New Zealand, two dispersals between Australia and New Zealand, and three dispersals from South America to North America.

Keywords—Amphitropic disjunction, Apioideae, cpDNA, ITS, phylogeny.

The genus *Lilaeopsis* Greene (Apiaceae subfamily Apioideae) consists of small, creeping, rhizomatous, perennial herbs occupying damp, marshy, or truly aquatic habitats, with fifteen species and four infraspecific taxa currently recognized (Table 1). The group is difficult taxonomically because the plants have a simplified and generally similar vegetative morphology. In all species the leaves are linear, hollow, and transversely septate, being derived from the rachis-axis of a compound leaf, with the septae corresponding to the positions of pinnae insertion in pinnatifid leaves (Kaplan 1970; Charlton 1992). These rachis leaves are different from those typical of other umbellifers (such as carrot, dill, parsnip, and celery) where they are generally pinnately compound and dissected.

The taxonomic history of *Lilaeopsis* is detailed by Affolter (1985) and underscores the problems in classifying plants having a reduced vegetative morphology. Affinities to both Araliaceae (i.e. *Hydrocotyle* L.) and Apiaceae have been proposed, with the phylogenetic position and monophyly of *Lilaeopsis* only confirmed recently (Downie et al. 2000, 2001, 2008; Petersen et al. 2002). Through extensive field investigations, cultivation of living material of most species in a common-garden coupled with the growth of many collections under different environmental treatments, and statistical analyses, Affolter (1985) showed that the shape and size of the rachis leaves of *Lilaeopsis* are readily modified in response to various degrees of submergence and light intensity. Fruit characters, so important in umbellifer classification, continued to receive emphasis, but recognition of their extensive variation in some taxa resulted in merging many previously recognized species. As examples, six previously described species and several infraspecific taxa were consolidated within the single polymorphic species *L. macloviana* from South America; the three New Zealand species recog-

nized by Hill (1928) were consolidated within the single polymorphic taxon *L. novae-zelandiae*; and three additional species from Australia were interpreted as intergrading forms of *L. polyantha*. In general, vegetative characters were deemed of little value for distinguishing among species, and fruit characters of the three polymorphic species were observed to be extremely variable, sometimes showing overlapping variation between species. To confound matters, specimens are often impossible to identify to species without mature fruits, so in some instances geography must be used to circumscribe them. As stated by Affolter (1985), "Morphological simplification has reduced the number of characters available to the taxonomist; phenotypic plasticity has diminished the utility of the few characters which remain." Further consolidation of species was also considered by Affolter (1985), but "would carry the process a step too far and would obscure real differences that exist among the South American, New Zealand, and Australian populations."

Molecular systematic studies indicate that *Lilaeopsis* is a member of Apiaceae tribe Oenantheae (Downie et al. 2000). While no unique morphological synapomorphy supports the monophyly of Oenantheae (Petersen et al. 2002), the plants do share certain traits, such as glabrous stems and leaves, clusters of fibrous or tuberous-thickened roots, globose to broadly ovate spongy-thickened fruits, and a preference for wet habitats (Downie et al. 2008). The "spongy cells" within the fruits are storage tracheids, nearly isodiametric in form and bigger in diameter than typical tracheids. They enhance buoyancy and facilitate dispersal in aquatic environments when the fruits dry out and these cells become filled with air (Briquet 1897; Hill 1927; Affolter 1985). Like *Lilaeopsis*, some plants of the tribe possess rachis or rachis-like leaves, such as *Oxypolis* Raf., *Ptilimnium* Raf., *Cynosciadium* DC., and *Limnosciadium* Mathias & Constance. The genus *Lilaeopsis* is unequivocally

Taxon	Distribution
L. attenuata (Hook. & Arn.) Fern. subsp. *attenuata*	Argentina
L. attenuata subsp. *ulei* (Pérez-Mor.) Affolter*	Brazil
L. brasiliensis (Glaz.) Affolter	Argentina, Brazil, Paraguay
L. brisbanica A. R. Bean	Australia
L. carolinensis J. M. Coult. & Rose	U. S. A. (Atlantic and Gulf coasts) South America (Argentina, Brazil, Paraguay, Uruguay) Europe (Portugal, Spain)
L. chinensis (L.) Kuntze	Canada and U. S. A. (Atlantic and Gulf coasts)
L. fistulosa A. W. Hill*	southeastern Australia
L. macloviana (Gand.) A. W. Hill	western South America (Colombia to Tierra del Fuego, Falkland Islands)
L. masonii Mathias & Constance	U. S. A. (California)
L. mauritiana G. Petersen & Affolter	Mauritius, Madagascar
L. novae-zelandiae (Gand.) A. W. Hill	New Zealand
L. occidentalis J. M. Coult. & Rose	Canada and U. S. A. (Pacific coast)
L. polyantha (Gand.) H. Eichler	southeastern Australia
L. ruthiana Affolter	New Zealand
L. schaffneriana (Schltdl.) J. M. Coult. & Rose subsp. *recurva* (A. W. Hill) Affolter	U. S. A. (Arizona), Mexico (Sonora)
L. schaffneriana subsp. *schaffneriana*	Mexico, northwestern South America
L. tenuis A. W. Hill*	Brazil

monophyletic. All species are true aquatics or occupy wet habitats. They are all tiny, perennial, rhizomatous herbs, with linear to spatulate, simple, septate rachis leaves. The inflorescences are few-flowered, the umbels are simple, and the schizocarp lacks a free carpophore. Such attributes are rare in the subfamily Apioideae and, when considered collectively, unambiguously circumscribe the genus. The results of molecular systematic studies also demonstrate strong support for its monophyly (Petersen et al. 2002; Downie et al. 2008); interspecific relationships within *Lilaeopsis*, however, have yet to be elucidated.

Lilaeopsis combines both American amphitropic (or bipolar) and amphiantarctic (or amphi-South Pacific) patterns of disjunction (Affolter 1985; Table 1). *Lilaeopsis* occurs in the temperate zones of both North and South America, with two taxa extending into the tropics at high elevations: *L. macloviana* exists along the Andes from Colombia south to Tierra del Fuego; and *L. schaffneriana* subsp. *schaffneriana* is distributed in Mexico and the Andes of northwestern South America. *Lilaeopsis* was introduced in the Iberian Peninsula, where it is now naturalized (Affolter 1985); these plants have been referred to as either *L. carolinensis* (Affolter 1985) or *L. attenuata* (Tutin 2001; Almeida and Freitas 2001). In the southern hemisphere, *Lilaeopsis* is widely disjunct, occurring predominantly in cool temperate regions of South America, Australia, and New Zealand, with isolated populations occurring in the southwestern and southern Indian Ocean. On the island of Mauritius, *L. mauritiana* is known from only a single locality and may be a local endemic (Petersen and Affolter 1999). *Lilaeopsis* has been reported from Madagascar based on a single, sterile specimen (Raynal 1977; Affolter 1985) that has been identified provisionally as "*L. aff. mauritiana* G. Petersen & Affolter?" (Sales et al. 2004). Sterile, unidentified specimens

of *Lilaeopsis* have also been collected from the Kerguelen Archipelago (Affolter 1985).

Both northern and southern hemispheric origins for *Lilaeopsis* have been suggested. Dawson (1971), for example, indicated that *Lilaeopsis* probably originated in the northern hemisphere, whereas Hill (1929) postulated an Antarctic origin, with two major subsequent migration routes northward (one route leading to New Zealand and Australia, and the other leading into and through the Andes of South America into North America). Raynal (1977) described *Lilaeopsis* as a "genuinely Gondwanian genus." The southern hemisphere has traditionally been considered to exhibit a vicariant history, with the fragmentation of Gondwana leading to the division of its ancestral biota (Sanmartín and Ronquist 2004). However, recent biogeographic studies based on molecular dating estimates have revealed that many plant transoceanic disjunctions are relatively recent, thus better explained by long-distance dispersal rather than by vicariance (Les et al. 2003; Sanmartín and Ronquist 2004; de Queiroz 2005). In explaining the disjunction of *L. carolinensis* in North and South America, Affolter (1985) speculated that its origin was probably in South America, with subsequent introduction via long-distance dispersal by migrating birds to North America. Such intercontinental dispersal by birds is regarded as a viable explanation for widely disjunct aquatic plant distributions (Raven 1963; Les et al. 2003). However, in explaining amphitropic distribution patterns in other genera of Apiaceae and taxa from other families having transoceanic disjunctions in the southern hemisphere, the predominant direction of dispersal appears to have been from the northern to southern hemisphere, even for those genera that are much diversified in the southern hemisphere (reviewed in Spalik et al. 2010). A phylogenetic hypothesis for *Lilaeopsis* is necessary to elucidate its historical biogeography, specifically its place of origin and direction(s) of long-distance dispersal.

The major objectives of this study are to provide an estimate of species-level relationships within the genus *Lilaeopsis*, especially among its New World members, and to better understand the historical biogeographic processes that have shaped its biodiversity. Since most morphological characters of *Lilaeopsis* are either reduced relative to other umbellifers or readily modified by the environment, they offer relatively little data for phylogeny estimation. Therefore, molecular data are essential. To reach these objectives, we analyze the nuclear ribosomal ITS and cpDNA *rps16* intron and *rps16-trnK* intergenic spacer regions, as previous studies have demonstrated the utility of these loci in resolving interspecific relationships in Apiaceae tribe Oenantheae (Petersen et al. 2002; Downie et al. 2008).

Materials and Methods

Leaf material for DNA extraction was obtained primarily from voucher specimens prepared by Affolter during the course of his monographic study of *Lilaeopsis* (Affolter 1985). Additional material was obtained from other herbarium specimens (AK, ARIZ, ASU, GA, ILL, ILLS, ISU, LSU, MICH, MO, TEX, and UC) and through fieldwork in southeastern Arizona. Four species of *Lilaeopsis* are sold in the commercial aquarium trade (*L. brasiliensis, L. macloviana, L. mauritiana*, and *L. novae-zelandiae*) and are commonly marketed as water-umbel, water chives, microsword, and grasswort; therefore, material of these species (five accessions) was obtained from two aquarium supply companies (Tropica Aquarium Plants, Denmark, and Florida Aquatic Nurseries, Florida). For four other accessions, DNA and leaf material were supplied to us directly (G. Petersen, University of Copenhagen, Denmark). In total, 13 species of *Lilaeopsis*

were represented by 54 accessions. Three taxa (*L. attenuata* subsp. *ulei*, *L. fistulosa,* and *L. tenuis*) were not included in the molecular study for lack of adequate material: *Lilaeopsis attenuata* subsp. *ulei* is restricted to Serra do Itatiaia, Brazil; *L. fistulosa* is known only from a few locations in Eastern New South Wales, Australia; and *L. tenuis* is known only from two collections from southeastern Brazil (Affolter 1985). Source and voucher information for all accessions of *Lilaeopsis* are presented in Appendix 1.

As outgroups, we included representatives of five closely related genera (six accessions) of Apiaceae tribe Oenantheae (*Atrema* DC., *Cynosciadium, Neogoezia* Hemsl., *Ptilimnium,* and *Trepocarpus* Nutt. ex DC.; Appendix 1). Previous phylogenetic studies have indicated that *Lilaeopsis* is a member of the North American Endemics clade of tribe Oenantheae, along with these five genera plus *Daucosma* Engelm. & A. Gray ex A. Gray, *Limnosciadium*, and *Oxypolis* (Hardway et al. 2004; Downie et al. 2008). This clade represents a group of taxa that is primarily distributed in North America, but also includes *Lilaeopsis* with a broader distribution. All phylogenetic trees were rooted with *Atrema, Neogoezia*, and *Trepocarpus*, as the aforementioned phylogenetic studies have revealed that these three genera comprise a clade that is more distantly related to *Lilaeopsis* than either *Cynosciadium* or *Ptilimnium*.

Total genomic DNA was extracted using a DNeasy plant mini kit (QIAGEN, Valencia, California), using 12–22 mg of dried leaf material following experimental strategies described elsewhere (Downie and Katz-Downie 1996, 1999; Lee and Downie 2006; Spalik and Downie 2006). Amplification of the entire ITS region (ITS1, 5.8S rDNA, ITS2) was attained using primers 18S-for (Feist and Downie 2008) and C26A (Wen and Zimmer 1996). For some accessions the two spacer regions were each amplified separately using the following primer pairs: 18S-ITS1-F and 5.8S-ITS1-R for ITS1, and ITS-3N and C26A for ITS2 (Spalik and Downie 2006). The ITS sequences were obtained for 57 accessions of *Lilaeopsis* and outgroups. Amplifications of the *rps16* intron and *rps16-trnK* intergenic spacer regions were obtained for most accessions using primer pair 5'exon(rps16) and 3'exon(rps16) for the intron and primer pairs rps16C and trnK or rps16–2 and trnK for the intergenic spacer region (Downie et al. 2008; Fig. 1). For those accessions where the spacer region could not be amplified with these primers, three internal primers were used (3'exon-1, trnK-1R, and trnK-1; Downie et al. 2008). In addition, 14 primers (L1-L14) were designed specifically for this study in order to amplify or sequence both noncoding regions (primer sequences are provided in Fig. 1). For some accessions, primer pairs L3 and L2 or L3 and 3'exon(rps16) were used to obtain intron data; however, because primer L3 overlaps the 5'exon/intron boundary, the first 30 bp of sequence at the 5' end of the intron could not be obtained. The cpDNA *rps16* intron and *rps16-trnK* sequence data were obtained for 40 accessions of *Lilaeopsis* and outgroups, with 37 accessions common to both ITS and cpDNA studies. In general, DNA extractions from herbarium specimens, particularly those more than 20 yrs old, resulted in poor or no PCR products. Many of these DNAs were amplified repeatedly, under different stringency conditions and using varying quantities of DNA, but failed to work. Of 135 DNA extractions attempted, only about half of these yielded PCR products of sufficient concentration to be sequenced and included in the study. Technical difficulties in working with *Lilaeopsis* DNAs have also been reported by Fiedler et al. (in press). All ITS and cpDNA sequences obtained have been deposited in GenBank (Appendix 1).

Nucleotide sequences of the ITS and cpDNA regions were each aligned initially using the default pairwise and multiple alignment parameters in Clustal X (gap opening cost = 15.00; gap extension cost = 6.66; DNA transition weight = 0.50; Jeanmougin et al. 1998), then rechecked and adjusted manually as necessary. Gaps were positioned to minimize nucleotide mismatches. Ambiguously aligned regions were excluded from the analysis. These aligned ITS and cpDNA data matrices are available in TreeBASE (study number S11209), as well as in Bone (2007). Characteristics of the aligned ITS and cpDNA sequences, separately and combined, were obtained with uncorrected pairwise nucleotide distances calculated in PAUP* version 4.0b10 (Swofford 2002). The percentage of data matrix cells scored as missing data in the ITS and cpDNA matrices was 0.8% and 1.5%, respectively. These regions represented a portion of the 5.8S gene (for those accessions where the two spacer regions had to be amplified separately) and several small areas within the cpDNA intron and intergenic spacer that could not be sequenced with the primers at hand.

The three data matrices (ITS, cpDNA, and combined ITS/cpDNA) were each analyzed using maximum parsimony (MP) and Bayesian inference (BI) methods. In the MP analysis implemented using PAUP*, characters were treated as unordered and all character transformations were equally weighted. Heuristic MP searches were replicated 1,000 times with random stepwise addition of taxa, tree-bisection-reconnection (TBR) branch swapping, and saving multiple trees. Gap states were treated as
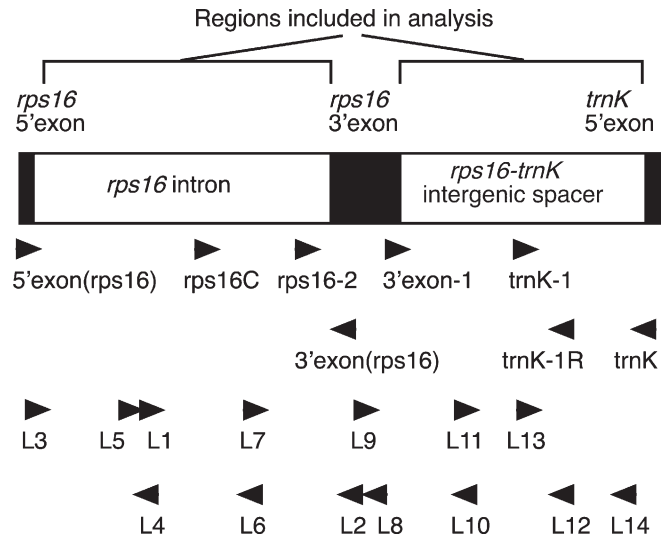

FIG. 1.   Map of the 1.8 kb locus of *Lilaeopsis* cpDNA showing the positions of genes *rps16* and *trnK*(UUU) 5'exon. The gene *rps16* is interrupted by an intron; an intergenic spacer separates gene *rps16* from gene 5'*trnK*. The two cpDNA regions sequenced in the phylogenetic analyses are indicated by brackets. The arrows represent the directions and approximate positions of the primers used in PCR amplifications and/or DNA sequencing. Fourteen primers, labeled L1 through L14, were developed specifically for this study; their sequences, written 5' to 3', are presented below. The eight remaining primers have been used in previous studies of Apiaceae tribe Oenantheae and their details are presented in Downie et al. (2008). **L1**: TATC(G/A)C(G/A)CGGGAATC(G/T)A(C/G)CGTT(C/T)A; **L2**: CTTTCTCTTCGGGATCGAACATCA; **L3**: AAAGCAACGTGCGACTTGAAGGAC; **L4**: TAAACGCTCGATTCCCG(C/T)G(C/T)GATA; **L5**: TCAAAGTGTATCGCACGGGAATCG; **L6**: TCATTTGTACCCATAACTCAAGTTGG; **L7**: TCCAACTTGAGTTATGGGTACAAATG; **L8**: AGCGGGAACGTTTAAATAACTTTGA; **L9**: AAGGAAGAGATCTTCGGAACGTGG; **L10**: AACCGACCAAATGAAGGAACTC; **L11**: TGGGAGTTCCTTCAATTGGTCG; **L12**: TTTGTTCGATACACTGTTGTCA; **L13**: TGTAGTGCCAATCCAACACAAGCC; **L14**: AGAAATGTCAAATTTATAGACCACCTCTTAG.

missing data. Unambiguous alignment gaps were scored as presence/absence characters using the simple indel coding method of Simmons and Ochoterena (2000). Gaps of equal length in more than one sequence were coded as the same presence or absence character state if they could not be interpreted as different duplication or insertion events. Indels of similar location, but with different lengths, were coded as different binary characters. Bootstrap values were calculated from 100 replicate analyses using TBR branch swapping and simple stepwise addition of taxa. Bremer (1994) support values were calculated using TreeRot version 3 (Sorenson and Franzosa 2007). Prior to combining the ITS and cpDNA data, the incongruence length difference (ILD) test of Farris et al. (1995) was carried out using the partition-homogeneity test of PAUP* to examine the extent of conflict between datasets. This test was executed with 1,000 replicate analyses, using the heuristic search option, simple stepwise addition of taxa, and TBR branch swapping.

The BI analysis was implemented using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Prior to analysis, MrModeltest version 2.2 (Nylander 2004) was used to select an evolutionary model of nucleotide substitution that best fits these data, as selected by the AIC estimator. The settings appropriate for this best-fit model (SYM + I for ITS and GTR + G for cpDNA) were put into a MrBayes block in PAUP* (nst = 6, rates = propinv and nst = 6, rates = gamma, respectively). In the BI analysis of combined data (with scored gaps), both models of nucleotide substitution were used for their respective molecular partitions, while a standard model for unordered characters was used for gap data. The priors on state frequencies, and rates and variation across sites were estimated automatically from the data assuming no prior knowledge about their values. Two independent analyses were each run simultaneously for one million generations, with tree sampling occurring every 100 generations. Starting trees were chosen at random. One thousand trees were discarded (as "burn-in") before stationarity was reached, prior to determining the posterior probability (PP) values from the remaining trees.

The biogeographic history of *Lilaeopsis* was reconstructed using simplified, fully resolved trees of species relationships obtained from analyses of combined ITS/cpDNA data. The differences between these trees were in the ancestral distributions assigned to species *L. carolinensis* and *L. schaffneriana*. Each terminal represented a major lineage supported by these phylogenetic results; however, three species (*L. mauritiana*, *L. macloviana*, and *L. masonii*) were not included as terminals in the biogeographic analysis: *L. mauritiana* arises from within a paraphyletic *L. brasiliensis*; and *L. macloviana* and *L. masonii* are subsumed within *L. occidentalis*. Outgroups were the same as included in the phylogenetic study. Four unit areas were defined: (A) North America (Canada, U. S. A., and Mexico); (B) South America; (C) Australia; and (D) New Zealand. The occurrence of *Lilaeopsis* in Europe (Spain and Portugal) is recent (Affolter 1985; Almeida and Freitas 2001), thus this region was not included as an ancestral area. Similarly, only few reports of dubiously identified material of *Lilaeopsis* from the Dominican Republic, Kerguelen Islands, and Madagascar precluded their inclusion in the biogeographic study. The dispersal-vicariance analysis was carried out using DIVA version 1.1, which reconstructs the distribution of ancestral areas that minimize dispersal events under a parsimony criterion (Ronquist 1996, 1997). Two optimizations were performed: first, with an unconstrained number of unit areas for each ancestral node and second, with this number restricted to two areas (Ronquist 1996, 1997; Downie et al. 2008). The rationale for the second optimization is that in an unconstrained analysis, the ancestral distribution at or near the base of the tree may be inferred to be widespread and include most or all individual unit areas inhabited by the terminals because of uncertainty (Ronquist 1996). Because we were interested in inferring the ancestral distributions if the group had a more restricted (and likely realistic) distribution, we repeated the analysis by limiting the number of ancestral areas assigned to each node to two.

Results

ITS Analysis—Sequence characteristics of the ITS region are provided in Table 2. The length of the entire ITS region across 57 accessions ranged from 594–608 bp, and alignment of these sequences resulted in a matrix of 634 positions, including 41 unambiguous gaps. Twenty-eight of these gaps were parsimony informative, with all but one of them one to three bp in size. The largest gap, of eight bp, characterized all accessions of *L. attenuata, L. carolinensis, L. chinensis*, and *L. schaffneriana*. The number of parsimony informative positions was 179, with the ITS-2 region containing more informative positions (95) than either ITS-1 (76) or 5.8S (eight). Uncorrected pairwise ITS sequence divergence values ranged from identity (for several conspecific taxa) to 23.7% (between *L. brasiliensis* and *P. nuttallii*). Within *Lilaeopsis*, ITS sequence divergence values ranged from identity to 9.6% (the latter between *L. occidentalis* and *L. chinensis*). Pairwise sequence comparisons between *L. occidentalis* and *L. masonii* accessions ranged from identity to 0.17%, those between *L. occidentalis* and *L. macloviana* accessions ranged from 0.33% to 1.0%, and

those between *L. brasiliensis* and *L. mauritiana* accessions also ranged from 0.33% to 1.0%. The highest levels of infraspecific sequence divergence (2.7%) occurred between accessions of *L. novae-zelandiae*. Ranges for other infraspecific divergence values were 0–1.2% for the four accessions of *L. macloviana*, 0–0.34% for the seven accessions of *L. occidentalis*, 0–1.2% for the five accessions of *L. brasiliensis*, 0–0.33% for the three accessions of *L. mauritiana*, and 0–0.17% for the ten accessions of *L. schaffneriana*. The two accessions of *L. schaffneriana* subsp. *schaffneriana* from Mexico had identical ITS sequences to two accessions of *L. schaffneriana* subsp. *recurva* from Arizona.

The MP analysis of the ITS data matrix recovered 15 minimal length 452-step trees (CI = 0.757 and 0.690, with and without uninformative characters, respectively; RI = 0.918). The strict consensus of these trees is presented in Fig. 2, with accompanying bootstrap and Bremer support values. The MP analysis of the ITS matrix plus 28 binary-scored indel characters recovered 15 trees, each of 488 steps (CI = 0.758 and 0.698, with and without uninformative characters, respectively; RI = 0.920). The strict consensus of these trees (not shown) differed from that presented in Fig. 2 by showing a monophyletic *L. brasiliensis* arising from within a paraphyletic *L. mauritiana*, *L. macloviana* accessions 2925 and 2518 comprising a clade, and *P. nuttallii* 2405 and *C. digitatum* 1804 also comprising a clade. Bootstrap support values for identical clades were similar in both analyses. To reveal the distribution of indels throughout the phylogeny and to show relative branch lengths, the pattern of indel distribution was mapped onto a single, arbitrarily selected tree derived from MP analysis of ITS sequences and scored gaps (Fig. 3). Patterns of indel distribution support the monophyly of *Lilaeopsis* and several species or species groups. A single unique indel supports the monophyly of the Australasian species *L. brisbanica, L. novae-zelandiae, L. polyantha*, and *L. ruthiana*, and four additional synapomorphic indels support the subsequent major dichotomy in this species group. Two synapomorphic indels support the branch leading to *L. carolinensis, L. attenuata, L. schaffneriana*, and *L. chinensis*. *Lilaeopsis chinensis* is the only species supported by a uniquely occurring indel. Four indels were homoplastic, each occurring 2–4 times on the tree. With the exceptions of a slightly greater resolution among the ten included accessions of *L. schaffneriana* and the union of outgroups *P. nuttallii* and *C. digitatum* into a weakly-supported clade, the majority-rule consensus tree derived from the BI analysis was topologically identical to that tree inferred through MP without scored gaps. The PP values are presented in Fig. 2 for those nodes occurring in both MP and BI trees.

As a result of phylogenetic analyses of ITS data, seven major clades are circumscribed within *Lilaeopsis* that correspond to species or species groups. These include: (1) *L. carolinensis*, (2) *L. attenuata*, (3) *L. schaffneriana*, (4) *L. chinensis*, (5) *L. brasiliensis* and *L. mauritiana* species group, (6) *L. brisbanica, L. novae-zelandiae, L. polyantha*, and *L. ruthiana* species group, and (7) *L. macloviana, L. masonii*, and *L. occidentalis* species group. The first four of these major clades comprised a well-supported monophyletic group in all analyses (93% BS, 1.00 PP), with this group characterized by eight bp and one bp alignment deletions. The clade of *L. macloviana, L. masonii,* and *L. occidentalis* was a sister group to the clade comprising all remaining *Lilaeopsis*. With the exception of the *L. carolinensis* clade, which was supported weakly in both MP and BI analyses (64% BS, 0.69 PP), support for the remaining clades comprising three or more accessions was moderate to high (BS values ≥ 75% and

TABLE 2.   Sequence characteristics of the ITS and cpDNA (*rps16* intron and *rps16-trnK* intergenic spacer) regions, separately and combined.

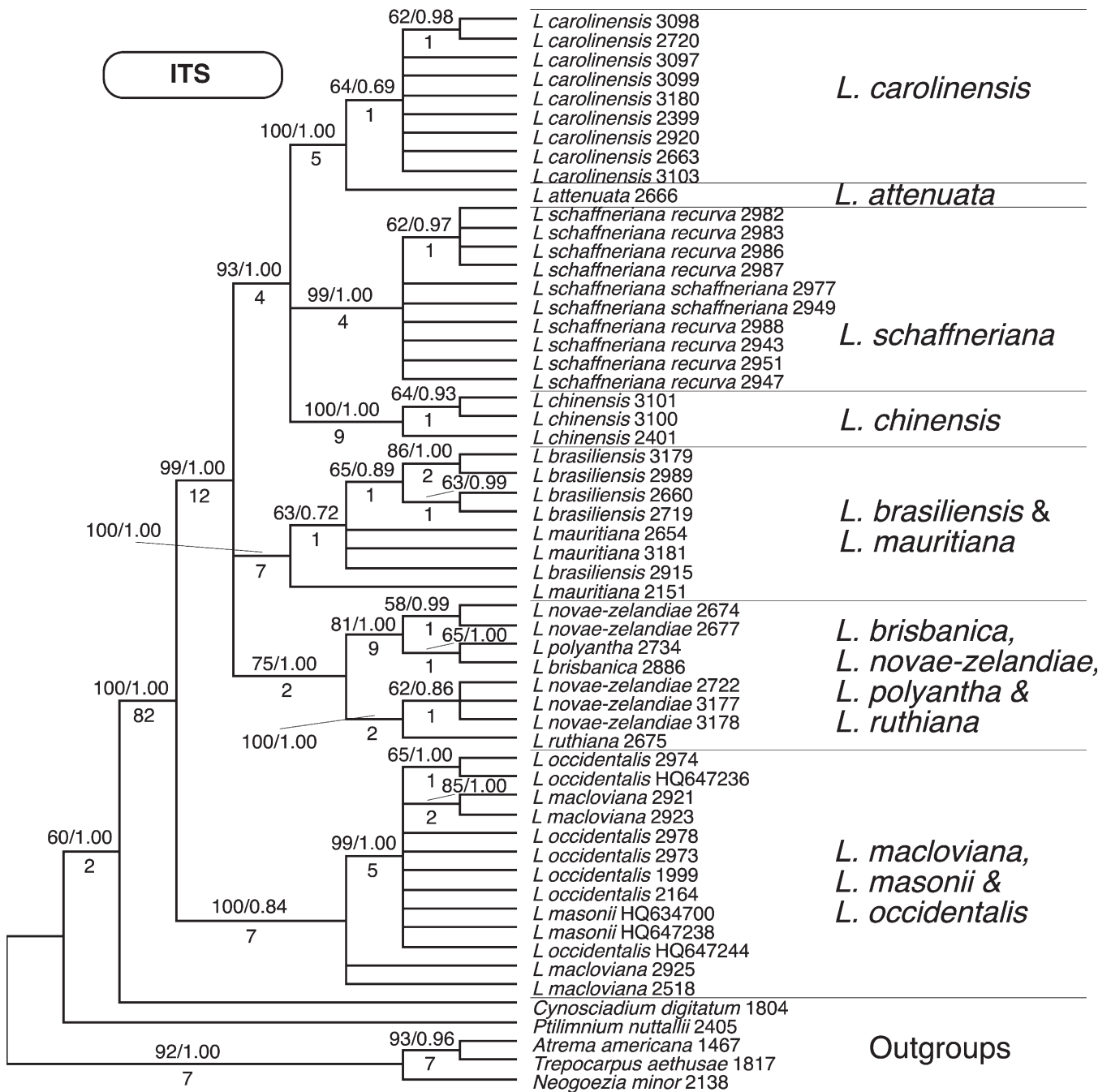| Sequence characteristic	ITS	cpDNA	Combined
No. of terminals	57	40	37
Length variation (range in bp)	594–608	1,506–1,566	2,113–2,172
No. of aligned positions	634	1,630	2,264
No. of excluded positions	0	40	40
No. of constant positions	372	1,436	1,851
No. of autapomorphic positions	83	66	129
No. of parsimony informative positions	179	88	244
No. of unambiguous gaps	41	47	56
No. of parsimony informative gaps	28	28	45
Max. pairwise sequence divergence (%) (*Lilaeopsis* only/all accessions)	9.6/23.7	1.7/4.6	3.8/9.4

FIG. 2. Strict consensus of 15 minimal length 452-step trees derived from maximum parsimony (MP) analysis of 57 nrDNA ITS sequences obtained from 13 species of *Lilaeopsis* and 5 outgroups (CI = 0.757 and 0.690, with and without uninformative characters, respectively; RI = 0.918). The majority-rule consensus tree derived from Bayesian inference analysis of these data was topologically identical to this MP strict consensus tree. Numbers above nodes represent MP bootstrap and BI posterior probability values, respectively. Numbers below nodes represent Bremer support values.

PP values ≥ 0.84). However, not all species were monophyletic in all analyses. *Lilaeopsis brasiliensis* was monophyletic when gaps were included as additional characters in the MP analysis (Fig. 3), but not when gaps were excluded or in the BI analysis. In all trees, *L. mauritiana* was paraphyletic relative to *L. brasiliensis*. *Lilaeopsis novae-zelandiae* comprised two major lineages, with the single included accession of *L. ruthiana* a sister group to one of these lineages, and the clade of *L. brisbanica* and *L. polyantha* a sister group to the other. Within the *L. macloviana*, *L. masonii* and *L. occidentalis* clade, neither *L. occidentalis* nor *L. macloviana* comprised a monophyletic group.

Furthermore, two accessions of *L. macloviana* from South America (2518 and 2925) were basal within this clade relative to North American *L. occidentalis* and *L. masonii* and the two other accessions of *L. macloviana* from South America.

cpDNA Analysis—Sequence characteristics of the cpDNA noncoding regions are provided in Table 2. The concatenated *rps16* intron and *rps16-trnK* intergenic spacer regions for 40 included accessions varied in length from 1,506 bp (*L. brasiliensis*) to 1,566 bp (*L. occidentalis*). Alignment of these sequences resulted in a matrix of 1,630 positions, with 47 unambiguous gaps. Twenty-eight of these gaps were parsimony informative,
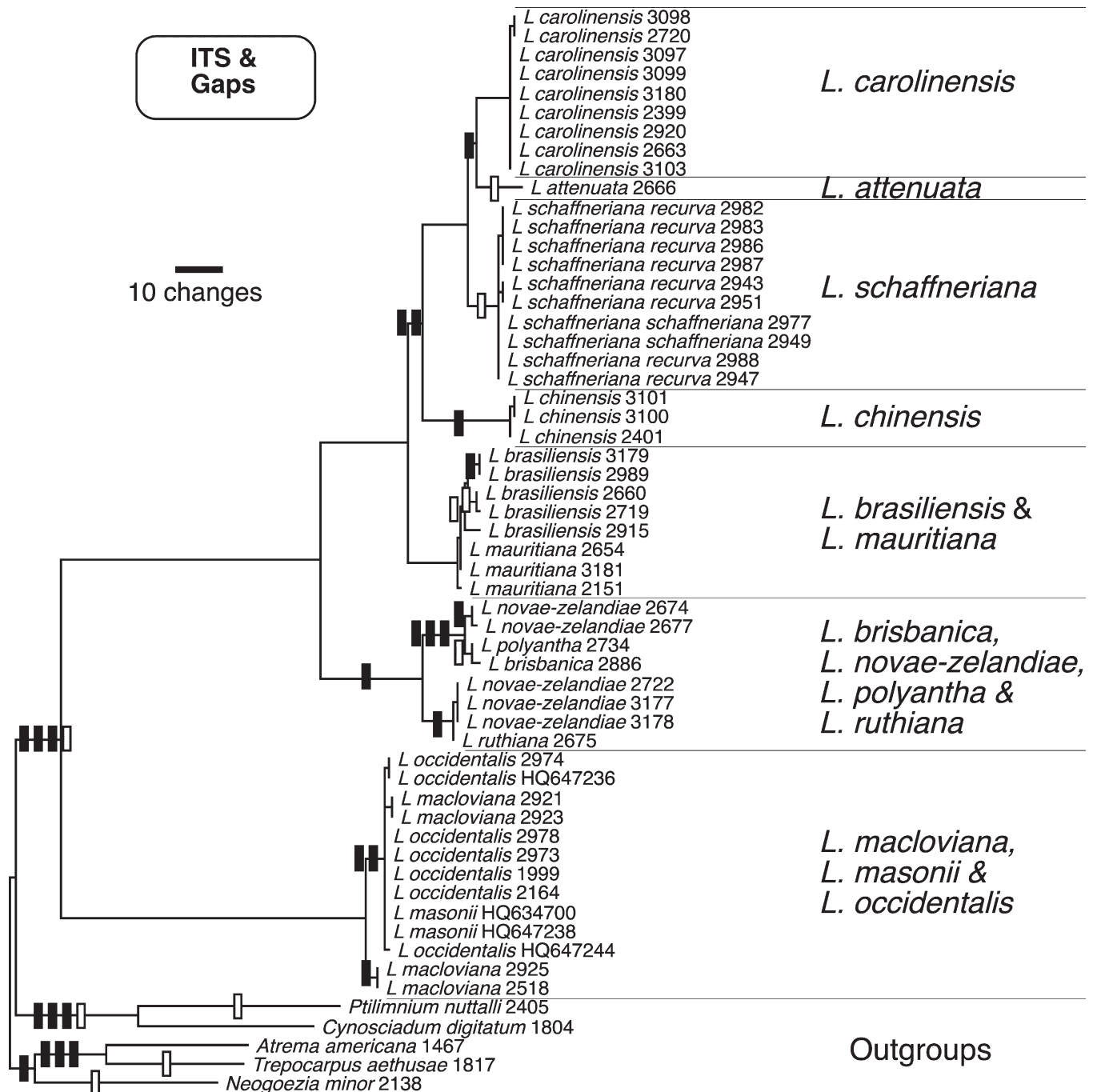
FIG. 3. One of 15 minimal length 488-step trees derived from maximum parsimony analysis of 57 nrDNA ITS sequences and 28 binary-scored informative gaps obtained from 13 species of *Lilaeopsis* and five outgroups (CI = 0.758 and 0.698, with and without uninformative characters, respectively; RI = 0.920). The pattern of indel distribution is indicated (solid bar, synapomorphic indel; open bar, homoplastic indel). Scale bar indicates numbers of inferred changes along each branch.

with 16 of them a single bp in size. The remaining informative gaps ranged between two and 26 bp, with the largest gap occurring only in *Trepocarpus* and *Atrema*. The next largest informative gaps, of 18, 14, and 11 bp, supported other outgroup lineages. Upon the exclusion of 40 ambiguously aligned positions, the number of variable positions was 154, with the *rps16-trnK* intergenic spacer region having a greater number of variable positions (85) than that of the intron (69). Uncorrected pairwise sequence divergence values across all ingroup and outgroup accessions ranged from identity for several conspecific taxa to 4.6% (between *L. carolinensis* and *P. nuttallii*); among *Lilaeopsis* cpDNAs, maximum pairwise interspecific sequence divergence values approached 1.7% (between *L. occidentalis* and *L. carolinensis*). These values were much lower than those of the ITS region; indeed, for the same pairwise comparisons, sequence divergence values for the ITS region were four to five times higher than those values inferred for cpDNA. Similarly, relative to the length of their respective unambiguous alignments, the proportion of parsimony informative nucleotide positions was approximately

five times greater for ITS (28%) than for cpDNA (6%). The number of parsimony informative gaps, however, was identical for both ITS and cpDNA data matrices (28). Pairwise cpDNA sequence divergence estimates for *L. schaffneriana* ranged from identity to 0.14%, with the single accession of *L. schaffneriana* subsp. *schaffneriana* from Mexico having an identical cpDNA sequence to those of four accessions of *L. schaffneriana* subsp. *recurva* from Arizona. Pairwise comparisons between *L. brasiliensis* and *L. mauritiana* accessions ranged from identity to 0.54%, and those between *L. occidentalis* and *L. macloviana* accessions ranged between 0.68% and 0.86%.

The MP analysis of the cpDNA data matrix recovered 26 minimal length 185-step trees (CI = 0.887 and 0.824, with and without uninformative characters, respectively; RI = 0.934). The strict consensus of these trees is presented in Fig. 4, with accompanying bootstrap and Bremer support values. The MP analysis of the cpDNA data matrix plus 28 binary-scored indel characters recovered 36 trees, each of 224 steps (CI = 0.857; CI excluding uninformative characters = 0.798; RI = 0.928). The strict consensus of these trees (not shown) was

consistent with that presented in Fig. 4. Differences between them included the clade of *L. brasiliensis* 2989 and *L. brasiliensis* 3179, the placement of *L. brasiliensis* 2719 in a clade with *L. brasiliensis* 2660 and all four accessions of *L. carolinensis*, and the placement of *L. macloviana* 2925 as one branch of a trichotomy along with the clade of all three accessions of *L. occidentalis* and the clade of all remaining accessions of *Lilaeopsis*. Patterns of indel distribution (not shown) are similar to those exhibited by ITS data. Nine indels were homoplastic, each occurring two to four times on a single, arbitrarily selected cpDNA derived tree. The majority of indels supported the monophyly of *Lilaeopsis* and the outgroup lineages. *Lilaeopsis chinensis* and *L. occidentalis* were the only species represented by two or more accessions that were supported by uniquely occurring indels. *Lilaeopsis carolinensis* was supported by a single homoplastic indel. The BI majority-rule consensus tree was almost fully congruent to that inferred using MP (omitting scored gaps), with the only exception of *L. macloviana* 2925 being a sister group to a clade comprising all other accessions of *Lilaeopsis* (including *L. occidentalis*).
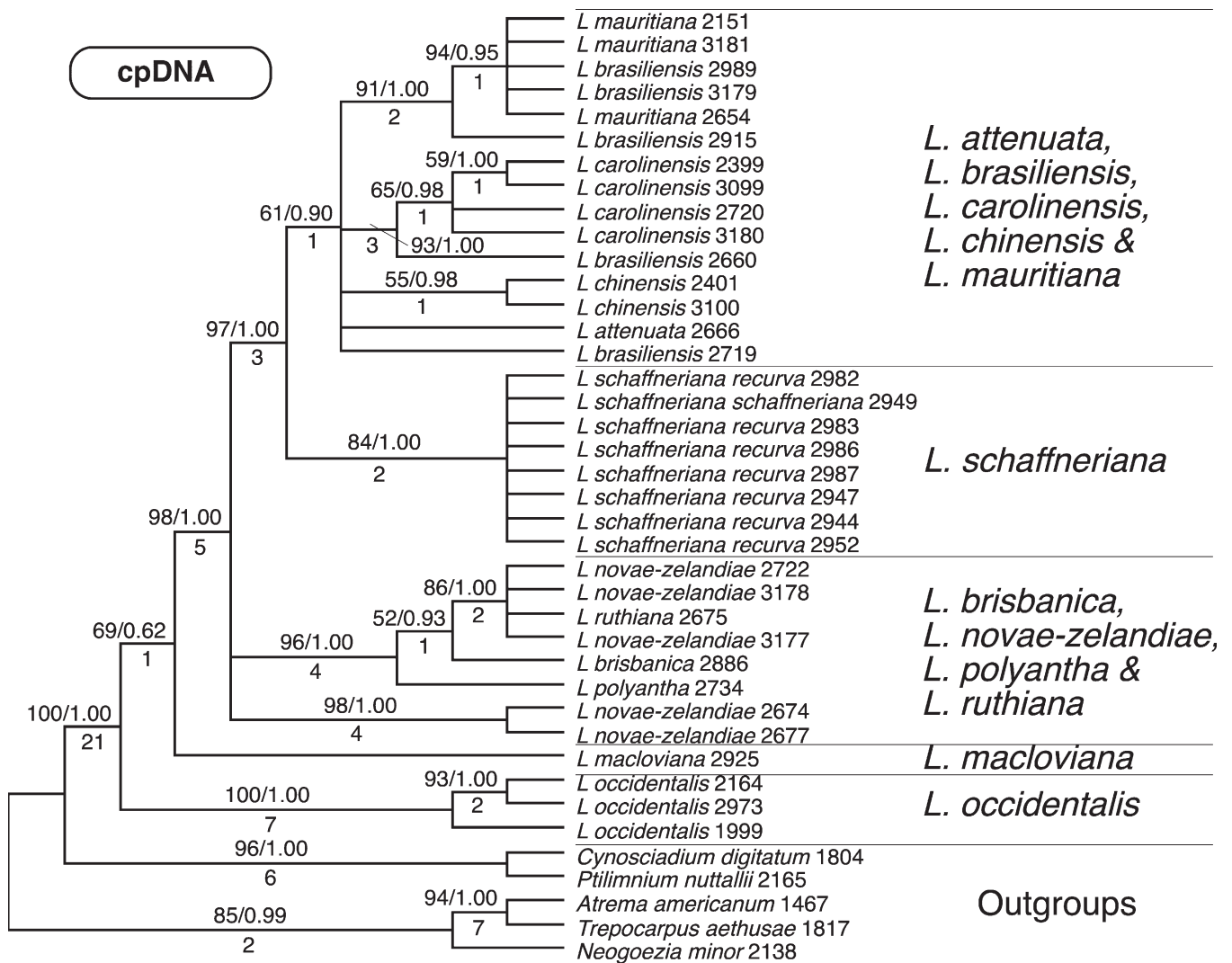

Fig. 4.   Strict consensus of 26 minimal length 185-step trees derived from maximum parsimony (MP) analysis of 40 cpDNA *rps16* intron and *rps16-trnK* intergenic spacer sequences representing 12 species of *Lilaeopsis* and five outgroups (CI = 0.887 and 0.824, with and without uninformative characters, respectively; RI = 0.934). The majority-rule consensus tree derived from BI analysis of these data was nearly topologically congruent to this MP strict consensus tree. Numbers above nodes represent MP bootstrap and BI posterior probability values, respectively. Numbers below nodes represent Bremer support values.

The trees obtained through MP and BI analyses of cpDNA sequences were less resolved and their branches generally more poorly supported than those inferred using ITS data. The previously delimited *L. carolinensis, L. attenuata*, and *L. chinensis* clades along with the *L. brasiliensis* and *L. mauritiana* species group comprised a large polytomy that was supported weakly (61% BS, 0.90 PP, and a Bremer support value of 1) in the cpDNA-derived trees. This large clade was a well-supported sister group to *L. schaffneriana*. The previously delimited *L. brisbanica, L. novae-zelandiae, L. polyantha*, and *L. ruthiana* species group comprised two lineages basal to the clade of all aforementioned taxa, with the single accession of *L. macloviana* and a clade of three accessions of *L. occidentalis* comprising successively sister branching lineages with ITS. The separation of *L. occidentalis* from *L. macloviana*, however, is poorly supported. Major differences between the ITS and cpDNA trees include the relative position of *L. macloviana*, the placement of *L. brisbanica* and *L. polyantha* relative to the two lineages of *L. novae-zelandiae*, and collapse of the *L. brasiliensis* and *L. mauritiana* group.

Combined ITS and cpDNA Analysis—Results of a partition-homogeneity test on a set of 37 accessions common to both ITS and cpDNA analyses revealed that these data matrices yield significantly incongruent phylogenetic estimates ($p = 0.01$). We acknowledge, however, that serious doubts have been raised regarding the value of this test as a criterion for deciding whether data should be combined into a single phylogenetic analysis (Yoder et al. 2001; Barker and Lutzoni 2002) and, in the absence of information suggesting that past hybridization events or other factors may have caused this discordance, combine all molecular data into a single data matrix for simultaneous consideration. Such an analysis also provided the greatest number of relationships receiving strong support. *Ptilimnium nuttallii* was not included in the combined analysis because different accessions were used in the partitioned analyses, although the species is unequivocally monophyletic (Feist and Downie 2008). *Lilaeopsis masonii* was also not included in the combined analysis because cpDNA data were unavailable. Alignment of ITS and cpDNA sequences for 33 accessions of *Lilaeopsis* and four outgroup taxa resulted in a matrix of 2,264 positions, with 40 positions excluded from subsequent analyses because of alignment ambiguity (Table 2). Of the remaining positions, 373 were variable and 244 of these were parsimony informative. Forty-five of 56 unambiguous alignment gaps were also parsimony informative. Uncorrected pairwise sequence divergence values across all accessions ranged from identity to 9.4%, the latter between *L. brasiliensis* and the outgroup *Trepocarpus aethusae*. Within *Lilaeopsis*, interspecific sequence variation ranged from 0.09% (between *L. ruthiana* and *L. novae-zelandiae*) to 3.8% (between *L. occidentalis* and *L. chinensis*). The next lowest values in interspecific comparisons were between *L. brasiliensis* and *L. mauritiana* (0.15–0.48%), between *L. brisbanica* and *L. polyantha* (0.48%), between *L. macloviana* and *L. occidentalis* (0.74–0.86%), and between *L. polyantha* and *L. novae-zelandiae* (0.78–1.2%). *Lilaeopsis carolinensis* and *L. attenuata* sequences differed by 1.2% of nucleotides. Maximum pairwise sequence divergence values for all interspecific comparisons averaged 2.2%. Infraspecific variation ranged from identity (for some conspecific accessions of *L. carolinensis, L. schaffneriana, L. novae-zelandiae*, and *L. occidentalis*) to 1.3% of nucleotides (*L. novae-zelandiae*). For each species where two or more accessions were available, pairwise infraspecific sequence divergence values were as follows: *L. brasiliensis* (0.05–0.55%); *L. carolinensis* (0–0.10%); *L. chinensis* (0.10%); *L. mauritiana* (0.05–0.19%); *L. novae-zelandiae* (0–1.3%); *L. occidentalis* (0–0.15%); and *L. schaffneriana* (0–0.10%). DNA data from the single included accession of *L. schaffneriana* subsp. *schaffneriana* were identical to that from one accession of *L. schaffneriana* subsp. *recurva*.

The MP analysis of the combined ITS and cpDNA matrix (excluding scored gaps) resulted in 12 minimal-length trees of 541 steps each (CI = 0.810 and 0.747, with and without uninformative characters, respectively; RI = 0.900), and the strict consensus of these trees, with accompanying branch support values, is presented in Fig. 5. Repeating the MP analysis with the 45 informative gaps scored as additional characters resulted in trees whose strict consensus was almost topologically identical to that inferred without scored gaps (CI = 0.794 and 0.736, with and without uninformative characters; RI = 0.898). The only substantive difference between these strict consensus trees was a monophyletic *L. mauritiana* arising from within a paraphyletic *L. brasiliensis* when gaps were included as additional characters (Fig. 5, inset); however, support for the monophyly of *L. mauritiana* was weak. The majority-rule consensus tree derived from the BI analysis (excluding scored gaps) was almost identical to the MP strict consensus tree inferred without scored gap characters (PP values are presented in Fig. 5), with the only difference being the union of *L. brisbanica* and *L. polyantha* as a weakly supported clade in the former. The BI majority-rule consensus tree obtained from all data (including scored gaps) is presented in Fig. 6A, while Fig. 6B presents a single tree derived from these same data. The only major difference between the results of the MP and BI analyses of all data is the relative position of *L. chinensis*.

Phylogenetic analyses of the combined data set resulted in better resolved trees than either of the partitioned analyses. The combined data set also provided the greatest number of relationships receiving strong support. On the basis of these combined data, seven major clades are confirmed within *Lilaeopsis*: (1) *L. carolinensis*, (2) *L. attenuata*, (3) *L. schaffneriana*, (4) *L. chinensis*, (5) *L. brasiliensis* and *L. mauritiana* species group, (6) *L. brisbanica, L. novae-zelandiae, L. polyantha*, and *L. ruthiana* species group, and (7) *L. occidentalis* and *L. macloviana* species group (*L. masonii* was not included in the combined analysis). Support for each of the clades comprising two or more taxa was high (BS ≥ 95%, PP ≥ 0.95, Bremer support 5–9 steps). *Lilaeopsis macloviana* and *L. occidentalis*, distributed in western South America and western North America, respectively, collectively comprise a well-supported clade sister group to all other members of the genus. *Lilaeopsis macloviana* and *L. occidentalis* also represent sister taxa, although only one accession of *L. macloviana* was included in the combined analysis. Combined sequence divergence estimates between these two species, however, were not high (0.74–0.86%), and within the range of those values obtained for infraspecific comparisons in other taxa. The Australasian species *L. brisbanica, L. novae-zelandiae, L. polyantha*, and *L. ruthiana* also comprise a well-supported clade. *Lilaeopsis novae-zelandiae* is not monophyletic, however, with three accessions of this species allying with *L. brisbanica, L. polyantha*, and *L. ruthiana* in one lineage, and the remaining two accessions of the species comprising the other lineage. *Lilaeopsis ruthiana* and the clade of three accessions of *L. novae-zelandiae* ally most strongly; the placements of *L. brisbanica* and *L. polyantha* with respect
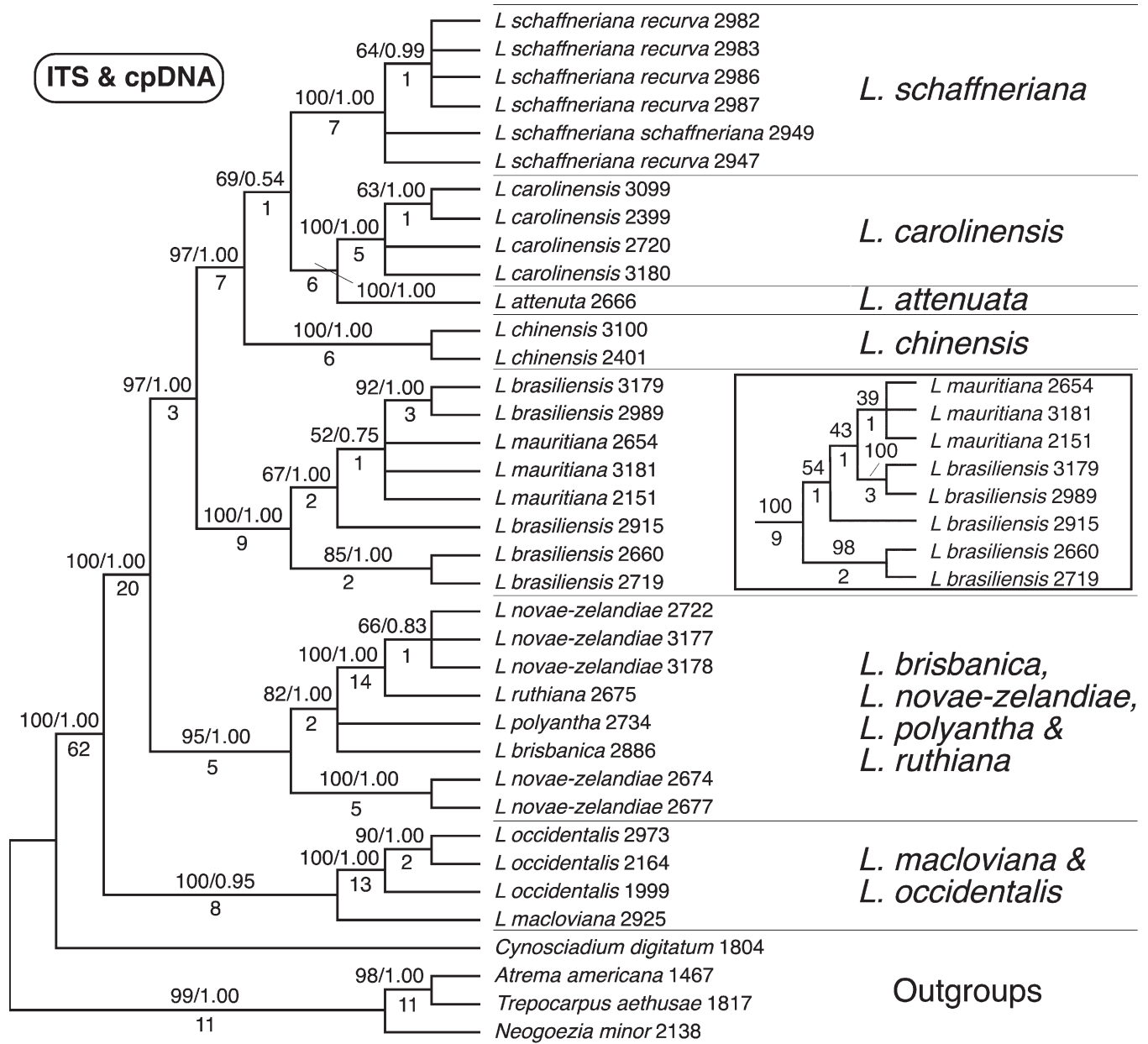
FIG. 5.   Strict consensus of 12 minimal length 541-step trees derived from maximum parsimony (MP) analysis of combined nrDNA ITS and cpDNA sequences from 37 accessions representing 12 species of *Lilaeopsis* and four outgroups (CI = 0.810 and 0.747, with and without uninformative characters, respectively; RI = 0.900). The majority-rule consensus tree derived from Bayesian inference analysis of these data was topologically congruent to this MP strict consensus tree. Repeating the MP analysis with 45 informative gaps scored as additional characters results in a strict consensus tree of similar topology with the exception of a monophyletic *L. mauritiana* arising from within a paraphyletic *L. brasiliensis* (inset). Numbers above nodes represent MP bootstrap and BI posterior probability values, respectively. Numbers below nodes represent Bremer support values.

to the two lineages of *L. novae-zelandiae* vary between the ITS and cpDNA analyses, however. *Lilaeopsis schaffneriana, L. carolinensis, L. attenuata*, and *L. chinensis* collectively comprise a well-supported clade, with *L. schaffneriana* subsp. *schaffneriana* from Mexico and *L. schaffneriana* subsp. *recurva* from Arizona showing no to little sequence divergence.

DIVA Analysis—Two sets of DIVA analyses were performed: the first, with terminals *L. carolinensis* and *L. schaffneriana* coded with both North and South America (AB) to reflect their current distributions; and the second, restricting the ancestral distribution of each of these species to only one of these areas (A or B), for it is possible that the other area was colonized by a recent independent dispersal. *Lilaeopsis*

carolinensis was proposed by Affolter (1985) as having a South American origin because its North American populations occur almost exclusively along the Gulf and Atlantic coasts, whereas those populations from South America are also found a great distance inland. The absence of inland localities in North America might reflect a relatively recent arrival, either by along-shore currents or shore-birds (Affolter 1985). Both subspecies of *L. schaffneriana* occur in Arizona and Mexico, whereas few collections of *L. schaffneriana* subsp. *schaffneriana* have been reported from northwestern South America. Based on this limited information, the center of origin of *L. schaffneriana* might possibly be North America. In all DIVA analyses, the ancestral distribution of *L. occidentalis* was coded as
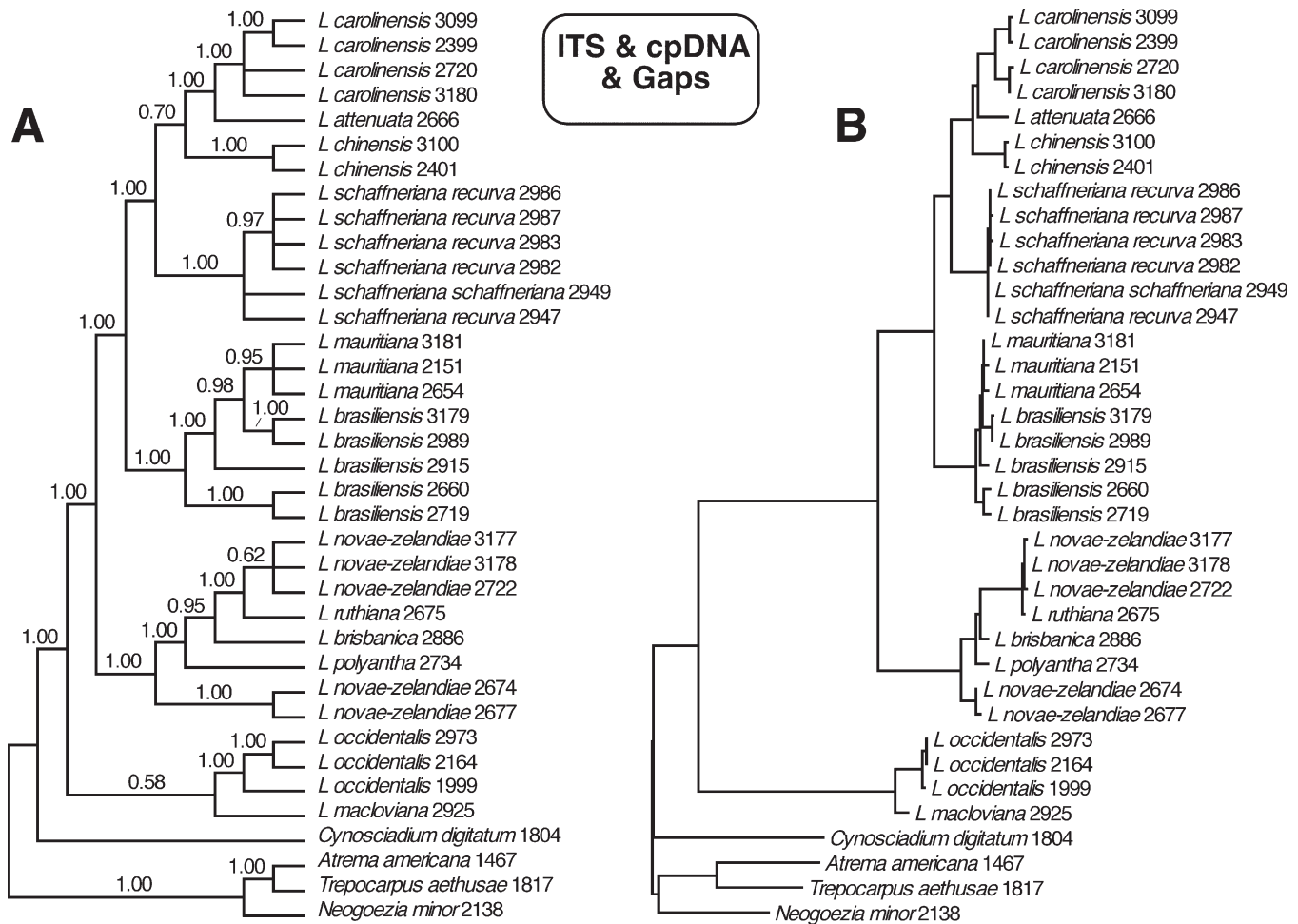
FIG. 6. Bayesian inference trees derived from analyses of combined ITS and cpDNA data (including scored gaps). A. Majority-rule consensus tree, with posterior probability values indicated; B. Single tree, showing relative branch lengths.

South America in accordance with the ITS phylogenies that showed *L. occidentalis* (and *L. masonii*) arising from within a paraphyletic *L. macloviana* from South America. We presumed that plants of *L. occidentalis, L. macloviana,* and *L. masonii* represent one polymorphic species, of which the earlier name *L. occidentalis* would apply (see Discussion).

In the first set of analyses, unrestricted optimal DIVA reconstructions, where maxareas = four (the total number of unit areas inhabited by the terminals), required seven dispersal events (not shown). For one ancestral node, however, the reconstruction was ambiguous with this node comprising three different optimal distributions, including an ancestral distribution for *Lilaeopsis* that represented three of the areas occupied by the terminals. Because none of the extant species of *Lilaeopsis* are widespread among these areas (nor is it supposed that the ancestral taxa were either), another optimization was carried out where the number of ancestral areas assigned to each node was restricted to two. In this optimization, seven dispersals were still required to explain the present-day distribution of these taxa, but fewer alternative ancestral areas were inferred (Fig. 7, upper tree). The DIVA analysis resulted in a South American origin of the genus *Lilaeopsis* following a dispersal of its ancestor from North America. A subsequent dispersal event to either Australia or

New Zealand was inferred, and if we assume the former scenario, the reconstruction then suggests two consecutive dispersals from Australia to New Zealand and three dispersals from South America to North America. If we assume the latter scenario, a dispersal from South America to New Zealand is followed by a dispersal from New Zealand to Australia and then back again to New Zealand.

In the second set of analyses, where ancestral areas were restricted to South America for *L. carolinensis* and to North America for *L. schaffneriana*, unrestricted optimal DIVA reconstructions required six dispersal events. For all deep ancestral nodes, however, the reconstructions were ambiguous with two nodes comprising five to nine different optimal distributions, including an ancestral distribution for *Lilaeopsis* that represented all of the areas occupied by the terminals. Restricting the number of ancestral areas assigned to each node to two also required six dispersals, but fewer alternative ancestral areas were inferred (Fig. 7, lower tree). In this scenario, ancestral area reconstructions for *Lilaeopsis* included either South America or a broader region encompassing both North and South America. This tree resulted in more ambiguities concerning the location of dispersals between North and South America, and consequently, more possible scenarios.
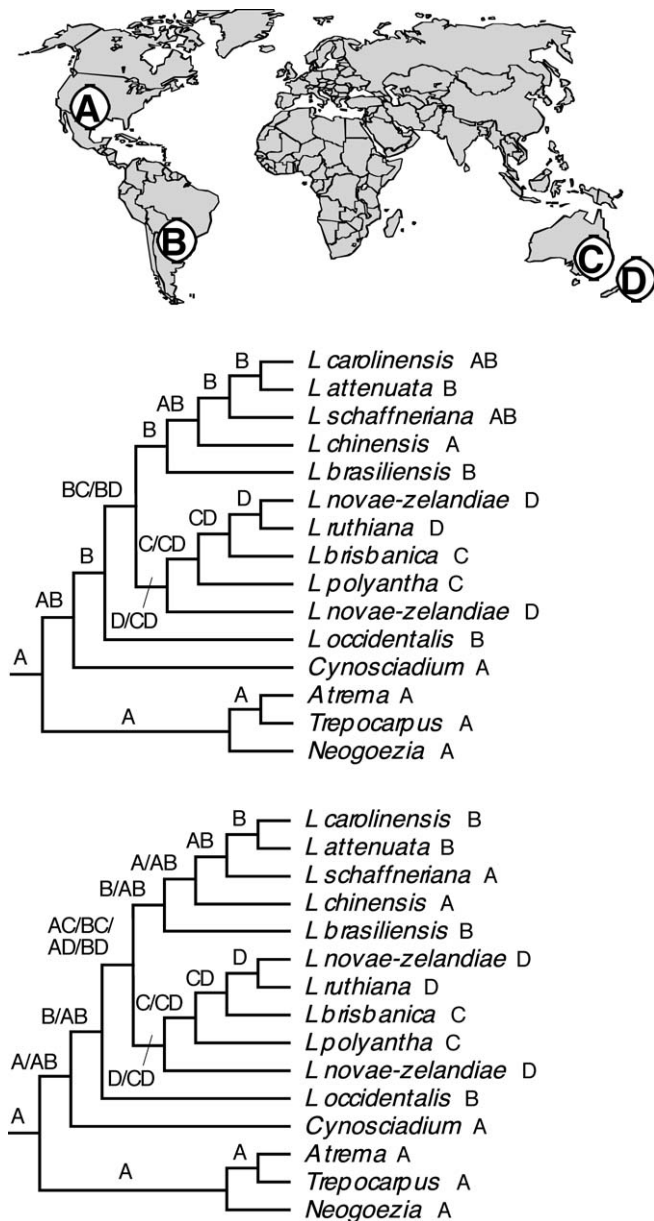
Fig. 7. Results of dispersal-vicariance (DIVA) analysis for *Lilaeopsis* and outgroups. The cladograms represent simplified, fully resolved trees of species relationships obtained from phylogenetic analyses of combined ITS and cpDNA data, with each terminal representing a major lineage revealed by these analyses. The trees differ with regard to the ancestral areas assigned to *L. carolinensis* and *L. schaffneriana*. Letters along internal branches indicate ancestral areas inferred with DIVA and correspond to the areas indicated on the map. *Lilaeopsis occidentalis* includes both *L. macloviana* and *L. masonii* and its ancestral distribution was coded as South America; *L. mauritiana* was not included as a terminal (see text for further explanation).

Discussion

Phylogenetic Resolutions—The phylogenetic results presented herein do not support the purported close relationship among sympatric species *L. attenuata, L. brasiliensis*, and *L. carolinensis* (Hill 1927; Purper and Gui Ferreira 1971; Affolter 1985). *Lilaeopsis attenuata* and *L. carolinensis* ally as well-supported sister taxa in trees derived from both ITS and combined ITS/cpDNA data, whereas in all trees *L. brasiliensis* is more closely allied to *L. mauritiana*. In the cpDNA-derived trees, however, these three species (along with *L. chinensis* and

L. mauritiana) comprise a clade, but resolution of relationships is poor and weakly supported because of few parsimony informative characters. Total ITS and cpDNA sequence divergence between *L. attenuata* and *L. carolinensis* is 1.2%, and while this value is below the maximum average of 2.2% for other interspecific comparisons in *Lilaeopsis*, it is much higher than most infraspecific comparisons and, therefore, supports the specific status of each of these taxa. We maintain *L. attenuata* and *L. carolinensis* as separate species. Both of these species can usually be differentiated readily in the field: the former with its linear or tapering, terete leaves, and dorsal and intermediate mericarp ribs rounded in cross section; the latter with its spatulate (rarely linear) leaves, and dorsal and intermediate ribs triangular in cross section. The presence of broadly spatulate leaves in plants of *L. carolinensis* and *L. brasiliensis*, in which a lamina has been restored by flattening and expansion of the distal portion of the rachis, has occurred twice independently during evolution of the genus. Spatulate leaves are also present in some populations of *L. chinensis*, but these are narrower or strap-shaped.

Affolter (1985) reported that populations of *L. carolinensis* from the eastern U. S. A. cannot be distinguished morphologically from those of temperate South America. While the leaves of this amphitropic species are indeed variable, the range of variation exhibited by plants from both continents is similar. Moreover, ITS sequences for *L. carolinensis* accessions from the U. S. A., Argentina, and Portugal are identical. The plants of coastal Portugal and northwestern Spain, identified by some as *L. attenuata* (Almeida and Freitas 2001; Tutin 2001), are probably all *L. carolinensis*, as indicated previously by Affolter (1985). The lack of ITS sequence divergence among populations of *L. carolinensis* from three continents may be a consequence of low mutation rates and recent dispersals, or anthropogenic introductions.

Petersen and Affolter (1999) described *Lilaeopsis mauritiana* for plants restricted to a single locality on the island of Mauritius. Its affinity to other species, whether those of the New World or Australasia, could not be inferred because of its distinctive fruits. Petersen and Affolter (1999) considered *L. mauritiana* endemic to the island, although they did not rule out completely that the species might have been recently introduced. The results of our investigations show that *L. mauritiana* is allied closely with *L. brasiliensis* from South America, and that recognition of *L. mauritiana* as a distinct species is not entirely clear. In trees derived from analyses of combined ITS/cpDNA data, accessions of each species are commingled, and both MP and BI analyses of combined molecular data with scored gaps weakly supported a monophyletic *L. mauritiana* arising from within a paraphyletic *L. brasiliensis*. Sequence divergence estimates of combined data between accessions of *L. brasiliensis* and *L. mauritiana* are among the lowest in interspecific comparisons and are within the range of those values determined for *L. brasiliensis* only. These molecular data suggest that these morphologically unique plants of Mauritius might actually be aberrant members of *L. brasiliensis*.

The fruit anatomy of *L. brasiliensis*, however, is different from that of *L. mauritiana*. Spongy cells are present in all five ribs of the former and absent from the latter. In *L. brasiliensis* the fruit ribs are angled, whereas they are variously rounded in *L. mauritiana*. The complete absence of spongy cells has also been observed in other species, such as *L. macloviana, L. polyantha*, and *L. ruthiana*, although these species show differences in their rib morphologies and some populations do indeed

produce spongy cells in some or all of their ribs (Petersen and Affolter 1999). Affolter (1985) has reported that several species of *Lilaeopsis* produce different fruit types in response to different microhabitats, so perhaps the plants from Mauritius have lost the ability to produce spongy tissue. For example, the widespread South American species *L. macloviana* exhibits a tremendous polymorphism in fruit structure, with fruits lacking spongy cells entirely, with it confined to the lateral ribs only, or with abundant spongy cells throughout all five ribs. The fruit ribs may be low and obscure, to prominent and broadly rounded or projecting. Populations of *L. brasiliensis* are known from river banks, ditches, seepage areas, and marshes or bogs (Affolter 1985), whereas *L. mauritiana* occurs in or along a moderately flowing, clear-watered stream (Petersen and Affolter 1999). *Lilaeopsis brasiliensis* has never been known to produce fruits lacking spongy tissue, but it may be possible that the different habitat of Mauritius has induced this response. The rapid loss of dispersal abilities of these plants on Mauritius may also explain the differences in fruit structure between these two species. Whether *L. mauritiana* should be maintained as a distinct species, or treated as some infraspecific rank of *L. brasiliensis*, or even subsumed under *L. brasiliensis*, remains the subject of further sampling of these taxa from both South America and Mauritius.

Lilaeopsis chinensis (eastern grasswort), a state-listed rare species, is found along the Atlantic and Gulf coasts of North America (Affolter 1985; Moore et al. 2009). The three accessions included in this study, all from the southeastern U. S. A., comprise a monophyletic group. In North America, the species is sympatric with *L. carolinensis* and they share several vegetative traits, including a flattening of the distal portion of the leaves. Indeed, in the absence of mature fruits, these species may be confused when their leaves are of similar shape and size, yet Hill (1927) and Affolter (1985) found no evidence that these two taxa should be considered a single species. Our results support the segregation of *L. chinensis* from *L. carolinensis*.

Lilaeopsis schaffneriana is distributed in southeastern Arizona and adjacent Sonoran Mexico, northern and central Mexico, and northwestern South America. A single, sterile collection is known from Santo Domingo, Dominican Republic and was only tentatively identified as this species because of its vegetative similarity and close proximity to *L. schaffneriana* from Mexico (Affolter 1985). Two subspecies are currently recognized, with subsp. *recurva* (Huachuca water umbel) being federally endangered (Titus and Titus 2008). Differences between the two subspecies include the overall shape of their desiccated, mature fruits and their geographic separation. All examined accessions of *L. schaffneriana* comprise a monophyletic group in the phylogenetic analyses, with no to little sequence divergence among them. *Lilaeopsis schaffneriana* is distantly related to *L. macloviana* from western South America, a sympatric species in the northern portion of its range where some populations are almost indistinguishable from those of *L. schaffneriana*.

Lilaeopsis occidentalis is distributed along the Pacific coast of North America, from the Queen Charlotte Islands southward to Marin County, California, and is almost entirely confined to salt water or brackish habitats, frequently below the level of high tides (Affolter 1985). The absence of spongy cells from the dorsal and intermediate fruit ribs of this species is a useful character separating it from the vegetatively similar *L. schaffneriana*, where spongy cells are present in all five ribs. The dorsal and intermediate ribs of *L. occidentalis* show considerable variation in the extent to which they protrude above the surface of the mericarp: low, rounded and obscure, to winglike and prominent. The results of the phylogenetic analyses do not indicate a close relationship between *L. occidentalis* and *L. schaffneriana*. Instead, results based on ITS sequences only show that accessions of *L. occidentalis* are inextricably linked with those of *L. macloviana*, a species distributed widely in western South America, from Colombia south to Tierra del Fuego, and eastward to the Falkland Islands. Results of the combined ITS/cpDNA data revealed a sister group relationship between *L. occidentalis* and *L. macloviana*, albeit only a single accession of *L. macloviana* was included.

Lilaeopsis macloviana occurs in a large variety of habitats, from sea level in the brackish water of intertidal zones, along margins of ditches and lakes, to wet meadows and bogs up to 4,700 m elevation (Affolter 1985). In the northern portion of its range, the species extends into the tropics along the Andes. It is polymorphic, showing considerable variation in vegetative organs and fruit morphology and anatomy, and prior to Affolter's monograph it was treated as six species and several infraspecific taxa. The conspicuous variation in vegetative morphology exhibited by these plants is accounted for by phenotypic plasticity in response to the variety of microenvironments in which they grow (Affolter 1985). A continuous series of fruit types is apparent according to the distribution of spongy cells, ranging from abundant in all five ribs to none whatsoever, and several different fruit types may be present in a single collection. The shape and the size of the fruits, as well as the prominence of dorsal and intermediate ribs and the number of vittae, are also variable within individual collections. Our sampling of this polymorphic species is sparse, with only four accessions included in the ITS study; however, they represent collections from throughout its range (i.e. Argentina, Bolivia, Peru, and Falkland Islands). *Lilaeopsis occidentalis* exhibits similar variability as *L. macloviana* in the degree to which dorsal and intermediate ribs protrude above the surface of the mericarp. Its fruits have spongy tissue confined only to the two lateral ribs, a condition also occurring in *L. macloviana*. Both species have leaves borne in clusters at the apex of vertical rhizome branches (although they may also occur individually and along horizontal rhizomes in *L. occidentalis*). Overall, the two species are similar morphologically, and the range of variation exhibited by *L. occidentalis* can largely be accommodated within that variability seen in *L. macloviana*. The close relationship between these two species is further supported by a shared chromosome number of *n* = 22, a polyploid number also known only for *L. masonii* (Affolter 1985). All other species of *Lilaeopsis* for which cytological data are available have a base chromosome number of *x* = 11 (Affolter 1985), a cytotype that is prevalent within subfamily Apioideae (Moore 1971). The habitat of *L. occidentalis* (brackish water of intertidal zones) also falls within that favored by plants of *L. macloviana* growing at sea level. Pairwise nucleotide divergence estimates between accessions of *L. occidentalis* and *L. macloviana* were generally low and these accessions are mixed in the ITS trees. In addition to the molecular and phylogenetic results presented herein, morphological, fruit anatomical, cytological, and ecological data all suggest that these plants probably best represent one polymorphic species, of which the earlier name *L. occidentalis* would apply. This species has an amphitropic distribution in the western hemisphere, with extensions into the tropics

along the Andes. Such a widespread geographic range is also shown by *L. carolinensis*, an Atlantic coastal species.

Lilaeopsis masonii (Mason's lilaeopsis) is a California state-listed rare species that has recently been subsumed under *L. occidentalis* (Fiedler et al. in press). Traditionally, *L. masonii* was considered restricted almost exclusively to the Sacramento/San Joaquin River Delta region of California and was distinguished from the more widespread *L. occidentalis* by its narrower, usually shorter leaves with few, obscure septa (Mathias and Constance 1977; Affolter 1985). In contrast, plants of *L. occidentalis* were described as more robust and distributed along the Pacific coast, although inland populations with smaller leaves were noted (Affolter 1985). Subsequent field surveys by Fiedler and colleagues expanded the range of *L. masonii* and showed that the leaf vegetative characters traditionally used to separate it from *L. occidentalis* overlap considerably (reviewed in Fiedler et al. in press). Both species have similar vegetative and fruit morphologies, are sympatric and grow in similar brackish habitats, and share the same unusual polyploid chromosome number. Additional evidence provided by Fiedler et al. (in press) for recognition of these two species as one taxon included essentially identical ITS sequences and a high degree of genetic similarity, as discerned through AFLP analyses. We agree with Fiedler et al. (in press) that the rare *L. masonii* should no longer be recognized as a separate taxon and that it, like *L. macloviana*, be subsumed within *L. occidentalis*.

Affolter (1985) consolidated the three New Zealand species of *Lilaeopsis* recognized by Hill (1927, 1928, 1929) into the single polymorphic taxon *L. novae-zelandiae*. The fruit of this species shows continuous variation in shape, size, distribution and presence of spongy tissue, and shape of the ribs. In addition, Affolter described *L. ruthiana* from New Zealand, which differs from *L. novae-zelandiae* by its relative uniformity in fruit structure. In *L. ruthiana* the mericarps are generally semicircular in cross section, the ribs are low and rounded, and spongy cells are absent from all five ribs or scarcely present in the lateral ribs only. Some fruits of *L. novae-zelandiae*, however, have a similar external appearance to that of *L. ruthiana*. Furthermore, Clayton (1998) has observed that *L. ruthiana* is almost indistinguishable from *L. novae-zelandiae* and suggested that its taxonomic distinction may not be justified. Affolter also merged the three Australian species recognized by Hill into another polymorphic species, *L. polyantha*. The Australian species *L. brisbanica* was erected for specimens previously ascribed to both *L. novae-zelandiae* and *L. polyantha* (Affolter 1985; Bean 1997). In describing *L. brisbanica*, Bean (1997) compared these specimens to only the typical form of *L. polyantha* and not the broadened circumscription of *L. polyantha,* as recognized by Affolter. As such, the diagnostic features of *L. brisbanica* fall within the range of variation seen in *L. polyantha* sensu Affolter. There are few, if any, vegetative characters useful for distinguishing among *L. novae-zelandiae*, *L. polyantha*, and *L. ruthiana* and they are often impossible to tell apart in the absence of fruits (Affolter 1985). As in other species of *Lilaeopsis*, vegetative features display considerable variation in response to the microhabitat in which they are found. *Lilaeopsis novae-zelandiae* and *L. polyantha* show overlapping variation among fruit characters, with fruits of some populations of the former impossible to key apart from some populations of the latter; thus, these two species are best circumscribed geographically (Affolter 1985).

The four Australasian species of *Lilaeopsis* included in this study, *L. brisbanica, L. novae-zelandiae, L. polyantha*, and *L. ruthi-*

ana, collectively form a well-supported monophyletic group in trees resulting from both ITS and combined ITS/cpDNA data. In trees derived from cpDNA sequences only, with or without scored gaps, these Australasian taxa comprise two separate clades, a result of too few phylogenetically informative characters. In the combined trees, the five accessions of *L. novae-zelandiae* separated into two lineages, with *L. brisbanica, L. polyantha,* and *L. ruthiana* allying with one of them. Within this Australasian clade, sequence divergence estimates for combined ITS/cpDNA data ranged from identity to 1.3%, with the greatest differences found between the two lineages of *L. novae-zelandiae*; otherwise, divergence estimates for some interspecific comparisons were low (e.g. 0.09% between *L. ruthiana* and *L. novae-zelandiae*), falling into the range for infraspecific comparisons in other taxa. The relationships among these Australasian taxa are not easily resolved. One could suppose either a monophyletic *L. novae-zelandiae* to subsume *L. brisbanica, L. polyantha,* and *L. ruthiana*, or maintain each of these Australasian species as distinct and recognize *L. novae-zelandiae* accessions 2674 and 2677 as a new species. The latter accessions, however, are not particularly distinct, either morphologically or geographically, and accession no. 2677 (*Affolter 182*) was collected from the type locality of the species. The vegetative similarities and overlapping patterns of fruit variation observed among these Australian taxa, coupled with low sequence divergence estimates observed in some interspecific comparisons and the paraphyly of *L. novae-zelandiae*, suggest that the entire group be treated as a single, polymorphic species. However, before such a treatment is invoked, further molecular and morphological studies of these Australasian species are warranted, given the limited sampling herein of such a taxonomically complex group.

Biogeography—Because a great proportion of the distributional range of *Lilaeopsis* parallels the Gondwanan disjunction in the southern hemisphere, the genus was assumed to have a southern hemispheric and ancient origin (Raynal 1977). However, biogeographic studies of other angiosperm genera exhibiting amphitropic distribution patterns with transoceanic disjunctions in the southern hemisphere have revealed that the predominant direction of dispersal was from the northern to the southern hemisphere, even for those groups that are much diversified in the latter (Raven 1963); moreover, such long-distance dispersals were likely relatively recent events (reviewed in Spalik et al. 2010). Genera of Apiaceae inferred to have ancestral distributions in the northern hemisphere with subsequent recent, long-distance dispersals to the southern hemisphere include *Sanicula* L. (Vargas et al. 1998, 1999), *Osmorhiza* Raf. (Wen et al. 2002; Yoo et al. 2002), *Chaerophyllum* L./*Oreomyrrhis* Endl. (Chung et al. 2005), *Daucus* L. (Spalik and Downie 2007; Spalik et al. 2010), *Eryngium* L. (Calviño et al. 2008), and *Apium* L. (Spalik et al. 2010). Other Apiaceae genera showing American amphitropic disjunctions include *Ammoselinum* Torr. & A. Gray and *Spermolepis* Raf. (Constance 1963), but their ancestral areas and directions of dispersal/migration have yet to be determined through modern biogeographic reconstructions. Prior to this study, we were unaware of any umbellifer genus exhibiting an American amphitropic distribution pattern with transoceanic disjunctions in the southern hemisphere that may have had its origin in the southern hemisphere.

Of the DIVA analyses presented, we favor the tree assuming terminal polymorphisms (AB) for both *L. carolinensis* and *L. schaffneriana* (Fig. 7, upper tree), in accord with their present

distributions, rather than inferring a single ancestral region for each of these lineages that may be regarded as speculative (Fig. 7, lower tree). The optimal solution of DIVA suggests that South America is indeed the ancestral area for *L. carolinensis*, with a dispersal to North America occurring relatively recently at a terminal branch. A similar scenario may be invoked for *L. schaffneriana*. The results of DIVA indicate that *Lilaeopsis* probably originated in South America, following a dispersal of its ancestor from North America. A subsequent dispersal from South America to Australia (or New Zealand), dispersals from Australia to New Zealand (or between New Zealand and Australia), and three dispersal events from South America to North America were also inferred.

A minimum of seven dispersal events is required to explain the present-day distribution of *Lilaeopsis*, and the isolated and scattered presence of *Lilaeopsis* in coastal Portugal and Spain, Mauritius, Madagascar, the Dominican Republic, and the Kerguelen Archipelago are recent introductions of possibly anthropogenic origins. Biogeographic studies, including those of several genera of Apiaceae, have suggested that bird-mediated oceanic dispersal underlies the distribution of many trans-Pacific disjunct plant groups, especially those favoring wetland and aquatic habitats (Vargas et al. 1998, 1999; Winkworth et al. 2002; Les et al. 2003; Sanmartín and Ronquist 2004; Chung et al. 2005; de Queiroz 2005). Similarly, amphitropic disjunctions in North and South America have also been explained by long-distance dispersal via migrating birds (Raven 1963; Cruden 1966). Fruits of *Lilaeopsis* can retain their buoyancy in both fresh and salt water for many months, with only a minimal loss of seed viability, and their dispersal by sea currents or birds may have facilitated their transport to new regions (Affolter 1985). The optimal solution of DIVA indicates multiple dispersals of *Lilaeopsis* between North and South America, and while such intercontinental disjunctions can be simply explained by long-distance dispersal by migratory birds, a more or less continuous overland migration between continents can also be invoked. Affolter (1985) indicated two avenues of migration of *Lilaeopsis* between North and South America: one on the eastern sides of the continents, the other between the northern Andes and southwestern North America. "Island hopping" across exposed regions of the continental shelf along the Gulf and Atlantic coasts during episodes of sea level lowering during the Pleistocene glaciations afforded opportunities for plant migration, with their intervening range wiped out with the final rise in sea level (Flint 1971; Affolter 1985). "Mountain hopping" through the American Cordillera was an important migratory route for temperate species moving across the American tropics, and such a pathway would have become available first during the Miocene and through the Pliocene (Constance 1963; Raven 1963; Cruden 1966). Raven (1963) hypothesized that American amphitropic plant disjunctions would have occurred from the Pliocene onwards, as neither species nor the habitats to which they are adapted probably existed before this time. This timeframe is in accordance with molecular dating for several apioid umbellifer genera and other taxa exhibiting amphitropic distribution patterns (Spalik et al. 2010). During the Pleistocene, wetland habitats from northern South America to central Mexico were extensive, maximizing the exchange of *Lilaeopsis* species between North and South America (Affolter 1985). The presence of *L. macloviana* at elevations up to 4,700 m in the Andes indicates that these plants are physiologically

capable of overland migration along the Cordilleran system. *Lilaeopsis schaffneriana* occurs in the northern Andes, central and northern Mexico, and adjacent Arizona, and may eventually be found to be fairly continuously distributed through the montane neo-tropics.

Lilaeopsis is well represented in cool temperate regions of the southern hemisphere, with 10 species occurring in South America and Australasia (Australia, Tasmania, and New Zealand). Additional populations are known from Mauritius, Madagascar, and the Kerguelen Archipelago, and no doubt these inconspicuous plants will eventually be reported from other scattered islands of the South Atlantic and South Indian Oceans. The presence of *Lilaeopsis* in Mauritius and Madagascar, a species representing either a local endemic (*L. mauritiana*) or an aberrant form of *L. brasiliensis*, is likely a recent introduction. In most phylogenies inferred herein, the accessions of *L. mauritiana* and *L. brasiliensis* are commingled, implying a recent split of the Mauritius taxon. One optimal solution of DIVA indicates that *Lilaeopsis* was dispersed from South America to New Zealand, with a subsequent dispersal westward from New Zealand to Australia and then back to New Zealand again. Dawson (1971) hypothesized that *Lilaeopsis* and other Apiaceae that occur in wet habitats in New Zealand arrived there by overseas, long-distance dispersal, probably a result of transport by migratory birds, a scenario reported repeatedly to explain many other southern hemisphere plant disjunctions (Sanmartín and Ronquist 2004; de Queiroz 2005). Winkworth et al. (2002) have postulated that many contemporary alpine species of New Zealand are recent arrivals, reaching New Zealand or diversifying there in the late Tertiary, and then traveling to other southern hemispheric landmasses. In contrast, others have reported that many alpine plant groups entered New Zealand via Australia, following the direction of the prevailing westerlies (e.g. Raven 1973; Pole 1994). Another optimal solution of DIVA indicates that *Lilaeopsis* was dispersed from South America to Australia, with subsequent dispersals eastward to New Zealand. Further resolution of biogeographic relationships will come from increased study of these Australasian plants.

Cladistic analysis of molecular data from both the chloroplast and nuclear genomes of *Lilaeopsis* has revealed infrageneric relationships heretofore unknown and demonstrated that several previously recognized species inadequately reflect natural groupings. The genus has probably had a South American origin following a dispersal of its ancestor from North America. In other angiosperms having a similar distribution pattern, the predominant direction of dispersal appears to have been the opposite, from the northern to southern hemisphere. Further studies of the genus are in order, especially to confirm the taxonomic changes proposed herein. However, because of their inconspicuous nature and grass-like appearance in sterile condition, these plants are often overlooked in the field and not well represented in herbaria, thus a substantial effort will be required. The extent of variability in fruit characters exhibited by several species also needs to be reexamined to produce a classification that is both functional and reflects the phylogenetic history of the plants. Three taxa have yet to be considered in molecular study (*L. attenuata* subsp. *ulei*, *L. fistulosa*, and *L. tenuis*), and their inclusion in future analyses should reveal their phylogenetic placements and taxonomic status, as well as confirm or revise the biogeographic scenarios inferred herein.

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APPENDIX 1. List of *Lilaeopsis* and outgroup specimens examined, with source and voucher information and GenBank accession numbers. Two GenBank numbers for cpDNA indicate separate sequences for the *rps16* intron and *rps16-trnK* intergenic spacer regions, respectively; a single GenBank number for cpDNA indicates contiguous sequence from the *rps16* intron to *trnK* (i.e., including the *rps16* 3'exon).

Lilaeopsis attenuata (Hook. & Arn.) Fern. subsp. *attenuata*: Argentina, Corrientes, Dep. Mburucuyá, Estancia Santa Teresa, cult. University of Michigan Botanical Gardens, *Affolter 115* (MICH, GA), DNA accession no. 2666 (cpDNA: GU144637, GU144611; ITS: EF177710). *L. brasiliensis* (Glaz.) Affolter: Argentina, Corrientes, Sloping ground towards a swamp, 11 June 1980, *Crucecita s. n.* (UC 1538838), DNA accession no. 2915 (cpDNA: GU144638, GU144612; ITS: GU128107). Brazil, Santa Catarina, between Matos Costa and Caçador, cult. University of Michigan Botanical Gardens, *Affolter 102* (MICH, GA), DNA accession no. 2660 (cpDNA: GU144639, GU144613; ITS: EF177712). Brazil, origin unknown, *Casselmann s. n.*, 1984, DNA and leaf material supplied by G. Petersen, *Petersen GPL3* (C, ILL), DNA accession no. 2719 (cpDNA: EF185224; ITS: EF177711). South America, origin unknown, cultivated material identified as *L. novae-zelandiae* obtained from Florida Aquatic Nurseries, Florida, *Bone s. n.* (ILL), DNA accession no. 2989 (cpDNA GU144640, GU144614; ITS: GU128108). South America, origin unknown, cultivated material obtained from Tropica Aquarium Plants, Denmark, *Troels 040, Bone 107* (ILL), DNA accession no. 3179 (cpDNA: GU144641, GU144615; ITS: GU128109). *L. brisbanica* A. R. Bean: Australia, Queensland, Brisbane River, SW of Brisbane, 19 October 1993, *Bean 6778* (MO 05061944), DNA accession no. 2886 (cpDNA: GU144607; ITS: HQ822270). *L. carolinensis* J. M. Coult. & Rose: Argentina, Corrientes, Dep. Mburucuyá, Estancia Santa María, cult. University of Michigan Botanical Garden, *Affolter 114* (MICH, GA), DNA accession no. 2663 (ITS: EF177713). Portugal, Coimbra, cult. University of

California Botanical Garden, *Constance C-1227* (MO 2260055), DNA accession no. 2920 (ITS: GU128110). U. S. A., Louisiana, St. John Parish, rare on stumps in canal along Hwy 51, 12 April 1992, *Montz & Montz 5696* (LSU), DNA accession no. 3097 (ITS: GU128111). U. S. A., Louisiana, St. Charles Parish, New Orleans District, Bonnet Carre Spillway, 25 April 1981, *Montz 5191* (LSU), DNA accession no. 3098 (ITS: GU128112). U. S. A., Louisiana, St. Charles Parish, uncommon in slip along Burchell Canal, Bayou Gauche, 20 July 1994, *Montz 6836* (LSU), DNA accession no. 3099 (cpDNA: GU144642, GU144616; ITS: GU128113). U. S. A., Louisiana, East Baton Rouge Parish, growing just beneath and on the surface of the Capitol Lakes in the shallows near the bank, 21 March 1995, *Walker 6* (LSU), DNA accession no. 3103 (ITS: GU128114). U. S. A., cultivated, origin unknown, *Bogner s. n.*, 1985, DNA and leaf material supplied by G. Petersen, *Petersen GPL4* (C, ILL), DNA accession no. 2720 (cpDNA: EF185225; ITS: AF466276). U. S. A., North Carolina, New Hanover Co., Wilmington, 31 May 1987, *MacDougal 2071* (MO 05033978), DNA accession no. 2399 (cpDNA: GU144643, GU144617; ITS: GU128115). Origin unknown, cultivated material identified as *L. macloviana* obtained from Tropica Aquarium Plants, Denmark, *Troels 040D, Bone 109* (ILL), DNA accession no. 3180 (cpDNA: GU144644, GU144618; ITS: GU128116). *L. chinensis* (L.) Kuntze: U. S. A., Louisiana, St. Marys Parish, along edge of Wax Bayou, April 1989, *Givens 5167* (LSU), DNA accession no. 3101 (ITS: GU128117). U. S. A., Louisiana, St. Tammany Parish, Pearl River Wildlife Management Area south of Hwy 90, marshy environment, 20 April 1991, *Kantz 22* (LSU), DNA accession no. 3100 (cpDNA: GU144645, GU144619; ITS: GU128118). U. S. A., North Carolina, New Hanover Co., west bank of Cape Fear River, 31 May 1987, *MacDougal 2068* (MO 05033977), DNA accession no. 2401 (cpDNA: GU144608; ITS: EF177714). *L. macloviana* (Gand.) A. W. Hill: Argentina, Tierra del Fuego, Estancia Cullen, spring along road to Puesto Beta, 7 January 1971, *Goodall 3234* (UC 150110), DNA accession no. 2921 (ITS: GU128119). Bolivia, Dep. Oruro, Prov. L. Cabrera, 28 February 1986, *Beck 11830* (UC 1549364), DNA accession no. 2925 (cpDNA: GU144609; ITS: GU128120). Falkland Islands, East Falkland, San Carlos, White Rincon, moist sand at top of beach, 24 January 1964, *Moore 649* (UC 297946), DNA accession no. 2923 (ITS: GU128121). Peru, Cuzco, 15 km S of Cuzco on road to Urcos, cult. University of Michigan Botanical Gardens, *Affolter 119* (MICH, GA), DNA accession no. 2518 (ITS: EF177715). *L. masonii* Mathias & Constance: U. S. A., California, Sacramento Co., Twitchell Island, 24 August 2007, *Fiedler et al. s. n.* (SFSU) (ITS: HQ634700). U. S. A., California, Solano Co., Liberty Island, 23 August 2007, *Fiedler et al. s. n.* (SFSU) (ITS: HQ647238). *L. mauritiana* G. Petersen & Affolter: Mauritius, Le Val Nature Park, *Windeløv s. n.,* 3 May 1992, DNA and leaf material supplied by G. Petersen, *Petersen GPL8* (C, ILL), DNA accession no. 2151 (cpDNA: EF185226; ITS: AF466277). Mauritius, Le Val Nature Park, *Affolter 000* (GA), DNA accession no. 2654 (cpDNA: GU144646, GU144620; ITS: GU128122). Mauritius, cultivated material obtained from Tropica Aquarium Plants, Denmark, *Troels 040B, Bone 106* (ILL), DNA accession no. 3181 (cpDNA: GU144647, GU144621; ITS: GU128123). *L. novae-zelandiae* (Gand.) A. W. Hill: New Zealand, North Island, Taranaki, nr mouth of Kaupokonui Strm, coast WNW of Manaia, *Affolter 168* (MICH, GA), DNA accession no. 2674 (cpDNA: GU144648, GU144622; ITS: GU128124). New Zealand, South Island, Otago, Otago Peninsula, Tomahawk Lagoon, *Affolter 182* (MICH, GA), DNA accession no. 2677 (cpDNA: GU144649, GU144623; ITS: GU128125). New Zealand, origin unknown, cultivated, DNA and leaf material supplied by G. Petersen, *Petersen GPL9* (C, ILL), DNA accession no. 2722 (cpDNA: EF185227; ITS: AF466278). New Zealand, North Island, Auckland Ecological Region, Tamaki Ecological District, east bank of the Tamaki River, 5 November 2000, *Gardner 10225* (AK), DNA accession no. 3177 (cpDNA: GU144650, GU144624; ITS: GU128126). New Zealand, origin unknown, cultivated material obtained from Tropica Aquarium Plants, Denmark, *Troels 040A, Bone 108* (ILL), DNA accession no. 3178 (cpDNA: GU144651, GU144625; ITS: GU128127). *L. occidentalis* J. M. Coult. & Rose: U. S. A., California, Del Norte Co., S side of the Klamath River, 28 August 1974, *Mastroqiuseppe 157* (TEX), DNA accession no. 2974 (ITS: GU128128). U. S. A., California, Marin Co., Bodega Head, July 10, 2007, *Fiedler et al. s. n.* (SFSU) (ITS: HQ647236). U. S. A., Oregon, Douglas Co., East Gardiner, N of Blacks Island, NE of RR bridge at mouth of Smith River, alluvial sand, gravel and muck, *Hill & Dutton 32982* (ILLS 203634), DNA accession no. 1999 (cpDNA: EF185228; ITS: AY360242). U. S. A., Oregon, Douglas Co., Tahkenitch Lake, along U.S. Hwy. 101, 2 November 1995, *Halse 5010* (ASU), DNA accession no. 2978 (ITS: GU128129). U. S. A., Oregon, Lincoln Co., Kernville, in mud flat off Pacific Ocean, 22 August 1975, *Seigler 9820* (ILL), DNA accession no. 2164 (cpDNA: GU144652, GU144626; ITS: GU128130). U. S. A., Washington, Klickitat County, Lyle, on a shoal by the mouth of the Klickitat River, 14 September 1992, *Halse 4555* (TEX), DNA accession no. 2973 (cpDNA: GU144653, GU144627; ITS: GU128131). U. S. A., Washington, Mason Co.,

Mason Lake, 25 July 2008, *Fiedler et al. s. n.* (SFSU) (ITS: HQ647244). **L. polyantha (Gand.) H. Eichler:** Australia, New South Wales, Bombala, Oct. 1998, DNA supplied by G. Petersen, *Petersen GPL26* (C), DNA accession no. 2734 (cpDNA: GU144654, GU144628; ITS: GU128132). **L. ruthiana Affolter:** New Zealand, Canterbury, Lake Lyndon, cultivated, *Affolter 176* (MICH), DNA accession no. 2675 (cpDNA: GU144655, GU144629; ITS: GU128133). **L. schaffneriana (Schltdl.) J. M. Coult. & Rose subsp.** *recurva* **(A. W. Hill) Affolter:** U. S. A., Arizona, Cochise Co., Huachuca Mts., Coronado Natl. Forest, Scotia Canyon, in stream, 1993, *McLaughlin & Bowers 6378* (ARIZ), DNA accession no. 2943 (ITS: GU128134). U. S. A., Arizona, Cochise Co., Huachuca Mts., Sycamore Canyon above Sycamore Spring, SW slope of Lone Mt., Elev. 5650′, 1993, *Fishbein & Adondakis 1315* (ARIZ 306551), DNA accession no. 2944 (cpDNA: GU144656, GU144630). U. S. A., Arizona, Cochise Co., Leslie Canyon National Wildlife Refuge, cult. in greenhouse, 11 August 2005, *Bone 101* (ILL), DNA accession no. 2982 (cpDNA: GU144657, GU144631; ITS: GU128135). U. S. A., Arizona, Cochise Co., Ft. Huachuca Military Reservation, near Sierra Vista, Garden Canyon Cienega, 11 August 2005, *Bone 102* (ILL), DNA accession no. 2983 (cpDNA: GU144658, GU144632; ITS: GU128136). U. S. A., Arizona, Cochise Co., Ft. Huachuca Military Reservation, near Sierra Vista, Garden Canyon, 11 August 2005, *Bone 103* (ILL), DNA accession no. 2986 (cpDNA: GU144659, GU144633; ITS: GU128137). U. S. A., Arizona, Cochise Co., Ft. Huachuca Military Reservation, near Sierra Vista, Sawmill Springs, 11 August 2005, *Bone 104* (ILL), DNA accession no. 2987 (cpDNA: GU144660, GU144634; ITS: GU128138). U. S. A., Arizona, Cochise Co., Coronado National Memorial, McClure Spring, 12 August 2005, *Bone 105* (ILL), DNA accession no. 2988 (ITS: GU128139). U. S. A., Arizona, Pima Co., Bingham Cienega Preserve, growing in ditch in marsh, *Titus s. n.* (ARIZ 356998), DNA accession no. 2951 (ITS: GU128140). U. S. A., Arizona, Santa Cruz Co., San Rafael State Park, along the Santa Cruz River, 2001, *McLaughlin & Lewis 9434* (ARIZ 359233), DNA accession no. 2952 (cpDNA: GU144661, GU144635). Mexico, Sonora, Los Fresnos Cienega, 32 miles N of Cananea, 23 June 1990, *Warren, Anderson & Saucedo s. n.* (ARIZ 292307), DNA accession no. 2947 (cpDNA: GU144610; ITS: EF177716). **L. schaffneriana subsp.** *schaffneriana:* Mexico, Chihuahua, 1988, *Laferriere 2208* (ARIZ 305701), DNA accession no. 2949 (ITS: GU128141). Mexico, Tláhuac District, 1 September 1989, *Jimenez-Osorino s. n.* (TEX), DNA accession no. 2977 (cpDNA: GU144662, GU144636; ITS: GU128142).

OUTGROUPS: *Atrema americanum* **DC.:** U. S. A., Texas, Williamson Co., 4 miles S of Jarrell on I-35, 18 May 1988, *Nesom & Grimes 6415* (MO 3691937), DNA accession no. 1467 (cpDNA: EF185207; ITS: AY360232). *Cynosciadium digitatum* **DC.:** U. S. A., Illinois, Jackson Co., Shawnee National Forest, 27 May 1993, *Phillippe 21886* (ILLS 183947), DNA accession no. 1804 (cpDNA: EF185220; ITS: AY360237). *Neogoezia minor* **Hemsl.:** Mexico, Oaxaca, Sierra de San Felipe between Oaxaca and Ixtlán de Juárez, 1 August 1963, *Molseed 278* (ISU 1060), DNA accession no. 2138 (cpDNA: EF185234; ITS: AY360244). *Ptilimnium nuttallii* **(DC.) Britt.:** U. S. A., Mississippi, Monroe Co., ca. 3 miles W of Aberdeen, 6 June 1996, *MacDonald 9514* (MO 05082318), DNA accession no. 2405 (ITS: EF177757). U. S. A., Oklahoma, Rogers Co., Claremore, 12 June 1974, *Jones 3030* (ILL), DNA accession no. 2165 (cpDNA: EF185259). *Trepocarpus aethusae* **Nutt. ex DC.:** U. S. A., Illinois, Alexander Co., Horseshoe Lake Conservation Area, 8 July 1996, *Basinger 10891* (ILLS 194558), DNA accession no. 1817 (cpDNA: EF185280; ITS: AY360264).