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PHYLOGENETIC RELATIONSHIPS AMONG *POLYSCIAS* (ARALIACEAE) AND CLOSE RELATIVES FROM THE WESTERN INDIAN OCEAN BASIN

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As currently circumscribed, *Polyscias* is the second largest genus in Araliaceae, with ca. 150 species, and is found throughout much of the Old World tropics. Recent studies have shown that the genus is paraphyletic, including as many as eight additional genera in a broad “*Polyscias* s.l.” clade. This study focuses on relationships among the species of *Polyscias* native to the western Indian Ocean basin (IOB), which form one of the major subclades of *Polyscias* s.l. By expanding the sample of IOB taxa by a factor of four and combining data from both nuclear (ITS) and chloroplast (*trnL-trnF*) markers, we have confirmed the close relationship of the IOB species to the Malesian members of *Polyscias* and *Gastonia* and also to the Pacific “*Tetraplasandra* group” (*Tetraplasandra*, *Reynoldsia*, and *Munroidendron*). Within the IOB clade, *Polyscias* is paraphyletic with regard to both *Cuphocarpus* and the remaining samples of *Gastonia*. Moreover, both *Cuphocarpus* and *Gastonia* are themselves polyphyletic. On the basis of the molecular phylogenetic reconstruction, we propose eight subgroups within the IOB clade, each of which is geographically coherent, and we describe morphological characters shared by members of each group. To strengthen our earlier proposals for generic circumscriptions and to assess *Polyscias* and its close relatives from beyond the IOB region, future studies should explore the remaining groups within *Polyscias* s.l. (e.g., the “*Arthropphyllum* group” and “section *Polyscias* group”), with a special emphasis on species from Malesia.

Keywords: Araliaceae, *Polyscias*, *Gastonia*, *Cuphocarpus*, Indian Ocean basin, Madagascar.

Introduction

Araliaceae (the ginseng family) is comprised of 41 genera and some 1350–1400 species, most of which are found in the tropical Southern Hemisphere, with centers of species richness in New Caledonia, Australasia, Madagascar, Southeast Asia, and South America (Plunkett et al. 2004; see also Wen et al. 2001). The two largest genera within Araliaceae are the pantropical *Schefflera* J. R. Forst. & G. Forst. and the paleotropical *Polyscias* J. R. Forst. & G. Forst., with ca. 650 (–900) and ca. 150 species, respectively. Together, these two genera account for more than half the species diversity in the family. Recent studies indicate, however, that neither genus is monophyletic (Plunkett et al. 2001, 2004; Wen et al. 2001). Because these two cases of nonmonophyly involve nearly every major clade in Araliaceae, it is clear that intergeneric relationships in the family cannot be understood until infrageneric phylogenies of both *Schefflera* and *Polyscias* are completed. Several studies have already been conducted on some members of *Polyscias* (Eibl et al. 2001; Plunkett et al. 2001), and an initial analysis of *Schefflera* is now in preparation. This study focuses on a group of species in *Polyscias* that occurs in the Indian Ocean basin (IOB), including Madagascar, the Mascarene and Comoro archipelagos, and adjacent continental Africa.

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Polyscias, as traditionally defined, includes all araliads with imparipinnate leaves, panicles of umbels, articulated pedicels, and five- to eight-merous flowers (Bentham 1867; Seemann 1868; Hutchinson 1967). This definition has been broadened over the past several decades to include all taxa with imparipinnate leaves and articulated pedicels (Stone 1965a, 1965b; Smith and Stone 1968; Bernardi 1971; Philipson 1978, 1979; Lowry 1989), which has necessitated the inclusion of several other genera (namely, *Bonnierella* R. Vig., *Botryopanax* Hutch., *Eupteron* Miq., *Gelibia* Hutch., *Kissodendron* Seem., *Nothopanax* Miq., *Palmervandenbroekia* Gibbs, *Sciadopanax* Seem., and *Tieghemopanax* R. Vig.). As currently circumscribed, *Polyscias* ranges across the South Pacific (from Tahiti to Fiji, New Caledonia, and neighboring island groups), extending through Australasia and Malesia and then into the IOB and Africa, reaching its western limit in Sao Tome and Principe. A broad phylogenetic study of *Polyscias* and its close relatives showed that as many as nine currently recognized genera (*Arthropphyllum* Blume, *Gastonia* Comm. ex Lam., *Munroidendron* Sherff, *Meryta* Forst., *Polyscias*, *Pseudopanax* K. Koch, *Reynoldsia* A. Gray, *Schefflera pro parte*, and *Tieghemopanax* R. Vig.) are included in a large clade referred to as “*Polyscias* s.l.” (Plunkett et al. 2001). Within this clade, eight informal groups were recognized: the *Tieghemopanax* group, *Tetraplasandra* group, *Arthropphyllum* group, *Meryta* group, section *Polyscias* group, *Polyscias tennantii* group, *Gastonia* group, and *Polyscias fulva* group. The last three groups in this list are largely or exclusively comprised of taxa

from the IOB and, in turn, are most closely related to the Malesian and Polynesian groups. The broader paraphyly of *Polyscias* s.l. is mirrored even within the IOB groups, which include members from two additional genera, *Gastonia* and *Cuphocarpus* (Wen et al. 2001; Plunkett et al. 2004). However, sampling of IOB *Polyscias* species by Plunkett et al. (2001) and in other recent studies has been rather limited, including no more than 12 of the 40 described species. Recent intensive botanical exploration and collecting, especially in Madagascar, has resulted in the discovery of many additional species of *Polyscias*, and we conservatively estimate that there are no fewer than 80 species of this genus from the IOB. Given these remarkable discoveries, together with the preliminary results suggested by our earlier work on *Polyscias*, we provide a more detailed analysis using both a broader set of IOB taxa and data from an additional molecular marker.

In our earlier study, we collected ITS sequence data because this marker had been successfully applied to phylogenetic studies in both Araliaceae and Apiaceae (Wen and Zimmer 1996; Mitchell and Wagstaff 1997; Downie et al. 1998; Katz-Downie et al. 1999; Wen et al. 2001). Its utility has also been established in a study that focused on another subclade of *Polyscias*, the *Tieghemopanax* group (Eibl et al. 2001). Sequences from the *trnL-trnF* region have been less widely used to date, but they were helpful in providing a complement to ITS data in both the study of *Tieghemopanax* (Eibl et al. 2001) and the broad reassessment of relationships within Araliaceae (Plunkett et al. 2004). In these studies, *trnL-trnF* data yielded only low to moderate levels of variability. However, those characters that were variable were also highly consistent and phylogenetically informative, helping to resolve relationships and refine the placement of problematic taxa where ITS data proved equivocal. Using these two molecular markers, we herein employ a much expanded sample that represented 74 species to test the relationships indicated by the earlier ITS studies (Plunkett et al. 2001, 2004; Wen et al. 2001), with a particular emphasis on the placement of the IOB group of *Polyscias* within the broader *Polyscias* s.l. clade and the relationships among the species within this group.

Material and Methods

From the 83 plant accessions used in this study (table 1), two 79-taxon data matrices were assembled for both ITS and *trnL-trnF* sequences, representing a total of 74 species (in 14 genera) for each matrix. This sample included 41 accessions of *Polyscias* native to the IOB, together with nine accessions from two additional IOB araloid genera (*Gastonia* and *Cuphocarpus*). Of the 33 remaining accessions, 29 represent the other major clades of *Polyscias* s.l. (as defined by Plunkett et al. [2001]) and the broader *Polyscias-Pseudopanax* and *Aralia* groups (*sensu* Plunkett et al. 2004). The final four samples (*Delarobrea paradoxa*, *Myodocarpus fraxinifolius*, *Apiopetalum velutinum*, and *Mackinlaya macrosciadea*) were included for outgroup comparison on the basis of earlier studies (Plunkett and Lowry 2001), which demonstrated that these taxa fall within the order Apiales but outside the core group of Araliaceae.

Total DNA was extracted from leaf tissue preserved in silica gel and/or CTAB salt solution using Doyle and Doyle's (1987) method as modified by Soltis et al. (1991) or using the DNeasy Plant Mini Kit (QIAGEN, Valencia, Calif.). For each sample, nuclear ITS and plastid *trnL-trnF* sequences were PCR amplified following the protocols described by Plunkett et al. (2004). The entire ITS region, including ITS1, the 5.8S coding region, and ITS2, was amplified using the forward primer ITS5 and the reverse primer C26A, sometimes in combination with internal primers ITS3 and N5.8S-r (White et al. 1990; Downie and Katz-Downie 1996; Wen and Zimmer 1996; Plunkett et al. 2004). For *trnL-trnF*, primers c and f, sometimes in combination with primers d and e (Taberlet et al. 1991), were used to obtain the region spanning the *trnL* intron and the *trnL-trnF* intergenic spacer. PCR products were cleaned using the QIAquick PCR Cleanup kit (QIAGEN) and sequenced directly using cycle-sequencing reactions and automated sequencing, following the protocols described by Chandler and Plunkett (2004) and Plunkett et al. (2004).

Raw sequence data were assembled, edited, and aligned visually using Sequencher (version 4.1, GeneCodes). Gaps were treated as missing data, and the data sets were analyzed with maximum parsimony (MP) using PAUP* (version 4; Swofford 2002). Each MP analysis was performed using random addition with 1000 replicates and tree-bisection-reconnection (TBR) branch swapping. Preliminary parsimony searches of the ITS and combined data sets yielded many thousands of trees due to the presence of several large polytomies. Because computer memory was exhausted by such searches, we followed the strategy outlined by Plunkett et al. (2001, 2004) to increase efficiency in exploring tree space. In this approach, an initial search of 1000 replicates (with random addition, ACCTRAN optimization, and MULPARS in effect) was executed, saving no more than 100 trees per replicate. The resulting trees were loaded as starting trees for a second analysis, setting a MAXTREE limit of 50,000 and swapping to completion. The strict consensus of the second analysis was then loaded as a topological constraint for an additional series of searches (1000 replicates, aborting each replicate after finding 1000 suboptimal trees) and saving only those shortest trees not agreeing with the constraint (however, no additional topologies were found). Bootstrap analyses (Felsenstein 1985) were performed with 1000 replicates, using full heuristic searches and TBR branch swapping and saving no more than 1000 trees per replicate. Congruence between the separate data sets was assessed both by visual inspection of resulting trees and by use of the incongruence length difference (ILD) test of Farris et al. (1995), implemented as the partition heterogeneity test in PAUP*.

Results

The ITS data matrix, which included sequence data from the noncoding ITS1 and ITS2 regions and the intervening 5.8S coding region, varied in length from 604 to 623 bp across the 79 taxa sampled. Alignment of these sequences required the addition of ca. 79 insertion/deletion (indel) gaps, most of which were a single base pair long but ranged up to 8 bp. The aligned length of the matrix was 665 bp, of which

204 bp (30.1%) were potentially parsimony informative, 334 bp (50.2%) were invariable, and the remaining 127 bp (19.1%) were variable in only a single taxon. The nucleotide composition was A = 19.1%, C = 36.7%, G = 25.1%, and T = 19.1%. A single ITS indel was coded as a binary character and added to the data matrix.

The *trnL-trnF* data matrix represents the entire length of the *trnL* intron and the *trnL-trnF* intergenic spacer (plus flanking sequences from the adjacent coding regions), ranging in length from 878 to 926 bp. Alignment of these sequences required the addition of ca. 44 indel gaps, ranging from 1 to 23 bp long. The aligned length used in the analyses was 1004 bp long, of which 840 bp (83.6%) were invariable, 84 bp (8.4%) were potentially parsimony informative, and the remaining 80 bp (8.0%) were variable in a single taxon. The nucleotide composition was A = 33.6%, C = 18.6%, G = 17.5%, and T = 30.2%. Four *trnL-trnF* indels were coded as binary characters and included in the data matrix.

The average rate of sequence evolution (based on Kimura two-parameter pairwise distances) across all taxa in the ITS data set (6.6%) was 5.5 times greater than that of the *trnL-trnF* data set (1.2%). For ITS, the highest level of variation (29.5%) was found between *Polyscias repanda* and the outgroup taxon *Mackinlaya macrosciadea*. The greatest divergence among taxa currently assigned to the *Polyscias* s.l. group was 11.3%, between *Polyscias macgillivrayi* and *Munroidendron racemosum*. Among the IOB *Polyscias*, the greatest distance was between *P. repanda* and *Cuphocarpus briquetianus* (8.3%). Four sets of accessions were found to have identical sequences: *Polyscias multibracteata* versus *Polyscias andrearum* (Lowry-3899), *C. briquetianus* versus *Cuphocarpus humbertii*, and between both samples of *Polyscias tennantii* and of *Cuphocarpus aculeatus*.

Across all taxa in the *trnL-trnF* data set, the greatest variation (7.8%) was between two outgroup taxa, *Myodocarpus fraxinifolius* and *M. macrosciadea*. Among the taxa representing *Polyscias* s.l., the greatest divergence was 2.7%, between *Polyscias australiana* and *C. briquetianus*. Considering only IOB taxa, the highest level of variation was found between *Polyscias "schatzii"* and *C. briquetianus* (1.8%). The lowest level of variation among all taxa in the *trnL-trnF* data set was identity, found in 47 different pairs of sequences (all within *Polyscias* s.l., of which 39 pairs represented IOB taxa).

Cladistic analysis of the ITS data set produced 50,000 most parsimonious (MP) trees of 817 steps (fig. 1). Excluding uninformative characters, the Consistency Index (CI) was 0.512, and the Retention Index (RI) was 0.730. Cladistic analysis of the *trnL-trnF* data matrix produced 984 MP trees of 212 steps long with a CI of 0.811 and a RI of 0.918 (fig. 2). Although the *trnL-trnF* data set provided relatively few variable characters, it supplied synapomorphies that helped to resolve branches that were poorly supported or unresolved using ITS data alone. In addition, the trees based on *trnL-trnF* sequences had higher CI and RI values than those generated from the ITS data, a fact that reflects the low number of homoplasious characters in *trnL-trnF*. Thus, the two data sets are highly complementary: the more rapidly evolving ITS data set provides greater resolution (but has higher levels of homoplasy), whereas the *trnL-trnF* data set provides more consistent characters (but yields less resolution).

Results from the partition heterogeneity test ($P = 0.006$) suggest that the separate data may be heterogeneous. However, the ILD test does not take into account relative strength of incongruent clades, and we note that visual inspection of the cladograms resulting from the separate analyses reveals highly similar topologies. With the exception of the placement of *Polyscias "andapensis,"* all other cases of topological differences are restricted to weakly supported clades (bootstrap [BS] < 50%). For this reason, we have analyzed the combined data set, following the "total evidence" approach of Kluge (1989; see also Wenzel 1997; Wenzel and Siddall 1999). Trees based on the separate analyses (figs. 1, 2) are also provided for those who wish to take a more conservative approach.

The combined analysis produced 41,721 MP trees, each of 1049 steps. The CI (0.546) and RI (0.754) values of these trees were greater than those based on ITS data alone, reflecting the more consistent characters derived from *trnL-trnF*. The strict consensus tree (fig. 3) confirms the paraphyly of *Polyscias* (in its current circumscription) but the monophyly of *Polyscias* s.l. We recognize four larger clades among the non-IOB species of *Polyscias* s.l., all with moderate to strong BS support: the section *Polyscias* group (BS = 100%, but as sister to the *Meryta* group, BS < 50%), the *Arthrophyllum* group (BS = 76%), the *Tieghemopanax* group (BS = 98%), and a combined *Tetraplasandra* + New Guinea clade (BS = 95%). The IOB species form a monophyletic group, but support is weak (BS < 50%). Support is considerably stronger for a larger clade that unites IOB *Polyscias* to the New Guinea + *Tetraplasandra* groups (BS = 84%). Within the IOB clade, *Polyscias pentamera* is sister to a trichotomy, comprising all of the remaining species. This trichotomy includes the *Polyscias fulva* group (BS = 80%), the *Cuphocarpus* p.p. + Mascarene Islands groups (BS < 50% but 99% and 96%, respectively, for the two subclades), and a large polytomy of 33 samples (BS < 50%).

Discussion

On the basis of ITS data from 12 exemplar taxa, Plunkett et al. (2001) proposed three groups comprising species from the IOB region (the *Polyscias tennantii* group, *Polyscias fulva* group, and *Gastonia* group), which together formed a single large clade. To expand the sampling of *Polyscias* in this geographical area and to test the monophyly of the IOB clade, this study included 29 newly sequenced accessions from among the ca. 80 species of *Polyscias* now known from this region, along with six representatives of two closely related genera, *Gastonia* (from which three of the seven species from the region were sampled) and the Malagasy endemic *Cuphocarpus* (from which three of the five species were analyzed). Parsimony analysis of the combined data set confirms only one of the three groups recognized in the initial ITS study of *Polyscias* s.l. (Plunkett et al. 2001), the *P. fulva* group. The *P. tennantii* group no longer appears in these expanded trees, and with the addition of five species from the Mascarene Islands, the composition of the original *Gastonia* group has changed so significantly that we hereby refer to it as the "Mascarene Islands group" to reflect the geographic range of its members. On the basis of the separate and combined

Table 1

Species Names for the DNA Accessions Used in This Study, with Native Range, Source, and Voucher Information

Taxon	Native range	Source and voucher/accession no.	GenBank accession no.
Indian Ocean basin <i>Polyscias</i> :			
<i>Polyscias "abrahamiana"</i> Lowry, ined.	Madagascar	Andasibe, Madagascar (<i>Labat</i> 3065, P)	ITS: AF229686, <i>trnL-trnF</i> : AF382148
<i>Polyscias albersiana</i> Harms	Tanzania	W Usambara Mts., Tanzania (<i>Lowry</i> 5153, MO)	ITS: AF229687, <i>trnL-trnF</i> : AF393883
<i>Polyscias amplifolia</i> Harms	Madagascar	Ambodisatrana, Madagascar (<i>McPherson</i> 17212, MO)	ITS: AF393851, <i>trnL-trnF</i> : AF393884
<i>Polyscias "andapensis"</i> Lowry, ined.	Madagascar	Anjanaharibe-Sud Reserve, Madagascar (<i>McPherson</i> 17269, MO)	ITS: AF393852, <i>trnL-trnF</i> : AF393885
<i>Polyscias andrearum</i> Bernardi	Madagascar	Ankirindro, Madagascar (<i>Schatz</i> 3899, MO)	ITS: AF393853, <i>trnL-trnF</i> : AF393886
<i>P. andrearum</i> Bernardi	Madagascar	Ambodisatrana, Madagascar (<i>McPherson</i> 17200, MO)	ITS: AF393854, <i>trnL-trnF</i> : AF393887
<i>Polyscias "anjozorobeensis"</i> Lowry, ined.	Madagascar	Anjozorobe, Madagascar (<i>Lowry</i> 4368, MO)	ITS: AF393855, <i>trnL-trnF</i> : AF393888
<i>Polyscias ariadnes</i> Bernardi	Madagascar	Ambodisatrana, Madagascar (<i>McPherson</i> 17161, MO)	ITS: AF393856, <i>trnL-trnF</i> : AF393889
<i>Polyscias aubrevillei</i> (Bernardi) Bernardi	Madagascar	Marojejy Natl. Park, Madagascar (<i>Morat</i> 4056, P)	ITS: AF393857, <i>trnL-trnF</i> : AF393890
<i>Polyscias baretiana</i> Bernardi	Madagascar	Masoala Peninsula, Madagascar (<i>Aridy</i> 281, MO)	ITS: AF229690, <i>trnL-trnF</i> : AF382149
<i>Polyscias boivimii</i> (Seem.) Bernardi	Madagascar	Ihosal, Madagascar (<i>Haeuermans</i> 204, P)	ITS: AY430380, <i>trnL-trnF</i> : AY430391
<i>Polyscias carolorum</i> Bernardi	Madagascar	Anjambalava, Madagascar (<i>Lowry</i> 5181, MO)	ITS: AF393880, <i>trnL-trnF</i> : AY430392
<i>Polyscias coriacea</i> W. Marais	Réunion	Plaine des Palmistes, Réunion (<i>Lowry</i> 4979, MO)	ITS: AF393859, <i>trnL-trnF</i> : AF393892
<i>Polyscias dichroostachya</i> Baker	Mauritius	Corps de Garde Mt., Mauritius (<i>Florens-Herb.</i> MAU 23405, MO)	ITS: AY430381, <i>trnL-trnF</i> : AY430393
<i>Polyscias farinosa</i> Harms	Ethiopia	Simean Mts., Ethiopia (<i>Puff</i> 990511, W)	ITS: AY430382, <i>trnL-trnF</i> : AY430394
<i>Polyscias felicitis</i> Bernardi	Comoro Islands	Forêt de la Grille, Grande Comore, Comoro Islands (<i>Labat</i> 3092, P)	ITS: AF393860, <i>trnL-trnF</i> : AF393893
<i>Polyscias fraxinifolia</i> Philipson	Madagascar	Zahamena Natl. Park, Madagascar (<i>Andrianjafy</i> 272, MO)	ITS: AF393861, <i>trnL-trnF</i> : AF393894
<i>Polyscias fulva</i> (Hieron.) Harms	Tropical Africa	Bvumba Mts., Zimbabwe (<i>Lowry</i> 4806, MO)	ITS: AF229699, <i>trnL-trnF</i> : AF382150
<i>Polyscias "longipedunculata"</i> Lowry, ined.	Madagascar	Orangéa, Madagascar (<i>Service Forestier</i> 27653, P)	ITS: AF393862, <i>trnL-trnF</i> : AF393895
<i>Polyscias "lucienneae"</i> Lowry, ined.	Madagascar	Analamazaotra-Perinet Reserve, Madagascar (<i>Dorr</i> 4343, MO)	ITS: AF393863, <i>trnL-trnF</i> : AF393896
<i>Polyscias madagascariensis</i> Harms	Madagascar	Zahamena Natl. Park, Madagascar (<i>Ratovoson</i> 176, MO)	ITS: AF393864, <i>trnL-trnF</i> : AF393897
<i>P. madagascariensis</i> Harms	Madagascar	Antoetra, Madagascar (<i>Jongkind</i> 884, MO)	ITS: AF393865, <i>trnL-trnF</i> : AF393898
<i>Polyscias "mangindranoensis"</i> Lowry, ined.	Madagascar	Tsaratana Massif, Madagascar (<i>Lowry</i> 5474, MO)	ITS: AY430383, <i>trnL-trnF</i> : AY430395
<i>Polyscias mauritiana</i> Marais	Mauritius	Montagne Cocotte, Mauritius (<i>Florens-Herb.</i> MAU 23406, MO)	ITS: AY430384, <i>trnL-trnF</i> : AY430396
<i>Polyscias mayottensis</i> Lowry, O. Pascal & Labat	Comoro Islands	Saziley, Mayotte, Comoros Islands (<i>Labat</i> 2935, MO)	ITS: AF229703, <i>trnL-trnF</i> : AF393901
<i>Polyscias "modestei"</i> Lowry, ined.	Madagascar	Ankirindro, Madagascar (<i>Schatz</i> 3898, MO)	ITS: AF393867, <i>trnL-trnF</i> : AF393900
<i>Polyscias multibracteata</i> Harms	Madagascar	Zahamena Natl. Park, Madagascar (<i>Rabenantoandro</i> 142, MO)	ITS: AF393868, <i>trnL-trnF</i> : AF393902
<i>Polyscias muraltiana</i> Bernardi	Madagascar	Masoala Peninsula, Madagascar (<i>Rabenantoandro</i> 215, MO)	ITS: AF393869, <i>trnL-trnF</i> : AF393903
<i>Polyscias myrsine</i> Bernardi	Madagascar	Zahamena Natl. Park, Madagascar (<i>Ratovoson</i> 144, MO)	ITS: AF393870, <i>trnL-trnF</i> : AF393904
<i>Polyscias "orientalis"</i> Lowry, ined.	Madagascar	Ankirindro, Madagascar (<i>Schatz</i> 3925, MO)	ITS: AF229708, <i>trnL-trnF</i> : AF393905

Table 1
(Continued)

Taxon	Native range	Source and voucher/accession no.	GenBank accession no.
<i>Polyscias ornifolia</i> Harms	Madagascar	Zahamena Natl. Park, Madagascar (Ratovoson 162, MO)	ITS: AF393871, <i>trnL-trnF</i> : AF393906
<i>Polyscias pentamera</i> (Baker) Harms	Madagascar	Ambatovy, Madagascar (McPherson 17519, MO)	ITS: AF393872, <i>trnL-trnF</i> : AF393907
<i>Polyscias</i> “ratovosonii” Lowry, ined.	Madagascar	Tsaratana Massif, Madagascar (Razamalaka 24, MO)	ITS: AY430385, <i>trnL-trnF</i> : AY430397
<i>Polyscias repanda</i> Baker	Madagascar	Réserve Naturelle de Mare Longue, Réunion (Lowry 4984, MO)	ITS: AF393873, <i>trnL-trnF</i> : AF393908
<i>Polyscias</i> “richardii” Lowry, ined.	Madagascar	Tsaratana Massif, Madagascar (Lowry 5395, MO)	ITS: AY430386, <i>trnL-trnF</i> : AY430398
<i>Polyscias</i> “schatzii” Lowry, ined.	Madagascar	Ankirindro, Madagascar (Schatz 3900, MO)	ITS: AF229714, <i>trnL-trnF</i> : AF393909
<i>Polyscias sessiliflora</i> Marais	Réunion	Col de Bébour, Réunion (Lowry 4981, MO)	ITS: AF229717, <i>trnL-trnF</i> : AF393912
<i>Polyscias stuhlmannii</i> Harms	Tanzania	W. Usambara Mts., Tanzania (Lowry 5152, MO)	ITS: AF393876, <i>trnL-trnF</i> : AF393913
<i>Polyscias tennantii</i> Bernardi	Madagascar	Andasibe, Madagascar (Labat 3074, P)	ITS: AF229720, <i>trnL-trnF</i> : AF393914
<i>P. tennantii</i> Bernardi	Madagascar	Ambatovy, Madagascar (McPherson 17504, MO)	ITS: AF393858, <i>trnL-trnF</i> : AF393891
<i>Polyscias zanthoxyloides</i> Harms	Madagascar	Ambatovy, Madagascar (McPherson 17536, MO)	ITS: AF393877, <i>trnL-trnF</i> : AF393915
Other <i>Polyscias</i> :			
<i>Polyscias australiana</i> (F. Muell.) Philipson	Australia (Queensland), New Guinea	Near Bellenden Ker Park, N Queensland, Australia (Plunkett 1500, MO)	ITS: AF229688, <i>trnL-trnF</i> : AF396410
<i>Polyscias balansae</i> (Baill.) Harms	New Caledonia	Mé Ori, New Caledonia (Lowry 4801, MO)	ITS: AF229689, <i>trnL-trnF</i> : AF382165
<i>Polyscias</i> cf. <i>belensis</i> Philipson	New Guinea	N of Timika, W Papua (New Guinea), Indonesia (Lowry 5269, MO)	ITS: AF393875, <i>trnL-trnF</i> : AF393911
<i>P. cf. belensis</i> Philipson	New Guinea	N of Timika, W Papua (New Guinea), Indonesia (Lowry 5275, MO)	ITS: AF393874, <i>trnL-trnF</i> : AF393910
<i>Polyscias bellendenkerensis</i> (F.M. Bailey) Philipson	Australia (Queensland)	Mt. Bartle Frere, northern Queensland, Australia (Plunkett 1538, MO)	ITS: AF229691, <i>trnL-trnF</i> : AF396411
<i>Polyscias</i> “crenata” (Pancher & Sebert) Lowry, ined.	New Caledonia	Baie de Tina, New Caledonia (Lowry 4664, MO)	ITS: AF229694, <i>trnL-trnF</i> : AF382169
<i>Polyscias</i> “jaffrei” Lowry, ined.	New Caledonia	Kopéto Massif, New Caledonia (Lowry 4740, MO)	ITS: AF229700, <i>trnL-trnF</i> : AF382176
<i>Polyscias</i> “lecardii” (R. Viguier) Lowry, ined.	New Caledonia	Mandjéla, New Caledonia (Lowry 4754, MO)	ITS: AF229701, <i>trnL-trnF</i> : AF382178
<i>Polyscias macgillivrayi</i> (Seem.) Harms	Australia (Queensland), E Malesia, Solomon Islands, Micronesia	Cape Kimberly, northern Queensland, Australia (Plunkett 1536, MO)	ITS: AF229702, <i>trnL-trnF</i> : AF396412
<i>Polyscias mollis</i> (Benth.) Harms	Australia (Queensland)	Bellenden Ker Park, northern Queensland, Australia (Plunkett 1507, MO)	ITS: AF229705, <i>trnL-trnF</i> : AF396413
<i>Polyscias nodosa</i> (Blume) Seem.	Malesia	Cult., Bogor Bot. Gard., Java (Lowry 5286, MO)	ITS: AY430387, <i>trnL-trnF</i> : AY430399
<i>Polyscias</i> “nothisii” Lowry, ined.	New Caledonia	Near Pouembout, New Caledonia (Lowry 4739, MO)	ITS: AF229707, <i>trnL-trnF</i> : AF382180
<i>Polyscias purpurea</i> C.T. White	Australia (Queensland)	Licuala State Forest Park, northern Queensland, Australia (Plunkett 1496, MO)	ITS: AF229711, <i>trnL-trnF</i> : AY430400
<i>Polyscias</i> “regalis” Bernardi ex Lowry, ined.	New Caledonia	Plateau de Dogny, New Caledonia (Lowry 4720, MO)	ITS: AF229712, <i>trnL-trnF</i> : AF382183

Table 1
(Continued)

Taxon	Native range	Source and voucher/accession no.	GenBank accession no.
<i>Polyscias scutellaria</i> (Burm. f.) Fosberg	Unknown, likely Melanesia; widely cult.	Cultivated, Flecker Bot. Gard., Cairnes, Queensland, Australia (<i>Plunkett</i> 1491, MO)	ITS: AF229716, <i>trnL-trnF</i> : AF396414
Other Araliaceae:			
<i>Aralia nudicaulis</i> Linn.	North America	Wen and Zimmer 1996	ITS: U41674, <i>trnL-trnF</i> : AF382157
<i>Arthrophyllum diversifolium</i> Blume	Malesia	Cult., Bogor Bot. Gard., Java (<i>Lowry</i> 5288, MO)	ITS: AY389027, <i>trnL-trnF</i> : AY393708
<i>Arthrophyllum</i> “mackeei” Lowry, ined.	New Caledonia	Haute Yaté, New Caledonia (<i>Lowry</i> 4670, MO)	ITS: AF229736, <i>trnL-trnF</i> : AF382158
<i>Cuphocarpus aculeatus</i> Decne. & Planch.	Madagascar	Sainte Luce, Madagascar (<i>Lowry</i> 5013, MO)	ITS: AF229737, <i>trnL-trnF</i> : AF393920
<i>Cuphocarpus aculeatus</i> Decne. & Planch.	Madagascar	Ambila-Lemaitso, Madagascar (<i>Lowry</i> 5125, MO)	ITS: AF393866, <i>trnL-trnF</i> : AF393899
<i>Cuphocarpus briquetianus</i> Bernardi	Madagascar	Tsaratanana Massif, Madagascar (<i>Lowry</i> 5329, MO)	ITS: AY430378
<i>C. briquetianus</i> Bernardi	Madagascar	Zahamena Natl. Park, Madagascar (<i>Rakotonandrasana</i> 562, MO)	<i>trnL-trnF</i> : AY430388
<i>Cuphocarpus humbertii</i> Bernardi	Madagascar	Marotolana, Madagascar (<i>Ratovoson</i> 479, MO)	ITS: AY430379, <i>trnL-trnF</i> : AY430389
<i>Gastonia cutispongia</i> Lam.	Réunion	Cult., Univ. de la Réunion, Réunion (<i>Lowry</i> 4976, MO)	ITS: AF229722, <i>trnL-trnF</i> : AF382161
<i>Gastonia duplicata</i> Thouars ex Baill.	Madagascar	Masoala Peninsula, Madagascar (<i>Aridy</i> 299, MO)	ITS: AF393878, <i>trnL-trnF</i> : AF382163
<i>Gastonia rodriguesiana</i> Marais	Rodrigues Island	Cultivated, Royal Botanic Garden Kew (acc. 662–86.06150)	ITS: AF229723, <i>trnL-trnF</i> : AF382162
<i>Gastonia spectabilis</i> (Harms) Philipson	New Guinea, Solomon Isl., Australia	N of Timika, West Papua (New Guinea), Indonesia (<i>Lowry</i> 5257, MO)	ITS: AY389036, <i>trnL-trnF</i> : AY430390
<i>Meryta balansae</i> Baill.	New Caledonia	Plateau de Dogny, New Caledonia (<i>Lowry</i> 4733, MO)	ITS: AF396417, <i>trnL-trnF</i> : AF382160
<i>Meryta</i> “ <i>pedunculata</i> ” Lowry, ined.	New Caledonia	Rivière Bleue Park, New Caledonia (<i>Lowry</i> 4756, MO)	ITS: AF396419, <i>trnL-trnF</i> : AF382159
<i>Munroidendron racemosum</i> (C.N. Forbes) Sherff	Hawaii	Cultivated, Missouri Bot. Gard. (<i>Plunkett</i> 1342, WS)	ITS: AF229738
<i>M. racemosum</i> (C.N. Forbes) Sherff	Hawaii	Natl. Trop. Bot. Gard. (<i>Lorence</i> 7628, PTBG)	<i>trnL-trnF</i> : AY393743
<i>Reynoldsia sandwicensis</i> A. Gray	Hawaii	Cult., Honolulu Bot. Gard., Hawaii (<i>Plunkett</i> 1359, WS)	ITS: AF229739, <i>trnL-trnF</i> : AY393765
<i>Schefflera bailloni</i> R. Vig.	New Caledonia	Roches d’Ouaième, New Caledonia (<i>Lowry</i> 4782, MO)	ITS: AF396420, <i>trnL-trnF</i> : AF396415
<i>Schefflera elegantissima</i> (Veitch ex Masters) Lowry & Frodin	New Caledonia; widely cult.	Mt. Mou, New Caledonia (<i>Lowry</i> 4715, MO)	ITS: AF229730, <i>trnL-trnF</i> : AF396416
<i>Schefflera gabriellae</i> Baill.	New Caledonia	Mt. Dzumac, New Caledonia (<i>Lowry</i> 4648, MO)	ITS: AF229731
<i>Schefflera gabriellae</i> Baill.	New Caledonia	Katrikoin, New Caledonia (<i>Lowry</i> 4792, MO)	<i>trnL-trnF</i> : AY393769
<i>Tetraplasandra oahuensis</i> Harms	Hawaii	Cult., Natl. Trop. Bot. Gard. (<i>Lorence</i> 81588, 710053.001, PTBG)	ITS: AF396421, <i>trnL-trnF</i> : AF393921
Outgroup:			
<i>Apiopetalum velutinum</i> Baill.	New Caledonia	Mt. Mou, New Caledonia (<i>Lowry</i> 4700, MO)	ITS: AF229742, <i>trnL-trnF</i> : AY393698
<i>Delarbrea paradoxa</i> Vieill. ssp. <i>paradoxa</i>	Malesia, Solomon Isl., Vanuatu, New Caledonia, Norfolk Isl.	Ponandou R., New Caledonia (<i>Lowry</i> 4766, MO)	ITS: AF229750

Table 1
(Continued)

Taxon	Native range	Source and voucher/accession no.	GenBank accession no.
<i>Delarbrea paradoxa</i> Vieill. ssp. <i>paradoxa</i>	Malesia, Solomon Isl., Vanuatu, New Caledonia, Norfolk Isl.	Katrikoin, New Caledonia (<i>Lowry</i> 4791, MO)	<i>trnL-trnF</i> : AF382152
<i>Mackinlaya macrosciadea</i> (F. Muell.) F. Muell.	Australia (Queensland)	Cultivated, Huntington Bot. Gard. (<i>Plunkett</i> 1365, WS)	ITS: AF229744, <i>trnL-trnF</i> : AY393701
<i>Myodocarpus fraxinifolius</i> Brongn. & Gris	New Caledonia	Mé Ori, New Caledonia (<i>Lowry</i> 4803, MO)	ITS: AY389026, <i>trnL-trnF</i> : AY393702

Note. Names and combinations in quotation marks indicated as “ined.” will be published elsewhere. Herbarium acronyms follow Holmgren et al. (1990).

analyses of the two data sets, this study provides clear evidence to support the interpretation of Plunkett et al. (2001) that *Polyscias*, as currently circumscribed, is paraphyletic. Increased sampling also provides greater insights into the relationships involving *Gastonia*, which appears to be polyphyletic. Its members are found in three distinct clades, with three species belonging to the IOB clade (*Gastonia cutispongia* and *Gastonia rodriguesiana* in the Mascarene Islands group and *Gastonia duplicata* in an unresolved position) and a fourth species, *Gastonia spectabilis* from Australasia, in the New Guinea group (i.e., outside the main IOB *Polyscias* clade). Our results further indicate that *Cuphocarpus* is also polyphyletic within the IOB clade (figs. 1–3). To evaluate the phylogenetic and evolutionary implications of our findings, we discuss each of the major subgroups within the IOB *Polyscias* clade separately.

The Status of *Gastonia*

Philipson (1970) redefined *Gastonia* and its presumed close relatives (*Indokingia* Hemsl., *Peekeliopanax* Harms, *Munroidendron*, *Reynoldsia*, and *Tetraplasandra*) to include all araliads with imparipinnate leaves that lack an articulation between the pedicel and the flower (although several species of *Polyscias* from Madagascar and the Mascarenes likewise have unarticulated pedicels; cf. Bernardi 1971, 1980). Although these genera appear to be morphologically coherent, Philipson (1970) suggested that they could be divided into two groups: (1) a Polynesian endemic group, which includes *Munroidendron*, *Reynoldsia*, and *Tetraplasandra*, and (2) *Gastonia*, which represents all species from the Solomon Islands westward to Madagascar and the Comoro Islands, within which he included the two monotypic genera *Peekeliopanax* and *Indokingia*. Philipson (1970) distinguished these two groups on the basis of differences in their fruits. Radiating stigmatic arms characterize the species of *Gastonia*, whereas members of the Polynesian group have styles that are fused to form a conical stylopodium. Molecular evidence supporting this distinction was recently published by Costello and Motley (2001), who examined the three Polynesian genera using data from ITS, the 5S spacer, and morphology (in particular, ovary position) to reconstruct their phylogeny. They also included *G. rodriguesiana* (an endemic

to the island of Rodrigues in the Mascarene archipelago) in their study, which was used as the sole outgroup. This sampling strategy did not allow Costello and Motley (2001) to assess the monophyly of *Gastonia* or its relationship to either the *Tetraplasandra* group or *Polyscias* s.l. However, in an unpublished study with increased sampling, A. Costello and T. J. Motley (personal communications) have independently demonstrated the nonmonophyly of *Gastonia*, with species currently assigned to *Gastonia* and *Reynoldsia* forming a paraphyletic group. Our study, however, with a much greater sampling of both *Polyscias* (especially in the IOB but also throughout its range) and *Gastonia*, suggests that *Gastonia* is not paraphyletic with regard to the *Tetraplasandra* group but rather broadly polyphyletic within *Polyscias* s.l. Two newly sequenced taxa from New Guinea (*G. spectabilis* and *Polyscias* cf. *belensis*) form a subclade that is sister to the *Tetraplasandra* group, but the Mascarene species of *Gastonia* (*G. rodriguesiana* and *G. cutispongia*) plus *G. duplicata* from Madagascar and the Comoros clearly belong to two different subclades within the IOB group of *Polyscias*. This indicates that only the Malesian/Pacific species of *Gastonia* represent appropriate outgroups for the *Tetraplasandra* group. Overall, the discrepancies between our results and those of Costello and Motley (2001; A. Costello and T. J. Motley, unpublished data) are minor and probably result from sampling differences and the choice of outgroups. Although we have now sampled four of the nine species of *Gastonia*, the paucity of samples from *Polyscias* (and its other close relatives) from Malesia represents a serious limitation for understanding the relationship between the Pacific taxa and the IOB group. A combined analysis building on the strengths of Costello and Motley's (2001; A. Costello and T. J. Motley, unpublished data) work on the *Tetraplasandra* group and our analysis from throughout *Polyscias* s.l., together with increased sampling of taxa from Malesia, will eventually be required to resolve the full extent of the polyphyly in *Gastonia*.

Cuphocarpus

The Malagasy endemic genus *Cuphocarpus* is distinguished from *Polyscias* and other related genera by the presence of a unilocular gynoeium (Bernardi 1971, 1980), although *Cuphocarpus* otherwise resembles these groups. In our analyses,

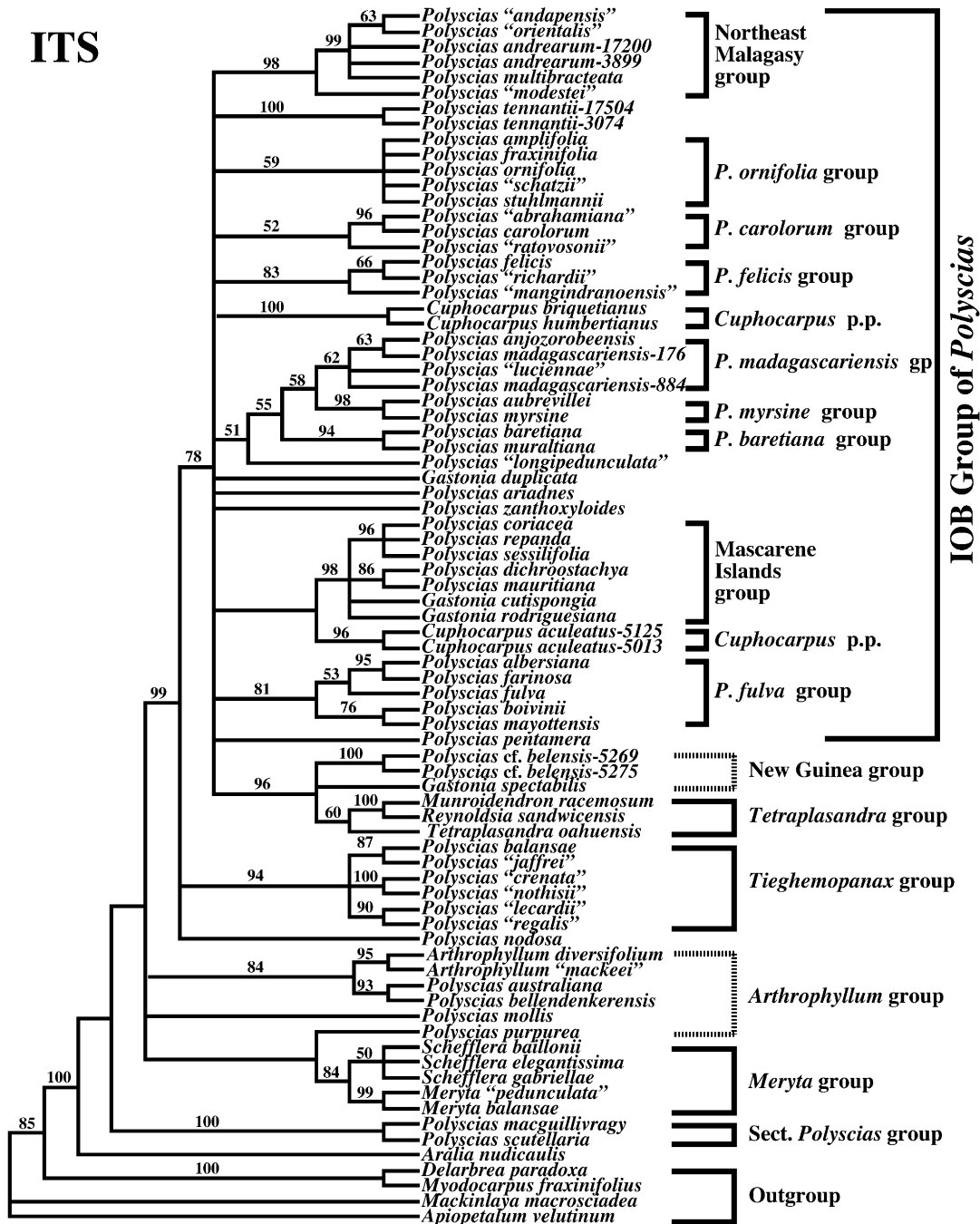


Fig. 1 Strict consensus of 50,000 shortest trees resulting from the parsimony analysis of 79 ITS sequences; tree length = 817 steps; Consistency Index = 0.512; Retention Index = 0.730. Bootstrap values of 50% or greater are provided above the nodes.

Cuphocarpus clearly falls within the IOB *Polyscias* clade (figs. 1–3), indicating that it does not warrant recognition as a distinct genus. Moreover, as currently circumscribed, *Cuphocarpus* appears to be polyphyletic. The widespread eastern coastal species *Cuphocarpus aculeatus* occupies a position sister to the Mascarene Islands group, whereas the two montane species sampled (*Cuphocarpus briquetianus* and *Cuphocarpus humbertianus*) are placed in a well-separated (and

highly supported) subclade. Morphologically, *C. aculeatus* differs from other members of *Cuphocarpus* in having sessile flowers arranged in spikes (vs. pedicellate flowers in umbels), larger leaves, and a much more robust habit. Furthermore, *C. aculeatus* usually occupies open, disturbed habitats at low elevation, whereas the other species grow only in montane primary forests. We have not been able to sample the two remaining species, *Cuphocarpus commersonii* and *Cuphocar-*

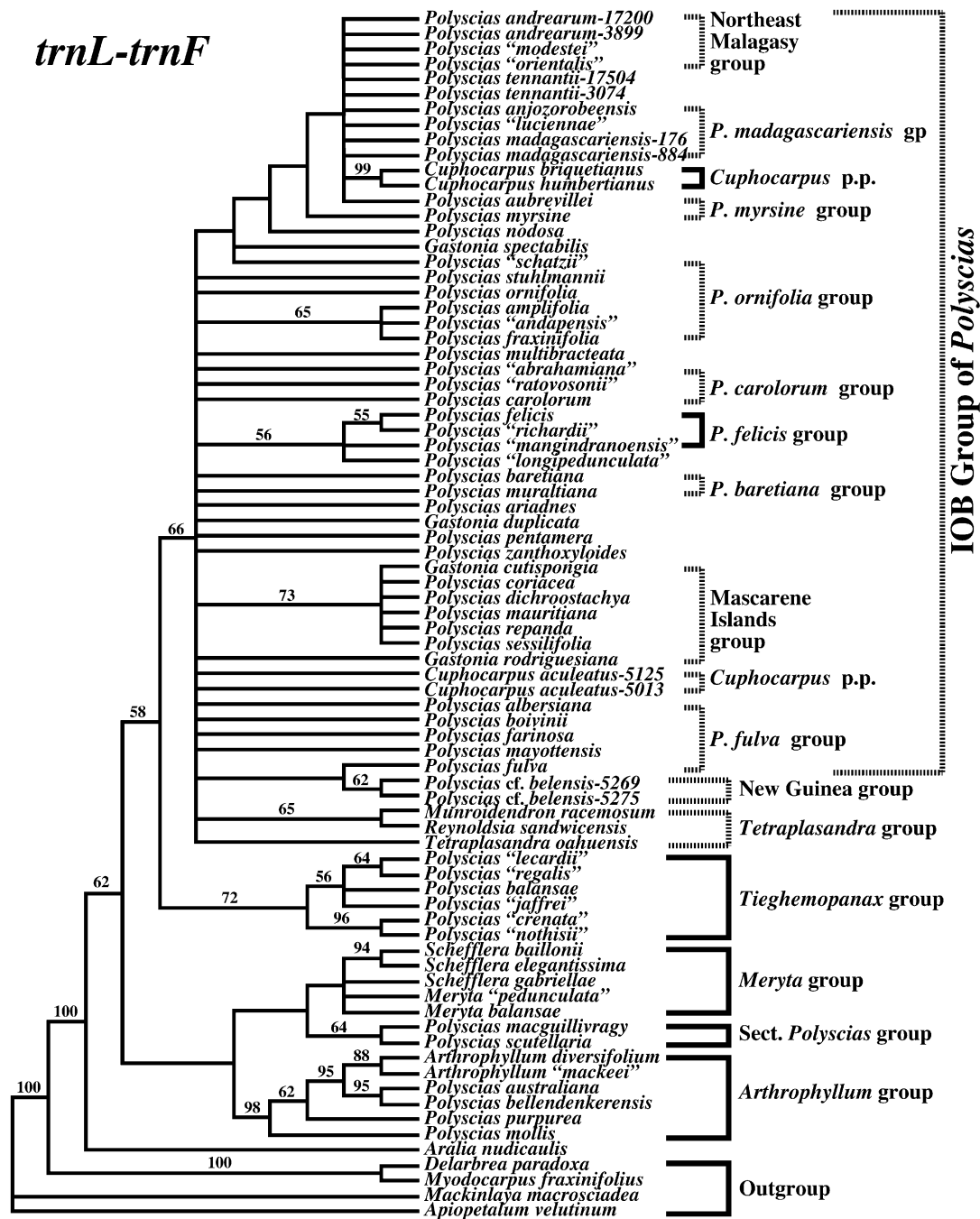


Fig. 2 Strict consensus of 984 shortest trees resulting from the parsimony analysis of 79 *trnL-trnF* sequences; tree length = 212 steps; Consistency Index = 0.811; Retention Index = 0.918. Bootstrap values of 50% or greater are provided above the nodes.

pus leandrianus, but considering their morphologies (which closely resemble the two upland species included in our study) and their distributions (which are also restricted to high-elevation sites), these two taxa probably belong to the montane *Cuphocarpus* group.

Northeast Malagasy Polyscias Group

Members of this strongly supported group (BS = 94%; fig. 3), which comprises *Polyscias* “*andapensis*,” *Polyscias*

multibracteata, *Polyscias andrearum*, *Polyscias* “*orientalis*,” and *Polyscias* “*modestei*,” are all small trees whose distribution is restricted to the humid forests of northeastern Madagascar, an area known for its high concentration of local endemism (G. Schatz and P. Lowry, unpublished data). These species all share relatively short terminal inflorescences and styles that are strongly recurved in fruit, with the exception *P. multibracteata*, which has larger inflorescences. Within the northeast Malagasy group, *P. andapensis* and *P. modestei*

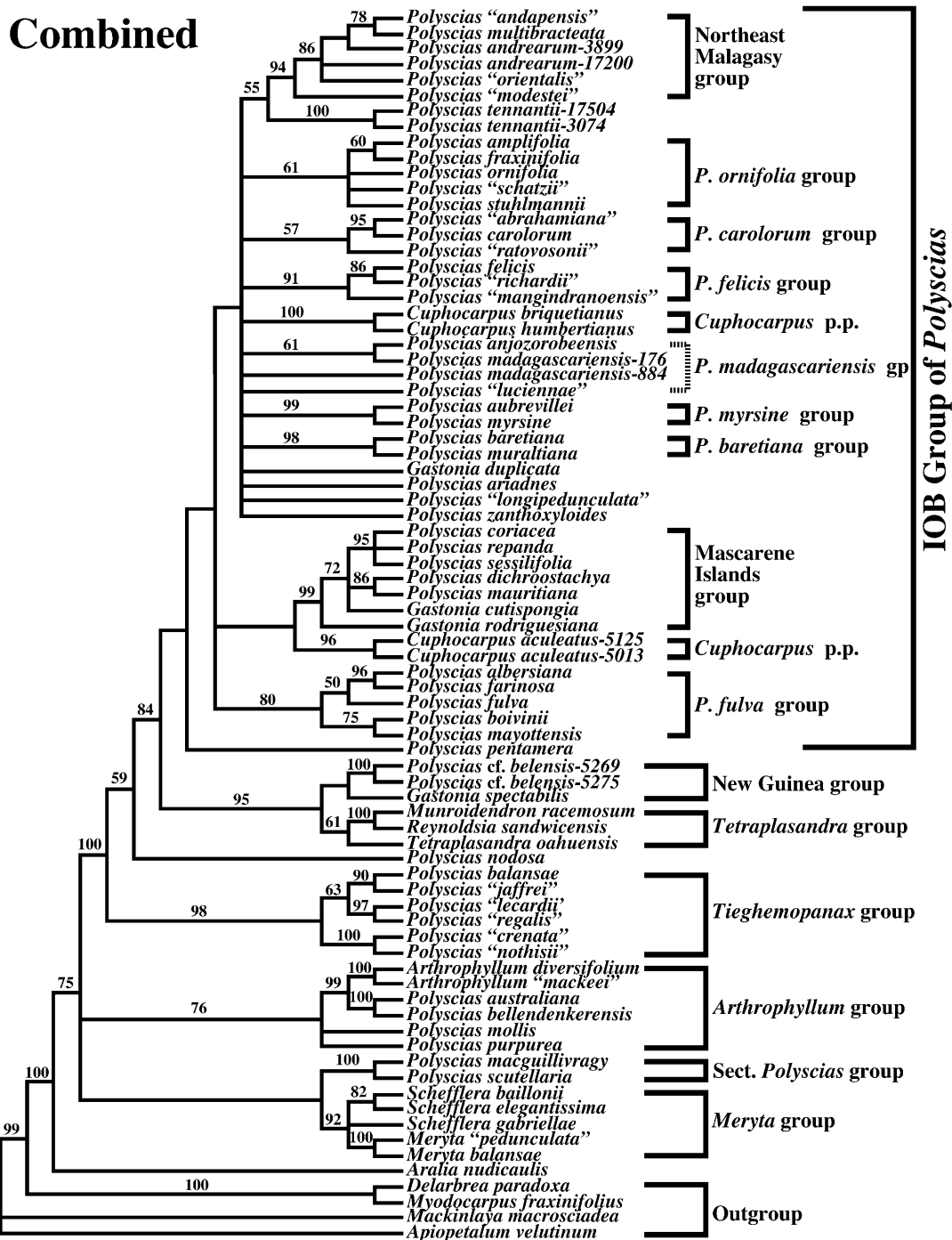


Fig. 3 Strict consensus of 41,721 shortest trees resulting from the parsimony of the combined analysis; tree length = 1049 steps; Consistency Index = 0.546; Retention Index = 0.754. Bootstrap values of 50% or greater are provided above the nodes.

have uni- to trifoliolate leaves, whereas *P. "orientalis"* has (1-)3-7 leaflets, *P. andrearum* has 7-9, and *P. multibracteata* has 13-25. *Polyscias "andapensis"* is also distinctive in having a bicarpellate gynoeceium, whereas *P. andrearum* has 2(-3) carpels, *P. multibracteata* has 3-5 carpels, and *P. "orientalis"* and *P. "modestei"* have 5 carpels.

Polyscias felicis Group

This well-supported clade (BS = 91%; fig. 3) includes two species known only from the Tsaratanana massif in northern Madagascar (*Polyscias "richardii"* and *Polyscias "mangindranoensis"*) and one from the Comoro Islands (*Polyscias felicis*). Their inflorescences are very similar in morphology

and organization, with long secondary axes bearing numerous slender umbellules along most of their length. However, each species has very distinctive leaves: *P. felicis* has small, ovate leaflets with an acuminate apex, but in *P. "mangindra-noensis,"* the leaflets are larger and elliptic with a rounded apex, and in *P. "richardii,"* they are very large and obovate, likewise with a rounded apex.

Polyscias myrsine Group

This group (BS = 99%; fig. 3) is comprised of two species (*Polyscias aubrevillei* and *Polyscias myrsine*) that are quite distinct from one another morphologically. *Polyscias aubrevillei* is a small treelet growing at high-elevation sites in northern Madagascar, whereas *P. myrsine* occurs throughout much of the humid part of the island, usually at low to mid-elevations, and is a larger tree (8–15 m tall). *Polyscias aubrevillei* has an exclusively bicarpellate gynoeceium and leaves with (5–)7–9 small, emarginate leaflets that are 2–3 cm long; *P. myrsine* has 3–4 carpels and leaves with 13–19 acute to rounded leaflets that are 10–12 cm long. Despite these morphological and ecological differences, however, a close relationship between these two species was indicated in both the ITS and combined analyses (figs. 1, 3).

Polyscias baretiana Group

The two species in this group (*Polyscias muraltiana* and *Polyscias baretiana*) form a well-defined species pair (BS = 98%; fig. 3). Both share a number of features and are found only in low- to midelevation humid forests in northeastern Madagascar. The two species are both shrubs or treelets 1–5 m tall and have slender inflorescences bearing flowers with pubescent pedicel articulations, although in *P. baretiana*, the rim formed by the articulation has minute bractlets, whereas these structures are lacking in *P. muraltiana*. The venation pattern of the leaves appears to be very similar in both species, but *P. baretiana* has 7–9 leaflets, whereas *P. muraltiana* has exclusively unifoliolate leaves. The ovaries of *P. baretiana* have (3–)4 carpels, but those of *P. muraltiana* have (4–)5 carpels.

Mascarene Islands Group

The Mascarene Islands are home to three species of *Gastonia* (Marais 1990), each endemic to one of the three islands that make up the archipelago (Réunion, Mauritius, and Rodrigues), plus 12 species of *Polyscias*. Seven of these 15 species were sampled for this study, all of which fall within the well-supported Mascarene Islands clade (BS = 99%; fig. 3). In this clade, *Gastonia rodriguesiana* from Rodrigues Island (the oldest in the archipelago) is sister to the remaining lineages. Our analysis shows that *G. cutispongia* from Réunion, the youngest island, forms a trichotomy with two other clades, both of which are well supported. The first of these groups (BS = 86%; fig. 3) comprises two species endemic to Mauritius (*Polyscias dichroostachys* and *Polyscias mauritiana*), and the second (BS = 95%; fig. 3) contains three species (*Polyscias coriacea*, *Polyscias sessiliflora*, and *Polyscias repanda*) restricted to Réunion.

Polyscias fulva Group

This well-supported clade of five species (BS = 81% in ITS tree, 67% in the combined analysis; figs. 1, 3) forms a morphologically coherent assemblage. Three of its members (*Polyscias farinosa*, *P. fulva*, and *Polyscias albersiana*) are from continental Africa (Bamps 1974), one (*Polyscias mayottensis*) is endemic to the islands of Mayotte and Moheli in the Comoros (Lowry et al. 1999), and the fifth (*Polyscias boivinii*) is restricted to dry habitats in western Madagascar (Bernardi 1971). Members of this group (and several apparently related taxa from Africa and Madagascar that have not yet been sampled) are characterized by a consistently bicarpellate gynoeceium and the presence of stellate-farinoso indument on the young shoots, inflorescences, and usually the leaves. The widespread *P. fulva* is a small to large tree, often of open and secondary habitats, and is characterized by its paniculate inflorescences with racemes as the ultimate units, whereas the closely related *P. farinosa* and *P. albersiana* (BS = 96%; fig. 3) from the mountains of Ethiopia and Tanzania, respectively, have their flowers organized in umbellules. *Polyscias mayottensis* and *P. boivinii* are likewise closely related to each other (BS = 75%; fig. 3), a finding that confirms the ideas of Lowry et al. (1999), who suggested that they form a morphologically distinctive group with two other species from western Madagascar, *Polyscias baehniana* (Bernardi) Bernardi and *Polyscias floccosa* (Drake) Bernardi.

In addition to these well-supported clades, several additional groups can be discerned from our study but with only moderate to low bootstrap values. The *Polyscias ornifolia* group (BS = 59%–61%; figs. 1, 3) includes five species from our sample (*Polyscias amplifolia*, *Polyscias fraxinifolia*, *P. ornifolia*, *Polyscias "schatzii,"* and *Polyscias stublmannii*), the first four of which are endemic to Madagascar (Bernardi 1971; P. Lowry, unpublished data) and the last of which is restricted to the Eastern Arc mountains of eastern Tanzania and adjacent Kenya (Bamps 1974). *Polyscias fraxinifolia* and *P. amplifolia* form a species pair (BS = 60% in the combined analysis; fig. 3) of small trees (4–9 m) that share a characteristic light brown to grayish white corticate-lenticillate surface on both the branches and the lower parts of the primary inflorescence axes. Both species have gynoeceia with five carpels and a well-developed calyx rim, although *P. fraxinifolia* has once-pinnate leaves with nine to 13 leaflets, whereas *P. amplifolia* has very large trifoliolate leaves up to 1 m long. The three remaining members of the group, which form an unresolved polytomy in our analysis, share several interesting features. *Polyscias ornifolia* and *P. "schatzii"* (as well as three other undescribed species) have a distinctive cupule-like pseudocalyx below the ovary, which is apparently unique within the genus. The only African species, *P. stublmannii*, closely resembles *P. ornifolia* in its general habit and leaf morphology, although its inflorescences are more robust and the pedicels do not bear a pseudocalyx below the ovary.

In the ITS tree, the *Polyscias madagascariensis* group (BS = 62%; fig. 1) is comprised of three species, *Polyscias "anjozorobensis,"* *Polyscias "lucienneae,"* and *P. madagascariensis*, all of which are restricted to the humid eastern escarpment of Madagascar and have spinulose leaflet margins. *Polyscias madagascariensis* is more widely distributed, but

the other two species are narrow endemics. Although support for this clade breaks down in the combined analysis (fig. 3), these plants share a number of features. In particular, *P. madagascariensis* and *P. "anjozorobensis"* closely resemble one another. They are both andromonoecious, with inflorescence branches that terminate in umbellules of hermaphroditic flowers and usually bear oppositely arranged lateral umbellules of staminate flowers that open only after the protandrous hermaphroditic flowers have completed their pollen-receptive ("female") phase. *Polyscias "anjozorobensis"* is a treelet 3–4 m tall with leaves that are once pinnate, whereas *P. madagascariensis* is a liana, shrub, or treelet that can reach ca. 3.5 m in height and has two to three pinnate leaves.

The combined analyses indicates a limited amount of support for the *Polyscias carolorum* group (BS = 57%; fig. 3), which is comprised of *P. carolorum*, *Polyscias "abrahamiana"*, and *Polyscias "ratovosonii"*. However, our analyses strongly support a sister species relationship between the widespread eastern Malagasy species *P. carolorum* and the locally endemic *P. "abrahamiana"*, which share several features, including overall inflorescence structure, short styles, and similarly shaped fruits. *Polyscias "ratovosonii"*, from the mountains of northern Madagascar, is morphologically very distinct and may not be closely related to the other species.

Proposed Taxonomic Realignments

It is widely held that taxonomic realignments should reflect phylogenetic relationships. On the basis of this reasoning, Plunkett et al. (2001) put forward two approaches to reassess *Polyscias*: (1) grouping *Polyscias* and the eight other genera falling into the *Polyscias* s.l. clade into a single, very large genus or (2) realigning generic limits to match more closely the clades recovered on the basis of molecular data. They suggested that the first option, while rendering *Polyscias* monophyletic, would merely transfer the classification problems of this group to a lower taxonomic level. The alternative approach, partitioning *Polyscias* and its close relatives into several smaller genera, would achieve the goal of monophyly without creating either a large "super-genus" or an excessive number of very small genera. A major part of this study focuses on the taxonomic realignment of *Polyscias* with a special emphasis on the IOB region. Parallel studies have already been completed for the *Tieghemopanax* group (Eibl 2000; Eibl et al. 2001) and the *Tetraplasandra* group (Costello and Motley 2001; A. Costello and T. J. Motley, unpublished data), but to date, intensive studies have not yet been initiated for the *Arthrophyllum* group or the section *Polyscias* group nor have we obtained a sufficiently large representation of species from Malesia. With the IOB group, we have resolved three well-supported clades of three or more species: the Northeast Malagasy group, the Mascarene Islands group, and the *P. fulva* group. In addition, we have identified several other clades that have less support and/or are comprised of only two species (e.g., the *P. ornifolia* group, *P. carolorum* group, *P. myrsine* group, and *P. baretiana* group). All species of *Polyscias*, *Gastonia*, and *Cuphocarpus* sampled from the IOB form a monophyletic group. Unfortunately, support for this clade is quite low (BS < 50%). Despite the need for major taxonomic realignments among these IOB species (and genera),

specific recommendations for such changes appear to be premature. This study has, however, made several major contributions to understanding the IOB clade, of which the polyphyly of both *Gastonia* and *Cuphocarpus* are among the most important. In addition, this study confirms the close relationship of the IOB clade to both the New Guinea clade (at present comprised of *Polyscias* aff. *belensis* and *G. spectabilis*) and the *Tetraplasandra* group (representing exclusively Pacific taxa).

Further studies are clearly warranted and would benefit from using both additional molecular markers and increased taxon sampling. Despite the utility of ITS and *trnL-trnF*, data from another rapidly evolving sequence could help provide better resolution. In a recent study of interspecific and intergeneric relationships of Myodocarpaceae (comprised of *Myodocarpus*, *Delarbraea*, and *Pseudosciadium*), the addition of nuclear ETS rDNA sequences provided much greater resolution than ITS and *trnL-trnF* alone (Sprenkle 2002; T. S. Sprenkle, G. M. Plunkett, and P. P. Lowry II, unpublished manuscript), and in a study of the Asian Palmate clade of Araliaceae, use of the *waxy* gene alone yielded sufficient resolution to assess relationships among (and often within) the genera of that clade (Mitchell and Wen 2004). These or other markers, therefore, may also be useful for resolving relationships involving the IOB clade of *Polyscias*.

Increasing sample size should be an equally important goal of future studies. Within the IOB group, expanded species sampling from Madagascar may help resolve the placement of many species whose position in the IOB group has been left unresolved. Over the past 5–10 yr, intensive fieldwork has led to discoveries that have nearly doubled our current estimate of Malagasy *Polyscias* species from 32 (Bernardi 1971, 1980) to ca. 60 (P. Lowry unpublished data), with more undoubtedly yet to be found. Adding taxa to a poorly resolved cladogram often compounds the lack of resolution. In IOB *Polyscias*, however, we suggest that increased sampling may lead to better resolution. For example, in our study, the sample of *Polyscias "longipedunculata"* is left unresolved in a large polytomy of 14 clades, but recent field collections have led to the discovery of at least two additional species thought to be closely related to *P. "longipedunculata"*. Inclusion of such new species in future studies may help to uncover additional clades and increase overall resolution.

Finally, sampling must be increased from two geographic regions: Malesia and the Seychelles Islands. There are roughly 15 species of *Polyscias* s.l. in Malesia, nearly all endemic, and a greater representation of these taxa is necessary to resolve the relationship between the IOB clade and the *Tetraplasandra* group from the Pacific. The placement of our only two Malesian representatives (in the New Guinea group) as sister to the *Tetraplasandra* group is well supported, but the relationship of this larger clade (New Guinea + *Tetraplasandra* groups) to the IOB group is left poorly supported. Within the IOB group, we currently have samples from all geographic regions except the Seychelles. The strong geographic structuring that characterizes most of the IOB subclades indicates that this group is well suited for exploring biogeographic patterns among the landmasses of the western Indian Ocean and Malesia, but a complete picture of these relationships cannot be examined until all regions are represented.

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