American Journal of Botany 95(8): 1015–1029. 2008.

EVOLUTIONARY RELATIONSHIPS IN THE SHOWY MISTLETOE FAMILY (LORANTHACEAE)[1]

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Loranthaceae (73 genera and ca. 900 species) comprise mostly aerial hemiparasitic plants. Three monotypic genera considered relicts are root parasites. The family is diverse in tropical areas, but representatives are also found in temperate habitats. Previous classifications were based on floral and inflorescence morphology, karyological information, and biogeography. The family has been divided into three tribes: Nuytsiae, Elytrantheae (subtribes Elytranthinae and Gaiadendrinae), and Lorantheae (subtribes Loranthinae and Psittacanthinae). Nuytsiae and Elytrantheae are characterized by a base chromosome number of $x = 12$, whereas subtribes Loranthinae ($x = 9$) and Psittacanthinae ($x = 8$) numbers are derived via aneuploid reduction. To elucidate the phylogeny of the family, we analyzed sequences from five genes (nuclear small and large subunit rDNA and the chloroplast genes *rbcL*, *matK*, and *trnL-F*) representing most genera using parsimony, likelihood, and Bayesian inference. The three root parasites, *Nuytsia*, *Atkinsonia*, and *Gaiadendron*, are supported as successive sister taxa to the remaining genera, resulting in a monophyletic group of aerial parasites. Three major clades are resolved each corresponding to a subtribe. However, two South American genera (*Tristerix* and *Notanthera*) and the New Zealand genus *Tupeia*, which were previously classified in subtribe Elytranthinae, are weakly supported as part of a clade representing the South American subtribe Psittacanthinae.

Key words: *matK*; parasitic plants; phylogeny; *rbcL*; ribosomal DNA; Santalales; *trnL-F* intergenic spacer.

Among the 12 clades of parasitic plants, the sandalwood order (Santalales) encompasses the widest array of habits including nonparasites, root parasites, and several variants of aerial (stem) parasites (Malécot and Nickrent, 2008; Der and Nickrent, 2008). Aerial parasitism has arisen five times in different lineages within the order (Nickrent, 2002; Vidal-Russell and Nickrent, 2008), each of which includes plants commonly referred to as "mistletoes." A mistletoe is a hemiparastic shrub that attaches to a host stem and is also a member of Loranthaceae, Misodendraceae, "Santalaceae," or Viscaceae (thus, the term describes a habit among some members of santalalean clades). The success of this life form is apparent in that nearly all of the major species-level radiations have taken place in mistletoes, such as the genera *Phoradendron* and *Viscum* (Viscaceae) and *Amyema* and *Psittacanthus* (Loranthaceae). The genus *Thesium* ("Santalaceae") is the sole exception with over 300 mainly Old World species of root hemiparasites. With 73 genera and over 900 species, Loranthaceae (loranths) have the greatest number of mistletoes in Santalales. Although a few species occur in temperate Europe, Asia, Australia, New Zealand, and South America, its greatest diversity is in the Old and New World tropics, particularly in seasonally dry habits of Africa and Australia. The last classification that treated the family worldwide was by Engler (1897) and generic concepts among loranths have changed dramatically since that time. We present here the first comprehensive multigene phylogeny (sampling 60 of the 73 genera) for this important santalalean family.

Characteristics of Loranthaceae—Most loranths have dichlamydous flowers (i.e., with two perianth whorls) where the highly reduced calyx is referred to as the calyculus. When present it is found as a small lobe or rim at the top of the inferior ovary. The corolla can be choripetalous (petals free) or gamopetalous (petals fused), and petal number varies from four to nine. There are as many stamens as petals, and the degree of fusion to the corolla differs among the genera. The anthers are two- or four-locular, the majority being basifixed. Pollen grains have a characteristic triangular to trilobate shape. The ovary is inferior and uni- to plurilocular, and it does not have true ovules. A mamelon arises from the base of the ovary where the embryo sacs are formed. Because these mistletoes do not have ovules, technically they also do not have true seeds, although this term is used for the functional unit. Fruits are pseudoberries that contain one viscin-coated seed. Chromosome numbers in the family are conservative and normally characterize groups of related genera. The base chromosome number for the family is $x = 12$, but other numbers are observed such as $x = 8$, 9, and 10 (Barlow and Wiens, 1971). Most members of Loranthaceae are stem-parasitic plants, but three monotypic genera are root parasites: *Nuytsia floribunda* from Western Australia, *Atkinsonia ligustrina* from eastern Australia and *Gaiadendron punctatum* from Central and South America.

Compared to other angiosperms, Loranthaceae can be seen as highly specialized (owing to its parasitic habit). However, previous authors suggested plesiomorphic and apomorphic features for the family that will be addressed in the context of the phylogeny reported here. Plesiomorphies include terrestrial root parasitism, the presence of a haustorial system with epicortical roots (Hamilton and Barlow, 1963; Kuijt, 1969), and inflorescences composed of aggregations of cymose inflorescence units or triads (Barlow, 1966; Barlow and Wiens, 1973). Floral plesiomorphic conditions include an ovary that is partially chambered into loculi at the base with ovular lobes (Maheshwari et al., 1957);

[1] Manuscript received 4 March 2008; revision accepted 23 May 2008.

The authors thank the numerous collectors listed in Table 1 who helped obtain specimens for this project. Thanks to MO for access to specimens, G. Amico for his useful comments, and S. Sipes for generously allowing use of her automated DNA sequencer. The authors appreciate the useful comments provided by two anonymous reviewers that improved this manuscript. Financial support was provided by a fellowship from the Fulbright Commission Argentina (R.V.-R.), the SIUC Graduate School and grants from the National Science Foundation (D.L.N.).

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doi:10.3732/ajb.0800085

small, open, pale, actinomorphic, choripetalous, six-merous, entomophilous, hermaphroditic flowers; and anthers that are dorsifixed and versatile (Barlow and Wiens, 1973).

Life history of Loranthaceae—All species in Loranthaceae depend on a biotic agent for pollination. This dependence is evidenced by their floral morphology distinguished by two main syndromes: entomophily and ornithophily. Flowers in the first group are usually small (2–10 mm), white or greenish, and choripetalous, whereas in the last group, the flowers are typically large (30–160 mm), gamopetalous, and brightly colored. Small flowers are found in the New Zealand genus *Tupeia*, *Cecarria* from New Guinea, *Barathranthus* from Asia, and in many of the $x = 8$ taxa from the New World tropics. In the New World group, which includes genera such as *Struthanthus*, *Cladocolea*, *Dendropemon*, *Phthirusa*, and *Oryctanthus*, these floral reductions have made alpha taxonomy difficult; thus their relationships at the specific and generic level are often not clear.

Bird-pollinated flowers in Loranthaceae may be entirely red, or they may display banding patterns of contrasting colors, such as orange, yellow, green and even black. In many species, pollen is released in a burst and is deposited on the pollinator. Examples of bird-pollinated flowers can be seen in *Helixanthera*, *Taxillus*, *Macrosolen*, and *Dendrophthoe*.

Because of the close affinities between these parasitic plants and their dispersal vectors and pollinators, birds are thought to have contributed to the diversification of loranthaceous mistletoes (Feehan, 1985; Restrepo et al., 2002). Loranthaceae, like nearly all mistletoes, are highly dependent on their dispersers for reaching a suitable host. Different assemblages of birds have been described as dispersers of Loranthaceae in Africa, Australia, Asia, and South America (Docters van Leeuwen, 1954; Davidar, 1983; Liddy, 1983; Watson, 2001; Restrepo et al., 2002); however, a unique interaction has been observed in the temperate forest of South America where an arboreal marsupial disperses the seeds of *Tristerix* (Amico and Aizen, 2000). The association of the plants with their dispersers is very close, and it has been proposed that in some cases these species have coevolved (Reid, 1991).

All aerial Loranthaceae have viscous seeds, an essential adaptation that permits attachment to the host branch. Soon thereafter, germination begins and the radicular end of the embryo forms an attachment disc (the holdfast). Actual penetration of the host branch follows, then an internal haustorial system (endophyte) forms where the parasite establishes a connection to the host xylem. In most loranths the shoot system develops from the epicotyl, whereas in *Tristerix aphyllus* the seedling axis degenerates and shoot growth is adventitious from the internal endophyte.

Taxonomy and classification—Delimitation of genera within the family has long presented taxonomic difficulties. Tieghem (1894) accepted 118 genera of Loranthaceae, yet just three years later Engler (1897) demoted all of these to various sections within one genus, *Loranthus*. Subsequent work by Danser (1929, 1933) provided the basic framework for today's classification. He improved Engler's system by recognizing a number of new genera and by reinstating many of those proposed by Tieghem. More recent work has been focused at the continental scale, for example Balle (1954) and Polhill and Wiens (1998) in Africa; Barlow (1966, 1974) in Asia, Australia, and New Zealand; and Kuijt (1988, 2003 and reference therein; Feuer and Kuijt, 1979) in the New World. Danser (1933) divided the family into three tribes: Nuytsieae, which includes only *Nuytsia*, characterized by a unilocular ovary and spreading cotyledons; Elytrantheae with plurilocular ovaries and with cotyledons that spread during germination; and Lorantheae, with unilocular ovaries and the cotyledons hidden in the endosperm during germination. Tribe Elytrantheae was subdivided into two subtribes: Elytranthinae, which includes stem parasites with baccate fruits and immobile anthers, and Gaiadendrinae, which are root parasites with a drupaceous fruit and dorsifixed versatile anthers. Tribe Lorantheae was subdivided into two subtribes: Loranthinae and Psittacanthinae, both of which lack endosperm.

Chromosome counts for most of the loranth genera were made by Barlow and Wiens (1971). Based on these cytological data, Barlow and Wiens (1971, 1973) reclassified some genera and split the assemblage of *Phrygilanthus* into separate genera following previous concepts. Danser's tribes and subtribes were retained, some South American genera were transferred from subtribe Loranthinae to Psittacanthinae and an unnamed tribe was added that included *Ileostylus* and *Muellerina*. Loranthinae are characterized by a base chromosome number $x = 9$ and Psittacanthinae by $x = 8$. The other two tribes (Elytrantheae and Nuytsieae) have $x = 12$.

Past molecular work has shown that Loranthaceae are monophyletic and sister to a clade composed of Schoepfiaceae and Misodendraceae (Nickrent and Duff, 1996; Nickrent et al., 1998; Nickrent and Malécot, 2001; Vidal-Russell and Nickrent, 2008). Cabrera (2002) constructed a molecular phylogeny of 43 genera of Loranthaceae that used the chloroplast gene *matK*. He found that the western Australian root parasite *Nuytsia* was sister to all other genera, followed by the eastern Australian endemic *Atkinsonia*. All the New World genera, except *Gaiadendron* and *Tristerix*, formed a well-supported clade, but taxon sampling from South America was limited. He recovered a clade that included several genera in subtribe Loranthinae. Within it, taxa were grouped with moderate support according to geographical distributions, thus distinguishing an Australasian and two African clades.

A more recent publication reported results of a molecular analysis for 47 genera in Loranthaceae based on nuclear ITS and chloroplast *trnL-F* (Wilson and Calvin, 2006). As seen in Cabrera (2002), *Nuytsia* was resolved as sister to all other genera in Loranthaceae, but most of the other nodes were not supported. Those clades that were supported (i.e., with bootstrap values >80) had already been recovered by Cabrera (2002).

No modern classification (either traditional or molecular) has included all genera of the family. The current study expands taxon sampling and uses more sequence information, thus providing the basis to establish such a classification. The evolutionary relationships between genera will be addressed, and the resulting phylogeny will be used to examine character evolution in the family with particular emphasis focused on floral features.

MATERIALS AND METHODS

Taxon sampling—Sixty of the 73 genera of Loranthaceae were sampled, which represents the full distribution of the family worldwide (Table 1). Although sequence variation in the chloroplast gene *rbcL* was generally conservative among genera, it was included to gain resolution at deeper levels in the phylogeny; sampling thus included only exemplars of major clades. Missing taxa are mainly from southeast Asia, some of which are very rare, and others are thought to be extinct. DNA extraction was attempted but without success from herbarium specimens of some of these rare taxa. Misodendraceae (two

species) and Schoepfiaceae (four species) were used as outgroups. GenBank numbers for all species are shown in Table 1; 202 sequences are newly reported for this study. Alignments and phylogenetic trees are available through TreeBASE (http://treebase.org) study number S2095.

DNA isolation and amplification—DNA was extracted from silica-dried or herbarium specimens using a 2× CTAB method (Nickrent, 1994). Polymerase chain reactions (PCR), purification, and sequencing were performed as described in Vidal-Russell and Nickrent (2007). The chloroplast gene *matK* was amplified with 78f (Vidal-Russell and Nickrent, 2007) and 163f (5′ AGG TTA CTA ATT GTG AAA CG 3′) as forward primers and 1564r (5′ ATG ATT RAC TAG ATC GTT GA 3′) as the reverse primer. For the other chloroplast regions, published primers were used (*trnL-F*: Taberlet et al. [1991]; *rbcL*: Vidal-Russell and Nickrent [2008]). The nuclear small subunit ribosomal DNA (SSU rDNA) was amplified with primers reported in Vidal-Russell and Nickrent (2008) and approximately 2000 bp of the large subunit ribosomal DNA (LSU rDNA) were amplified in two pieces with the primers 27f (Vidal-Russell and Nickrent, 2008) and S5f (5′ CGT GCA AAT CGT TCG TCT 3′) as forward and S6r (5′ CGC CAG TTC TGC TTA CCA 3′) and 2134r (5′ GGA CCA TCG CAA TGC TTT GT 3′) as reverse.

Phylogenetic analysis—Sequences were aligned manually in the program Se-Al version 2.0a11 (Rambaut, 2004). With protein-coding genes, the translated amino acid sequences were used to aid alignment. Phylogenetic tree inferences for each gene individually and for the concatenated data set were obtained through maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). In MP analyses, heuristic searches were performed with 1000 random addition sequences holding 10 trees for each iteration and by using the tree-bisection-reconnection (TBR) algorithm for branch swapping. A maximum limit of 100 000 trees was imposed. A parsimony ratchet method (Nixon, 1999) was performed with 20 independent searches of 200 iterations and randomly weighting 25 characters per iteration for SSU rDNA using the programs PAUPRat version 1 (Sikes and Lewis, 2001) and PAUP* version 4.0b10 (Swofford, 2003).

Nodal support for MP was obtained through maximum parsimony bootstrap resampling (MPBS) run for 100 replications. For each bootstrap pseudoreplicate, heuristic searches were performed with 100 random addition sequences holding 10 trees for each iteration, with the TBR branch-swapping algorithm. When trees longer than the most parsimonious tree were found, only 10 were saved per replicate. For *matK*, *rbcL*, LSU rDNA, and the concatenated nuclear data set, analyses could not proceed because of the high number of best trees found during a particular replicate. In these cases, a time limit of 60 min per additional replicate was imposed.

For ML, the model of molecular evolution appropriate for each individual gene partition was selected using the program Modeltest version 3.6 (Posada and Crandall, 1998) using the hierarchical likelihood ratio test and the Akaike information criterion (Posada and Buckley, 2004). Frequently both methods selected the same model, but when they differed, the model with fewer parameters was used. Model selection can be found in Table 2. Heuristic tree searches were conducted starting with a neighbor joining tree, then by performing TBR branch swapping. Nodal support was obtained through bootstrap resampling (MLBS), with 100 replications using the program GARLI version 0.951 (Zwickl, 2006).

Bayesian inference was performed in the program MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with models chosen for each partition by the program MrModeltest version 2.2 (Nylander, 2004) (Table 2). Two independent analyses were run with four chains each. The Markov chain Monte Carlo was set to run for five million generations, saving trees and parameters every 100th generation. The run was set to stop if topological convergence was reached between the two runs, which was determined by the presence of a standard deviation in split frequencies that was lower than 0.01 (discarding 25% as burn-in). Upon run completion, inspection of the likelihood scores vs. generation plots showed that these scores had always reached stationary before the first 25% of the samples; thus discarding this fraction as burn-in was conservative. Model parameters were estimated as part of the analysis. Uniform prior probabilities were assigned to all parameters except the state frequencies for which a Dirichlet prior distribution was assigned. When more than one partition was analyzed (i.e., the concatenated data set), parameter estimations were unlinked allowing partitions to evolve at different rates.

Morphological analysis—Flower characteristics associated with pollination syndromes (i.e., corolla color, flower symmetry, petal fusion) were coded for all genera. This matrix was used for parsimony character reconstruction on the consensus tree of the concatenated data set using the program Mesquite version 2.01 (Maddison and Maddison, 2007).

RESULTS

Statistics relating to the results of the various analyses of the separate and concatenated partitions are shown in Table 2. Although the level of resolution between the different gene partitions differed, there were no conflicting topologies that received high support. Among the five individual gene partitions, the lowest percentage of informative characters was from SSU rDNA followed by LSU rDNA, *rbcL*, *trnL-F*, and finally *matK*. Although the percentage of informative sites for the nuclear gene partition was less than half that of the chloroplast partition, the number of shortest trees recovered with MP was similar (384 and 336, respectively). Because the SSU rDNA partition gave very little resolution of relationships among genera, it is not shown. The combined SSU and LSU rDNA (nuclear) tree was less resolved than the LSU rDNA partition alone. For this reason, only the LSU rDNA partition tree is discussed next.

Nuclear LSU ribosomal gene partition—Parsimony analysis of the nuclear LSU partition did not recover *Nuytsia* as sister to the remaining genera but as one of four clades that arise from a polytomy along the spine of the tree (Fig. 1). The second clade of the polytomy is composed of *Alepis* and *Peraxilla* (clade A), and the third clade contains seven genera traditionally classified as subtribe Elytranthinae (clade B). The fourth clade (C) includes the remaining loranth genera but receives only moderate support and is not recovered using the chloroplast genes. Clade C is composed of three clades that arise from a polytomy, the first of which is *Atkinsonia*, the second *Gaiadendron* plus clade D, and the third the remaining loranths. Clade D contains two $x = 12$ genera (*Tupeia* [New Zealand] and *Notanthera* [South America]), *Desmaria* (South America) with $x = 16$, and the $x = 8$ clade (E) that includes seven genera. Although *Tristerix* and *Ligaria* do not occur with clade D and E taxa (as they do with the chloroplast genes), they resolve with little support in the next most derived position on the tree. In an unresolved position in clade F is clade G containing *Loranthus* and *Cecarria*. Clade I includes six Australasian and Indomalayan genera that have traditionally been considered part of tribe Lorantheae. The remaining loranths (clade J) include Asian taxa such as *Scurrula*, *Taxillus*, and *Helixanthera* as well as the remaining loranths that are mainly African.

Concatenated chloroplast gene partitions—Results of the analysis of the data set composed of the three concatenated chloroplast genes placed the three root parasites, *Nuytsia*, *Atkinsonia*, and *Gaiadendron* in a successive grade at the base of the other Loranthaceae (Fig. 2). The next clade is composed of a polytomy of four clades, the first of which contains the New Zealand endemics *Peraxilla* and *Alepis* (clade A), which is sister to six genera traditionally classified as subtribe Elytranthinae (clade B). The genus *Tupeia* is part of this polytomy instead of being sister to *Desmaria* as seen with the nuclear partition (Fig. 1). The third clade of the polytomy, clade D, contains South American endemics with $x = 12$, 16, and 10 base chromosome numbers (*Notanthera*, *Tristerix*, *Desmaria*, and *Ligaria*) and clade E composed of eight $x = 8$ genera of New World mistletoes. Clade F, the fourth of the polytomy, contains all the remaining loranths, most

TABLE 1. Taxa included in phylogenetic analyses with voucher information (DNA accession number [DNA acc.] from collection maintained by D. L. Nickrent at SIUC) and GenBank accession numbers.

Name	Source	Collector(s)	Herb.	DNA acc.	SSU rDNA	LSU rDNA	*matK*	*trnL-F*
Actinanthella menyharthii (Engl & Schinz ex Schinz) Balle	Rhodesia	*D. Wiens 4638*	MO [a]	4375	EU544313*	EU544352*	EU544408*	N/A
Aetanthus nodosus Engl.	Ecuador	*Garmendia & Igual 1227*	MO	4561	EU544314*	N/A	EU544409*	N/A
Agelanthus sansibarensis (Engl.) Polhill & Wiens	Kenya	*S. A. Robertson S.N.*	none	2987	U59946	EU544353*	EU544410*	N/A
Alepis flavida (Hook. f.) Tiegh.	New Zealand	*B. Molloy S.N.*	SIU	2743	L24139	EF464474*	EF464508	EF464481
Amyema glabrum (Domin.) Dans.	Australia	*D. L. Nickrent 2794*	SIU	2794	AF039073	EU544354*	EU544411*	EU544476*
Amyema queenslandicum (Blakely) Dans.	Australia	*D. L. Nickrent 2784*	SIU	2784	EU544315*	EU544355*	EU544412*	N/A
Amylotheca duthiana (King) Dans.	Sarawak, Malaysia	*D. L. Nickrent 4022*	SIU	4022	EU544316*	EU544356*	EU544413*	EU544477*
Atkinsonia ligustrina (A. Cunn. ex F. Muell.) F. Muell.	Australia	*D. Watson 4458*	CANB	4343	EF464464	EF464475	DQ787444	DQ788714
Bakerella Tiegh.	Madagascar	*S. Razafimandimbison S. S. 332*	SIU	4161	EU544318*	EU544358*	EU544415*	EU544479*
Barathranthus axanthus (Korth.) Miq.	Sarawak, Malaysia	*D. L. Nickrent 4029*	SIU	4029	EU544317*	EU544357*	EU544414*	EU544478*
Benthamina alyxifolia (F. Muell. ex Benth.) Tiegh.	Australia	*W. Forstreuter Be.al.01*	SIU	4127	EU544319*	EU544359*	EU544416*	EU544480*
Berhautia senegalensis Balle	Gambia	*M. Jones S.N.*	SIU [a]	4576	EU544320*	EU544360*	EU544417*	N/A
Cecarria obtusifolia (Merr.) Barlow	Queensland, Australia	*B. Hyland 16493*	QRS	4562	EU544321*	EU544361*	EU544418*	EU544481*
Cladocolea gracilis Kuijt	Mexico	*A. C. Sanders & P. A. Fryxell 4172*	MO [a]	3066	EU544322*	EU544362*	EU544419*	EU544482*
Dactyliophora novae-guineae Danser	Queensland, Australia	*B. Hyland 16461*	QRS	4563	EU544323*	EU544363*	EU544420*	EU544483*
Decaisnina triflora (Spanoghe) Tieghem.	Papua New Guinea	*D. L. Nickrent et al. 4491*	LAE	4491	EU544324*	EU544364*	EU544421*	EU544484*
Dendropemon bicolor Krug. & Urb	Puerto Rico	*D. L. Nickrent 2700*	ILL	2700	AF039075	EU544365*	EU544422*	N/A
Dendrophthoe longituba (Elm.) Dans	Sarawak, Malaysia	*D. L. Nickrent 4010*	SIU	4010	AY957441	EU544366*	EU544423*	EU544485*
Dendrophthoe curvata (Blume) Miquel.	Sarawak, Malaysia	*D. L Nickent & C. Calvin 4012*	SIU	4012	EU544325*	EU544367*	EU544424*	N/A
Desmaria mutabilis (P. & E.) Jacks	Chile	*G. Amico S.N.*	BCRU	4510	EF464465	EF464476	EF464509	EF464486
Diplatia furcata Barlow	Queensland, Australia	*D. L. Nickrent 2824*	SIU	2824	L24088	EU544368*	EU544425*	EU544486*
Emelianthe panganensis (Engl.) Danser	Tanzania	*E. Mboya 594*	MO [a]	4889	EU544326*	EU544369*	EU544426*	EU544487*
Englerina ramulosa (Sprague) Polhill & Wiens	Kenya	*S. A. Robertson S.N.*	SIU	2984	L24140	EU544370*	EU544427*	N/A
Erianthemum dregei (Eckl. & Zeyh.) Tiegh.	Kenya	*S. A. Robertson S.N.*	SIU	2985	L25679	EU544371*	EU544428*	EU544488*
Gaiadendron punctatum (R. & P.) G. Don.	Costa Rica	*S. Sargent S.N.*	SIU	2729	L24143	DQ790209	DQ787445	DQ788715
Globimetula dinklagei (Engl.) Dans.	Gabon	*J. Wieringa 2858 & 3250*	WAG	3087	AF039076	EU544372*	EU544429*	EU544489*
Helicanthes elastica (Desr.) Danser	Kerala, India	*Pradeep 5342*	SIU [a]	2839	EU544328*	EU544375*	EU544432*	N/A
Helixanthera coccinea Dans.	Sarawak, Malaysia	*D. L. Nickrent 4019*	SIU	4019	AF039077	EU544373*	EU544430*	EU544490*
Helixanthera cylindrica (Jack) Dans.	Sarawak, Malaysia	*P. C. Yii & Yulaihi S 72056*	SIU	4037	EU544327*	EU544374*	EU544431*	N/A
Ileostylus micranthus (Hook. f.) Tiegh.	New Zealand	*B. Molloy S.N.*	SIU	2741	EU544329*	EU544376*	EU544433*	EU544491*
Lepidaria cf. forbesii Tiegh.	Sarawak, Malaysia	*D. L. Nickrent 4044*	SIU	4044	EU544330*	EU544378*	EU544434*	EU544492*
Lepeostegeres lancifolius Dans.	Sarawak, Malaysia	*P.C. Yii & Julaihi S 72091*	SIU	4041	N/A	EU544379*	EU544435*	N/A
Ligaria cuneifolia (Ruiz & Pavón) Tiegh.	Chile	*G. Amico S.N.*	BCRU	4567	L24152	EF464477	EF464510*	DQ442940
Loranthus europaeus L.	Italy	*U. Kuhlmann S.N.*	SIU	2849	L24153	EU544380*	EU544436*	EU544493*
Loranthus odoratus Wall.	Nepal	*M. Devkota 301*	KATH	4977	EU544331*	EU544381*	N/A	EU544494*
Loxanthera speciosa Bl.	Sarawak, Malaysia	*D. L. Nickrent 4026*	SIU	4026	EU544332*	EU544382*	EU544437*	EU544495*
Lysiana filifolia Barlow	Queensland, Australia	*D. L. Nickrent 4449*	SIU	4449	EU544333*	EU544383*	EU544438*	EU544496*
Macrosolen cochinchinensis (Lour.) Tiegh.	Sarawak, Malaysia	*P.C. Yii & Yulaihi S 72052*	SIU	4038	EU544334*	EU544384*	EU544439*	EU544497*
Moquiniella rubra (Spreng. f.) Balle	South Africa	*K. Steiner 2836*	SIU	3042	AF039078	DQ790207	DQ790171	EF464489
Muellerina eucalyptoides (DC) Barlow	NSW, Australia	*D. Watson S.N.*	SIU	4310	EU544335*	EU544385*	EU544440*	EU544498*
Notanthera heterophylla (R. & P.) G. Don.	Chile	*C. Aedo 7202*	MA	4372	EF464466	EF464478	EF464511	N/A

TABLE 1. Continued.

Name	Source	Collector(s)	Herb.	DNA acc.	SSU rDNA	LSU rDNA	*matK*	*trnL-F*
Notanthera heterophylla (R. & P.) G. Don.	Chile	*G. Amico S.N.*	BCRU	4582	N/A	N/A	N/A	DQ442939
Nuytsia floribunda (Labill.) R. Br.	Western Australia	*B. Lamont S.N.*	SIU	2747	DQ790103	DQ790210	DQ787446	N/A
Nuytsia floribunda (Labill.) R. Br.	Western Australia	*A. Markey S.N.*	SIU	3080	N/A	N/A	N/A	DQ788716
Oedina pendans (Engl. & Krause) Polhill & Wiens	Tanzania	*R. E. Gereau & C. J. Kayombo 4213*	MO [a]	4329	EU544336*	EU544386*	EU544441*	EU544499*
Oliverella rubroviridis Tiegh.	Zambia	*N. B. Zimba et al. 1097*	MO [a]	4330	EU544337*	EU544387*	EU544442*	N/A
Oncella ambigua (Engl.) Tiegh.	Kenya	*S. A. Robertson & K. Medley 5459*	MO [a]	4673	EU544338*	N/A	EU544443*	N/A
Oncocalyx sulfurens (Engl.) Wiens & Polh.	Kenya	*W. Forstreuter 9117*	SIU	2850	EU544339*	EU544388*	EU544444*	EU544500*
Oryctanthus occidentalis L.) Eichler.	Costa Rica	*D. L. Nickrent 2763*	SIU	2763	L24408	EU544389*	EU544445*	EU544501*
Peraxilla tetrapetala (L. f.) Tiegh.	New Zealand	*B. Molloy S.N.*	SIU	2744	EU544340*	EU544390*	EU544446*	EU544502*
Phragmanthera crassicaulis (Engl.) Balle	Gabon	*J. Wieringa 2506*	WAG	3037	EU544341*	EU544391*	EU544447*	EU544503*
Phthirusa pyrifolia (H.B.K.) Eichler.	Costa Rica	*D. L. Nickrent 2762*	SIU	2762	L24412	EU544392*	EU544448*	EU544504*
Plicosepalus sagittiflorus (Engl.) Danser	Kenya	*W. Forstreuter S.N.*	SIU	2852	EU544342*	EU544393*	EU544449*	N/A
Psittacanthus calyculatus (DC) G. Don.	Mexico	*D. Wiens S.N.*	SIU	4043	L24414	EU544394*	EU544450*	N/A
Septulina glauca (Thunb.) Tieg.	South Africa	*D. L. Nickrent 4089*	SIU	4089	EU544346*	EU544398*	N/A	EU544506*
Scurrula ferruginea (Jack) Dans.	Sarawak, Malaysia	*D. L. Nickrent 4008*	SIU	4008	EU544343*	EU544395*	EU544451*	EU544505*
Scurrula parasitica L.	Sarawak, Malaysia	*D. L. Nickent & C. Calvin 4004*	SIU	4004	EU544345*	EU544397*	EU544453*	N/A
Scurrula pulverulenta (Wall.) G. Don.	Nepal	*M. Devkota 661*	KATH	4159	EU544344*	EU544396*	EU544452*	N/A
*Socratina bemarivensi*s (H. Lecomte) S. Balle	Madagascar	*C. C. H. Jongkind et al. 3548*	MO [a]	4179	EU544347*	EU544399*	EU544454*	EU544507*
Sogerianthe sessiflora (Danser) Danser	Papua New Guinea	*D. L. Nickrent et al. 4467*	LAE	4467	EU544348*	EU544400*	EU544455*	EU544508*
*Sprague*a*nella rhamnifoli*a (Engl.) Balle	Kenya	*S. A. Robertson, D. Wiens, C. Calvin 5452*	MO	4674	N/A	EU544401*	EU544456*	N/A
Struthanthus oerstedii (Oliv.) Standley et Calderon	Costa Rica	*S. Sargent S.N.*	none	2728	L24421	EU544402*	EU544457*	EU544509*
Struthanthus woodsonii Cufod.	Costa Rica	*D. L. Nickrent 2761*	SIU	2761	EU544349*	EU544403*	EU544458*	EU544510*
Tapinanthus constrictiflorus (Engl.) Dans.	Gabon	*Y. Wieringa 2860*	WAG	3088	L24422	EU544404*	EU544459*	EU544511*
Taxillus chinensis (DC) Dans.	Sabah, Malaysia	*D. L. Nickrent 4032*	SIU	4032	EU544350*	EU544405*	EU544460*	EU544512*
Tolypanthus involucratus Roxb.) Tiegh.	Bhutan	*Grierson & Long 3557*	GH [a]	4907	N/A	N/A	EU544461*	N/A
Tripodanthus acutifolius (Ruiz & Pavon) Tiegh.	Brazil	*Wasum et al. 7586*	MO [a]	2969	L24424	EU544406*	EU544462*	EU544513*
Tristerix corymbosus (L.) Kuijt.	Chile	*V. Melzheimer S.N.*	SIU [a]	4129	EF464467	N/A	EF464512	N/A
Tristerix corymbosus (L.) Kuijt.	Chile	*G. Amico S.N.*	BCRU	4572	N/A	EF464479	N/A	
Tristerix corymbosus (L.) Kuijt.	Chile	*G. Amico S.N.*	BCRU	4597A	N/A	N/A	N/A	EF464493
Tristerix corymbosus (L.) Kuijt.	Argentina	*G. Amico S.N.*	BCRU	4575E	N/A	N/A	N/A	N/A
Tupeia antarctica (Forst. f.) Cham. et Schlecht	New Zealand	*B. Molloy S.N.*	SIU	2742	L24425	DQ790208	DQ790172	EF464494
Vanwykia remota (Baker & Sprague) Wiens	Tanzania	*T. Fison 91/1*	MO [a]	4331	EU544351*	EU544407*	EU544463*	EU544514*
Misodendrum linearifolium DC	Argentina	*D. E. Bran*	SIU	2829	L24397	N/A	N/A	N/A
Misodendrum linearifolium DC	Argentina	*G. Amico 136*	BCRU	4591	N/A	DQ790211	DQ787438	DQ788712
Misodendrum punctulatum Banks ex DC	Argentina	*G. Amico S.N.*	BCRU	3031	N/A	N/A	N/A	N/A
Misodendrum punctulatum Banks ex DC	Argentina	*G. Amico S.N.*	BCRU	4593	N/A	N/A	DQ787443	DQ788711
Quinchamalium chilense Lam.	Argentina	*R. Vidal-Russell S.N.*	SIU	4503	EF464469	N/A	EF464514	EF464491
Schoepfia vacciniiflora Planch. ex Hemsl.	Panama	*G. McPherson and P. M. Richardson 15981*	MO [a]	3069	N/A	N/A	EF464515	N/A
Schoepfia fragrans Wall.	China	*Tsi Zhanhuo 91–417*	MO [a]	5009	N/A	N/A	N/A	DQ788718
Schoepfia schreberi Gmelin	Bahamas	*D. L. Nickrent 2599*	ILL	2599	L24418	AF389261	DQ787447	DQ788717

[a] DNA derived from an herbarium specimen

* Sequence generated for this study

of which have $x = 9$ as their base chromosome number. This clade is composed of a polytomy of clade G (*Cecarria* and *Loranthus*), clade H (*Ileostylus* and *Muellerina* with $x = 11$), and a clade containing the remaining loranths. As with the nuclear LSU rDNA tree (Fig. 1), clade I includes six genera of Australasia and Indomalaya classified in tribe Lorantheae. Both these partitions show that the two accessions of *Amyema* are not monophyletic. Unlike the LSU rDNA tree, the genus *Barathranthus* is not included in clade I. Clade J is composed of 25 genera of Asian and African Loranthaceae. Clades recovered within clade J using the chloroplast gene partition agree in some cases with those obtained from LSU rDNA but not in others. Clades in common include *Scurrula* plus *Taxillus*, *Englerina* plus *Tapinanthus*, and *Socratina* plus *Vanwykia*.

Concatenated five-gene analysis—The five-gene analysis yielded one tree (Fig. 3) with greater resolution than those obtained from either the nuclear (Fig. 1) or chloroplast (Fig. 2) partitions. As with the chloroplast gene tree (Fig. 2), *Nuytsia* is resolved with high support as sister to all other Loranthaceae. The next two taxa to diverge in stepwise manner are *Atkinsonia* and *Gaiadendron*. Clade A, with *Alepis* and *Peraxilla* and clade B with the group of six genera including *Amylotheca*, emerge next and are sister. This clade is sister to all remaining loranths. Generally, clade D is similar in composition to the equivalent clades in the nuclear and chloroplast trees, but here the genera *Tristerix*, *Ligaria*, and *Tupeia* are included, albeit as part of a polytomy. As before, clade D contains a strongly supported clade E of $x = 8$ New World mistletoes. The large clade F contains clade H, which is sister to the remaining loranths and is composed of the $x = 11$ taxa, *Ileostylus* and *Muellerina*. Clade G with *Loranthus* and *Cecarria* is sister to a large clade containing clades I and J. Clade I includes the seven Australasian and Indomalayan genera, although the sister relationship of *Barathranthus* to this clade is poorly supported. Clade J contains the remaining loranths from Asia and Africa. Although the relationships among African genera are not well resolved, *Actinanthella*, *Agelanthus*, *Berhautia*, and *Oncocalyx* are consistently recovered as part of the same clade as are *Emelianthe* and *Globimetula*.

Morphological analyses—The most parsimonious reconstruction for the Loranthaceae ancestor is a yellow, choripetalous, actinomorphic flower. Characters associated with bird pollination, such as gamopetalous corollas, evolved several times such as in *Alepis* (clade A), *Lysiana* (clade B), *Ligaria* (clade D), *Psittacanthus* (clade E), and in most members of clades I and J. Taxa with gamopetalous corollas display a variety of flower colors, although most are red with a banded pattern of a contrasting color or colors. Zygomorphic flowers are found almost exclusively in clade J composed mainly of African taxa, a group known to have reached a high degree of specialization for bird pollination. A summary of pollination types and base chromosome numbers plotted on a simplified molecular phylogeny of the family is shown in Fig. 4.

DISCUSSION

This study represents the first multigene molecular phylogeny of Loranthaceae with robust sampling at the generic level. In comparison to similar intergeneric analyses within other families of Santalales, resolution of relationships among loranths proved to be particularly difficult, mainly owing to less phylogenetic signal in genes typically used at this level. For example, both *rbcL* and SSU rDNA sequences were useful for resolving relationships among genera in "Santalaceae" and Viscaceae (Der and Nickrent, 2008). This higher resolution is partly attributable to the general increase in substitution rates seen in more evolutionarily derived groups (i.e., Viscaceae). It appears that within loranths morphological and karyotypic character evolution has proceeded such that clear differences can be seen between many genera, but this differentiation is not marked by comparable changes in the nuclear and chloroplast genomes. A similar discordance was found for *Argyroxiphium* (Asteraceae) of Hawaii, which differs markedly in morphology from its tarweed ancestors in California but lacks concomitant genetic differentiation (Baldwin, 1997). The weak support for the relationships of the main aerial parasite clades (A+B, E, and F) and some monotypic genera (*Desmaria*, *Notanthera*, *Tupeia*) can probably be attributed to rapid radiations that occurred

TABLE 2. Summary of tree statistics from parsimony analyses and model of molecular evolution selected by hierarchical likelihood ratio test for each gene partition (SSU rDNA, LSU rDNA, *rbcL*, *matK*, *trnL-F*) and concatenate analyses (nuclear, chloroplast and five genes).

Statistic	SSU rDNA	LSU rDNA	Nuclear	*rbcL*	*matK*	*trnL-F*	Chloroplast	5 Genes
Sampling (no. taxa)	69	63	65	28	70	58	70	70
Alignment length (bp)	1855	2285	4140	885	1565	1622	4082	8231
Number of indels scored					9		9	9
Variable characters	336 (18%)	693 (30%)	1001 (24%)	189 (21%)	931 (59%)	519 (32%)	1639 (40%)	2682 (32%)
Informative characters (incl. indels)	156 (8%)	279 (12%)	430 (10%)	125 (14%)	627 (40%)	267 (16%)	1001 (24%)	1465 (18%)
Number of MP trees	ratchet	1084	384	1519	1379	100000	336	180
Tree length	748–750	1773	2553	340	2668	1108	4123	6904
CI		0.494	0.490	0.670	0.531	0.648	0.573	0.532
CI excluding uninformative characters		0.317	0.322	0.586	0.456	0.523	0.478	0.414
RI		0.576	0.561	0.710	0.714	0.626	0.690	0.636
ML model	trN+I+Γ	trN+I+Γ	trN+I+Γ	TIM+I+Γ	TVM+Γ	TIM+I+Γ	GTR+I+Γ	GTR+I+Γ
BI model	GTR+I+Γ	GTR+I+Γ				GTR+I+Γ		
1st codon position				F81+ Γ	GTR+Γ			
2nd codon position				JC	GTR+Γ			
3rd codon position				GTR+Γ	GTR+Γ			

Notes: BI = Bayesian inference, CI = consistency index, incl. = including, ML = maximum likelihood, MP = maximum parsimony, RI = retention index

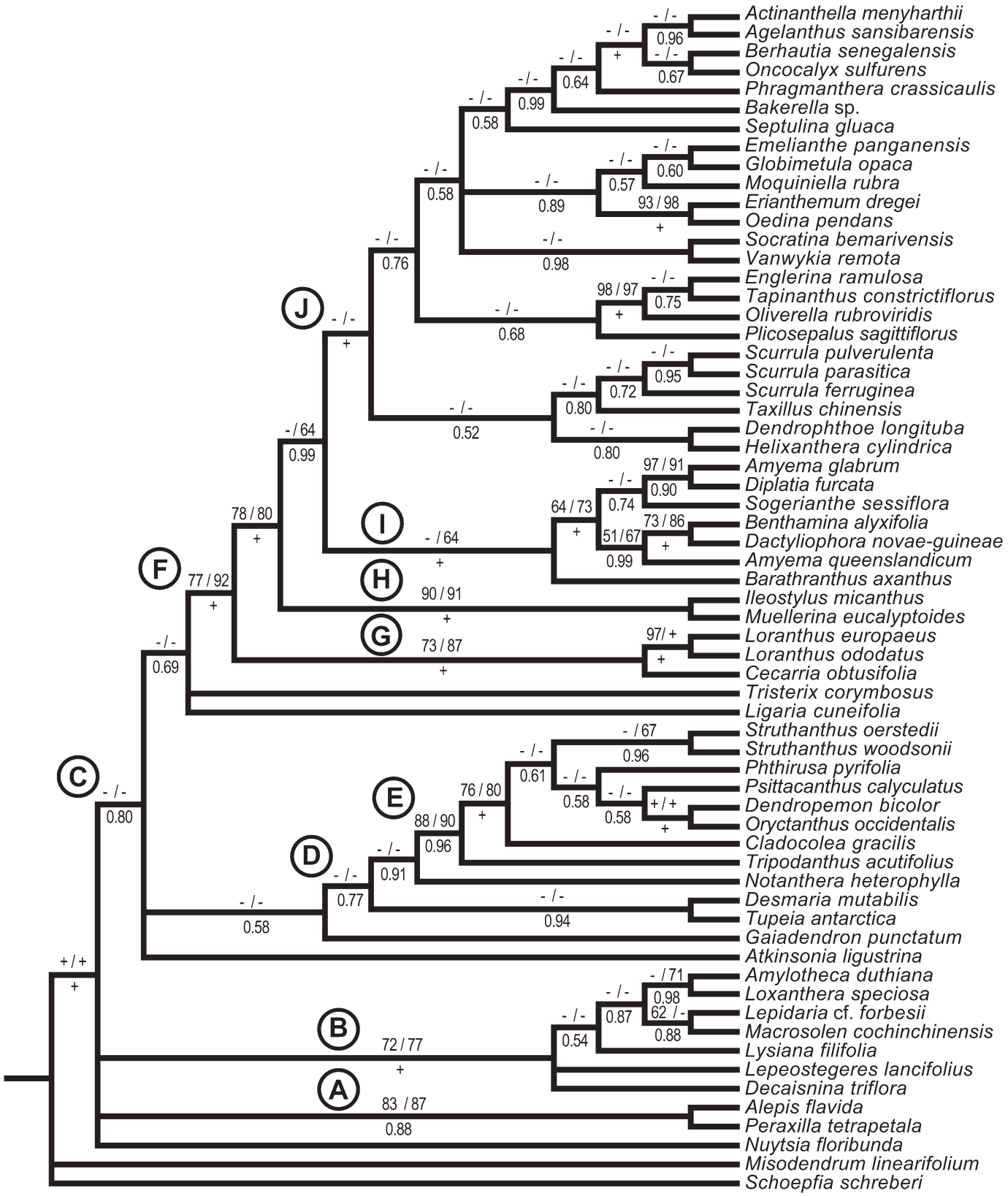

Fig. 1. Majority rule consensus tree from a Bayesian analysis of nuclear LSU rDNA sequences of Loranthaceae and outgroups. Nodal support is given above the branches as bootstrap values for parsimony, likelihood, and below as posterior probabilities (a plus sign indicates 100 or 1.0).

when this habit evolved in the Oligocene (Vidal-Russell and Nickrent, 2008).

Results obtained in this study are in general agreement with the earlier classification by Danser (1933), which was later modified by Barlow and Wiens (1971, 1973). However, the molecular phylogenetic trees presented here resulted in rearrangement of some genera, suggesting a need to change the circumscription of some tribes. Each clade will be discussed, including how it correlates with previous classifications and noting morphological features characteristic of that clade.

Nuytsia floribunda has always been recognized as different from other loranths and was classified in its own tribe by Danser (1933), a relationship supported by molecular data that placed it as sister to all other Loranthaceae (Figs. 2 and 3). This monospecific genus is a small tree of western Australia with a winged, wind-dispersed achene—a fruit type unique in the family. Moreover, *Nuytsia* nonspecifically parasitizes the roots of nearby plants using one of the most unusual haustorial types in Santalales. The haustorium contains sclerenchymatous prongs that act like scissors that transversely sever the host root (Beyer et al., 1989; Calladine et al., 2000). Some of its characteristics, like being phanerocotylar (cotyledons that spread during germination) and its base chromosome number of $x = 12$, resemble tribe Elytranthinae, while others, such as its unilocular ovary, resemble tribe Lorantheae.

The other root parasites, *Atkinsonia* from eastern Australia and *Gaiadendron* from Central and South America, are the only members of subtribe Gaiadendrinae in Tribe Elytrantheae (Danser, 1933). *Atkinsonia* is a small shrub, whereas *Gaiadendron* can be a shrub or an aerial parasite (Kuijt, 1963). Our data indicate that these two genera diverged early in the evolutionary history of the family after *Nuytsia*. The three root parasites do not form a clade, but they are successive sister taxa to the remaining members of the family. This topology suggests that aerial parasitism arose once in Loranthaceae, not four times as proposed by Wilson and Calvin (2006).

Tribe Elytrantheae is characterized by a plurilocular ovary and by fusion of the nucellus with the middle of the ovary, thus producing an enlarged mamelon. All genera are phanerocotylar and have a base chromosome number $x = 12$. This tribe has traditionally included two subtribes: Gaiadendrinae (just described) and Elytranthinae, the latter with 15 extant genera (Table 3), four of which were not sampled in this study. If the subtribe is circumscribed without *Notanthera*, *Tristerix*, and *Tupeia* (i.e., our clades A and B), it is strongly supported as monophyletic. The two New Zealand genera, *Alepis* and *Peraxilla* (clade A), long considered to be closely related (Barlow, 1966), are sister to the other clade B taxa. Within clade B, the sister relationship of *Amylotheca* and *Loxanthera* is well supported. These two genera, as well as *Decaisnina* have racemes of flowers in triads, whereas *Lepidaria* and *Macrosolen* have monads in spikes, racemes, and capitula. All genera in clades A and B have epicortical roots except *Lysiana*, which produces localized infections. The inflorescence of this genus is reduced to a two-flowered, axillary umbel. Another specialized feature of *Lysiana* is the high degree of fusion of the nucellus with the ovary wall where it is not possible to distinguish ovules from the mamelon (Cocucci and Venturelli, 1982). On the other hand, *Lysiana* and *Peraxilla* have an ovary with four loculi (Bhatnagar and Johri, 1983), which is considered a plesiomorphy. In other genera of this clade, the loculi of the ovary disappear (e.g., *Macrosolen*), or they are retained only in the basal portion of the ovary (e.g., *Amylotheca*).

Tribe Lorantheae is characterized by unilocular ovaries and a highly reduced or even missing mamelon (Cocucci and Venturelli, 1982; Bhatnagar and Johri, 1983). Previous classifications subdivided the tribe into two subtribes, Psittacanthinae and Loranthinae, with Psittacanthinae composed of all South American taxa plus *Ligaria* and *Desmaria* (Table 3). In this study, genera with $x = 8$ are recovered as monophyletic (clade E). Other South American genera (*Tristerix*, *Notanthera*, *Ligaria*, and *Desmaria*) together with the New Zealand genus *Tupeia*, are present in clade D, but the relationships are not well resolved (Fig. 3). Among all loranth clades, clade D has the greatest heterogeneity in base chromosome number: *Tupeia*, *Tristerix*, and *Notanthera* with $x = 12$, *Ligaria* with $x = 10$, *Desmaria* with $x = 16$, and the remaining South American genera with $x = 8$. Resolving the relationships and biogeography of this clade is crucial to an understanding of the history of the entire family.

Tupeia differs from the other New Zealand genera, *Alepis* and *Peraxilla*, in clade A, by lacking epicortical roots and by having a raceme of triads. However, the three genera share the $x = 12$ base chromosome number. In *Tupeia* the infection type is localized, and the endophyte grows within the host cortex, a type also found in *Tristerix* among clade D taxa (Kuijt, 1982; Calvin and Wilson, 2006).

Kuijt (1985) placed the following small-flowered (2–10 mm) genera of South and Central America in an assemblage separate from the other New World genera: *Cladocolea*, *Dendropemon*, *Ixocactus*, *Panamanthus*, *Phthirusa*, *Oryctanthus*, *Oryctina*, and *Struthanthus*. *Oryctina*, *Ixocactus*, and *Panamanthus* were not sampled in this study, but the remaining five genera of clade E appear in two well-supported clades that arise from a trichotomy, the third member of which is a clade containing the large-flowered (>30 mm) genera *Aetanthus* and *Psittacanthus*. *Struthanthus* is highly supported as sister to *Cladocolea*, a result that is not surprising given their morphological similarity. Kuijt (1981) placed them together in the *Cladocolea-Struthanthus* complex, differentiating these taxa by inflorescence features: *Cladocolea* with racemes of single flowers and *Struthanthus* with racemes of triads. The other small-flowered genera, *Dendropemon*, *Oryctanthus*, and *Phthirusa*, form a clade, which is in agreement with relationships proposed by Kuijt (1991). The first two genera are characterized by indeterminate racemes or spikes of bracteolate monads, while *Phthirusa* has an indeterminate raceme or spike of bracteolate triads (Kuijt, 1981).

The large-flowered genera *Aetanthus* and *Psittacanthus* form a well-supported clade in agreement with their similar inflorescence and floral morphology. *Psittacanthus* is a large genus with 119 species (Kuijt, 2008, in press) with various inflorescence types, all probably derived from a raceme of triads with pedicellate flowers (Kuijt, 1981). These three New World clades (i.e., the two small-flowered and the large-flowered clades) are highly supported as sister to *Tripodanthus*, which has medium sized (15–35 mm) white flowers arranged in a determinate raceme of triads. Given this topology, some hypotheses about floral evolution can be made. Among the clade D genera, several have reduced flowers that are likely insect-pollinated (*Tupeia*, *Desmaria*, *Notanthera*, *Tripodanthus*, and *Notanthera*). Thus, no matter how the *Tristerix* plus *Ligaria* clade resolves relative to this polytomy, it appears that traits related to insect pollination are plesiomorphic whereas tubular, bird-pollinated flowers evolved secondarily twice, i.e., in the ancestors of the *Tristerix-Ligaria* and *Psittacanthus-Aetanthus* clades.

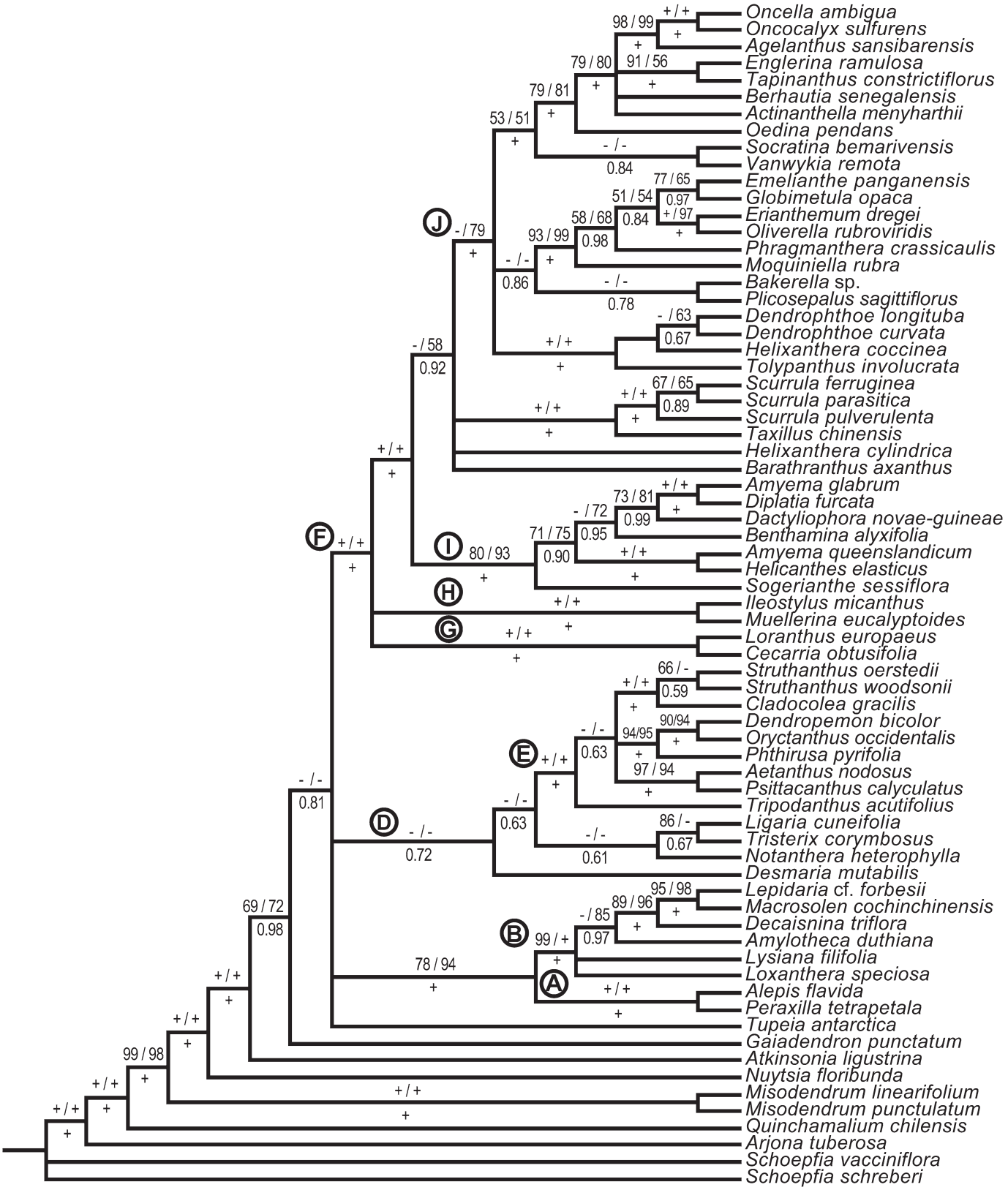

Fig. 2. Majority rule consensus tree from a Bayesian analysis of the chloroplast genes *rbcL*, *matK*, and *trnL-F* from Loranthaceae and outgroups. Nodal support is given above the branches as bootstrap values for parsimony, likelihood, and below as posterior probabilities (a plus sign indicates 100 or 1.0).

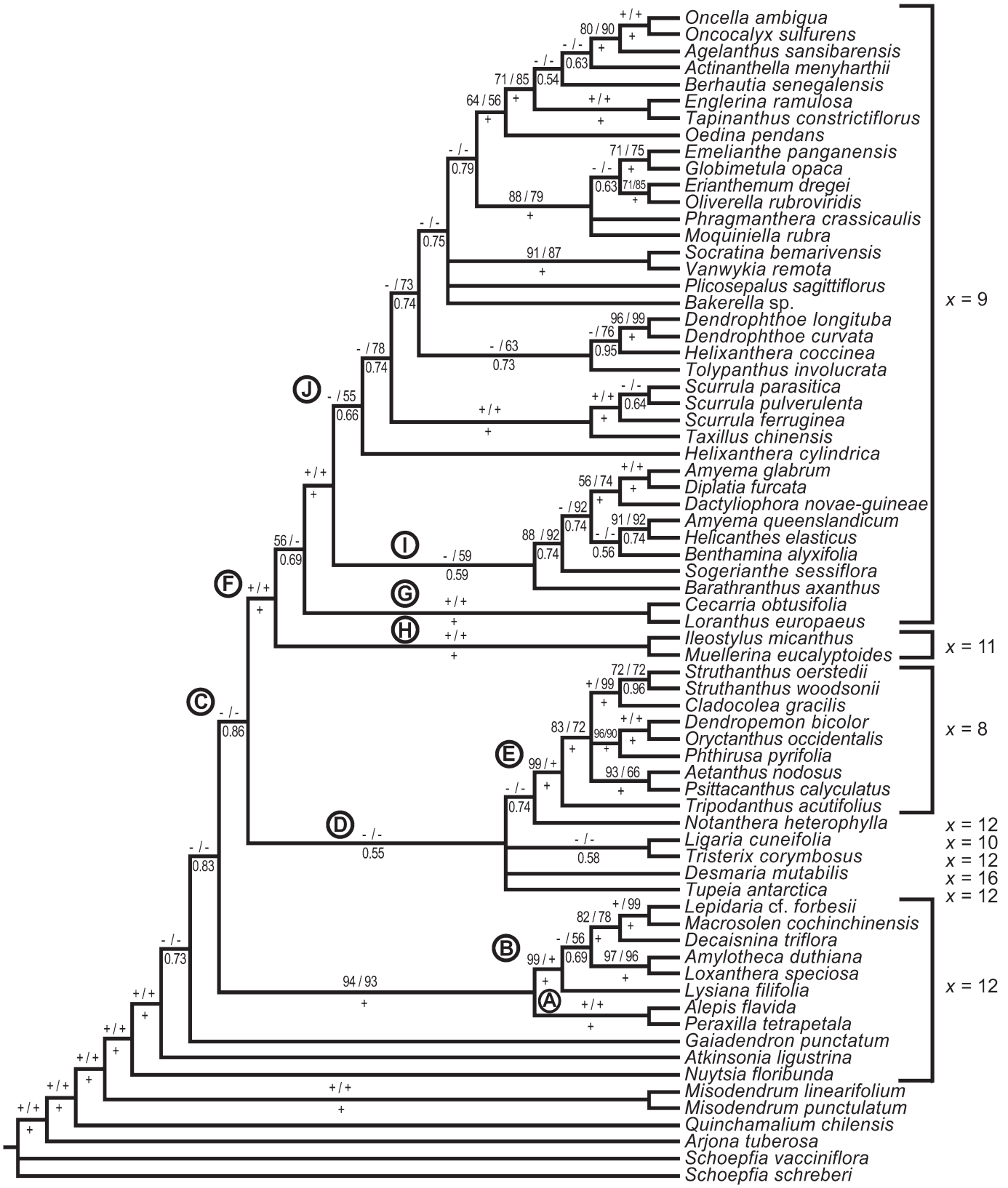

Fig. 3. Majority rule consensus tree from a Bayesian analysis of the concatenated data set that includes the five genes for Loranthaceae and outgroups. Nodal support is given above the branches as bootstrap values for parsimony, likelihood, and below as posterior probabilities (a plus sign indicates 100 or 1.0). Base chromosome numbers are indicated to the right.

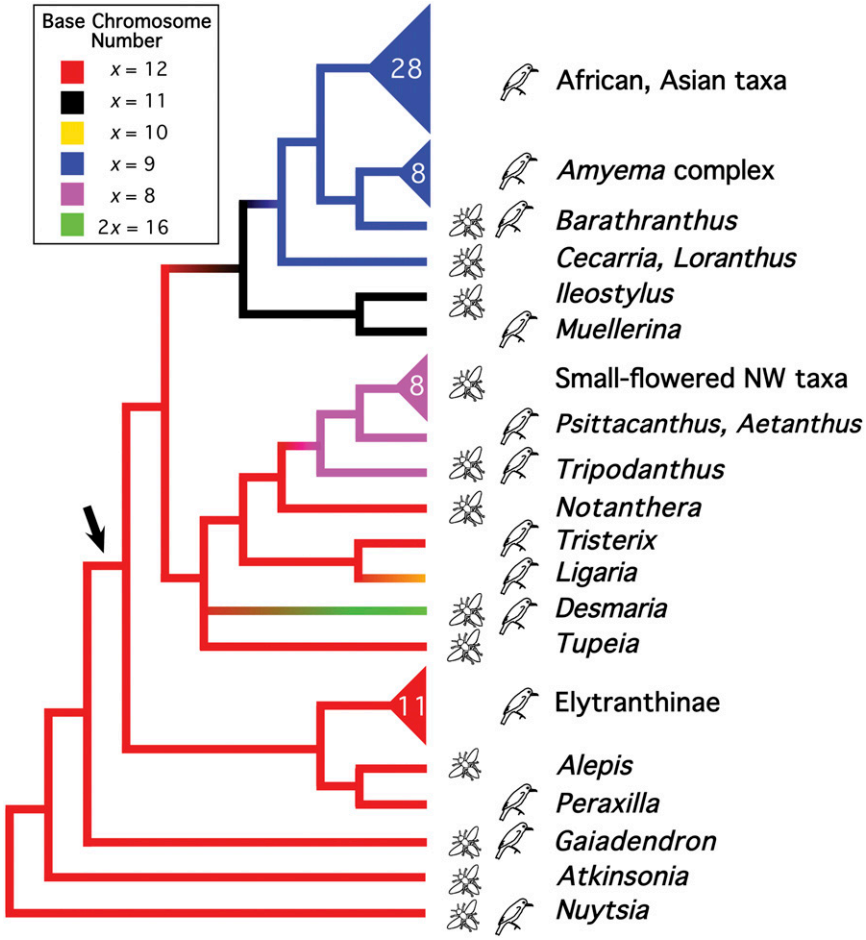

Fig. 4. Loranthaceae phylogenetic tree (simplified from Fig. 3) showing different base chromosome numbers and pollination types. Larger clades have been collapsed and the number of genera in the clade indicated in the terminal triangle. According to this reconstruction, stem parasitism arose once in the family (large arrow). Optimization of pollination type indicates that exclusively bird-pollinated clades arose multiple times independently.

Ileostylus and *Muellerina* (clade H) are sister to subtribe Loranthinae. These two genera have been recognized as a separate (but unnamed) tribe by Barlow and Wiens (1971), mainly because they have a base chromosome number of $x = 11$, unique for the family. Danser (1933), who included both genera in Loranthinae, indicated that *Ileostylus* was the "most primitive" in that subtribe. *Ileostylus* and *Muellerina* both are characterized by epicortical roots arising from the base of the plant and indeterminate inflorescences with racemes of triads. The four species of *Muellerina* are endemic to eastern Australia, whereas the single species, *Ileostylus micranthus*, is endemic to New Zealand. Clade H taxa represents the second of two apparently independent aneuploid reductions derived from $x = 12$ ancestors (the other being clade E). The third aneuploid reduction (to $x = 9$) is seen in all members of subtribe Loranthinae (Barlow and Wiens, 1971). Clade G containing *Cecarria* and *Loranthus* is highly supported; however, its position on the tree is not well resolved as it alternates between being sister to *Ileostylus* and *Muellerina* or sister to the remaining Loranthinae. Barlow and Wiens (1973) proposed that *Cecarria* represents a relict near the stem of the Old World line, a position not in disagreement with the phylogeny reported here. *Cecarria* has localized haustoria, but at least in some species of *Loranthus*, epicortical roots from the base of the plant can be formed. With the exception of these two genera, subtribe Loranthinae (clades I plus J) is highly supported as monophyletic.

The *Amyema* complex (*Amyema*, *Dactyliophora*, *Benthamina*, *Diplatia*, *Sogerianthe*) is well supported (clade I) and includes genera characterized by large inflorescences and flowers in triads; however, this general type is frequently reduced in various taxa (Danser, 1933; Kuijt, 1981). Most genera have epicortical roots but some *Amyema* species produce localized infections. *Diplatia* is the only genus in this clade that has endophytic growth (cortical strands). The two species of *Amyema* (*A. glauca* and *A. queenslandica*) sampled in this study did not form a clade, thus suggesting that the entire genus of 92 species is not monophyletic, a result in agreement with Calladine and Waycott (1999). Additional molecular work is required to establish affinities among all its components.

Within clade J, a clade with *Dendrophthoe* and *Tolypanthus* (from Indomalaya and Asia) are sister to another large clade (18 genera) found in Africa and Madagascar. These two genera have epicortical roots, as does *Helixanthera coccinea*. As a genus, *Helixanthera* is not monophyletic as evidenced by *H. cylindrica* being sister to the remaining taxa in clade J. This genus, with ca. 50 species, is widespread from Asia to Africa. As with *Amyema*, these results suggest that further work should be done to test the monophyly of the genus.

TABLE 3. Classification of genera with number of species, base chromosome number (*x*), and the phylogenetic clade label to which they belong in the phylogenetic analysis of the concatenated data set.

Taxon	No. species	*x*	Clade
Tribe Nuytsieae			
Nuytsia	1	12	*Nuytsia*
Tribe Elytrantheae			
Subtribe Gaiadendrinae			
Atkinsonia	1	12	*Atkinsonia*
Gaiadendron	1	12	*Gaiadendron*
Subtribe Elytranthinae			
Alepis	1	12	A
Amylotheca	4	12	B
Cyne	6	12	n.s.
Decaisnina	25	12	B
Elytranthe	10	12	n.s.
Lampas	1		n.s.
Lepeostegeres	9	12	n.s.
Lepidaria	12	12	B
Loxanthera	1	12	B
Lysiana	6	12	B
Macrosolen	25	12	B
Notanthera	1	12	D
Peraxilla	2	12	A
Trilepidea	1	extinct	n.s.
Tristerix	11	12	D
Tupeia	1	12	D
Tribe unnamed			
Muellerina	4	11	H
Ileostylus	1	11	H
Tribe Lorantheae			
Subtribe Loranthinae			
Actinanthella	2	9	J
Agelanthus	59	9	J
Amyema	92	9	I
Bakerella	16	9	J
Barathranthus	3	9	I
Benthamina	1	9	I
Berhautia	1	9	J
Cecarria	1	9	G
Dactyliophora	3	9	I
Dendrophthoe	30	9	J
Diplatia	3	9	I
Distrianthes	1	9	n.s.
Emelianthe	1	9	J
Englerina	25	9	J
Erianthemum	16	9	J
Globimetula	13	9	J
Helicanthes	1	9	I
Helixanthera	ca. 50	9	J
Loranthus	2	9	G
Moquiniella	1	9	J
Oedina	4	9	J
Oliverella	3	9	J
Oncella	4	9	J
Oncocalyx	13	9	J
Papuanthes	1	9	n.s.
Pedistylis	1	9	n.s.
Phragmanthera	34	9	J
Plicosepalus	12	9	J
Scurrula	56	9	J
Septulina	2	9	n.s.
Socratina	2	9	J
Sogerianthe	4	9	I
Spragueanella	2	9	n.s.
Tapinanthus	30	9	J
Taxillus	35	9	J
Thaumasianthes	2		n.s.
Tolypanthus	4	9	J
Trithecanthera	4	9	n.s.
Vanwykia	2	9	J
Subtribe Psittacanthinae			
Aetanthus	10	8	E
Cladocolea	25	8	E
Dendropemon	25	8	E
Desmaria	1	16	D
Ixocactus	3	8	n.s.
Ligaria	2	10	D
Oryctanthus	10	8	E
Oryctina	6	8	n.s.
Panamanthus	1	8	n.s.
Phthirusa	60	8	E
Psittacanthus	50	8	E
Struthanthus	~50	8	E
Tripodanthus	2	8	E

Only weakly supported as monophyletic, the African/Malagasy genera are characterized by their highly specialized bird-pollination mechanisms. Several genera have zygomorphic flowers with vents in the corolla tube (used by birds to open the flower) and coiled, explosive filaments. Their inflorescences are indeterminate with monads, and the flowers are five-merous with the petals fused into a tube. Most genera belong to one of two well supported clades, the exceptions being *Plicosepalus*, *Socratina*, *Taxillus*, and *Vanwykia*, which were described as "primitive" among African genera by Polhill and Wiens (1998) because they retained more pleisomorphic characters and because their pollination mechanism is less specialized. Except for *Socratina*, whose haustorial connection is unknown, the other three genera have epicortical roots that emerge only from the base of the plant; other African genera have single haustorial attachments. *Taxillus* is a genus with ca. 30 species distributed mainly in Asia with only one species reaching Africa. The accession sampled in this study was from Malaysia; thus no statements can be made about relationships with the African species. In this study *Taxillus* was sister to *Scurrula*, which agrees with their highly similar floral morphology and their mutual possession of epicortical roots. *Vanwykia* was described as closely related to the taxilloid genera (*Taxillus*, *Socratina*, *Septulina*, and *Bakerella*), but in this study it is strongly supported as sister to *Socratina*. The latter genus is endemic to Madagascar and *Vanwykia* is found in eastern and southeastern Africa; therefore a dispersal event to Madagascar from a common ancestor with *Socratina* is implied. *Oedina*, *Agelanthus*, and *Oncocalyx* belong to one of the African clades that are able to form cortical stands within the host, and the last two can form secondary shoots from this endophytic system. The other genera in this clade are characterized by localized haustorial connections. In the other African clade, only *Moquiniella* forms cortical strands and apparently lacks the capacity to form secondary shoots. The remaining five genera in this clade have single haustorial attachments.

Biogeographic implications—The molecular phylogeny presented herein has obvious implications for the biogeographic history of Loranthaceae, particularly when viewed in relation to the chronogram reported in Vidal-Russell and Nickrent (2008). Space does not permit discussion here; however, formal biogeographic analyses have been conducted (Vidal-Russell, 2007), and this topic will be fully explored in a future publication. The following represent general observations and interpretations, particularly those that relate to the roles played by pollinating and fruit-dispersing birds during the evolutionary history of Loranthaceae. Of the three basalmost loranth lineages, *Atkinsonia* is exclusively insect pollinated, and *Nuytsia* and *Gaiadendron* are visited by both insects and birds. All three of these genera have flowers whose morphology matches the reconstruction of the ancestral entomophilous type (i.e., open, choripetalous, and yellow). We argue here that these early (Cretaceous) loranths were pollinated exclusively by insects and that their floral morphology was shaped by this selectional environment. Present-day visitation by birds is likely a secondary event where opportunistic birds obtain nectar from these flowers. In the Old World, Loranthaceae are primarily pollinated and dispersed by oscine birds (order Passeriformes, suborder Passeri). These birds, in the families Dicaeidae and Nectariniidae, open the flowers with their bills by pinching the apex or by "unzipping" the flower along a corolla slit (Davidar, 1983; Feehan, 1985). The close association with pollinating birds appears to have driven selection for various floral traits. Some African genera such as *Erianthemum*, *Actinanthella*, and *Oedina* are highly specialized for bird-pollination. Their flowers change color at maturation, and their tubular corollas split along the petal junctions to form window-like fenestrae. This fenestration results from tension generated by differential growth of the stamens, which are fused to the petals below the fenestrae but free above (Kirkup, 1998). The pollinating sunbirds insert their beaks through the fenestrae, thus triggering rapid flower opening, inward coiling of the filaments, and deposition of pollen on the bird's head. Simultaneously, the petals recurve and the style moves forward. Pollination and dispersal of Loranthaceae in Australia and New Zealand is performed by honeyeaters (Melphagidae), one of the first lineages to diverge within the oscines during the Eocene (Barker et al., 2004). *Peraxilla* from New Zealand has developed an explosive mechanism that is activated by the pollinating bird (Ladley et al., 1997).

In the New World, pollination functions are performed by both oscines and hummingbirds (order Apodiformes), whereas fruit dispersal is by suboscines (order Passeriformes, suborder Tyranni). Many hummingbird species visit loranth flowers (genera such as *Aetanthus*, *Ligaria*, *Psittacanthus*, and *Tristerix*) as do some species of *Diglossa*, nectar-robbing birds that are responsible for pollinating these flowers in the Andes (Graves, 1982; Amico et al., 2007). In addition, a large number of New World loranths are insect pollinated, particularly those in the $x = 8$ small-flowered clade.

Aerial parasitism in Loranthaceae is estimated to have evolved on Gondwana during the Oligocene, ca. 28 mya (Vidal-Russell and Nickrent, 2008) when Australia, Antarctica, and South America were still connected. The interaction between Melphagidae and Loranthaceae likely began at this time, fueling diversification and the subsequent dispersal of these aerial parasites to New Zealand. Two migrational waves, one from New Zealand and one from Australia, resulted in the spread and diversification of these loranth lineages throughout Australasia, Indomalaya, and eventually Africa. Migration of Asian genera in subtribe Loranthinae (e.g., *Amyema*, *Dendrophthoe*, and *Benthamina*) into Australia was probably effected by *Dicaeum* (presently the only species of mistletoe bird found in Australia), which is thought to have migrated into Australia in the Pliocene (Reid, 1988).

At present, the picture of biotic interactions among New World loranths and their pollinators is not clear, mainly owing to the paucity of fossils for hummingbirds, the oldest of which (30 mya) is from Europe (Mayr, 2004). Two groups within clade D (Fig. 3) are pollinated by birds: (1) *Tristerix* and *Ligaria* and (2) *Psittacanthus* and *Aetanthus*. Given the topology of the molecular tree, it is likely that these plants arrived at similar floral morphologies via independent interactions with these birds and that such interactions became established more recently than the situation with honeyeaters and loranths in Australia.

Upon arriving in Africa, the ancestral loranth ($x = 9$) underwent a massive adaptive radiation that generated over 20 genera of subtribe Loranthinae. This radiation can be linked to two sources: the development of the savannah habit during middle Miocene (Jacobs, 2004) and interaction with pollinating birds during the Oligocene (Barker et al., 2004; Beresford et al., 2005). Although mistletoes are found in tropical rainforests, they are particularly suited to more open habitats where full sun permits the active transpiration necessary to maintain more negative water potentials than their hosts. Floral features such as zygomorphy, bright and contrasting corolla colors, and explosive pollen dehiscence mechanisms evolved in response to interaction with sunbirds (Nectariniidae), which are responsible for pollinating many loranths in Africa. Sunbirds are sister to flowerpeckers (Ericson et al., 2003), and both families belong to a group of oscines that diversified and dispersed from Australia around 45 mya (Barker et al., 2004). These two bird families likely played an important role in the diversification and dispersal of Loranthaceae through Asia and into Africa during the Tertiary.

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