Phylogenetic relationships of Santalales with insights into the origins of holoparasitic Balanophoraceae

Huei-Jiun Su,[1] Jer-Ming Hu,[1] Frank E. Anderson,[2] Joshua P. Der[3] & Daniel L. Nickrent[4]

1 *Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, 106, Taiwan*
2 *Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901-6509, U.S.A.*
3 *Department of Biological Sciences, California State University Fullerton, Fullerton, California 92834-6850, U.S.A.*
4 *Department of Plant Biology, Southern Illinois University, Carbondale, Illinois 62901-6509, U.S.A.*
Author for correspondence: *Daniel L. Nickrent, nickrent@plant.siu.edu*
ORCID: HJS, http://orcid.org/0000-0001-5751-7260; JMH, http://orcid.org/0000-0003-2739-9077; FEA, http://orcid.org/0000-0002-5786-0314; JPD, http://orcid.org/0000-0001-9668-2525; DLN, http://orcid.org/0000-0001-8519-0517

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Abstract To date molecular data have not revealed the exact phylogenetic position of Balanophoraceae in relation to hemiparasitic Santalales. To elucidate the phylogeny of Santalales and the position of Balanophoraceae, three plastid genes (*matK*, *rbcL*, *accD*), three nuclear genes (SSU and LSU rDNA and *RPB2*) and one mitochondrial gene (*matR*) from 197 Santalales samples (including 11 Balanophoraceae species) were analyzed with parsimony, maximum likelihood and Bayesian inference methods. Our results demonstrate that Balanophoraceae is composed of two well-supported clades: a relatively slow-evolving one including *Dactylantus*, *Hachettea*, and *Mystropetalon* (Mystropetalaceae) and an extremely fast-evolving one composed of the remaining Balanophoraceae s.str. Support for monophyly of the two clades was low, thus it appears holoparasitism has arisen twice independently in Santalales. These two clades appeared during a time of great change in the order (ca. 100 Ma) when several major evolutionary innovations emerged, e.g., the root hemiparasites of Santalaceae s.l., the first aerial parasites (Misodendraceae), herbaceous root parasites (Schoepfiaceae), root parasitic Loranthaceae (the ancestors of aerial parasitic mistletoes), as well as the holoparasites in Balanophoraceae and Mystropetalaceae.

Keywords Balanophoraceae; molecular phylogeny; Mystropetalaceae; parasitic plant; Santalales

Supplementary Material Electronic Supplements 1 (Appendix S1; Figs. S1–S7) and 2 (Appendix 1 in tabular form) and alignment are available in the Supplementary Data section of the online version of this article at http://www.ingentconnect.com/content/iapt/tax

■ INTRODUCTION

The sandalwood order (Santalales) is worldwide in distribution and is the largest order of parasitic plants including ca. 179 genera and 2460 species. Unlike most parasitic angiosperms, many members are woody and habits include both root and stem parasites such as mistletoes. Placement of Santalales within the global angiosperm phylogeny has previously been uncertain despite strong support for monophyly of the clade (Soltis & al., 1999, 2003; Hilu & al., 2003). More recent analyses using chloroplast genes have shown that Santalales is strongly supported as sister to a clade referred to as Superasteridae that contains 12 other orders including Caryophyllales and Asterales (Moore & al., 2010; Ruhfel & al., 2014). Features that unite all Santalales includes simple leaves, valvate perianth, free-central pendulous placentation (including reduced derivations), and one-seeded fruits. Moreover, many members have C18 (and longer chain) acetylenic fatty acids such as santalbic acid (Aitzetmüller, 2012; Kubitzki, 2015).

Reviews of the complex taxonomic history of Santalales have been made (Reed, 1955; Kuijt, 1968, 2015; Malécot, 2002; Nickrent & al., 2010). From the 19th century to present, there has been a trend towards recognizing the mutual affinities among a core group composed of seven families in a single order, Santalales: Olacaceae, Misodendraceae, Loranthaceae, Opiliaceae, Eremolepidaceae, Santalaceae and Viscaceae (Kuijt, 1968; Cronquist, 1981). A number of other families, such as Medusandraceae (Wurdack & Davis, 2009), Dipentodontaceae (Peng & al., 2003), Grubbiaceae (Xiang & al., 2002) have also been included at one time or another but the current consensus is that they are not closely related.

The association of Balanophoraceae with Santalales dates to the middle of the 19th century. Eichler (1867) provided detailed descriptions and illustrations of female flowers among various genera of Balanophoraceae and Cynomoriaceae. He stated that Cynomoriaceae was allied with *Hippuris* L. (Saxifragales: Haloragaceae), in agreement with Hooker (1856) and that Balanophoraceae (minus *Mystropetalon* Harv.) were related

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to Misodendraceae and Loranthaceae. A connection to Santalales was maintained by Van Tieghem (1896), and Fagerlind (1948), both of whom were influenced by shared morphological reductions in the gynoecium. Kuijt (1968, 1969) speculated that these morphological similarities could be the results of convergent adaptions to parasitism. Some 20th century classifications included these parasites in Santalales (Engler & Gilg, 1912; Cronquist, 1981) whereas some did not (Kuijt, 1968; Takhtajan, 1997). In all of these more recent classifications, Cynomoriaceae was included in Balanophoraceae or allied with it. Takhtajan (1997) placed superorder Balanophoranae near Rafflesianae, both in Magnoliidae. Balanophorales was split into eight families that corresponded with the subfamilies and tribes of Harms (1935). Later Takhtajan (2009) moved Balanophoranae to Rosidae, placing it after Santalanae, and reunited the segregate families into Balanophoraceae. These dramatically different taxonomic concepts, even among treatments from the same individual, demonstrate well the uncertainty about affinities of this group of holoparasites.

Clarification of relationships within and between Santalales and Balanophoraceae began with the introduction of molecular data. The first molecular phylogenetic study of Santalales that included robust taxon sampling was by Nickrent & Duff (1996). This study established the basic topological structure of the phylogenetic tree with Olacaceae s.l. as basalmost followed by Misodendraceae, Loranthaceae, Opiliaceae, Santalaceae and Viscaceae. *Schoepfia* Schreb. was shown to be more closely related to Misodendraceae and "Eremolepidaceae" a component of Santalaceae s.l. Small-subunit rDNA sequences were used to generate this phylogeny and were also used to show a split between the Old and New World genera of Balanophoraceae. That study did not address any possible relationships between Santalales and Balanophoraceae, mainly because of high substitution rates in the holoparasites (Nickrent & Starr, 1994) that compromised via long-branch attraction (LBA) parsimony analyses with other angiosperms. Later work using both nuclear ribosomal and mitochondrial gene sequences showed that Cynomoriaceae was not closely related to Balanophoraceae and that the later was most closely related to Santalales (Nickrent & al., 2005). Both of these relationships were later confirmed in the large-scale study of parasite evolution by Barkman & al. (2007). Further confirmation of a Santalales affinity came from molecular phylogenies of nuclear *RPB*2 and B-class genes (Su & Hu, 2012). Despite these advances, the exact placement of Balanophoraceae, either sister to or within Santalales, was not resolved.

Relationships within Santalales (not including Balanophoraceae) have been explored in several molecular phylogenetic investigations: Olacaceae s.l. (Malécot & Nickrent, 2008), Loranthaceae (Vidal-Russell & Nickrent, 2008a), and Santalaceae s.l. (Der & Nickrent, 2008). The timing of the evolution of the mistletoe habit was examined using 36 representatives of Santalales (Vidal-Russell & Nickrent, 2008b). These data were summarized and a new classification of the order proposed by Nickrent & al. (2010). Although a consensus phylogeny was presented, this did not derive from an alignment containing all known sequences for the order.

In the present study, sequence data from the nucleus, chloroplast and mitochondrion have been assembled for all available Santalales including Balanophoraceae. For the first time, analyses were conducted with a 7-gene matrix to examine (1) support for all Santalales clades (families) and (2) the phylogenetic position of Balanophoraceae in the sandalwood order.

■ MATERIALS AND METHODS

Taxon sampling. — A total of 19 families, 148 genera and 180 species of Santalales were sampled, including 10 genera and 11 species of Balanophoraceae (Appendix 1, with voucher information and GenBank accession numbers). Six species from other core eudicots were used as the outgroups. Although some classifications include *Cynomorium* L. within or near Balanophoraceae (Cronquist, 1981; Takhtajan, 2009), it was not included in this study (justification in Electr. Suppl.: Appendix S1).

DNA extraction and gene amplifications. — For the newly obtained sequences in this study, genomic DNA was extracted from herbarium, fresh frozen or silica dried plant tissues using a standard CTAB method (Doyle & Doyle, 1987) or a modified CTAB method (Nickrent, 1994, 1997). Chloroplast *matK* and *accD* genes were amplified using primers and protocols reported in Rogers & al. (2008). Nuclear LSU rDNA genes were amplified according to Vidal-Russell & Nickrent (2008a). The nuclear SSU rDNA, mitochondrial *matR* and the homologs of *RPB2* sequences were amplified with the primers and conditions described in Su & Hu (2012). Direct sequencing of PCR products used various automated methods. Additionally, five sequences were extracted from four transcriptome assemblies included in the 1000 Plants Project (1KP; http://www.onekp.com) using BLAST, including *Dendropemon caribaeus* Krug & Urb. (*accD*, *RPB2*), *Phoradendron leucarpum* (Raf.) Reveal & M.C.Johnst. (*RPB2*), *Exocarpos cupressiformis* Labill. (*RPB2*), and *Daenikera corallina* Hürl. & Stauffer (*RPB2*).

Phylogenetic analyses. — Edited sequences were imported into Se-Al v.2.0a11 (Rambaut, 2007) and aligned manually. For the protein coding genes, the nucleotide sequences were translated into amino acid sequences and indels were introduced while maintaining sequence frame. All indels were treated as missing data. For nuclear ribosomal DNA, alignment was guided by reference to published higher-order structures. Individual gene alignments were saved as NEXUS files and then concatenated using Mesquite v.3.01 (Maddison & Maddison, 2011).

All of the individual gene datasets and the concatenated datasets were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods. For the ML and BI analyses, appropriate substitution models for each individual gene dataset were estimated using jModelTest v.2.1.3 (Posada, 2008, 2009). The ML topologies were performed under Genetic Algorithm for Rapid Likelihood Inference (Garli) v.2.0 (Zwickl, 2006). 100 search replicates with stepwise addition of taxa and all other options set to defaults. Rapid bootstrapping (BS) of 500 pseudo-replicates was performed in RAxML v.7.0.4 (Stamatakis, 2006) under the GTR+I+G model. The BI analyses were performed with

MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) and the best-fitting substitution model for the combined datasets were estimated using PartitionFinder v.1.1.1 (Lanfear & al., 2012) for each gene and codon position. Markov Chain Monte Carlo searches were performed with four chains of 2.5 (individual gene datasets) or 7 (concatenated dataset) million generations with a sample frequency of 500. The consensus tree and Bayesian inference posterior probabilities (BIPP) were calculated from trees with split frequencies less than 0.01.

For the MP analyses, heuristic searches were conducted by PAUP* v.4.0b10 (Swofford, 2002) with 1000 random addition replicates using tree bisection-reconnection (TBR) branch swapping with steepest descent option in effect. The maximum trees were set to 10,000 and 1 tree was held at each step. The MP bootstrap were evaluated using 100 random-addition replicates with the maximum trees retained set to 10,000, retaining only 10 trees of length ≥1 per replicate (nchuck = 10, chuckscore = 1).

Hypothesis testing. — Multiple tests were conducted to assess possible conflicts among different datasets and analytical approaches. Tree topologies and support values resulting from each dataset were compared to assess the possible conflicts. To test the effects of different data and different inference methods on the phylogenetic estimation, we performed the MP/ML analyses with varying gene content: (1) removing the fast-evolving taxa of Balanophoraceae in the 7-gene concatenated dataset and (2) removing the third-codon position of protein-coding genes in the non-plastid gene dataset.

Trees resulting from MP and ML analyses of the concatenated dataset supported different hypotheses of relationships among members of Balanophoraceae. Two simulation-based tests were conducted to assess whether monophyly of Balanophoraceae can be rejected under ML and if MP is being misled by LBA. A parametric bootstrapping test called SOWH (Swofford & al., 1996) was implemented to evaluate the hypothesis that a tree on which Balanophoraceae is monophyletic is the best explanation of the concatenated 7-gene dataset. The complexities of this dataset—i.e., alignment gaps in many of the genes, missing data for some genes for some taxa, and no plastid data for Balanophoraceae—complicated the simulations. To test this hypothesis, we estimated the best-fitting model for each gene in jModelTest as described above, ignoring models that included a proportion of invariant sites parameter (this parameter is not available in PAML; see below). The ML tree for the 7-gene dataset, partitioned by gene, was estimated in Garli under a topological constraint that enforced monophyly for Balanophoraceae. Each individual gene dataset was opened in PAUP*; the ML tree was loaded into PAUP* along with each individual-gene dataset. Taxa missing for each gene were deleted from the ML tree, producing a trimmed ML tree based on the full dataset that matched the complement of taxa for which data were available for each gene. Each of the seven resulting trimmed trees was loaded into PAML v.4.3 (Yang, 2007) along with the corresponding individual gene dataset, and branch lengths of the trimmed tree and substitution model parameters were estimated for each gene. The seven sets of trimmed tree topology, branch lengths and model parameter estimates were individually used to simulate 100 single-gene datasets in INDELible v.1.03 (Fletcher & Yang, 2009). These 100 simulated single-gene data matrices were concatenated using FASconCAT v.1.0pl (Kück & Meusemann, 2010). The resulting simulated 7-gene concatenated datasets did not include indels within genes, but did match the original concatenated dataset's pattern of missing data on a "by locus" basis (i.e., taxa missing data for genes in the original data also lacked these data in the simulated concatenated data matrices). The simulated datasets were analyzed in Garli with and without enforcing a topological constraint for Balanophoraceae monophyly. Garli analyses were conducted using five random stepwise addition search replicates, with data partitioned by gene and using the best-fitting substitution models for each gene as estimated for the original concatenated dataset. The resulting delta values (differences in log likelihood for the pair of ML trees estimated for each simulated dataset) were used as a null distribution for the hypothesis that a tree including a monophyletic Balanophoraceae is the true tree. The observed delta value (the difference in likelihood between the unconstrained ML tree for the original dataset and the best ML tree found under a Balanophoraceae monophyly constraint) was compared to this null distribution. If the observed delta was greater than 95% of the simulated delta values, the null hypothesis was rejected.

A similar test was used to investigate whether LBA could be misleading MP, causing it to recover a monophyletic Balanophoraceae. Following (Huelsenbeck & Crandall, 1997), data were simulated and concatenated as described above, but on the unconstrained ML tree estimated by Garli via an analysis of the original, 7-gene concatenated dataset with the data partitioned by gene. These 100 simulated concatenated datasets were analyzed with MP as described above. Trees resulting from MP analyses were passed through a Balanophoraceae monophyly filter in PAUP*. If LBA is misleading MP, we expect MP to return trees on which Balanophoraceae is monophyletic.

■ RESULTS

A total of 124 sequences were newly obtained in this study, which including 7 SSU rDNA, 23 LSU rDNA, 39 *RPB2*, 1 *rbcL*, 3 *matK*, 34 *accD* and 17 *matR* gene sequences. Statistics relating to the separate and combined gene datasets and subsequent phylogenetic analyses are given in Table 1. Among the seven individual gene datasets, the plastid *matK* gene contained the highest percentage of parsimony-informative characters (54%) whereas the nuclear SSU rDNA exhibited the lowest (25%). Although the level of resolution between the different datasets differed, the relationships among the major clades were largely congruent without a significant conflict (here we refer to BS > 60%) among the topologies (Electr. Suppl.: Fig. S1).

Phylogenetic analyses of combined datasets. — The topologies of the 7-gene concatenated dataset from ML/BI (Fig. 1, BI tree not shown) and MP (Electr. Suppl.: Fig. S2) are all highly similar to each other. The monophyly of Santalales is well supported by all three methods (MP/ML BS = 99%–100%, BIPP = 1.0) and the clades corresponding to each of the Santalales families (except for Balanophoraceae)

Table 1. Characteristics of different datasets.

Dataset	Taxa	Aligned length (bp)	PI sites	Tree length	MP trees	CI	RI	ML model
accD	86	1,449	680	3,091	104,109	0.496	0.676	GTR+I+G
matK	166	1,929	1,042	7,035	4,070	0.358	0.758	GTR+I+G
rbcL	130	1,437	449	2,598	1,430	0.389	0.686	TVM+I+G
SSU rDNA	179	1,841	455	3,165	7,498	0.345	0.629	GTR+I+G
LSU rDNA	133	2,166	737	5,255	120	0.341	0.549	GTR+I+G
RPB2	54	1,716	682	4,934	8	0.284	0.424	GTR+I+G
matR	54	2,346	629	2,136	15,566	0.740	0.657	TIM+G
Plastid combined	172	4,815	2,171	12,840	530	0.394	0.727	TVM+I+G
Non-plastid combined	182	8,069	2,503	15,707	7,320	0.374	0.532	GTR+I+G
Non-plastid combined (3rd codon removed)	182	6,715	1,725	10,562	30	0.395	0.575	GTR+I+G
7 genes combined	186	12,884	4,674	28,736	6,912	0.380	0.641	GTR+I+G & SYM+I+G
7 genes minus Bal. A	178	12,884	4,052	25,219	15,552	0.384	0.657	GTR+I+G

Abbreviations: PI, parsimony informative; MP, maximum parsimony; CI, consistency index; RI, retention index; ML, maximum likelihood; Bal., Balanophoraceae

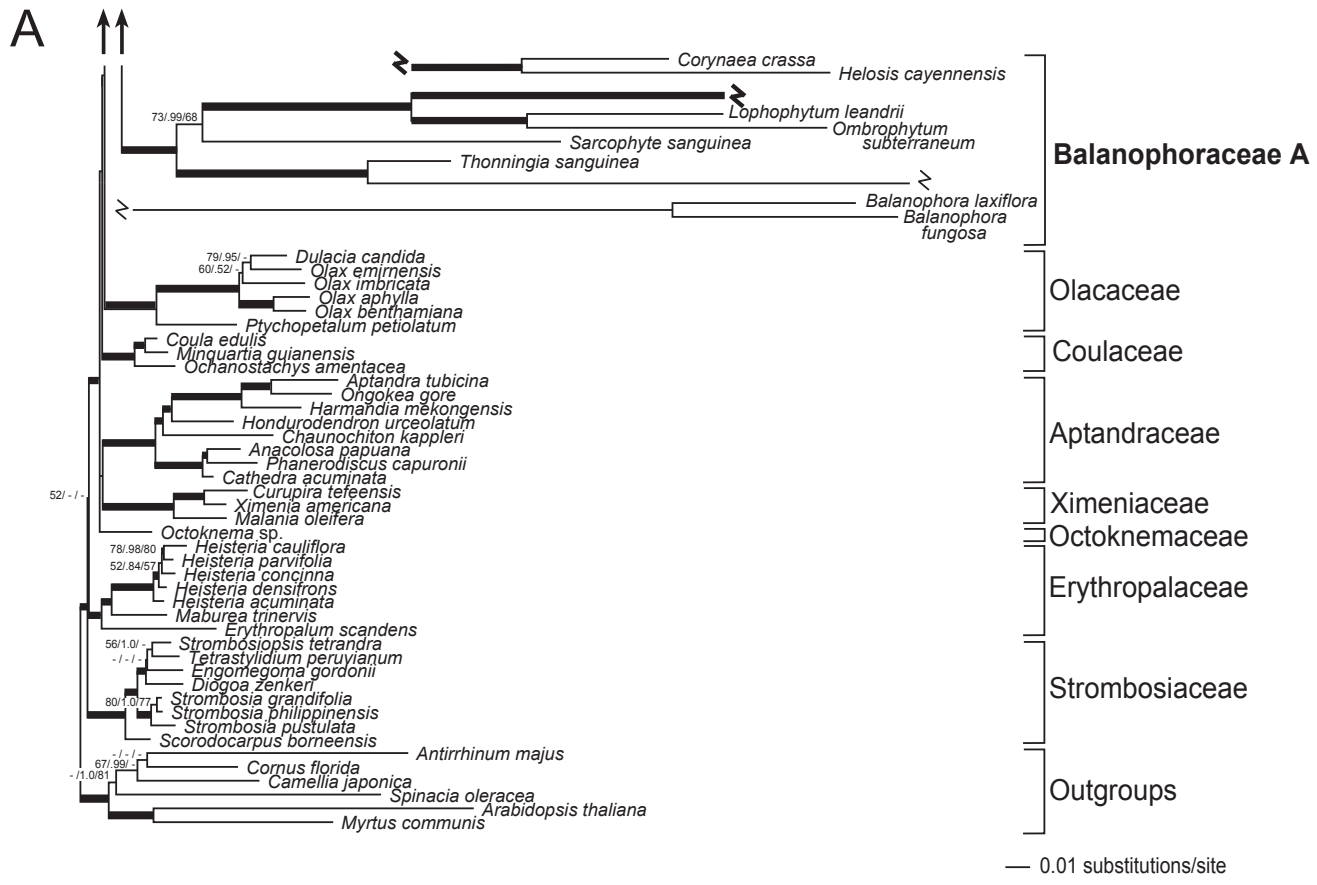
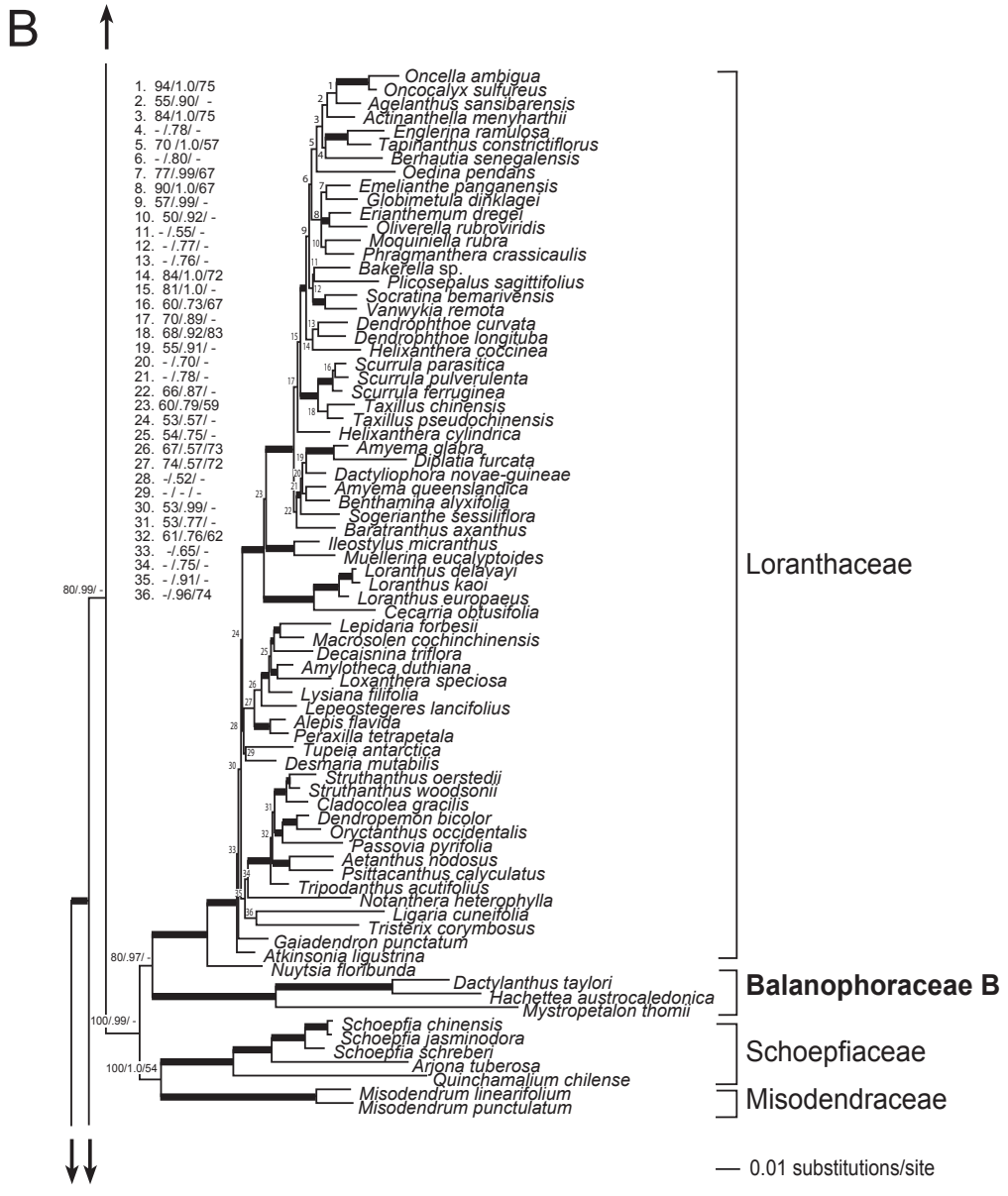


Fig. 1A–C. The ML phylogram inferred from the 7-gene-combined dataset for Santalales. Bootstrap support greater than 80% and Bayesian posterior probabilities greater than 0.95 are shown with bold lines. Lesser support values are given above the nodes: maximum likelihood bootstrap/Bayesian inference posterior probabilities/maximum parsimony bootstrap (MLBS/BIPP/MPBS). **A,** Bottom portion of the phylogram showing relationships among Olacaceae s.l. and Balanophoraceae s.str. Two of the long branches in Balanophoraceae were split given graphical constraints. **B,** Middle portion of the phylogram showing relationships among Misodendraceae, Schoepfiaceae, Balanophoraceae B (Mystropetalaceae), and Loranthaceae. So as not to obscure the Loranthaceae clades, nodes were numbered (1–36) and the support values indicated next to the tree. **C,** Upper portion of phylogram showing relationships among Opiliaceae and seven families sometimes classified as Santalaceae s.l.

all received bootstrap support greater than 95%. However, there are some topological differences between of ML/BI and MP, including the relationships among Olacaceae, Coulaceae, Aptandraceae, Octoknemaceae and Ximeniaceae, as well as the relationships among Thesiaceae, Comandraceae, Cervantesiaceae and Balanophoraceae. The position of Octoknemaceae (consisting of one accession of *Octoknema* Pierre) was placed at different positions on the trees (Fig. 1A; Electr. Suppl.: Fig. S2), as one of the basal lineages in Santalales with ML/BI (Fig. 1) or as sister to the "non-Olacaceae s.l." taxa with MP (Electr. Suppl.: Fig. S2). Some support for the latter position was obtained with ML/BI using a dataset where the fast-evolving Balanophoraceae taxa were removed (Electr. Suppl.: Fig. S3). The relationships among Olacaceae, Coulaceae, Aptandraceae and Ximeniaceae also varied between the ML/BI and MP trees. Sister relationships were found between Olacaceae and Aptandraceae and between Coulaceae and Ximeniaceae in the MP analysis (Electr. Suppl.: Fig. S2) and the ML analysis without fast-evolving Balanophoraceae (ML/MP BS = 53%–78%, BIPP = 0.95–1.0 in Electr. Suppl.: Fig. S3). However, in the ML analysis with all Balanophoraceae, Coulaceae was resolved as a sister to Olacaceae and the remaining "non-Olacaceae s.l. taxa; moreover, Ximeniaceae was sister to Aptandraceae. In both of these cases support for the clades was low.

In both the ML/BI and MP trees (Fig. 1A, B; Electr. Suppl.: Fig. S7) Balanophoraceae are resolved as two well-supported clades. The first, called Balanophoraceae A, is composed of seven genera including *Balanophora* J.R.Forst. & G.Forst. and the second, Balanophoraceae B, is composed of *Dactylanthus* Hook.f., *Hachettea* Baill. and *Mystropetalon*. These two clades have conspicuously different substitution rates resulting in the former with very long intertaxon branch lengths whereas the latter clade has branches comparable to other Santalales parasites (e.g., Viscaceae). The MP tree (Electr. Suppl.: Fig. S2)



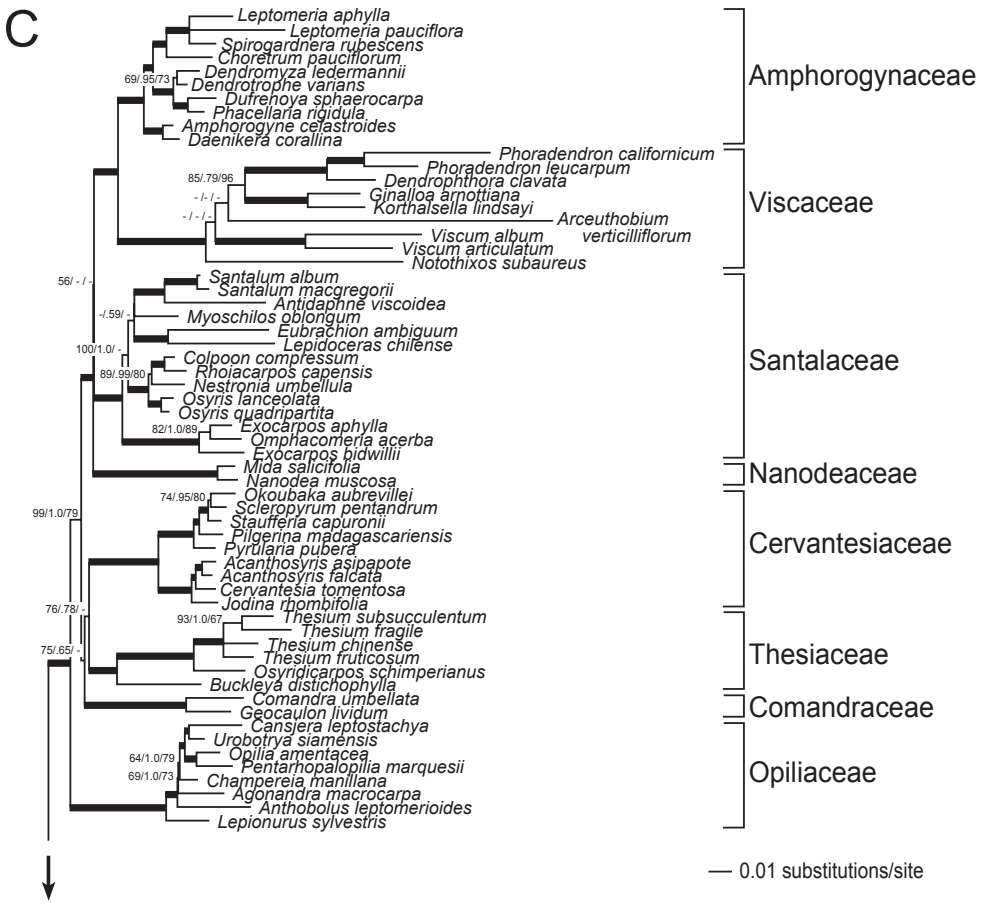
shows Balanophoraceae as monophyletic but with low support (MPBS < 50%). In contrast, Balanophoraceae are not monophyletic on the ML/BI tree (Fig. 1) with the B clade sister to Loranthaceae (MLBS = 80%, BIPP = 0.97) and the A clade sister to the "non-Olacaceae s.l." clade (MLBS = 94%, BIPP = 1.0). After removal of the A clade in both the ML (Electr. Suppl.: Fig. S3) and MP (Electr. Suppl.: Fig. S4) trees, the position of the B clade remained the same, either sister to Loranthaceae (MLBS = 93%, BIPP = 0.96) or grouped with Schoepfiaceae and Misodendraceae (MPBS = 96%).

The large mistletoe family Loranthaceae received strong support as monophyletic in all analyses with the root parasite *Nuytsia* R.Br. sister to the rest of the family. *Atkinsonia* F.Muell. also a root parasite from Australia, diverged next in both the ML/BI (Fig. 1B) and MP (Electr. Suppl.: Fig. S2) trees. The third root parasite, *Gaiadendron* G.Don. diverged next in the ML/BI tree but not on the MP tree. The topology of these two trees differed most in the positions of biogeographically key taxa such as *Tupeia* Cham. & Schltdl. from New Zealand and *Desmaria* Tiegh. and *Notanthera* G.Don. from South America. In general, several clades from the "spine" of these trees were poorly supported, thus resulting in low resolution.

The upper portion of the ML/BI (Fig. 1C) and MP (Electr. Suppl.: Fig. S2) trees consisting of Opiliaceae and Santalaceae s.l. were for the most part congruent. Opiliaceae (including *Anthobolus* R.Br. of former Santalaceae) was resolved with strong support as sister to the remaining clades. The monophyly of the other seven families received strong bootstrap support with ML/BI and MP but interrelationships differed in some cases. A sister relationship between Thesiaceae and Cervantesiaceae received moderate support (MLBS = 76%, BIPP = 0.78, MPBS < 50%) and Comandraceae was sister to them (MLBS = 75%, BIPP = 0.65, MPBS < 50%). Despite showing these three families in one clade on the shortest MP tree (Electr. Suppl.: Fig. S2), support for this clade was low.

Comparing the topologies based on the plastid and non-plastid gene datasets. — The phylogenetic relationships of the major clades based on ML and MP analyses of four non-plastid and three plastid genes are shown in Fig. 2. The individual genes contained different amounts of phylogenetic signal as shown by the number of parsimony-informative sites (Table 1) with the lowest being SSU rDNA and *rbcL* and the highest *matK* and LSU rDNA. *RPB*2 and *matR* had the lowest taxon sampling, which also influences comparisons of phylogenetic signal across genes and partitions (Electr. Suppl.: Fig. S1). Despite some incongruences between the ML and MP topologies in non-plastid and plastid data partitions, most of these conflicted relationships received weak bootstrap support (MP/ML BS < 60%).

Although support for clades representing the families Olacaceae s.l. and Santalaceae s.l. were generally high, relationships between these clades (i.e., along the "spine" of the



tree) were not well supported. Because the plastid genes are absent in Balanophoraceae, this partition could not be used to assess its position in the order (Fig. 2). For the four non-plastid genes (Fig. 2), a monophyletic family was not supported and the same topology and clades (A and B) as were seen on the 7-gene concatenated tree (Fig. 1) were obtained in the ML analysis. Conversely, Balanophoraceae was resolved as monophyletic, albeit weakly (MPBS = ca. 50%) on the MP tree (Fig. 2; Electr. Suppl.: Fig. S2). Exclusion of the third-codon position of *matR* and *RPB2* from the 4-gene non-plastid partition yielded similar results for the position of Balanophoraceae (Electr. Suppl.: Fig. S5). This exercise removed much of the phylogenetic signal, thus support for some of the relationships along the "spine" of the tree was reduced, particularly in the in the MP tree. For the ML tree, the same topology as seen in Fig. 2 was recovered.

Results of the SOWH test suggest that ML rejects Balanophoraceae monophyly. The observed difference in likelihood for the ML topology and the topology resulting from a search constrained to return a monophyletic Balanophoraceae was 42.4064. This delta value was much greater than all 100 delta values for data simulated on the constrained topology ($P < 0.01$; the largest simulated delta value was 0.0021). However, the Huelsenbeck test, used to determine whether LBA could be misleading MP to return a monophyletic Balanophoraceae even if the true tree had a polyphyletic Balanophoraceae, suggests that LBA is not misleading MP. Balanophoraceae monophyly was not recovered in any tree resulting from MP analysis of the 100 datasets simulated on the ML topology (on which Balanophoraceae was polyphyletic; data not shown).

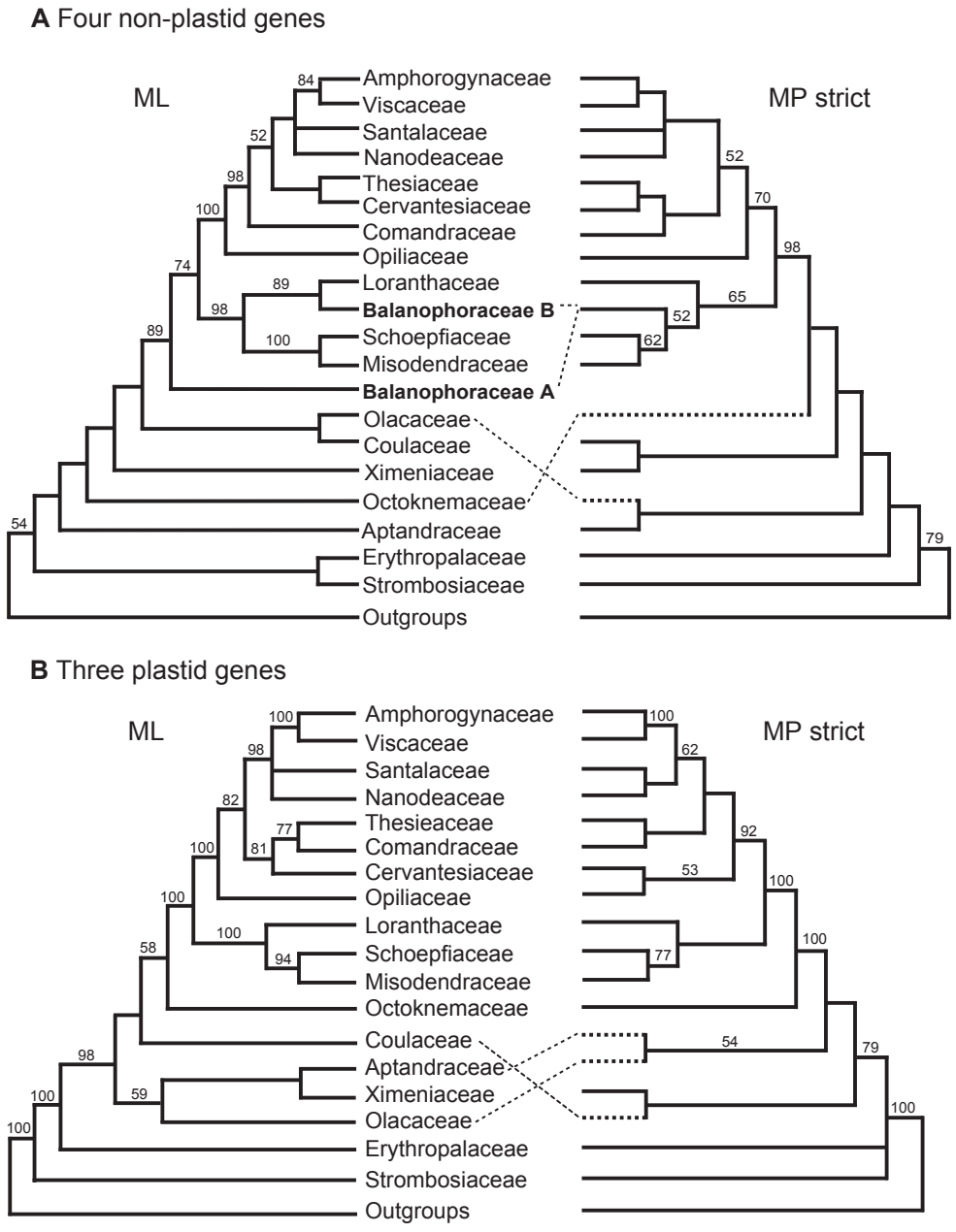


Fig. 2. Comparisons of tree topologies of Santalales for two gene partitions using maximum likelihood (ML) and strict consensus maximum parsimony (MP). Bootstrap support values are shown above the nodes. **A,** Trees derived from four non-plastid genes (SSU and LSU rDNA, *RPB2*, *matR*); **B,** trees derived from three plastid genes (*accD*, *matK*, *rbcL*).

■ DISCUSSION

This study represents the first molecular phylogenetic analysis to use comprehensive taxon sampling in Santalales, including Balanophoraceae. DNA sequences for 148 of the total 179 genera in the order were utilized, including 10 of 17 genera of Balanophoraceae. Although plastid genes could not be PCR-amplified from Balanophoraceae, three genes (*rbcL*, *matK*, *acc*D) were concatenated with nuclear *RPB*2, SSU and LSU rDNA and mitochondrial *matR* to maximize resolution. With newly generated sequences added to those from previous studies, resolution of the position of Balanophoraceae within Santalales has improved.

Relationships among the autotrophic and hemiparasitic clades of Santalales. — The phylogenetic relationships among the major clades in Santalales (minus Balanophoraceae) are generally congruent with those presented in previous molecular phylogenetic investigations (Der & Nickrent, 2008; Malécot & Nickrent, 2008; Vidal-Russell & Nickrent, 2008a, b). Each of these clades will be briefly discussed, particularly with reference to two classifications that have appeared after publication of the above molecular studies: the Angiosperm Phylogeny Website (Stevens, 2001–) and Kuijt (2015). The traditionally recognized family Olacaceae was shown to be polyphyletic by Malécot & Nickrent (2008) and eight clades were recognized as families in Nickrent & al. (2010): Strombosiaceae, Erythropalaceae, Octoknemaceae, Ximeniaceae, Aptandraceae, Coulaceae, Olacaceae and Schoepfiaceae (Fig. 1). Although interfamilial relationships were not fully resolved, all of these families were strongly supported by the molecular data.

With its six genera, Strombosiaceae was classified as a family by Nickrent & al. (2010) and Stevens (2001–) but not by Kuijt (2015) who lumped these genera, along with *Heisteria* Jacq., *Maburea* Maas, and *Brachynema* Benth. into a broadly defined Olacaceae. As discussed in Malécot & Nickrent (2008), molecular and morphological cladistic trees (Malécot & al., 2004) have points of agreement and disagreement regarding relationships within Strombosiaceae. Given that Olacaceae s.l. (as defined by Kuijt) still shows extreme morphological heterogeneity, we believe it is more prudent to use the molecular data to inform how to partition this variability in a phylogenetically meaningful way. Morphological features shared by members of this family were discussed in Nickrent & al. (2010) and are presented in more detail in Nickrent (1997–).

The present study includes three genera of Erythropalaceae: *Erythropalum* Bl., *Heisteria*, and *Maburea*. In addition, the genus *Brachynema* (not sampled here) was shown via molecular work by K. Wurdack (unpub.) to belong in this family, near *Maburea*. These genera have very similar leaf anatomy (Maas & al., 1992) and also have axile placentation, absent elsewhere in Santalales. Morphological features that support placing *Brachynema* in Santalales include the presence of a valvate perianth and an acrescent calyx. If *Brachynema* truly belongs in this family, then the already wide array of morphological variation in vegetative and reproductive features increases further. With regard to *Erythropalum*, Kuijt (2015) states "the systematic affinities of *Erythropalum* have yet to be resolved", yet both MP and ML trees reported here show strong support for its inclusion in this family. Kuijt (2015) indicates he followed Sleumer (1935) in removing *Erythropalum* from Santalales; however, a later publication by the same author includes the genus in Olacaceae (Sleumer, 1980).

The family Octoknemaceae, containing only the genus *Octoknema* was recognized by Nickrent & al. (2010), Stevens (2001–) and Kuijt (2015). Despite adding nuclear and mitochondrial sequence data for *Octoknema*, its position in Santalales remains poorly resolved. There appears to be conflict between the non-plastid and plastid partitions (Fig. 2) where the latter placed it as weakly supported as sister to the "non-Olacaceae" clades. This relationship was also seen in Malécot & Nickrent (2008) which had only two plastid genes (*rbcL*, *matK*) available for analysis. Although the tree inferred from *RPB2* plus SSU rDNA agreed with this placement, the tree resulting from *matR* did not, thus leaving the placement of Octoknemaceae ambiguous.

Ximeniaceae composed of *Curupira* Black, *Douradoa* Sleumer (not sampled here), *Malania* Chun & Lee, and *Ximenia* L. is a well-supported clade from both molecules and morphology. The family was recognized as composed here by Nickrent & al. (2010), Stevens (2001–) and Kuijt (2015). Its position in the Santalales phylogeny is variable depending upon the gene or method of analysis, often forming a weakly supported clade with Coulaceae (Fig. 1A; Electr. Suppl.: Figs. S1, S2). For *Ximenia*, positive evidence exists for root parasitism (DeFilipps, 1969); however, haustoria could not be seen on excavated roots of a large individual of *Curupira* (C. Clement, pers. comm.) nor on potted seedlings (Rodrigues, 1961). If some Ximeniaceae are parasitic and others are not, then it is possible that parasitism evolved more than once in the order. Alternatively, it may have evolved only once, but was then lost in some Ximeniaceae. The loss of parasitism once it has evolved in a lineage has never been documented and, given the selective advantage this trophic mode provides, this explanation seems less likely. Finally, it is possible that with additional searching, especially on young roots of older plants growing in the presence of host roots, haustoria will be found. Clearly additional basic research is required.

Aptandraceae was recognized as a family by Nickrent & al. (2010), Stevens (2001–) and in a modified form by Kuijt (2015). The topology of this clade (Fig. 1) and the one derived from a 4-gene analysis (Ulloa & al., 2010) were identical. The family is composed of two well-supported clades: the Aptandra clade (*Aptandra* Miers, *Chaunochiton* Benth., *Harmandia* Baillon, *Hondurodendron* Ulloa & al., *Ongokea* Pierre) and the Anacolosa clade (*Anacolosa* Bl., *Cathedra* Miers, *Phanerodiscus* Cavaco), both characterized by several morphological synapomorphies (Ulloa & al., 2010). Kuijt (2015) excluded *Anacolosa* and *Cathedra* stating these genera "lack such fundamental features", referring to anther dehiscence by reflexed valves or pores. Because he believed these two genera had "more regular anther structure", and did not describe their dehiscence, Kuijt (2015) placed them in Olacaceae. The variable anther morphology in Aptandraceae was discussed in Ulloa & al. (2010). In fact, the anther dehiscence in *Cathedra* is similar to

that seen in *Chaunochiton* and *Phanerodiscus*, i.e., porose (see Aptandraceae images in Nickrent, 1997–). Likewise, the slit or porose dehiscence is described and illustrated for *Anacolosa* in Capuron (1968). Thus, both molecular data and morphology strongly support recognition of Aptandraceae as circumscribed by Nickrent & al. (2010).

Coulaceae was circumscribed identically by Nickrent & al. (2010), Stevens (2001–) and Kuijt (2015). Composed of *Coula* Baillon, *Minquartia* Aublet and *Ochanostachys* Masters, it is strongly supported as monophyletic; however, its position in Santalales is not stable. ML trees for SSU rDNA and *matK* (Electr. Suppl.: Fig. S1) as well as the 7-gene ML tree with fast Balanophoraceae removed (Electr. Suppl.: Fig. S3) show Coulaceae as sister to Ximeniaceae but this relationship was not recovered in the 7-gene ML tree with all taxa included (Fig. 1). Haustoria were not found on excavated roots of *Ochanostachys amentacea* Mast. cultivated at the Rimba Ilmu Botanic Garden in Malaysia (Teo, 1997), thus at least this member of the family appears to be autotrophic.

Olacaceae s.str. is composed of *Dulacia* Sleumer, *Olax* L., and *Ptychopetalum* Benth. which follows Nickrent & al. (2010) and Stevens (2001–) but not Kuijt (2015). The latter author did not recognize Erythropalaceae nor Strombosiaceae and included those members, as well as the above three genera and *Brachynema* Benth, in a heterogeneous Olacaceae s.l. composed of 13 genera. Olacaceae s.str. is very well supported as monophyletic based on molecules and as discussed in Malécot & Nickrent (2008), have long been recognized for their morphological, anatomical, and palynological homogeneity. Haustorial parasitism has been documented for *Olax* (Pate & al., 1990) and *Ptychopetalum* (Anselmino, 1932).

The middle portion of the Santalales tree (Fig. 1B) is where much evolutionary change is taking place. Here is where the hemiparasites Santalaceae s.l., Misodendraceae, Schoepfiaceae and Loranthaceae originate, as well as the holoparasites in Balanophoraceae (discussed below). These clades apparently diverged from "Olacaceae-like" ancestors ca. 90–100 Ma (Vidal-Russell & Nickrent, 2008b; Bell & al., 2010; Naumann & al., 2013). Misodendraceae contains one genus, *Misodendrum* Banks ex DC., with eight species of aerial parasites of *Nothofagus* Blume in southern South America (Vidal-Russell & Nickrent, 2007). Time estimates using Bayesian relaxed molecular clock methods date the appearance of the family, and likely the first mistletoes, at ca. 80 Ma (Vidal-Russell & Nickrent, 2008b). This clade diverged from a clade of root parasites classified in Nickrent & al. (2010) as Schoepfiaceae. This family, composed of *Arjona* Comm. ex Cav., *Quinchamalium* Molina, and *Schoepfia* Schreber was recognized by Stevens (2001–) and Kuijt (2015), but the latter author includes only the genus *Schoepfia*. The sister relationship between Misodendraceae and Schoepfiaceae received strong support with most molecular partitions. Moreover, the inclusion of *Arjona* and *Quinchamalium* in Schoepfiaceae is strongly supported by molecular data and several morphological features discussed in Nickrent & al. (2010). These data were apparently dismissed by Kuijt (2015) who retained *Arjona* and *Quinchamalium* in their traditional family, Santalaceae s.l.

The Misodendraceae/Schoepfiaceae clade is sister to another composed of Loranthaceae and the Balanophoraceae B clade. This topology is obtained using the full 7-gene dataset as well as the non-plastid and plastid gene partitions (Figs. 1, 2). Relationships within Loranthaceae are largely congruent with those previously published (Vidal-Russell & Nickrent, 2008a), hence they will not be described in detail here. The Australian root parasite *Nuytsia* is strongly supported as sister to the remainder of the family. Although the major clades, classified as tribes and subtribes in Nickrent & al. (2010) were recovered, the "spine" of the Loranthaceae tree has a number of poorly resolved nodes that will require additional molecular markers to resolve. That classification was reported verbatim in Kuijt (2015). Loranthaceae was the last of the five mistletoe clades to evolve, ca. 28 Ma (Vidal-Russell & Nickrent, 2008b) and it subsequently underwent a massive adaptive radiation producing great generic and specific diversity.

The upper portion of the Santalales ML tree (Fig. 1C) contains Opiliaceae and Santalaceae s.l. as presented by Stevens (2001–). The classification for this group here follows Nickrent & al. (2010) where Santalaceae s.l. is composed of seven families: Comandraceae, Thesiaceae, Cervantesiaceae, Nanodeaceae, Santalaceae s.str., Viscaceae and Amphorogynaceae. The classification system of Kuijt (2015) is most similar to that of Pilger (1935) which contained a broadly defined Santalaceae as well as Eremolepidaceae and Viscaceae. Kuijt (2015) did not follow an existing nor propose a new tribal classification of Santalaceae. Moreover, advancements such as recognition of Amphorogyneae (Stauffer, 1969) or insights gained from molecular analyses (Der & Nickrent, 2008) were not incorporated, thus the Santalaceae treatment by Kuijt (2015) is not a classification but an alphabetical list of generic descriptions.

Opiliaceae is strongly supported as sister to the remaining clades. Although included in Olacaceae by many 19th century workers, the family Opiliaceae has been consistently recognized after the treatment by Sleumer (1935). Members have leaves with cystoliths, bisexual or unisexual flowers, and a superior ovary with one ovule (Kuijt, 2015). Seven of the ten genera in this family were sampled as well as the genus *Anthobolus* which was traditionally classified in Santalaceae near *Exocarpos* Labill. (Stauffer, 1959). As discussed in Der & Nickrent (2008) and Nickrent & al. (2010), the finding that *Anthobolus* is more closely related to Opiliaceae was surprising, although both have a superior ovary which differs from most Santalaceae s.l. with inferior ovaries. True ovules are seen in Opiliaceae but not in *Anthobolus* which has a central cone-shaped placenta and undifferentiated ovules (Stauffer, 1959). With regard to *Anthobolus*, Kuijt (2015) argued "its affinity with the Santalaceous *Exocarpos* is undeniable". It should be pointed out, however, that reductions in the placental-ovule complex in Opiliaceae have occurred. The ovule of *Agonandra* is not elevated on a free-central placenta but is basal (Hiepko, 2000). Taking this reduction trend further one can envision the condition seen in *Anthobolus*. The phylogenetic placement in Opiliaceae is consistent across nuclear and chloroplast genes and this relationship is seen not only with *A. leptomerioides* F.Muell. (sampled here) but also with *A. filifolius*

R.Br. (Nickrent, data not shown). It is worth considering that the morphological similarity to *Exocarpos* reflects convergence or atavism, as is prevalent in Santalaceae s.l. (Der & Nickrent, 2008).

The classification of Stevens (2001–) presents Santalaceae s.l. containing seven tribes or groups that correspond to the families of Nickrent & al. (2010). It is curious that the rank of family was accepted for the segregates of Olacaceae s.l. but was not followed for the equally polyphyletic Santalaceae s.l. As shown in the present multigene study, both groups have well-supported clades (here recognized at the familial rank) and both have poorly supported interrelationships among the families along the "spine" of the tree. Given the topology and support values for the nodes in the upper portion of the Santalales tree, it is not clear why Stevens (2001–) excluded Opiliaceae from Santalaceae s.l. As outlined in Nickrent & al. (2010), four secondary principles were followed (in addition to monophyly), one of which was stability, i.e., minimizing nomenclatural changes. Both Santalaceae and Viscaceae are very well-established in the literature, thus retaining these names causes the least disruption and preserves information about these clearly defined families. Three of the five remaining families, Amphorogynaceae, Cervantesiaceae and Thesiaceae are very strongly supported as monophyletic and each is well characterized morphologically. The remaining two families (Comandraceae, Nanodeaceae) are small, each containing just two genera. The former was sister to the Cervantesiaceae/Thesiaceae clade but with only moderate support (Fig. 1C). Similarly, Nanodeaceae was sister to a clade containing Santalaceae, Amphorogynaceae and Viscaceae but with low support. It is likely that additional sequences will eventually resolve the "spine" of the Santalales tree.

Phylogenetic placement of Balanophoraceae within Santalales. — The results of this study confirmed that Balanophoraceae are derived from within Santalales (Barkman & al., 2007; Nickrent & al., 2010; Su & Hu, 2012) and showed the presence of two distinct clades with widely differing substitution rates: a fast-evolving clade A and a relatively slowly evolving clade B. The MP tree recovered a monophyletic Balanophoraceae, with low support (Fig. 2; Electr. Suppl.: Fig. S2), whereas the ML tree placed clade B as sister to Loranthaceae (moderate support) and clade A two nodes deeper on the tree as sister to the non-Olacaceae s.l. clades (Fig. 1A–B). The data present here support a non-monophyletic Balanophoraceae, thus indicating that holoparasitism evolved independently two times in Santalales. Artifactual phylogenetic relationships resulting from LBA (Felsenstein, 1978) were demonstrated with another holoparasite group, Rafflesiaceae s.l. (Nickrent & al., 2004) where MP (as opposed to model based methods) was particularly susceptible. Among-site rate variation is a common characteristic of sequence evolution that results from different selective constraints on different sites (Yang & Kumar, 1996). In contrast to MP, the model-based analyses better accommodate different sequence evolution parameters such as base substitution heterogeneity and rate variation among nucleotide sites (Bos & Posada, 2005; Gadagkar & Kumar, 2005; Philippe & al., 2005). Given the branch lengths in the Balanophoraceae A clade, it is reasonable to expect such systematic error; however, results of the Huelsenbeck test indicate LBA was not misleading MP. For the three clade B genera, branch lengths on the phylogram (Fig. 1B) are not particularly long, in fact comparable to those seen among genera of Viscaceae. Moreover, after the removal of clade A taxa, the position of clade B in both the ML and MP tree did not change. When third-codon position of protein-coding genes are removed, which reduced some degree of substitution bias, the resulting relationships of both Balanophoraceae clade A and B remained constant (Electr. Suppl.: Fig. S5). Balanophoraceae clades A and B were also consistently separated following removal of the fastest evolving category of sites (Electr. Suppl.: Fig. S6) or by using amino acid sequences of the five protein-coding genes (Electr. Suppl.: Fig. S7). These results indicate that the Balanophoraceae clades were not influenced by LBA and that their topologies reflect actual phylogenetic affinity.

The recognition of at least two groups within Balanophoraceae is not unprecedented. As mentioned in the Introduction, Eichler (1867) separated *Mystropetalum* from the remaining Balanophoraceae. His association of the latter with Misodendraceae and Loranthaceae is amazingly similar to the molecular phylogenetic results reported here. Harms (1935) proposed dividing the genera into six subfamilies and this system was followed in a slightly modified form by Takhtajan (1997) whose classification included eight families, all split from Balanophoraceae s.l. Given the type genus occurs in clade A, we retain that family name Balanophoraceae Rich. (Richard 1822: 429) in the strict sense for *Balanophora* and 13 other genera. For clade B, two family names are available, Mystropetalaceae Hook.f. (Hooker, 1853: 94) for *Mystropetalon* and Dactylanthaceae (Engl.) Takht. (Takhtajan, 1987: 43) for *Dactylanthus* and *Hachettea*. Given earlier date of publication of the former, we include those three genera in Mystropetalaceae.

Phylogenetic relationships among genera of Balanophoraceae. — In Mystropetalaceae, *Hachettea* of New Caledonia is sister to *Dactylanthus* of New Zealand and that clade is sister to *Mystropetalon* of South Africa. All three of these genera have comparatively restricted distributions on Gondwanan landmasses. The time of divergence for this clade is approximately the late Cretaceous, in agreement with Naumann & al. (2013) who derived a date for the Santalales/Balanophoraceae clade of 109 Ma. The common ancestor, likely a woody root hemiparasite (as in Olacaceae), underwent a radiation that produced the common ancestor of Opiliaceae/Santalaceae s.l., the first aerial parasites (Misodendraceae), the first herbaceous perennial root hemiparasites (Schoepfiaceae – *Arjona*, *Quinchamalium*), the first root parasitic members of Loranthaceae (i.e., *Nuytsia*, *Atkinsonia*), and the holoparasites of Mystropetalaceae. The ancient ancestor of Mystropetalaceae was likely woody and hemiparasitic, but evolutionary changes along this branch produced the herbaceous holoparasites we see in the extant genera. Reductions and losses of floral parts seen in Balanophoraceae s.str. (below) are not as pronounced in Mystropetalaceae. For example, a perianth is present on the female flowers of all three genera (Hansen, 1986; Holzapfel, 2001; Hansen & Kubitzki, 2015). The morphological reductions of the gynoecium and a

shared *Allium* type of embryo sac inspired Fagerlind (1948) to propose a shared ancestry between "Balanophorales" and Viscaceae/*Helixanthera* Lour. (Loranthaceae). Morphological and anatomical features of the haustorium in *Mystropetalon* were considered to be similar to Santalales (Weber, 1986). Given the phylogenetic data, it is now important to determine if such apparent similarities represent synapomorphies.

Relationships among the genera Balanophoraceae s.str. are generally concordant with groups recognized in morphology-based classifications (Takhtajan, 1997, 2009; Hansen & Kubitzki, 2015). *Thonningia* Vahl. together with *Langsdorffia* Raddi were placed in Langsdorffieae by Harms and this tribe was elevated to subfamily by Takhtajan (2009). The former genus is found in tropical Africa whereas the latter has a disjunct distribution that includes Madagascar, New Guinea, and tropical America. Molecular data place *Langsdorffia malagasica* (Fawc.) B.Hansen as sister to *Thonningia* (Nickrent, unpub. data). As reflected in the classification by Harms (1935), Langsdorffioideae is related to Balanophoroideae (containing just *Balanophora*). This is borne out by the molecular data, chemical data such as the storage of balanophorin (as opposed to starch) in the tubers, and morphology where female flowers have one style. With ca. 16 species, *Balanophora* is the largest genus in the family and has a wide distribution from Madagascar to India, Indomalaya, Australia and Pacific islands. It is monoecious or dioecious with complex male and female inflorescence structure (Eberwein & al., 2009). The male flowers have a valvate perianth and anthers fused into a synandrium whereas the female flowers are the smallest among angioserms composed of as few as 50 cells (Hansen, 1972). The length of the branch leading to the two *Balanophora* species (Fig. 1) is exceptionally long, thereby reflecting the number of substitutional changes that accompanied the evolutionary trajectory of this genus. The neotropical genera *Lophophytum* Schott & Endl. and *Ombrophytum* Poepp. have long been known to be related (Harms, 1935) and *Lathrophytum* Eichl. may also be part of this group (Nickrent, unpub. molecular data). Their female flowers lack a perianth and have two styles. Another neotropical subfamily, Helosidoideae (Harms, 1935) contains *Corynaea* Hook.f., *Helosis* Rich. and three paleotropical genera *Ditepalanthus* Fagerl., *Rhopalocnemis* Junghuhn and *Exorhopala* Steenis (none sampled here). The latter genus was synonymized with *Helosis* by Eberwein & Weber (2004); however, molecular data confirming that these taxa are congeneric does not yet exist. The New World genus *Scybalium* Schott & Endl. placed in its own family Scybaliaceae by Takhtajan (1997) also belongs near *Corynaea* and *Helosis* (Nickrent, unpub. molecular data), thus supporting the classification of Harms (1935). The genera *Sarcophyte* Sparrm. and *Chlamydophytum* Mildbr. (latter unsampled) occur in tropical Africa and are characterized by having robust, paniculate male inflorescences. They were placed in subfamily Sarcophytoideae by Harms (1935) and Sarcophytaceae by Takhtajan (1997). The molecular data show *Sarcophyte* as intermediate in position between the Balanophoroideae/Langsdorffioideae clade and the Lophophytoideae/Helosidoideae clade.

Conclusions. — For the autotrophic and hemiparasitic members of Santalales, this study confirms the composition of and support for clades seen in previous molecular phylogenetic studies that were classified at the family level. Presented here is strong evidence that the holoparasitic family Balanophoraceae are derived from within Santalales. A relatively slow-evolving clade composed of *Dactylanthus*, *Hachettea*, and *Mystropetalon* (all Southern Hemisphere taxa) was shown to be sister to Loranthaceae and is here recognized as Mystropetalaceae. The other fast-evolving clade, composed of seven sampled genera, including *Balanophora*, has a wide distribution in tropical and subtropical areas and is here recognized as Balanophoraceae s.str. Our data, which support the non-monophyly of Balanophoraceae s.l., help to explain why the morphology-based classifications of this family have historically varied widely.

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Appendix 1. Samples used in the phylogenetic analyses. Following each species name is country of origin, collector & number, herbarium of voucher, DNA accession number and GenBank numbers in the following order: SSU rDNA, LSU rDNA, *RPB2*, *rbcL*, *matK*, *accD*, and *matR*. Sequences being reported here for the first time are indicated by *. Missing sequences are indicated by a dash (–). See Electronic Supplement 2 to view these data in tabular form.

Acanthosyris asipapote M.Nee: Bolivia, *M. Nees & I. Vargas 45009* (NY), DLN 4051, DQ329163, AF181776, –, DQ329171, DQ329182, DQ329193, –. ***Acanthosyris falcata*** Griseb.: Bolivia, *M. Nees 46690* (NY), DLN 4053, DQ329164, –, –, DQ329172, DQ329183, DQ329194, –. ***Actinanthella menyharthii*** (Engl. & Schinz) Balle: Zimbabwe, *D. Wiens 4638* (MO), DLN 4375, EU544313, EU544352, –, –, EU544408, –, –. ***Aetanthus noduosus*** (Desr.) Engl.: Ecuador, *F.M. Garmendia 1227* (MO), DLN 4561, EU544314, –, –, –, EU544409, –, –. ***Agelanthus sansibarensis*** (Engl.) Polhill & Wiens: Kenya, *S.A. Robertson s.n.* (SIU), DLN 2987, U59946, EU544353, –, EU544464, EU544410, –, –. ***Agonandra macrocarpa*** L.O.Williams: Costa Rica, *D.L. Nickrent 2764* (SIU), DLN 2764, L24079, DQ790205, –, DQ790130, DQ790169, DQ790237, –. ***Alepis flavida*** (Hook.f.) Tiegh.: New Zealand, *B. Molloy s.n.* (SIU), DLN 2743, L24139, EF464474, –, –, EF464508, *KP263290, –. ***Amphorogyne celastroides*** Stauffer & Hürl.: New Caledonia, *G.D. McPherson 18051* (MO), DLN 4564, EF584571, –, *KP263324, –, EF584614, –, –. ***Amyema glabra*** (Domin) Danser: Australia, *D.L. Nickrent 2795* (SIU), DLN 2795, AF039073, EU544354, –, EU544465, EU544411, –, (AY453106). ***Amyema queenslandica*** (Blakely) Danser: Australia, *D.L. Nickrent 2788* (SIU), DLN 2788, EU544315, EU544355, –, –, EU544412, –, –. ***Amylotheca duthiana*** (King) Danser: Malaysia, *D.L. Nickrent 4022* (SIU), DLN 4022, EU544316, EU544356, –, –, EU544413, –, –. ***Anacolosa papuana*** Schellenb.: Solomon Islands; Indonesia, *R. Regalado & M.Q. Sirikolo 692; A.C. Church 338* (MO; A), DLN 4247; Hu 1852, DQ790104, –, (*KP263325), DQ790144, DQ790181, DQ790250, –. ***Anthobolus leptomerioides*** F.Muell.: Australia, *B.J. Lepschi & L.A. Craven 4352* (CANB), DLN 4311, EF584572, –, –, EF584589, EF584615, *KP263291, –. ***Antidaphne viscoidea*** Poepp. & Endl.: Costa Rica, *S. Sargent s.n.* (SIU), DLN 2730, L24080, *KP263237, –, L26068, EF464500, *KP263292, –. ***Aptandra tubicina*** (Poepp.) Benth. ex Miers: Peru, *H. van der Werff & R. Vasquez 13846* (MO), DLN 4202, DQ790105, DQ790217, –, DQ790141, DQ790178, DQ790247, –. ***Arceuthobium verticilliflorum*** Engelm.: Mexico, *D.L. Nickrent 2065* (SIU), DLN 2065, L24042, EF464470, (AY566624), L26067, –, –, –. ***Arjona tuberosa*** Cav.: Argentina, *V. Melzheimer s.n.; Pisann 3594* (SIU; GH), DLN 4131; Hu 1860, EF464468, EF464480, *KP263326, EF464532, EF464513, –, *KP263264. ***Atkinsonia ligustrina*** (Lindl.) F.Muell.: Australia, *D. Watson 4458* (SIU), DLN 4343, EF464464, EF464475, –, EF464526, DQ787444, –, . ***Bakerella* sp.:** Madagascar, *S. Razafimandimbison 332* (MO), DLN 4161, EU544318, EU544358, –, EU544466, EU544415, –, . ***Balanophora fungosa*** J.R.Forst. & G.Forst.: Australia; Taiwan, *D.L. Nickrent 2825; J.-Y. Huang 20040203* (SIU; TAI), DLN 2825; Su 118, JN392868, *KP263238, JQ613269, –, –, –, JQ613244. ***Balanophora laxiflora*** Hemsl.: Taiwan, *H.-J. Su 043, 044* (TAI), Su 043, 044, JN392870, *KP263239, JQ613270, –, –, –, JQ613245. ***Baratranthus axanthus*** (Korth.) Miq.: Malaysia, *D.L. Nickrent 4029* (SIU), DLN 4029, EU544317, EU544357, –, –, EU544414, –, –. ***Benthamina alyxifolia*** (Benth.) Tiegh.: Australia, *W. Forstreuter Be.al.01* (SIU), DLN 4127, EU544319 , EU544359, –, –, EU544416, –, –. ***Berhautia senegalensis*** Balle: Gambia, *M. Jones s.n.* (SIU), DLN 4576, EU544320, EU544360, –, –, EU544417, –, –. ***Buckleya distichophylla*** (Nutt.) Torr.: U.S.A., *L.J. Musselman s.n.* (SIU), DLN 2735, X16598, EF464473, –, DQ329180, DQ329191, DQ329202, (DQ110331). ***Cansjera leptostachya*** Benth.: Australia, *D.L. Nickrent 2815* (SIU), DLN 2815, L24084, DQ790204, –, DQ790128, DQ790167, –, –. ***Cathedra acuminata*** (Benth.) Miers: Brazil, *J.A. Ratter & al. 6782* (MO), DLN 4244, FJ848847, –, –, DQ790145, DQ790182, DQ790251, –. ***Cecarria obtusifolia*** Merr.: Australia, *B. Hyland 16493* (QRS), DLN 4562, EU544321, EU544361, –, EU544467, EU544418, –, –. ***Cervantesia tomentosa*** Ruiz & Pav.: Bolivia, *L.J. Dorr & L.C. Barnett 6941* (MO), DLN 4273, DQ329165, *KP263240, *KP263327, DQ329173, DQ329184, DQ329195, (DQ110333). ***Champereia manillana*** (Blume) Merr.: Thailand, *W. Forstreuter s.n.* (SIU), DLN 3014, JQ613223, –, JQ613271, DQ790129, DQ790168, DQ790236, JQ613247. ***Chaunochiton kappleri*** (Sagot ex Engl.) Ducke: Costa Rica, *N. Zamora & al. 1928* (MO), DLN 3052, DQ790106, DQ790218, –, DQ790142, DQ790179, DQ790248, –. ***Choretrum pauciflorum*** A.DC.: Australia, *B. Lepschi & al. 4237* (CANB), DLN 4222, EF584573, –, –, EF464522, EF464503, *KP263293, –. ***Cladocolea gracilis*** Kuijt: Mexico, *A.C. Sanders & P.A. Fryxell 4172* (MO), DLN 3066, EU544322, EU544362, –, –, EU544419, –, –. ***Colpoon compressum*** P.J.Bergius: South Africa, *D.L. Nickrent 4084* (SIU), DLN 4084, EF584574, –, –, EF584590, EF584616, *KP263294, –. ***Comandra umbellata*** (L.) Nutt.: U.S.A., *G. Tonkovitch s.n.* (SIU), DLN 2739, L24772, DQ329170, *KP263241, *KP263328, DQ329181, DQ329192, DQ329203, –. ***Corynaea crassa*** Hook.f.: Costa Rica, *D.L. Nickrent & S.-C. Hsiao 3011* (SIU), DLN 3011, L24400, *KP263242, *KP263329, –, –, –, *KP263265. ***Coula edulis*** Baill.: Gabon, *J.J. Wieringa 3295* (WAG), DLN 3079, –, –, –, DQ790147, DQ790184, DQ790253, –. ***Curupira tefeensis*** G.A.Black: Brazil, *C. Clement s.n.* (INPA), DLN 4988, DQ790107, DQ790221, , DQ790150, DQ790187, DQ790256, –. ***Dactylanthus taylorii*** Hook.f.: New Zealand, *C. Ecroyd s.n.* (SIU), DLN 4071, AY957443, *KP263243, *KP263330, –, –, –, AY957447. ***Dactyliophora novae-guineae*** (F.M.Bailey) Danser: Australia, *B. Hyland 16461* (QRS), DLN 4563, EU544323, EU544363, –, –, EU544420, –, –. ***Daenikera corallina*** Hürl. & Stauffer : New Caledonia, *J. Munzinger 2054* (NOU), DLN 4876, EF464462, EF464472? or Josh, *KP263331, EF464523, EF464504, *KP263295, –. ***Decaisnina triflora*** (Span.) Tiegh.: Papua New Guinea, *D.L. Nickrent 4491* (WAU), DLN 4491, EU544324, EU544364, –, EU544468, EU544421, –, –. ***Dendromyza ledermannii*** (Pilg.) Stauffer: Papua New Guinea, *D.L. Nickrent 4466* (WAU), DLN 4466, EF464463, –, –, EF464524, EF464505, *KP263297, –. ***Dendropemon caribaeus*** Krug & Urb.: Puerto Rico, *D.L. Nickrent 2172* (SIU), DLN 2172, AF039075, EU544365, (*KP263332), EU544469, EU544422, (*KP263296), –. ***Dendrophthoe curvata*** (Blume) Miq.: Malaysia, *D.L. Nickrent 4012* (SIU), DLN 4012, EU544325, EU544367, –, (HQ317760), EU544424, –, –. ***Dendrophthoe longituba*** (Elmer) Danser: Malaysia, *D.L. Nickrent 4010* (SIU), DLN 4010, (HQ317760), EU544366, –, –, EU544423, –, (DQ110337). ***Dendrophthora clavata*** (Benth.) Urb.: Colombia, *M. Melampy s.n.* (SIU), DLN 2182, L24086, AF181813, –, L26069, EF584636, –, –. ***Dendrotrophe varians*** (Blume) Miq.: Australia; Malaysia, *D.L. Nickrent 2827; 4014* (SIU), DLN 2827; DLN 4014, L24087, –, –, EF464520, EF464501, –, –. ***Desmaria mutabilis*** (Poepp. & Endl.) Tiegh. ex T.Durand & B.D.Jacks.: Chile, *G. Amico s.n.* (SIU), DLN 4510, EF464465, EF464476, –, EF464527, EF464509, *KP263298, –. ***Diogoa zenkeri*** Exell & Mendonça: Gabon, *J.J. Wieringa 3288* (WAG), DLN 3078, DQ790108, DQ790223, *KP263333, DQ790152, DQ790189, DQ790258, *KP263266. ***Diplatia furcata*** Barlow: Australia, *D.L. Nickrent 2824* (SIU), DLN 2824, L24088, EU544368, –, –, EU544425, –, –. ***Dufrenoya sphaerocarpa*** (Danser) Stauffer: Indonesia, *G.G. Hambali s.n.* (SIU), DLN 2754, AF039071, –, –, EF584592, EF584617, *KP263299, –. ***Dulacia candida*** (Poepp.) Kuntze: Ecuador, *M.J. Macía & al. 553* (MO), DLN 4245, DQ790109, –, *KP263334, DQ790137, DQ790174, DQ790244, DQ110338. ***Emelianthe panganensis*** (Engl.) Danser: Tanzania, *E. Mboya 594* (MO), DLN 4889, EU544326, EU544369, –, –, EU544426, –, –. ***Englerina ramulosa*** (Sprague) Polhill & Wiens: Kenya, *S.A. Robertson s.n.* (SIU), DLN 2984, (L24140), EU544370, –, EU544470, EU544427, –, –. ***Engomegoma gordonii*** Breteler: Equatorial Guinea, *B. Senterra 18-81* (P), DLN 4555, DQ790110, –, –, DQ790153, –, –, –. ***Erianthemum dregei*** (Eckl. & Zeyh.) Tiegh.: Kenya, *S.A. Robertson s.n.* (SIU), DLN 2985, L25679, EU544371, –, –, EU544428, –, –. ***Erythropalum scandens*** Blume: Indonesia; China, *M. Chase 1328; H.-J. Su 055* (K; TAI), DLN 4165; Su 055, DQ790111, DQ790233, *KP263335, DQ790164, DQ790200, DQ790267, *KP263267. ***Eubrachion ambiguum*** (Hook. & Arn.) Engl.: Puerto Rico, *D.L. Nickrent 2699* (SIU), DLN 2699, L24141, AF389273, –, L26071, EF464498, *KP263300, –. ***Exocarpos aphyllus*** R.Br.: Australia, *A. Markey & B. Barlow s.n.* (SIU), DLN 3094, EF584575, –, –, EF584593, EF584618, *KP263301, –. ***Exocarpos bidwillii*** Hook.f.: New Zealand, *B. Molloy s.n.* (SIU), DLN 2745, L24142, *KP263244, (*KP263336), EF584594, EF584619, *KP263302, –. ***Gaiadendron punctatum*** (Ruiz & Pav.) G.Don: Costa Rica, *S. Sargent s.n.* (SIU), DLN 2729, L24143, DQ790209, –, L26072, DQ787445, DQ790238, DQ110339. ***Geocaulon lividum*** (Richardson) Fernald: U.S.A., *J. Fetzner s.n.* (SIU), DLN 3047, AF039072, *KP263245, –, EF584595, EF584620, *KP263303, –. ***Ginalloa arnottiana*** Korth.: Malaysia, *J. Beaman 9074* (SAR), DLN 2982, L24144, –, –, L26070, EF584637, –, –. ***Globimetula dinklagei*** (Engl.) Danser: Gabon, *J.J. Wieringa 2858* (WAG), DLN 3087, AF039076, EU544372, –, –, EU544429, –, –. ***Hachettea austrocaledonica*** Baill.: New Caledonia, *J.-M. Groult s.n.* (SIU), DLN 4181, AY957444, *KP263246, *KP263337, –, –, –, AY957448. ***Harmandia mekongensis*** Baill.: Indonesia, *Koizumi 1411* (KYO), DLN 5597, FJ848849, –, –, FJ848842, FJ848845, FJ848843, –. ***Heisteria acuminata*** (Humb. & Bonpl.) Engl.: Panama, *R. Perez 161835* (US), STRI:BCI 161835, –, –, –, GQ981760, GQ982009, –, –. ***Heisteria cauliflora*** Sm.: French Guyana, *M.F. Prévost 3796* (CAY), DLN 4254, DQ790112, DQ790229, –, DQ790160, DQ790196, DQ790264, –. ***Heisteria concinna*** Standl.: Panama, *C. Augspurger s.n.* (SIU), DLN 2732, L24146, DQ790230, *KP263338, DQ790161, DQ790197, –, –. ***Heisteria densifrons*** Engl.: French Guyana, *J.K. Munzinger & al. 497* (P), DLN 4232, DQ790113, DQ790231, –, DQ790162, DQ790198, –, –. ***Heisteria parvifolia*** Sm.: Cameroon, *M. Cheek 5985* (K), DLN 4166, –, DQ790232, –, AJ131771, AY042600, DQ790266, GU351220. ***Helixanthera coccinea*** (Jack) Danser: Malaysia, *D.L. Nickrent 4019* (SIU), DLN 4019, –, EU544373, –, –, EU544430, –, –. ***Helixanthera cylindrica*** (Jack ex Roxb.) Danser: Malaysia, *C. Calvin & al. B22* (SAR), DLN 4037, EU544327, EU544374, –, –, EU544431, –, –. ***Helosis cayennensis*** (Sw.) Spreng.: Costa Rica, *D.L. Nickrent & S.-C. Hsiao 3006; J. Gomez s.n.* (SIU), DLN 3006; DLN 3017, L25682, *KP263247, –, –, –, –, *KP263268. ***Hondurodendron urceolatum*** C.Ulloa,

Appendix 1. Continued.

Nickrent, Whitef. & D.L.Kelly: Honduras, *Fagen & al. DA/2MS 313* (MO), DLN 5555, FJ848848, , –, FJ848841, FJ848846, FJ848844, –. ***Ileostylus micranthus*** Tiegh.: New Zealand, *B. Molloy s.n.* (SIU), DLN 2741, EU544329, EU544376, –, EU544471, EU544433, –, –. ***Jodina rhombifolia*** (Hook. & Arn.) Reissek: Bolivia, *M. Nees 46673* (NY), DLN 4052, DQ329166, –, –, DQ329174, DQ329185, DQ329196, –. ***Korthalsella lindsayi*** (Oliv. ex Hook.f.) Engl.: New Zealand; Taiwan, *B. Molloy s.n.; C.-C. Wu s.n.* (SIU; TAI), DLN 2740; Su 119, L24150, (*KP263248), (*KP263339), L26073, –, –, –. ***Lepeostegeres lancifolius*** Danser: Malaysia, *Calvin & al. B27* (SAR), DLN 4041, –, EU544379, –, –, EU544435, –, –. ***Lepidaria forbesii*** Tiegh.: Malaysia, *D.L. Nickrent 4044* (SIU), DLN 4044, EU544330, EU544378, –, –, EU544434, –, –. ***Lepidoceras chilense*** (Molina) Kuijt: Chile, *C. Marticorena & R. Rodríguez 10043* (CONC), DLN 4065, EF464459, –, –, EF464519, EF464499, –, –. ***Lepionurus sylvestris*** Blume: Indonesia; unknown, *G. Hambali s.n.; M.W. Chase 1333* (SIU; K), DLN 2880; Kew DNA Bank 1333, DQ790101, DQ790206, *KP263340, DQ790131, DQ790170, –, *KP263269. ***Leptomeria aphylla*** R.Br.: Australia, *B.J. Lepschi 4875* (CANB), DLN 4609, –, –, –, EF584597, EF584622, *KP263305, –. ***Leptomeria pauciflora*** R.Br.: Australia, *A. Markey & B. Barlow s.n.* (SIU), DLN 3081, EF464460, EF464471, –, EF464521, EF464502, –, –. ***Ligaria cuneifolia*** (Ruiz & Pav.) Tiegh.: Chile, *G. Amico s.n.* (SIU), DLN 4567, L24152, EF464477, –, EF464528, EF464510, –, –. ***Lophophytum leandrii*** Eichler : Argentina, *M. Gonzalez 291* (TAI), Gonzalez 291, *KP263283, *KP263249, *KP263341, –, –, –, *KP263270. ***Loranthus delavayi*** Tiegh.: Taiwan, *C.-C. Wu 0033* (TAI), Wu 0033, JQ613220, –, –, HQ317767, –, –, JQ613248. ***Loranthus europaeus*** Jacq.: Italy, *U. Kuhlmann s.n.* (SIU), DLN 2849, L24153, EU544380, –, JQ933393, EU544436, –, –. ***Loranthus kaoi*** (J.M.Chao) H.S.Kiu: Taiwan, *H.-J. Su 021* (TAI), Su 021, JQ613221, –, JQ613272, –, *KP263261, –, JQ613249. ***Loxanthera speciosa*** Blume: Malaysia, *D.L. Nickrent 4026* (SIU), DLN 4026, EU544332, EU544382, –, –, EU544437, –, –. ***Lysiana filifolia*** Barlow: Australia, *D.L. Nickrent 4449* (SIU), DLN 4449, EU544333, EU544383, –, –, EU544438, –, –. ***Maburea trinervis*** Maas: Guyana, *R. Zagt s.n.* (P), DLN 4256, DQ790114, DQ790234, *KP263342, DQ790165, DQ790201, DQ790268, DQ110345. ***Macrosolen cochinchinensis*** (Lour.) Tiegh.: Malaysia; China, *C.Calvin & al. s.n.; H.-J. Su 052* (SAR; TAI), DLN 4038; Su 052, EU544334, EU544384, *KP263343, *KP263282, EU544439, –, *KP263271. ***Malania oleifera*** Chun & S.K.Lee: China, *Caoming 0340; H.-J. Su 051* (P; TAI), DLN 4158; Su 051, DQ790115, DQ790222, *KP263344, DQ790151, DQ790188, DQ790257, *KP263272. ***Mida salicifolia*** A.Cunn.: New Zealand, *C.C. Ogle 3413* (CHR), DLN 4233, EF584577, –, *KP263345, EF584598, EF584623, *KP263306, *KP263273. ***Minquartia guianensis*** Aubl.: Costa Rica, *D.L. Nickrent 2758* (SIU), DLN 2758, L24396, –, *KP263346, DQ790148, DQ790185, DQ790254, DQ110346. ***Misodendrum linearifolium*** DC.: Chile, *G. Amico 136* (BCRU), DLN 4591, L24397, DQ790211, –, L26074, DQ787438, –, –. ***Misodendrum punctulatum*** Banks ex DC.: Argentina, *R. Vidal-Russell 61, 62* (BCRU), Su 117, *KP263284, *KP263250, *KP263347, EF464531, DQ787443, (*KP263307), *KP263274. ***Moquiniella rubra*** A.Spreng.: South Africa, *K. Steiner 2836* (NBG), DLN 3042, AF039078, DQ790207, –, DQ790132, DQ790171, –, –. ***Muellerina eucalyptoides*** (DC.) Barlow: Australia, *D. Watson s.n.* (SIU), DLN 4310, EU544335, EU544385, –, EU544472, EU544440, –, –. ***Myoschilos oblongum*** Ruiz & Pav.: Chile, *M.F. Gardner & S.G. Knees 4387* (MO), DLN 4182, EF584578, –, –, EF584599, EF584624, *KP263308, –. ***Mystropetalon thomii*** Harv.: South Africa, *D.L. Nickrent 4091* (SIU), DLN 4091, AY957445, *KP263251, *KP263348, –, –, –, AY957449. ***Nanodea muscosa*** Banks ex C.F.Gaertn.: Argentina, *D.M. Moore 2302* (MO), DLN 4183, EF584579, –, –, EF584600, EF584625, *KP263309, –. ***Nestronia umbellula*** Raf.: U.S.A., *L.J. Musselman s.n.* (SIU), DLN 2736, L24399, –, –, EF584601, EF584626, *KP263310, DQ110348. ***Notanthera heterophylla*** (Ruiz. & Pav.) G.Don.: Chile, *C. Aeodo 7202* (MA), DLN 4372; 4582, EF464466, EF464478, –, EF464529, EF464511, –, –. ***Notothixos subaureus*** Oliv.: Australia, *D.L. Nickrent 2790* (SIU), DLN 2790, L24403, *KP263252, –, L26075, –, (*KP263311), . ***Nuytsia floribunda*** R.Br.: Australia, *B. Lamont s.n.; A. Markey & B. Barlow; L. Mucina s.n.* (SIU; TAI), DLN 2747; DLN 3080; Su 120, DQ790103, DQ790210, *KP263349, DQ790134, DQ787446, DQ790239, DQ110349. ***Ochanostachys amentacea*** Mast.: Indonesia, *M. Chase 1329* (K), DLN 4167, DQ790116, –, –, DQ790146, DQ790183 , DQ790252, –. ***Octoknema* sp.:** Equatorial Guinea, *B. Senterra SO 291* (P), DLN 4560, *KP263285, –, *KP263350, DQ790139, DQ790176, –, *KP263275. ***Oedina pendans*** (Engl. & K.Krause) Polhill & Wiens: Tanzania, *R.E. Gereau & C.J. Kayombo 4213* (MO), DLN 4329, EU544336, EU544386, –, –, EU544441, –, –. ***Okoubaka aubrevillei*** Pellegr. & Normand: Cameroon, *M. Cheek 6007* (K), DLN 4173, –, –, –, DQ329175, DQ329186, DQ329197, –. ***Olax emirnensis*** Baker: Madagascar, *G.E. Schatz & al. 3620* (MO), DLN 4035, DQ790119, DQ790214, –, DQ790136, DQ790173, DQ790243, . ***Olax imbricata*** Roxb.: China, *J.-M. Hu 1618* (TAI), Hu 1618, JQ613222, *KP263253, *KP263351, , –, –, JQ613246. ***Olax aphylla*** R.Br.: Australia, *D.L. Nickrent 2810* (SIU), DLN 2810, L24405, DQ790212, –, DQ792943, –, DQ790241, –. ***Olax benthamiana*** Miq.: Australia, *M. Chase 2176* (K), DLN 4168, DQ790118, DQ790213, –, DQ790135, AY042620, DQ790242, –. ***Oliverella rubroviridis*** Tiegh.: Zambia, *N.B. Zimba & al. 1097* (MO), DLN 4330, EU544337, EU544387, –, –, EU544442, –, –. ***Ombrophytum subterraneum*** (Aspl.) B.Hansen: Chile, *J.D. Mauseth 1987-506* (TEX), DLN 2983, L24406, *KP263254, –, –, , –, EU281127. ***Omphacomeria acerba*** (R.Br.) A.DC.: Australia, *B. Lepschi & B.R. Murray 4213* (CANB), DLN 4221, EF584580, –, –, EF584602, EF584627, *KP263312, –. ***Oncella ambigua*** (Engl.) Tiegh.: Kenya, *S.A. Robertson & K. Medley 5459* (MO), DLN 4673, EU544338, –, –, –, EU544443, –, –. ***Oncocalyx sulfureus*** (Engl.) Wiens & Polhill: Kenya, *W. Forstreuter 9117* (SIU), DLN 2850, EU544339, EU544388, –, –, EU544444, –, –. ***Ongokea gore*** (Hua) Pierre: Gabon, *F.J. Breteler & al. 14888* (WAG), DLN 4184, DQ790120, DQ790216, *KP263352, DQ790140, DQ790177, DQ790246, DQ110350. ***Opilia amentacea*** Roxb.: Australia, *D.L. Nickrent 2816* (SIU), DLN 2816, L24407, DQ790202, –, L26076, AY042621, *KP263313, –. ***Oryctanthus occidentalis*** (Kunth) Kuijt: Costa Rica, *D.L. Nickrent 2763* (SIU), DLN 2763, L24408, EU544389, –, –, EU544445, –, –. ***Osyridicarpos schimperianus*** (Hochst. ex A.Rich.) A.DC.: South Africa, *D.L. Nickrent 4110* (SIU), DLN 4110, EF584581, , –, EF584603, EF584628, *KP263315, –. ***Osyris lanceolata*** Hochst. & Steud.: South Africa, *D.L. Nickrent 2731* (SIU), DLN 2731, U42803, AF389274, –, EF464525, EF464506, –, –. ***Osyris quadripartita*** Salzm. ex Decne.: Spain, *D.L. Nickrent 4062* (SIU), DLN 4062, EF584582, (FJ588878), –, EF584604, AY042623, *KP263314, (AF520155). ***Passovia pyrifolia*** (Kunth) Tiegh.: Costa Rica, *D.L. Nickrent 2762* (SIU), DLN 2762, L24412, EU544392, –, –, EU544448, –, –. ***Pentarhopalopilia marquesii*** (Engl.) Hiepko: Gabon, *J.J.F.E. deWilde & R.W. deWilde-Bakhuizen 11212* (WAG), DLN 4180, DQ790102, DQ790203, –, DQ790127, DQ790166, –, –. ***Peraxilla tetrapetala*** (L.f.) Tiegh.: New Zealand, *B. Molloy s.n.* (SIU), DLN 2744, EU544340, EU544390, –, EU544473, EU544446, *KP263316, –. ***Phacellaria rigidula*** Benth.: China, *Y. Ding s.n.* (SIU), DLN 5042, EF584583, –, –, EF584605, (EF584629), –, –. ***Phanerodiscus capuronii*** Malécot, G.E.Schatz & Bosser: Madagascar, *G.E. Schatz & al. 3439* (MO), DLN 4204; Kew DNA Bank 9294, DQ790122, DQ790219, (*KP263353), DQ790143, DQ790180, DQ790249, –. ***Phoradendron californicum*** Nutt.: U.S.A., *J. Paxton s.n.* (SIU), DLN 2689, AF039070, AF181803, –, EF584613, EF584639, –, –. ***Phoradendron leucarpum*** (Raf.) Reveal & M.C.Johnst.: U.S.A., *D.L. Nickrent 2077* (ILL), DLN 2077, X16607, *KP263256, *KP263354, GQ997750, GQ997723, GQ997713, –. ***Phragmanthera crassicaulis*** (Engl.) Balle: Gabon, *J.J. Wieringa 2506* (WAG), DLN 3037, EU544341, EU544391, –, –, EU544447, –, –. ***Pilgerina madagascariensis*** Z.S.Rogers, Nickrent & Malécot: Madagascar, *R. Rabevohitra & al. 4485* (MO), DLN 4954, DQ329169, –, –, DQ329178, DQ329189, DQ329200, –. ***Plicosepalus sagittiflorus*** (Engl.) Danser: Kenya, *W. Forstreuter s.n.* (SIU), DLN 2852, EU544342, EU544393, –, –, EU544449, –, –. ***Psittacanthus calyculatus*** A.C.Sm.: Mexico, *D. Wiens s.n.* (SIU), DLN 4043, (L24414), EU544394, –, –, EU544450, –, –. ***Ptychopetalum petiolatum*** Oliv.: Gabon, *F.J. Breteler 14745* (WAG), DLN 4212, DQ790121, DQ790215, *KP263355, DQ790138, DQ790175, DQ790245, –. ***Pyrularia pubera*** Michx.: U.S.A., *L.J. Musselman s.n.* (SIU), DLN 2737, L24415, –, –, DQ329179, EF464507, DQ329201, –. ***Quinchamalium chilense*** Molina: Argentina; Bolivia, *R. Vidal-Russell s.n.; J.R.I. Wood 9149* (SIU; K), DLN 4503; Kew DNA Bank 9573, EF464469, *KP263257, *KP263356, EF464533, EF464514, *KP263317, *KP263276. ***Rhoiacarpos capensis*** (Harv.) A.DC. : South Africa, *D.L. Nickrent 4117* (SIU), DLN 4117, EF584584, –, , EF584606, EF584630, *KP263318, –. ***Santalum album*** L.: India; Taiwan, *R. Narayana s.n.; H.-J. Su 028* (SIU; TAI), DLN 2734; Su 028, JQ613224, AY957453, JQ613266, L26077, *KP263262, *KP263319, JQ613250. ***Santalum macgregorii*** F.Muell.: Papua New Guinea, *D.L. Nickrent 4499* (WAU), DLN 4499, EF584585, –, –, EF584607, EF584631, –, –. ***Sarcophyte sanguinea*** Sparrm.: South Africa, *D.L. Nickrent 4109* (SIU), DLN 4109, *KP263286, *KP263258, –, –, –, –, *KP263277. ***Schoepfia chinensis*** Gardner & Champ.: China, *H.-J. Su 054* (TAI), Su 054, *KP263287, –, *KP263357, HQ415145, *KP263263, –, *KP263278. ***Schoepfia jasminodora*** Siebold & Zucc.: Taiwan, *H.-J. Su 022* (TAI), Su 022, JQ613226, –, JQ613273, HQ415146, HQ415321, –, JQ613252. ***Schoepfia schreberi*** J.F.Gmel.: Bahamas, *D.L. Nickrent 2599* (ILL), DLN 2599, L24418, AF389261, –, L11205, AY957451, DQ790240, GU351300. ***Scleropyrum pentandrum*** (Dennst.) Mabb.: Thailand, *S. Suddee & al. 1007* (TCD), DLN 4347, DQ329167, –, –, DQ329176, DQ329187, DQ329198, –. ***Scorodocarpus borneensis*** (Baill.) Becc: Malaysia, *S.P. Teo s.n.; M.W. Chase 1331* (SIU; K), DLN 3028; Kew DNA Bank 1331, U59934, DQ790228, *KP263358, DQ790159, DQ790195, DQ790263, *KP263279. ***Scurrula ferruginea*** (Jack) Danser: Malaysia, *D.L. Nickrent 4008* (SIU), DLN 4008, EU544343, EU544395, –, –, EU544451, –, –. ***Scurrula parasitica*** L.: Malaysia, *D.L. Nickrent 4004* (SIU), DLN 4004, EU544345, EU544397, –, –, EU544453, –, –. ***Scurrula pulverulenta*** (Wall.) G.Don: Nepal, *M.*

Appendix 1. Continued.

Devkota 661 (KATH), DLN 4159, EU544344, EU544396, –, –, EU544452, –, –. ***Socratina bemarivensis*** (Lecomte) Balle: Madagascar, *C.C.H. Jongkind & al. 3548* (MO), DLN 4179, EU544347, EU544399, –, –, EU544454, –, –. ***Sogerianthe sessiliflora*** (S.Moore) Danser: Papua New Guinea, *D.L. Nickrent & al. 4467* (WAU), DLN 4467, EU544348, EU544400, –, –, EU544455, –, –. ***Spirogardnera rubescens*** Stauffer: Australia, *H.U. Stauffer & al. 5385* (Z), DLN 4546, EF464458, –, –, EF464518, EF464497, *KP263320, –. ***Staufferia capuronii*** Z.S.Rogers, Nickrent & Malécot: Madagascar, *R. Randrianaivo & al. 825* (MO), DLN 4956, DQ329168, –, –, DQ329177, DQ329188, DQ329199, –. ***Strombosia grandifolia*** Hook.f. ex Benth.: Gabon, *F.J. Breteler 15457* (WAG), DLN 4268, DQ790123, DQ790225, –, DQ790156, DQ790192, DQ790260, –. ***Strombosia philippinensis*** S.Vidal: Philippines, *D. Heuschkel, Honolulu B.G. 81.724* (SIU), DLN 2831, AF039079, DQ790226, –, DQ790157, DQ790193, DQ790261, –. ***Strombosia pustulata*** Oliv.: Gabon, *J.J. Wieringa 2781* (WAG), DLN 4054, DQ790124, DQ790227, *KP263359, DQ790158, DQ790194, DQ790262, DQ110360. ***Strombosiopsis tetrandra*** Engl.: Gabon, *J.J. Wieringa 3300* (WAG), DLN 4055, DQ790125, DQ790224, –, DQ790155, DQ790191, DQ790259, –. ***Struthanthus oerstedii*** (Oliv.) Standl.: Costa Rica, *S. Sargent s.n.* (SIU), DLN 2728, L24421, EU544402, –, –, EU544457, –, –. ***Struthanthus woodsonii*** Cufod.: Costa Rica, *D.L. Nickrent 2761* (SIU), DLN 2761, EU544349, EU544403, –, EU544474, EU544458, –, –. ***Tapinanthus constrictiflorus*** (Engl.) Danser: Gabon, *J.J. Wieringa 2860* (WAG), DLN 3088, (L24422), EU544404, –, –, EU544459, –, (DQ110361). ***Taxillus chinensis*** (DC.) Danser: Malaysia, *D.L. Nickrent 4032* (SIU), DLN 4032, EU544350, EU544405, –, –, EU544460, –, –. ***Taxillus pseudochinensis*** (Yamam.) Danser: Taiwan, *H.-J. Su 018* (TAI), Su 018, *KP263288, –, *KP263360, –, –, –, –. ***Tetrastylidium peruvianum*** Sleumer: Peru, *H. van der Werff & R. Vasquez 13875* (MO), DLN 4205, DQ790126, –, –, DQ790154, DQ790190, *KP263321, –. ***Thesium chinense*** Turcz.: Taiwan, *C.-C. Wu 0024* (TAI), Wu 033, JQ613225, *KP263259, JQ613267, –, –, –, JQ613251. ***Thesium fragile*** L.f.: South Africa, *D.L. Nickrent 4102* (SIU), DLN 4102, EF584586, –, –, EF584608, EF584632, *KP263322, –. ***Thesium fruticosum*** A.W.Hill: South Africa, *K. Steiner s.n.* (SIU), DLN 2845, EF584587, –, –, EF584609, EF584633, –, –. ***Thesium subsucculenta*** (Kammer) J.C.Manning & F.Forest: Canary Islands (Spain), *A. Santos Guerra s.n.* (TFNC), DLN 4374, EF584576, –, –, EF584596, EF584621, *KP263304, –. ***Thonningia sanguinea*** Vahl: Gabon; Ghana; Cameroon, *G. Walters & al. 961; H.H. Schmidt & al. 1619; J.-M. Onana 2927* (MO; K), DLN 4382; DLN 4215; Kew DNA Bank 19155, *KP263289, –, *KP263361, –, –, –, *KP263280. ***Tripodanthus acutifolius*** (Ruiz & Pav.) Tiegh.: Brazil, *Wasum & al. 7586* (MO), DLN 2969, L24424, EU544406, –, EU544475, EU544462, *KP263323, –. ***Tristerix corymbosus*** (L.) Kuijt: Chile, *V. Melzheimer s.n.; G. Amico s.n.* (SIU; BCRU), DLN 4129; DLN 4572, EF464467, EF464479, –, –, EF464512, –, –. ***Tupeia antarctica*** (G.Forst.) Cham. & Schltdl.: New Zealand, *B. Molloy s.n.* (SIU), DLN 2742, L24425, DQ790208, –, DQ790133, DQ790172, –, –. ***Urobotrya siamensis*** Hiepko: Thailand, *Geesink & al. 7807* (B), DLN 4369, EF584588, –, –, EF584611, EF584635, –, DQ110365. ***Vanwykia remota*** (Baker & Sprague) Wiens: Tanzania, *T. Fison 91/1* (MO), DLN 4331, EU544351, EU544407, –, –, EU544463, –, –. ***Viscum album*** L.: U.S.A., *P. Faber s.n.* (SIU), DLN 3024, U42821, AF389275, –, L26078, JN895000, –, –. ***Viscum articulatum*** Burm.f.: Australia; Taiwan, *D.L. Nickrent 2812; H.-J. Su 020* (SIU; TAI), DLN 2812; Su 020, JQ613228, *KP263260, (JQ613265), EF464517, EF464496, –, –. ***Ximenia americana*** L.: Bahamas, *D.L. Nickrent 2601; D. Owen s.n.* (ILL; TAI), DLN 2601; Su 121, L24428, DQ790220, *KP263362, GQ997898, GQ997871, GQ997860, *KP263281. ***Antirrhinum majus*** L.: AJ236047, AY423077, AY566619, L11688, AJ429342, GQ996966, AY453102. ***Arabidopsis thaliana*** (L.) Heynh.: X16077, X52320, Z19121, U91966, AF144378, AF05697, NC_001284. ***Camellia japonica*** L.: U42815, AY727975, AY566627, L12602, AF380074 , (KC143082), (AF421034). ***Cornus florida*** L.: X17370, AF297532, AJ556175, (L14395), (AY526237), GQ998074, (AY725883). ***Myrtus communis*** L.: (GU476479), EU002154, AJ556164, HM850194, AY525136, (GQ870669), GU351259. ***Spinacia oleracea*** L.: L24420, HQ843464, DQ058635, NC_002202, NC_002202, NC_002202, AY453110.