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Molecular Phylogenetic Relationships of Olacaceae and Related Santalales

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Abstract—As traditionally circumscribed, the family Olacaceae contains a morphologically diverse assemblage of genera that has historically caused much confusion regarding their classification. For example, Olacaceae contain parasites and nonparasites, climbing lianas and trees, and members with dichlamydous and monochlamydous perianths. This family is basalmost in the sandalwood order (Santalales), thus it represents the staging ground for many innovations that evolved in subsequent groups. The present molecular phylogenetic study has obtained DNA sequence data (nuclear SSU rDNA and chloroplast *rbcL* and *matK*) for all but two of the 28 genera in this group. Maximum parsimony and Bayesian analyses have resolved seven clades, well-supported by molecular and morphological characters. Root hemiparasitism appears to have first evolved in the clade containing *Ximenia* whereas clades between that one and the outgroup appear to be entirely autotrophic.

Keywords—chloroplast DNA, Olacaceae, parasitism, phylogeny, ribosomal DNA, Santalales.

The sandalwood order (Santalales) is the largest group of angiosperms with both woody and parasitic members. It includes ca. 160 genera and 2200 species that are typically classified in six families: Loranthaceae, Misodendraceae, Olacaceae, Opiliaceae, Santalaceae, and Viscaceae. Molecular phylogenetic work has clearly placed this order among the core eudicots, however, it emerges from a polytomy that also includes Caryophyllales, Gunnerales, rosids, and asterids (Soltis et al. 2000, 2003). This position suggests that Santalales are not related to more derived rosids, as portrayed in most traditional classifications, but that they are older, as previously suggested by Sleumer (1984a). Despite uncertainty about its global placement, previous phylogenetic analyses show that Santalales is a strongly supported monophyletic clade. Synapomorphies for the order include free central pendulous ovules and the presence of long chain polyunsaturated fatty acids (e.g. ximeninic or santalbic acid), both features that are otherwise rare or absent in other angiosperms. Santalales have the broadest range of nutritional modes seen in any order of angiosperm because it includes autotrophs, root parasites and stem parasites. For this reason, the sandalwoods are an ideal group to study the evolutionary acquisition of various parasitic habits.

Among the santalalean families, Olacaceae has long been regarded as the most "primitive" because it contains both root parasitic and nonparasitic species (Engler 1894; Engler and Gilg 1924; Sleumer 1935; Fagerlind 1948; Kuijt 1969). In addition to diverse nutritional modes, all genera of Olacaceae also show heterogeneity in anatomy, palynology and morphology (see for example Lobreau-Callen 1980; Baas 1982; Sleumer 1984b; van den Oever 1984). Historically, this extreme variation has prompted many workers to consider Olacaceae as a polyphyletic assemblage that could be split into multiple families (Sleumer 1935; Fagerlind 1948; Reed 1955; Kuijt 1968). More recently, molecular phylogenetic analyses provided evidence that Olacaceae is polyphyletic (Nickrent and Duff 1996; Nickrent et al. 1998; Nickrent and Malécot 2001). The latter study, which sampled 17 of the 28 genera in the family and used sequence data from nuclear small-subunit (SSU) rDNA (=18S) and chloroplast *rbcL*, documented the existence of eight clades of "Olacaceae". As previously shown (Nickrent and Duff 1996; Nickrent et al. 1998), *Schoepfia* was more closely related to Misodendraceae and Loranthaceae than to Olacaceae. From this work and following Judd et al. (2002), we consider that *Schoepfia* is best placed in its own familly, Schoepfiaceae Blume, that now also includes *Arjona* and *Quinchamalium* (Der and Nickrent 2008). Resolution among the remaining seven clades was poor such that all essentially emerged from a polytomy at the base of the order. A cladistic study that used 80 morphological characters and sampled all 28 genera of Olacaceae (as well as representatives from the other santalalean families) resulted in a paraphyletic Olacaceae (Malécot et al. 2004).

In this study, we used sequences from nuclear and chloroplast genes to assess relationships among a nearly comprehensive sampling of basal Santalales. Our goal is to use these data to 1) clarify phylogenetic relationships among all basal Santalales, 2) assess the monophyly of various groups within Olacaceae, and 3) identify morphological synapomorphies for various clades of Olacaceae.

Materials and Methods

Taxonomic Sampling—Twenty-six genera and 32 species of Olacaceae were sampled, including those that have at times been segregated into distinct families such as Aptandraceae, Erythropalaceae, and Octoknemaceae. All Olacaceae genera except *Harmandia* and *Douradoa* were included. The latter was described in 1984 and this extremely rare monospecific genus has been recollected only once, in 1976. Attempts to extract DNA from available herbarium specimens of *Harmandia* and *Douradoa* proved unsuccessful. *Brachynema* was excluded because the morphological study by Malécot (2002) showed that it is best placed among Ericales. Genera with fewer than eight species were represented by a single species, whereas attempts were made for larger genera to include two or more species. This sampling was determined partially by availability of material but is also justified in that the genera are well defined and that very few have ever been proposed to be paraphyletic (the exception is the type genus *Olax* which is paraphyletic when *Dulacia* is recognized as a distinct genus, as was done in Malécot 2002). The genus *Anacolosa* is here represented by *Anacolosa papuana*, whereas in our first study (Nickrent and Malécot 2001) material identified as *A. casearioides* (*Schatz et al. 3620*) proved to be *Olax emirnensis* (Malécot 2002; Malécot and Dubuisson 2004; Rogers et al. 2006). The remaining Santalales are represented by six genera of Opiliaceae, four of Loranthaceae, *Misodendrum*, and *Schoepfia*. In total, 43 taxa of Santalales were sampled (Appendix 1). Because of the ambiguous position of Santalales within the eudicotyledons, 15 outgroups were selected from members of Proteales, Caryophyllales, Saxifragales, Trochodendraceae, Rosales, Brassicales, Vitaceae, Cornales, and Solanales.

PCR and Sequencing—DNA was extracted from silica gel dehydrated or herbarium preserved leaf tissue as described in Nickrent (1994). SSU rDNA was amplified and sequenced using the 12 forward (5′-TCC TGC

CAG TAS TCA TAT GC-3′) and 1769 reverse (5′-CAC CTA CGG AAA CCT TGT T- 3′) primers. The *rbcL* gene was amplified and sequenced with the forward (5′–ATG TCA CCA CAA ACA GAR AC-3′) and 3′ reverse (5′-TAG TAA AAG ATT GGG CCG AG-3′) primers. For *matK*, the primers developed by Hilu and Liang (1997) were used, and one specifically designed for this study, 1316 rev (TCG AAG TAT ATA CTT TAT TCG). Typical PCR amplification reactions included: 1× buffer (Promega, Madison, Wisconsin; 10mM Tris HCl, 50mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 50 μM dNTP's, 1 unit Taq polymerase, 0.4 μM of each primer and ca. 30 ng of genomic DNA. PCR products were purified on 1.0% agarose gels, the bands of the appropriate size excised, and purified using a commercial kit (EZNA Cycle-Pure Kit, Omega BioTek Inc., Liburn, Georgia). For some samples, TA cloning was performed (Promega pGEM-T Easy, Promega Corp., Madison, Wisconsin) according to the manufacturer's protocol. The cycle sequencing reaction products were purified using Centri-Sep 100 spin columns with Sephadex (Princeton Separations, Inc. Adelphia, New Jersey). Direct sequencing was conducted using automated methods (ABI Prism 377 automated DNA sequencer, Applied Biosystems) according to manufacturer's protocols.

***Sequence Alignment*—**Electropherogram files were edited using either Sequence Navigator (Parker 1997), Sequencher 4.1 (Gene Codes 2000), or 4Peaks (Griekspoor and Groothius 2006). Edited sequences were then placed in Se-Al (Rambaut 2004) and aligned manually. For aligning *rbcL* and *matK*, the nucleotide sequences were translated into amino acid sequences and indels were introduced while maintaining sequence frame. All indels were treated as missing data. SSU rDNA from *Cathedra* and *Coula* and *matK* from *Engomegoma* were not obtained. Taken together, missing sequence and indels constituted 12.9% of the matrix. The 3-gene alignment is available from TreeBASE (study number S1843). Sequences for all three genes for the 15 outgroup taxa were obtained from GenBank and the newly generated sequences for Santalales taxa were deposited with GenBank (Appendix 1).

***Phylogenetic Reconstruction*—**Maximum parsimony (MP) and Bayesian inference (BI) analyses were performed for individual and combined partitions. In all analyses, gaps were coded as missing. MP analyses were performed in PAUP* (Swofford 2002) whereas BI was performed using MrBayes (Ronquist and Huelsenbeck 2003) with a model selected by MrModeltest (Nylander et al. 2004). MP analyses used heuristic searches, random addition sequence (200 replicates), and the Tree bisection reconnection (TBR) swapping algorithm. Homoplasy was estimated using the consistency and retention indices. Bootstrap (BS) support values (Felsenstein 1985) were obtained from 100 replicates with simple addition sequence and TBR. Bootstrap estimates for the SSU rDNA partition were obtained by setting a time limit to 1 min./replicate. For BI, two independent analyses with four chains each were performed for five million generations. Trees and parameters were saved every 100 generations, producing 50,000 trees each run. Model parameters were estimated as part of the analysis; uniform prior probabilitites were assigned to all except the state frequencies for which a Dirichlet prior distribution was assigned and estimated as part of the analysis. When more than one partition was analyzed (separate gene partitions and codon partitions), parameter estimations were unlinked and the rate of the priors was set to vary. The burn-in was determined by stationarity in the −ln likelihood score. The variance between runs in all cases was below 0.001, so runs were combined increasing the number of trees in the posterior probability distribution. The General Time Reversible model (GTR + I + Γ) was selected by MrModeltest for each of the three gene partitions. For the two protein-coding genes, first and second vs. third positions were analyzed separately, but all resulted in GTR + I + Γ or slightly less complex versions of this model (e.g. GTR + Γ or SYM + I + Γ).

Results

A total of 105 new sequences were obtained in this study: 26, 39, and 40 sequences for SSU rDNA, *rbcL* and *matK*, respectively. The concatenated SSU rDNA, *rbcL* and *matK* (3-gene) data set had 4690 aligned positions for 43 ingroup and 15 outgroup taxa. Of these, 1,815 positions were from SSU rDNA, 1,440 from *rbcL,* and 1,435 from *matK*. Thirteen positions, all in the V4 region of the SSU rDNA, were excluded because they could not be unambiguously aligned. Indel events in *matK* were often recognizable as tandem duplications, generally ranging from one to three codons in length. Some were autapomorphic whereas a number of others were synapomorphic; that is, shared among two or more related taxa.

Gene diversity statistics following MP analysis for the three separate gene partitions and the concatenated 3-gene data set is shown in Table 1. As would be expected, the number of variable and parsimony informative characters ranged from the lowest in SSU rDNA (171, 9.4%) to the highest in *matK* (653, 45.5%). The number of shortest trees is highest for SSU rDNA and lowest in *rbcL* (not *matK* as might be expected given its higher number of informative sites). The consistency indices for SSU rDNA and *rbcL* were approximately equivalent, but lower than *matK*.

The topologies of the individual MP gene trees are compared in Fig. 1. For SSU rDNA, little resolution was obtained for the "spine" of the tree such that the ingroup was not monophyletic. Only five of 45 clades received BS support of 90% or greater, but sufficient signal exists to obtain support for several clades, such as Opiliaceae, the *Olax/Ptychopetalum* clade, and the clade that includes *Anacolosa* and four other genera. For the *rbcL* partition, 19 of the 46 clades received BS support of 90% or greater. Although the shortest trees all show a monophyletic ingroup, this clade received < 50% BS support. As with SSU rDNA, the clades along the spine of the tree were poorly resolved. With its higher number of parsimony informative sites, the *matK* gene provided the highest level of resolution among the three partitions with 24 of the 46 clades receiving BS support of 90% or greater. BS support for the ingroup clade was 99% and a number of clades within Olacaceae were highly supported.

Because the individual gene trees were not conflicting in topology and generally differed only in the degree of resolution, the partitions were concatenated and analyzed together. MP analysis of the 3-gene matrix yielded ten most parsimonious trees of 5,691 steps, the strict consensus of which is shown in Fig. 2. Of the 52 total clades, 30 received BS support of 90% or greater. The BI tree (Fig. 3) recovered many of the same clades as the MP tree, thus the two will be discussed together, focusing upon clades of particular relevance (labeled A-J on Figs. 2, 3). Both MP and BI resolved a monophyletic Santalales (clade A) with 100% BS support (Fig. 2) and a PP of 1.0 (Fig. 3). Moderate (BS = 71) to high (PP = 1.0) support was obtained for a sister-group relationship between Santalales and the asterid clade.

As traditionally defined (i.e. including *Schoepfia*), Olaca-

Table 1. Gene diversity statistics for the various partitions and combined data sets. The SSU rDNA data set lacked sequences of *Cathedra* and *Coula*; the *matK* data set lacked *Engomegoma*. C.I = consistency index, C.I.- = consistency index minus uninformative characters, R.I. = retention index.

	No. Trees	Tree Length	C.I.	C.I.-	R.I.	No. Chars.	Conserv. Chars.	Variabl. Uninform.	Variabl. Inform.
SSU rDNA	1,061	842	0.538	0.3796	0.6209	1,799	1,426	202	171
rbcL	9	1,636	0.4682	0.3691	0.5733	1,440	864	230	346
matK	101	3,023	0.4995	0.435	0.6199	1,435	506	276	653
3-gene	10	5,691	0.4837	0.3968	0.5897	4,690	2,799	711	1,180

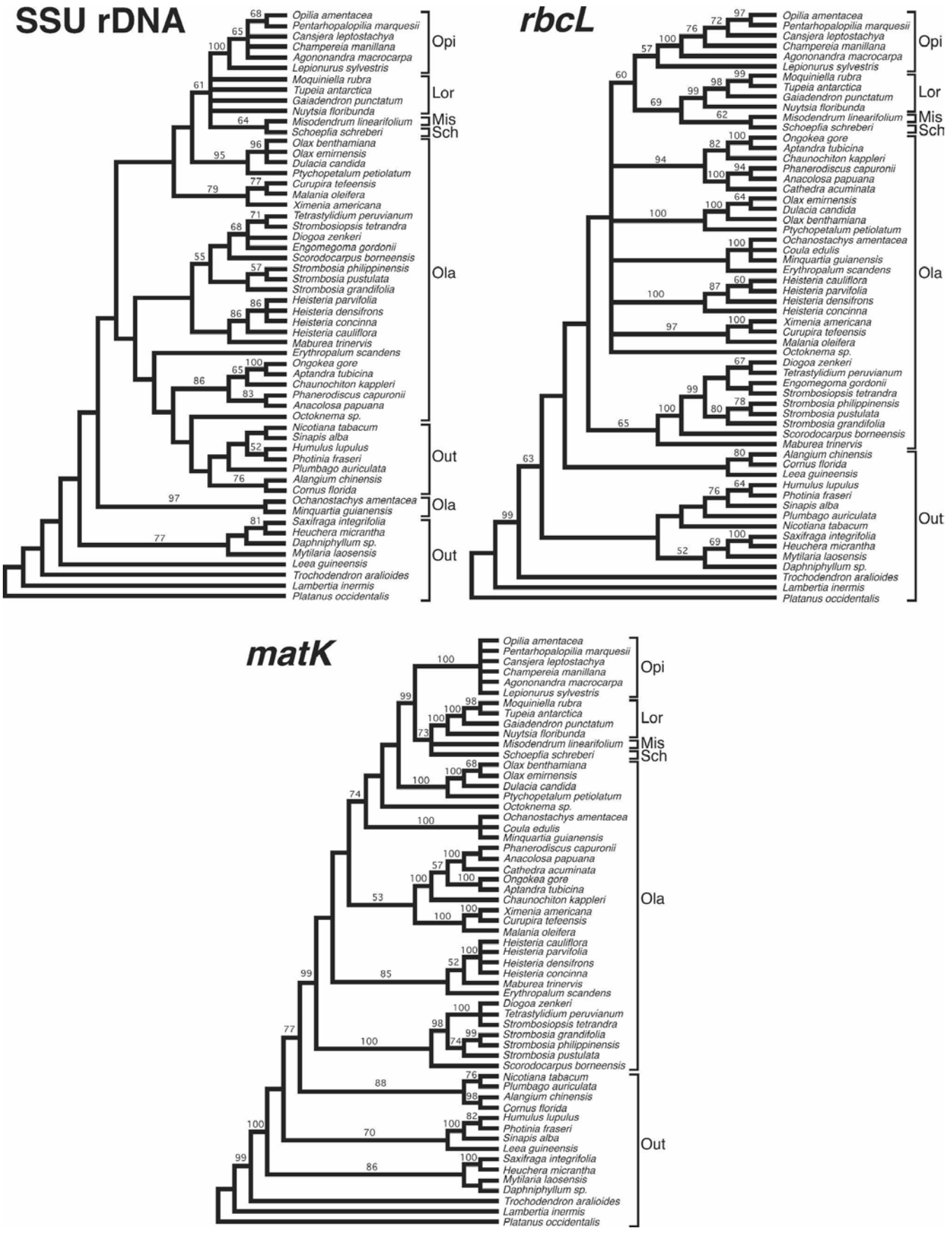

FIG. 1. Maximum parsimony trees for the three individual gene partitions, SSU rDNA, *rbcL*, and *matK*. Bootstrap values greater than 50% (100 replications) are given above the branches. Taxon abbreviations are: Opi = Opiliaceae, Lor = Loranthaceae, Mis = Misodendraceae, Sch = Schoepfiaceae, Ola = Olacaceae, Out = outgroups.

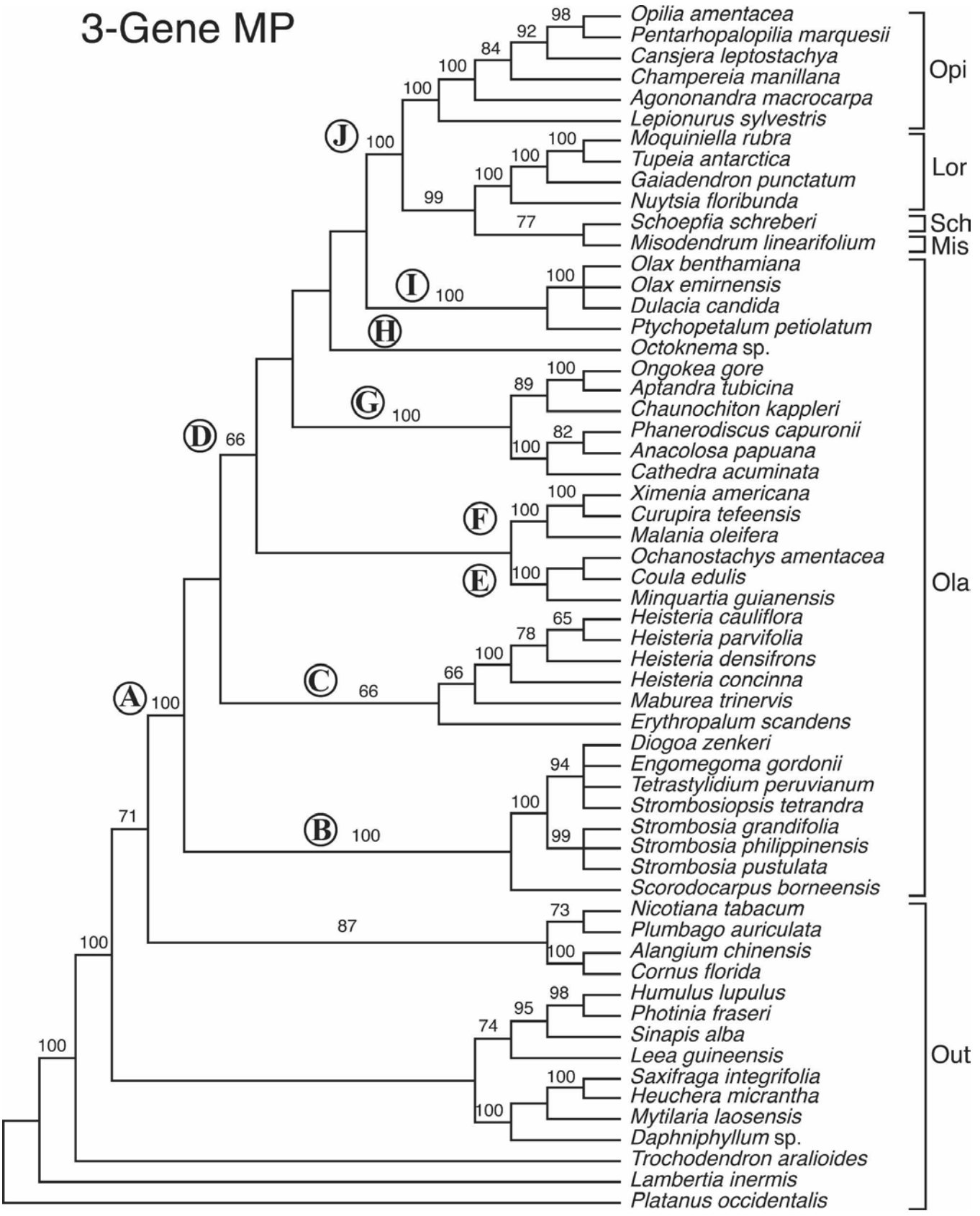

FIG. 2. Strict consensus of ten most parsimonious trees obtained from the 3-gene data set (concatenated nuclear SSU rDNA, *rbcL*, and *matK*) for basal Santalales. Bootstrap values greater than 50% (100 replications) are given above the branches. Circled letters identify clades of Olacaceae s. lat. (see discussion in text). Taxon abbreviations are as in Fig. 1.

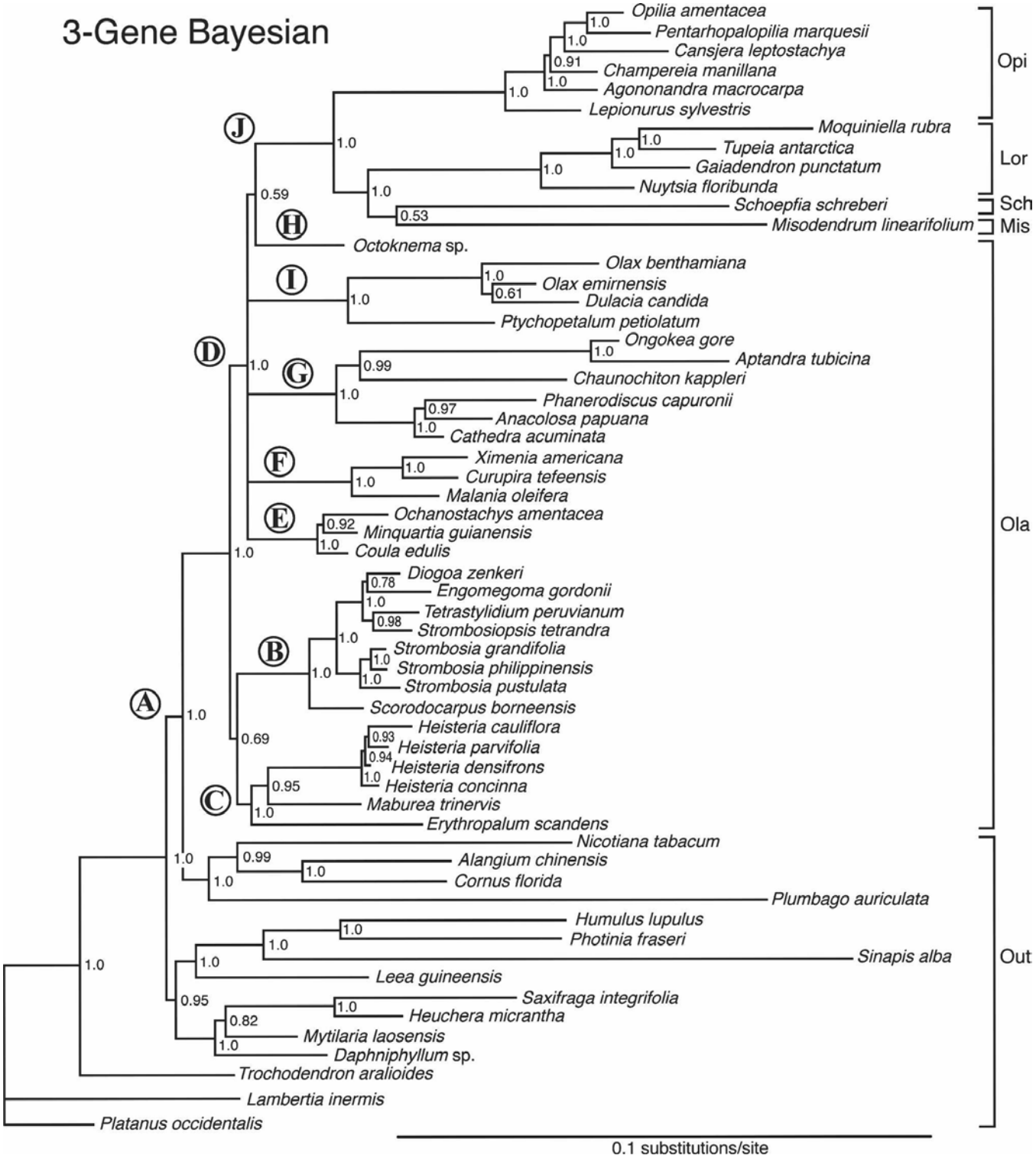

FIG. 3. Bayesian tree obtained from the 3-gene data set for basal Santalales. Posterior probabilities are given to the right of each node. Circled letters identify clades of Olacaceae s. lat. (see discussion in text). Taxon abbreviations are as in Fig. 1. Scale at bottom gives the number of substitutions per site.

ceae are polyphyletic in these analyses and the core of the family (i.e. without *Schoepfia*) is a paraphyletic group composed of seven lineages (Figs. 2, 3). Although support for these seven clades is generally high, relationships among them were not always resolved, as evidenced by the polytomy in the BI tree and by low BS support for a number of clades along the spine of the MP tree. Clade B (BS = 100, PP = 1.0) is composed of six arborescent genera with *Scorodocarpus borneensis* resolved as sister to a clade further composed of two well-supported clades. The first of these contains three species of *Strombosia* whose interrelationships were not resolved. The next clade, *Strombosiopsis, Tetrastylidium, Engomegoma* and *Diogoa* were also unresolved using MP. With BI, *Strombosiopsis* is sister to *Tetrastylidium*. Clade C (BS = 66, PP = 1.0) comprises four species of *Heisteria* and the two monospecific genera *Maburea* and *Erythropalum*.

A monophyletic *Heisteria* is supported as sister to *Maburea* with low to moderate support (BS = 66, PP = 0.95). Relatively low BS and PP support is also obtained for the lianescent *Erythropalum* as sister to the *Maburea* plus *Heisteria* clade (Fig. 2). Although the topology is not seen on any of the individual gene trees, weak MP BS support (52%) and a low BI PP (0.69) is obtained for a monophyletic B plus C clade.

Clade D received only poor MP BS support (i.e. 66%) but was resolved with high posterior probability (1.0) on the BI tree (Fig. 3). This clade contains the remainder of taxa classified in Olacaceae as well as Misodendraceae, Schoepfiaceae, Loranthaceae, and Opiliaceae.

Clades E and F are unresolved with BI (Fig. 3) but do occur as sister (but not supported) with MP (Fig. 2). Clade E (BS = 100, PP = 1.0) contains *Ochanostachys*, *Coula,* and *Minquartia*, a group of arborescent taxa that have traditionally been classified in tribe Couleae. For clade F (BS = 100, PP = 1.0), the pantropical *Ximenia* is sister to *Curupira* from South America and this clade is sister to *Malania*, a tree endemic to China.

Clade G (BS = 100, PP = 1.0) is composed of six genera of small to large trees, many of which show unusual accrescent structures upon fruiting (see Discussion). The clade contains two well-supported subclades, the first with *Anacolosa*, *Cathedra*, and *Phanerodiscus* and the second with *Aptandra, Chaunochiton,* and *Ongokea*. The former group has often been recognized as tribe Anacoloseae and the latter (*Aptandra* and *Ongokea*) as tribe Aptandreae. *Chaunochiton* has traditionally been placed in tribe Heisterieae.

Clade H is composed solely of *Octoknema*, an enigmatic taxon that has frequently been classified as separate from Olacaceae. MP analysis placed *Octoknema* as sister to clades I and J with BS less than 50% and BI shows it as sister only to clade J but with a low posterior probability. Because none of these nodes received strong support, *Octoknema* and clades I and J should be viewed as arising from a polytomy. Clade I (BS = 100, PP = 1.0), composed of *Ptychopetalum*, *Dulacia,* and *Olax* is well supported, but the monophyly of the latter two genera is not supported by MP or BI.

Among the clade J taxa, only *Schoepfia* has been considered a member of Olacaceae. This pantropical root-parasitic genus is resolved with moderate support (BS = 77, PP = 0.53) as sister to *Misodendrum*, an aerial parasite from Patagonia in South America. The *Schoepfia/Misodendrum* clade is sister to Loranthaceae (BS = 99, PP = 1.0), here represented by just four of the 73 genera in the family. The root parasite *Nuytsia* is basalmost followed by *Gaiadendron*, an arborescent mistletoe that can be either a root or stem parasite. *Moquiniella* from southern Africa and *Tupeia* from New Zealand are aerial parasites (mistletoes). The second major clade within clade J represents Opiliaceae (BS = 100, PP = 1.0), a well-characterized pantropical family of 10 genera of lianas, shrubs, and small trees. Within Opiliaceae, *Lepionurus*, a monotypic shrub from South-East Asia, is basalmost, followed by *Agonandra* from the neotropics. The remaining sampled Opiliaceae all occur in the western hemisphere.

Discussion

This study represents the first molecular phylogeny of Olacaceae with comprehensive taxon sampling. The trees reported here are congruent with previously published molecular trees, such as in Nickrent and Malécot (2001), but have increased resolution. This improvement, stemming from both greater taxon sampling and the addition of more sequence data, has increased our understanding of the major phylogenetic lineages in basal Santalales. When the molecular tree is compared with the cladogram reported in Malécot et al. (2004), some similarities and dissimilarities can be noted. The overall pattern of relationships among the families is similar, for example with the autotrophic Olacaceae occupying the basalmost position and Opiliaceae a more derived position on both trees. The morphological tree failed to capture the sister relationship between Misodendraceae and Schoepfiaceae, and the sister relationship of this clade to Loranthaceae. Within Olacaceae, both analyses recovered six clades (tribes): Ximenieae, Couleae, Olaceae (*Olax, Dulacia*, and *Ptychopetalum*), Aptandreae, Anacoloseae (*Anacolosa, Cathedra*, and *Phanerodiscus*), and Anacoloseae (*Diogoa, Engomegoma, Strombosiopsis*, and *Tetrastylidium*). The exact relationships among these tribes differed between the two analyses; however, support for these nodes on the morphological tree was low. In the following section we will integrate relevant morphological data that characterize the clades. Although the most parsimonious MP tree (Fig. 2) and BI tree (Fig. 3) give some indication of the general progression from the basalmost to more advanced clades, the presence of polytomies precludes fully discussing relationships among all seven olacaceous clades; this must be left to future work.

Clade B is composed of six genera with *Scorodocarpus borneensis* well-supported as sister to the remainder of the clade. Members of this subfamily share isostemonous pentamerous or tetramerous flowers (except *Scorodocarpus* where they are diplostemonous) without intercalary scales or staminodes, whereas clade C has diplostemonous or isostemonous flowers with intercalary scales or staminodes. The endocarp of the drupaceous fruit in clade B is woody and 1–2 mm thick whereas it is thin and crustaceous in clade C. The clade composed of the remaining five genera (*Diogoa*, *Engomegoma*, *Strombosia*, *Strombosiopsis,* and *Tetrastylidium*) is also well supported by molecular data and two anatomical synapomorphies: the presence of druses in leaf epidermal cells and opposite intervascular pits (Baas 1982). The genus *Strombosia* is well supported as monophyletic. Although the 3-gene MP tree shows the three species as emerging from a polytomy, the BI tree shows *S. grandifolia* as sister to *S. philippinensis*. The clade of four paucispecific genera, *Diogoa*, *Engomegoma*, *Tetrastylidium*, and *Strombosiopsis,* was well supported with molecular (94% BS) and morphological (85% BS in Malécot et al. 2004) data. These taxa have the synapomorphy of apiculate anther connectives (Malécot et al. 2004). Both molecular (BI tree, Fig. 3) and morphological data support a sister relationship between *Strombosiopsis* and *Tetrastylidium*, whereas these different analyses gave conflicting topologies for the other two genera.

Clade C is composed of three genera, *Erythropalum, Heisteria,* and *Maburea*. This clade received 66% BS support in the 3-gene analysis but had a posterior probability of 1.0. Numerous anatomically- or morphologically-based studies of Olacaceae have never clearly identified a group consisting of genera in this clade. Maas et al. (1992) stated that wood anatomy links *Maburea* to *Heisteria* whereas palynology (Lobreau-Callen 1980, 1982) and leaf anatomy (Baas 1982) link it to tribe Couleae. Based on such characters, the affinities of *Erythropalum* were never clear because of its highly divergent habit (a woody climber with axillary tendrils). Indeed these features have led many workers to isolate *Erythropalum* in its

own family, Erythropalaceae (Gagnepain 1910; Sleumer 1935). Members of clade C bear diplostemonous pentamerous flowers, sometimes isostemonous with staminodes or scales (*Erythropalum* and *Heisteria asplundii*) and fruits with thin crustaceous endocarps (Malécot et al. 2004).

Clade E received strong support from molecular data (BS = 100, PP = 1.0) and 90% BS support with morphological data (Malécot et al. 2004). This very homogeneous group of three genera was recognized as early as 1899 (Tieghem 1899a, b), by all subsequent workers (Sleumer 1935; Stauffer 1961), and has been circumscribed as tribe Couleae. The clade is marked by several synapomorphies such as the presence of leaf dendritic hairs, lignified epidermal cell secondary walls, presence of laticiferous channels in the leaves, epidermal cell druses, spike-like thyrsoid inflorescences, and diplostemonous to polystemonous pentamerous flowers (Malécot et al. 2004). Despite its clear diagnosability, the placement of Couleae among basal Santalales remains unclear.

Clade F is well supported as monophyletic based on analyses of molecular data (BS = 100, PP = 1.0), and weakly supported based on analyses of morphological data (BS = 63%, Malécot et al. 2004). As with clade E, the exact position of clade F in Santalales in not resolved. The most recently described member of this clade is *Malania oleifera* from China and Lee (1980) linked this taxon to *Ximenia*. This relationship was confirmed by van den Oever (1984) using wood anatomical features. These taxa also share basal petiole vascularization (a simple bundle, but not a complete vascular cylinder), fundamentally umbellate inflorescences (reduced to solitary flowers in some *Ximenia* species), and tetramerous flowers with two cycles of stamens. No molecular data were available to place *Douradoa* in clade F, however, the morphological cladistic analysis by Malécot et al. (2004) placed it in the corresponding clade with *Curupira*, *Malania*, and *Ximenia*. At least two synapomorphies characterize clade F: an incomplete vascular cylinder at the base of the petiole and a long conical style.

Clade G represents one of the most unusual groups of basal Santalales and is characterized by a remarkable diversity in flower and fruit evolution. This group of six genera is resolved as monophyletic with molecular (BS = 100, PP = 1.0) and morphological data, although the latter had low BS support. The presence of two locules in the ovary appears to be a synapomorphy for the clade. Other features shared by all members of this clade are the long cylindrical style, distal petiole vascular bundle (simple but not a complete cylinder with more or less complex additional strands), alternate intervascular pits, and pollen grains whose polar axis is shorter than the equatorial diameter (breviaxal or oblate). The molecular trees (Figs. 2, 3) yield two subclades, each with three genera, with all relationships well supported. The first clade contains *Cathedra, Anacolosa,* and *Phanerodiscus,* which have several synapomorphies: lignified guard cells, petals with apical thickenings, porose anther dehiscence, prolonged anther connectives, and diploporate pollen. The second clade, with *Chaunochiton, Aptandra*, and *Ongokea,* is characterized by long, thin petals, valvate anther dehiscence, and an accrescent calyx. The genus *Harmandia* was unavailable for molecular analysis but the morphological cladistic study by Malécot et al. (2004) placed it in this clade. Accrescence of floral tissues on fruits seems to be the rule in clade G, but the exact nature of the tissues involved varies. For example, in both *Anacolosa* and *Cathedra*, the floral disc is accrescent and surrounds the drupe at maturity. In *Phanerodiscus*, the "coupe induviale", a structure absent in the flower at anthesis, develops between the calyx and the disc (Capuron 1968; Malécot et al. 2003) and eventually matures into membranous, pseudo-inflated, laterally lobed vesicles. Given this variation, the family would be an excellent candidate to study from the perspective of floral homeotic gene evolution. The genera *Aptandra* and *Ongokea* are clearly closely related as evidenced by a number of synapomorphies such as fused staminal filaments, glandular tissue between the stamens and petals, and a concave apocolpium on the pollen. Moreover, the branches leading to these genera are comparatively longer than those of other taxa on the BI tree (Fig. 3), thus indicating their higher rates of molecular evolution.

Octoknema, the sole member of Clade H, is a genus of 6–7 species of trees from tropical Africa. The genus was placed in its own family by van Tieghem (1905). In subsequent works the family often included the genus *Okoubaka* (Louis and Léonard 1948; Takhtajan 1997) which was transferred to Santalaceae by Stauffer (1957) – a placement supported by molecular data (Nickrent and Malécot 2001). The MP tree (Fig. 2) includes this taxon in an unsupported sister group position with clades I and J. With BI (Fig. 3), *Octoknema* is sister to clade J but with essentially no support. This position stands in contrast to the morphological cladistic analysis of Malécot et al. (2004) where the genus was placed with taxa equivalent to our clades B, C, and E. Morphological features are either unusual for Santalales (e.g. dioecy, stellate pubescence, expanded stigmatic excrescences in the female flowers, and endocarp laminae), or provide conflicting links to various groups. For example, floral stellate trichomes and wood anatomy suggested a close relationship with Couleae (Mildbread 1935; Reed 1955), whereas further work on leaf and wood anatomy indicated this taxon was isolated from other Olacaceae (Baas 1982; van den Oever 1984). Studies of pollen ultrastructure (Lobreau-Callen 1982) indicated a more derived position within the order (near Opiliaceae), a result similar to that of Schultze-Motel (1964) who made it a tribe in subfamily Schoepfioideae. The reduction or loss of sepals in *Octoknema* is in agreement with other santalalean taxa such as those in clade J.

Clade I was strongly supported by molecular data (BS = 100, PP = 1.0) and was also recovered with morphological data, albeit without support. This clade, composed of *Dulacia, Olax,* and *Ptychopetalum,* was recognized as Olacaceae s. str. by van Tieghem (1896). This group of three genera share several anatomical, morphological, and palynological characters (Malécot et al. 2004). Although the 3-gene MP tree (Fig. 2) gives a polytomy for the two species of *Olax* and *Dulacia*, greater resolution is obtained with BI (Fig. 3). In this case, the New World taxon *Dulacia* is embedded within the Old World genus *Olax*, a position maintained with increased sampling within the genus (Malécot 2002). Thus, to maintain monophyly, we recommend including the 13 species of *Dulacia* in *Olax*. This genus was originally regarded as a section of *Olax* (Valeton 1886) based on its half-inferior vs. superior ovary and the presence of five staminodes and three stamens. The latter feature links this taxon to African members of *Olax* section *Triandreae*. With ca. 42 species distributed from Africa to New Caledonia, the genus *Olax* requires a modern taxonomic revision.

Clade J is resolved as monophyletic with high support (BS = 100, PP = 1.0) and relationships within this clade are

also fully resolved. The Schoepfiaceae/Misodendraceae clade is sister to Loranthaceae (BS = 99, PP = 1.0) and this clade is sister to Opiliaceae with 100% BS support and 1.0 PP. Opiliaceae are more closely related to more derived members of Santalales, e.g. Santalaceae and Viscaceae, than they are to Olacaceae. This has been shown in previous molecular phylogenetic studies of Santalales (Nickrent and Malécot 2001) and was recognized by Valeton (1886). *Schoepfia* is the sole member of Olacaceae s. lat. that occurs in clade J. As discussed in Malécot et al. (2004), this genus has been assigned to Loranthaceae (Candolle 1830), a tribe or subfamily in Olacaceae (Engler 1894), and to its own family (Tieghem 1896; Gagnepain 1910). In morphological cladistic analyses (Malécot et al. 2004), *Schoepfia* was sister to all Santalales families except Olacaceae. Previous molecular analyses (Nickrent and Duff 1996; Nickrent et al. 1998; Nickrent and Malécot 2001) have consistently placed this taxon as sister to Misodendraceae. In the present study, using three genes, this relationship receives low support, but the five gene study of Vidal-Russell and Nickrent (in press) shows strong support (BS = 99, PP = 1.0). As with *Misodendrum* and Loranthaceae, *Schoepfia* has a calyx reduced to a vestigial structure called a calyculus. On the basis of wood anatomy, Engler (1894) stated that *Schoepfia* was the "most evolved genus of Olacaceae." Its wood anatomy is also distinct from Olacaceae in that it possesses aliform-confluent parenchyma (Sleumer 1984b) and its tracheid to vessel length ratio was the most derived of all olacaceous genera examined (Reed 1955). All of these data strongly support recognition of Schoepfiaceae as distinct from Olacaceae.

The Evolution of Parasitism in Santalales—Among Olacaceae s. lat., actual root parasitism has been documented for only four genera: *Olax* (Barber 1907a; Pate et al. 1990), *Ptychopetalum* (Anselmino 1932), *Schoepfia* (Werth et al. 1979), and *Ximenia* (Heckel 1900; Barber 1907b). Positive evidence for nonparasitism (i.e. documented absence of haustoria) has been obtained for five olacaceous genera: *Heisteria* (Kuijt 1969), *Erythropalum, Ochanostachys, Scorodocarpus*, and *Strombosia* (Ping 1997). Studies of the germination of *Strombosia* and *Ongokea* by Heckel (1901, 1902) as well as those of *Curupira* by Rodrigues (1961) do not allow confirmation of the presence or absence of root haustoria. This is because these studies were carried out with plants grown in pots, thus their parasitic behavior as would occur in nature may not have been documented. Four of the five genera that have been shown to be nonparasitic (hence autotrophic) occur in clades B and C, thus it is likely that parasitic members do not exist in these clades. Moreover, given the reports for clade E, it can be assumed that all three genera (i.e. including *Minquartia*) are nonparasitic. The first clade for which positive evidence of parasitism exists is clade F. Unfortunately, neither the MP nor BI analyses resolved the polytomy from which clade F and E emerge. Moreover, no information exists on parasitism for clade G and *Octoknema*. Given the topology of the molecular trees, and assuming a single origin of root hemiparasitism, one can predict that all Ximenieae (i.e. *Curupira* and *Malania*), as well as all Aptandreae and *Octoknema* are root parasites. This would require resolution of the polytomy such that Ximenieae is sister to the remaining clades in the order. As argued in Malécot et al. (2004), reversals from root hemiparasitism to the autotrophic condition are considered unlikely given the selective advantage conferred by parasitism. Given this phylogenetic information, one may reconstruct the ancestral santalalean root parasite ancestor as being a small tree with relatively large fruits. This ancestral type likely occurred in dryer areas such as the margins of humid forests and savannas, as does the present day *Ximenia*.

Classification of Olacaceae—The concept that Olacaceae are a single phylogenetic unit has not been accepted by all workers, in particular Tieghem (1896,1897a, b) who divided it into a number of smaller families. Gagnepain (1910), who accepted van Tieghem's classification, wrote (translated from French): "As understood by Bentham and Hooker in Genera Plantarum, Olacacées constitutes a heterogeneous group; neither in the external characters, nor in the sexual characters, does one discover the affinities between genera which make a natural family; it is not even a family by sequence, with distinct tribes, having some affinity between them, but more a juxtaposition of genera often without place [...]".

Many of the clades described in the present work correlate with those diagnosed by Engler (1894) who classified them at the rank of tribe or family (Table 2). The classification of Olacaceae proposed by Sleumer (1935) was a modification of the Englerian system that remained essentially intact, even following his revision of Asian/Malesian (Sleumer 1980) and New World (Sleumer 1984a) Olacaceae. Since Sleumer's clas-

TABLE 2. Comparison of a traditional infrafamilial classification of Olacaceae with results from this molecular phylogenetic analysis (*Brachynema* excluded, see text).

Traditional classification – modified from Engler (1894), Sleumer (1984b), and Breteler et al. (1996)	Molecular clades (see Figs. 2, 3)
Subfamily Anacolosoideae Airy-Shaw	
Tribe 1. Couleae Engl.	
Coula Baill.	Clade E
Maburea Maas	Clade C
Minquartia Aubl.	Clade E
Ochanostachys Mast.	Clade E
Tribe 2. Heisterieae Dumort.	
Chaunochiton Benth.	Clade G
Heisteria Jacq.	Clade C
Tribe 3. Anacoloseae Engl.	
Anacolosa (Blume) Blume	Clade G
Cathedra Miers	Clade G
Diogoa Exell and Mendonca	Clade B
Engomegoma Breteler	Clade B
Phanerodiscus Cavaco	Clade G
Scorodocarpus Becc.	Clade B
Strombosia Blume	Clade B
Strombosiopsis Engl.	Clade B
Tetrastylidium Engl.	Clade B
Tribe 4. Ximenieae Engl.	
Ximenia L.	Clade F
Subfamily Olacoideae Sond.	
Tribe 5. Olaceae Horan.	
Curupira G.A.Black	Clade F
Douradoa Sleumer	Clade F ?
Dulacia Vell.	Clade I
Malania Chun and S.K.Lee	Clade F
Olax L.	Clade I
Ptychopetalum Benth.	Clade I
Tribe 6. Aptandreae Engl.	
Aptandra Miers	Clade G
Harmandia Pierre ex Baill.	Clade G ?
Ongokea Pierre	Clade G
Subfamily Schoepfioideae Engl.	
Schoepfia Schreber	Clade J
Dubious affinities	
Erythropalum Blume	Clade C
Octoknema Pierre	Clade H

sification, new evidence has been amassed, e.g. from palynology (Reed 1955; Feuer 1978; Lobreau-Callen 1980, 1982) and from anatomy (Reed 1955; Baas 1982; van den Oever 1984) and new genera have been named, e.g. *Maburea* (Maas et al. 1992) and *Engomegoma* (Breteler et al. 1996). But in all cases the authors attempted to incorporate their data into the existing Englerian classification or a slight modification of it (Table 2). In short, this heterogeneous assemblage of taxa posed real classification problems without an empirical phylogeny in place.

As recognized by early workers, and now confirmed by molecular phylogenetic analyses, Olacaceae are polyphyletic. This result is in agreement with the morphologically-based cladistic analysis of Malécot et al. (2004) where Olacaceae were shown to be paraphyletic. In addition to *Schoepfia* (here classified as Schoepfiaceae), the molecular trees provide evidence for seven additional clades that have all previously been placed in Olacaceae. The results obtained to date do not yield unequivocal evidence for the relationships among these seven clades, thus further resolution of relationships among basal Santalales will require additional molecular data. These phylogenetic analyses have improved our understanding of and provided the first step towards a reclassification of Olacaceae, a group of plants that have been taxonomically controversial for over a century.

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LITERATURE CITED

Anselmino, E. 1932. Die Stammpflanzen der Droge Muira-puama. *Notizblatt des Botanisches Gartens und Museums zu Berlin-Dahlem* 11: 623–626.

Baas, P. 1982. Leaf anatomy and classification of the Olacaceae, *Octoknema* and *Erythropalum*. *Allertonia* 3: 155–210.

Barber, C. A. 1907a. Studies in root parasitism. The haustorium of *Olax scandens*. *Memoirs of the Department of Agriculture of Indian Botany* 2: 1–47.

Barber, C. A. 1907b. Parasitic trees of southern India. *Proceedings of the Cambridge Philosophical Society* 14: 246–256.

Breteler, F. J., P. Baas, F. D. Boesewinkel, F. Bouman, and D. Lobreau-Callen. 1996. *Engomegoma* Breteler (Olacaceae) a new monotypic genus from Gabon. *Botanische Jahrbücher für Systematik* 118: 113–132.

Candolle, A. P. de. 1830. *Prodromus systematis naturalis regni vegetabilis, sive enumeratio contracta ordinum, generum, specierumque plantarum hucusque cognitarum, juxta methodi naturalis normas digesta* vol. 4. Paris: Treuttel et Würtz.

Capuron, R. 1968. *Matériaux pour l'étude de la flore forestière de Madagascar: Olacacées, Opiliacées et Santalacées arbustives ou arborescentes de Madagascar*. Atananarivo: Centre Technique Forestier Tropical Madagascar, Section Botanique.

Der, J. and D. L. Nickrent. 2008. A molecular phylogeny of Santalaceae (Santalales). *Systematic Botany* 33: in press.

Engler, A. 1894. Olacaceae. Pp. 231–242 in *Die natürlichen Pflanzenfamilien* vol. 3(1), eds. A. Engler and K. Prantl. Leipzig: Wilhelm Engelmann.

Engler, A. and E. Gilg. 1924. *Syllabus der Pflanzenfamilien*. Ed. 9–10. Berlin: Borntraeger.

Fagerlind, F. 1948. Beiträge zur Kenntnis der Gynäceummorphologie und Phylogenie der Santalales-Familien. *Svensk Botanisk Tidskrift* 42: 195–229.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Feuer, S. M. 1978. Aperture evolution in the genus *Ptychopetalum* Benth. (Olacaceae). *American Journal of Botany* 65: 759–763.

Gagnepain, F. 1910. Comment faut-il comprendre la famille des Olacacées? *Bulletin de la Société Botanique de France* 57: 373–380.

Gene Codes. 2000. Sequencher. Ann Arbor: Gene Codes Corporation.

Griekspoor, A. and T. Groothius. 2006. 4Peaks, ver. 1.7.1. Amsterdam: Netherlands Cancer Institute.

Heckel, E. 1900. Sur le parasitisme du *Ximenia americana* L. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* 131: 764–765.

Heckel, E. 1901. Sur le processus germinatif dans les genres *Onguekoa* et *Strombosia* de la famille des Olacacées. *Annales de l'Institut Botanico-Géologique Colonial de Marseille* 8: 17–27.

Heckel, E. 1902. Sur la germination des *Onguekoa* et des *Strombosia*. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* 134: 489–490.

Hilu, K. W. and H. Liang. 1997. The *matK* gene: sequence variation and application in plant systematics. *American Journal of Botany* 84: 830–839.

Judd, W., C. S. Campbell, E. A. Kellogg, P. F. Stevens, and M. J. Donoghue. 2002. *Plant systematics: a phylogenetic approach*. Ed. 2. Sunderland: Sinauer Associates.

Kuijt, J. 1968. Mutual affinities of Santalalean families. *Brittonia* 20: 136–147.

Kuijt, J. 1969. *The biology of parasitic flowering plants*. Berkeley: University of California Press.

Lee, S.-K. 1980. *Malania*, a new genus of oil-yielding plant. *Bulletin of the Botanical Laboratory of the North-East Forestry Institute* 6: 67–72.

Lobreau-Callen, D. 1980. Caractéres comparés du pollen des Icacinaceae et des Olacaceae. *Adansonia* 20: 29–89.

Lobreau-Callen, D. 1982. Structures et affinités polliniques des Cardiopterygaceae, Dipentodontaceae, Erythropalaceae et Octoknemataceae. *Botanische Jahrbücher für Systematik* 103: 371–412.

Louis, J. and J. Léonard. 1948. Octoknemaceae. Pp. 288–293 in *Flore du Congo Belge et du Ruanda-Urundi. Spermatophytes* vol. 1, eds. Comite Executif de la Flore du Congo-Belge & Jardin Botanique de l'Etat. Bruxelles: Jardin Botanique de l'Etat.

Maas, P. J. M., P. Baas, F. D. Boesewinkel, P. Hiepko, D. Lobreau-Callen, L. van den Oever, and B. J. H. ter Welle. 1992. The identity of "Unknown Z": *Maburea* Maas, a new genus of Olacaceae in Guyana. *Botanische Jahrbücher für Systematik* 114: 275–291.

Malécot, V. 2002. *Histoire, classification et phylogénie des Olacaceae Brown (Santalales)*. Ph.D. thesis. Paris, France: Université de Jussieu Paris VI.

Malécot, V., G. E. Schatz, and J. Bosser. 2003. Révision synoptique du genre *Phanerodiscus* Cavaco (Olacaceae) à Madagascar. *Adansonia* 25: 119–128.

Malécot, V., D. L. Nickrent, P. Baas, L. van den Oever, and D. Lobreau-Callen. 2004. A morphological cladistic analysis of Olacaceae. *Systematic Botany* 29: 569–586.

Malécot, V. and J.-Y. Dubuisson. 2004. L'utilisation des herbiers en phylogénie moléculaire. Pp. 53–65 in *Les herbiers un outil d'avenir – tradition et modernité*, eds. R. Pierrel and J.-P. Reduron. Nancy: AFCEV.

Mildbread, J. 1935. Octoknemaceae. Pp. 42–45 in *Die Natürlichen Pflanzenfamilien* vol. 16b, eds. A. Engler and K. Prantl. Leipzig: Wilhelm Engelmann.

Nickrent, D. L. 1994. From field to film: rapid sequencing methods for field collected plant species. *BioTechniques* 16: 470–475.

Nickrent, D. L. and R. J. Duff. 1996. Molecular studies of parasitic plants using ribosomal RNA. Pp. 28–52 in *Advances in parasitic plant research*, eds. M. T. Moreno, J. I. Cubero, D. Berner, D. Joel, L. J. Musselman, and C. Parker. Cordoba: Junta de Andalucia, Dirección General de Investigación Agraria.

Nickrent, D. L. and V. Malécot. 2001. A molecular phylogeny of Santalales. Pp. 69–74 in *7th International parasitic weed symposium*, eds. A. Fer, P. Thalouarn, D. M. Joel, L. J. Musselman, C. Parker, and J. A. C. Verkleij. Nantes: Faculté des Sciences.

Nickrent, D. L., R. J. Duff, A. E. Colwell, A. D. Wolfe, N. D. Young, K. E. Steiner, and C. W. dePamphilis. 1998. Molecular phylogenetic and evolutionary studies of parasitic plants. Pp. 211–241 in *Molecular*

systematics of plants II: DNA sequencing, eds. D. E. Soltis, P. S. Soltis, and J. J. Doyle. Boston: Kluwer Academic Publishers.

Nylander, J., F. Ronquist, J. Huelsenbeck, and J. Nieves-Aldrey. 2004. Bayesian phylogenetic analysis of combined data. *Systematic Biology* 53: 47–67.

Parker, S. R. 1997. Sequence Navigator. Multiple sequence alignment software. *Methods in Molecular Biology (Clifton, N.J.)* 70: 145–154.

Pate, J. S., J. Kuo, and N. J. Davidson. 1990. Morphology and anatomy of the haustorium of the root hemiparasite *Olax phyllanthi* (Olacaceae), with special reference to the haustorial interface. *Annals of Botany* 65: 425–436.

Ping, S. T. 1997. Root hemi-parasitism in Malayan Olacaceae. *Gardens Bulletin Singapore* 49: 1–13.

Rambaut, A. 2004. Se-Al Sequence alignment editor, ver. 2.0 a11. Oxford: Department of Zoology, University of Oxford.

Reed, C. F. 1955. The comparative morphology of the Olacaceae, Opiliaceae, and Octoknemaceae. *Memorias da Sociedade Broteriana* 10: 29–79.

Rodrigues, W. A. 1961. Ensaios preliminares de germinacao da castanha Curupira em laboratorio. *Publicacao Instituto National de Pesquisas da Amazonia–Botanica* 12: 1–22.

Rogers, Z. S., V. Malécot, and K. G. Sikes. 2006. A Monograph of Olax L. (Olacaceae) in Madagascar and the Comoro islands. *Adansonia série 3* 28: 71–100.

Ronquist, F. and J. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* 19: 1572–1574.

Schultze-Motel, W. 1964. Santalales. Pp. 1–66 in *A. Engler's Syllabus der Pflanzenfamilien* vol 2, ed. H. Melchior. Berlin: Borntraeger.

Sleumer, H. O. 1935. Olacaceae. Pp. 1–32 in *Die natürlichen Pflanzenfamilien* vol. 16b, eds. A. Engler & K. Prantl. Leipzig: Wilhelm Engelmann.

Sleumer, H. O. 1980. A taxonomic account of the Olacaceae of Asia, Malesia, and the adjacent areas. *Blumea* 8: 145–168.

Sleumer, H. O. 1984a. Olacaceae. Pp. 1–29 in *Flora malesiana* Series 1 vol. 10(1), ed. C. G. G. J. Van Steenis. The Hague: Martinus Nijhoff.

Sleumer, H. O. 1984b. Olacaceae. Pp. 1–159 in *Flora neotropica* monograph 38, ed. J. L. Luteyn. Bronx: New York Botanical Garden.

Soltis, D. E., P. S. Soltis, M. W. Chase, M. E. Mort, D. C. Albach, M. Zanis, V. Savolainen, W. H. Hahn, S. B. Hoot, M. F. Fay, M. Axtell, S. M. Swensen, L. M. Prance, W. J. Kress, K. C. Nixon, and J. S. Farris. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. *Botanical Journal of the Linnean Society London* 133: 381–461.

Soltis, D. E., A. E. Senters, M. J. Zanis, S. Kim, J. D. Thompson, P. S. Soltis, L. P. Ronse De Craene, P. K. Endress, and J. S. Farris. 2003. Gunnerales are sister to other core eudicots: implications for the evolution of pentamery. *American Journal of Botany* 90: 461–470.

Stauffer, H. U. 1957. Santalales-Studien I. Zur Stellung der Gattung *Okoubaka* Pellegrin et Normand. *Berichte der Schweizer Botanischen Gesellschaft* 67: 422–427.

Stauffer, H. U. 1961. Santalales-Studien. VIII. Zur Morphologie und Taxonomie der Olacaceae-Tribus Couleae. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* 106: 412–418.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (* and other methods), ver. 4.0.b10. Sunderland: Sinauer Associates.

Takhtajan, A. 1997. *Diversity and classification of flowering plants*. New York: Columbia University Press.

Tieghem, P. V. 1896. Sur les phanérogames à ovule sans nucelle, formant le groupe des innucellées ou santalinées. *Bulletin de la Société Botanique de France* 43: 543–577.

Tieghem, P. V. 1897a. Sur les inseminées à ovules sans nucelle formant la subdivision des innucellées ou santalinées. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* 1: 723–728.

Tieghem, P. V. 1897b. Sur les inseminées à nucelle pourvu d'un seul tegument, formant la subdivision des unitegminées ou Icacininées. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* 1: 871–876.

Tieghem, P. V. 1899a. Deux genres nouveaux pour la famille des Coulacées. *Bulletin du Muséum d'Histoire Naturelle* 5: 97–100.

Tieghem, P. V. 1899b. Sur les Coulacées. *Annales des Sciences Naturelles, Botanique, Série 8* 10: 125–136.

Tieghem, P. V. 1905. Sur le genre Octocnème considéré comme type d'une famille distincte, les Octocnémacées. *Journal de Botanique* 19: 45–58.

Valeton, T. 1886. *Critisch overzicht der Olacineae B. & H*. Dissertation de Doctor in Plant- en Dierkunke. Groningen, Netherland: Rijks Universiteit.

van den Oever, L. 1984. Comparative wood anatomy of the Olacaceae. Pp. 177–178 in *Proceedings of the Pacific Regional Wood Anatomy Conference*, ed. S. Sudo. Tsukuba: Tsukuba University.

Vidal-Russell, R. and D. L. Nickrent. The first mistletoes: origins of aerial parasitism in Santalales. *Plant Systematics and Evolution* (in press).

Werth, C. R., W. V. Baird, and L. J. Musselman. 1979. Root parasitism in *Schoepfia* Schreb. (Olacaceae). *Biotropica* 11: 140–143.

Appendix 1. Collection information for DNA samples analyzed. Herbarium specimens (deposited at SIUC) are followed by DNA accession numbers (samples archived at SIUC by D. L. Nickrent) and GenBank numbers for rDNA, *rbcL*, and *matK*, respectively.

Agonandra macrocarpa L. O. Williams, Costa Rica, *D. Nickrent 2764*; 2764; L24079; DQ790130; DQ790169. *Cansjera leptostachya* Benth., Australia, *D. Nickrent 2815*; 2815; L24084; DQ790128; DQ790167. *Champereia manillana* Merrill S.E., Asia, *W. Forstreuter s.n.*; 3014; L24746; DQ790129; DQ790168. *Lepionurus sylvestris* Blume, Java, *G. Hambali s.n.*; 2879; DQ790101; DQ790131; DQ790170. *Opilia amentacea* Roxb., Australia, *D. Nickrent 2816*; 2816; L24407; L26076; AY042621. *Pentarhopalopilia marquesii* (Engl.) Hiepko, Africa, *J. J. deWilde & B. deWilde 11212*; 4180; DQ790102; DQ790127; DQ790166. *Gaiadendron punctatum* (Ruiz. & Pav.) G. Don., Costa Rica, *S. Sargent s.n.*; 2729; L24143; L26072; DQ787445. *Moquiniella rubra* (Spreng. f.) Balle, S. Africa, *K. Steiner s.n.*; 3042; AF039078; DQ790132; DQ790171. *Nuytsia floribunda* (Labill.) R. Br., W. Australia, *B. Lamont s.n.*; 2747; DQ790103; DQ790134; DQ787446. *Tupeia antarctica* (Forst. f.) Cham. & Schlecht., New Zealand, *B. Molloy 2575*; 2742; L24425; DQ790133; DQ790172. *Schoepfia schreberi* Gmelin, Bahamas, *D. Nickrent 2599*; 2599; L24418; L11205; DQ787447. *Misodendrum linearifolium* DC, Argentina, *D. E. Bran s.n., G. Amico 136*; 2829, 4591; L24397; L26074; DQ787438. *Anacolosa papuana* Schellenberg, Solomon Islands, *Regalado & Sirikolo 692*; 4247; DQ790104; DQ790144; DQ790181. *Aptandra tubicina* (Poeppig) Miers, Peru, *H. van der Werff & Vasquez 13846*; 4202; DQ790105; DQ790141; DQ790178. *Cathedra acuminata* (Benth.) Miers, Brasil, *J. A. Ratter et al. 6782*; 4244; N/A; DQ790145; DQ790182. *Chaunochiton kappleri* (Engl.) Ducke, Costa Rica, *N. Zamora 1928*; 3052; DQ790106; DQ790142; DQ790179. *Coula edulis* Baill., Gabon, *J. Wieringa 3295*; 3079; N/A; DQ790147; DQ790184. *Curupira tefeensis* Black, Brazil, *C. Clement s.n.*; 4988; DQ790107; DQ790150; DQ790187. *Diogoa zenkeri* (Engl.) Exell & Mendonça, Gabon, *J. Wieringa 3288*; 3078; DQ790108; DQ790152; DQ790189. *Dulacia candida* (Poeppig) O. Kuntze, Ecuador, *M. J. Macía et al. 553*; 4245; DQ790109; DQ790137; DQ790174. *Engomegoma gordonii* Breteler, Equatorial Guinea, *B. Senterre 18-81*; 4555; DQ790110; DQ790153; N/A. *Erythropalum scandens* Blume, Java (at Bogor), *M. Chase 1328*; 4165; DQ790111; DQ790164; DQ790200. *Heisteria cauliflora* Smith, French Guyana, *M. F. Prevost 3796*; 4254; DQ790112; DQ790160; DQ790196. *Heisteria concinna* Standl., Costa Rica, *C. Augspurger s.n.*; 2732; L24146; DQ790161; DQ790197. *Heisteria densifrons* Engl., French Guyana, *J. Munzinger et al. 497*; 4232; DQ790113; DQ790162; DQ790198. *Heisteria parvifolia* Smith, W. Africa, *M. Cheek 5985, M. Chase 843*; 4166; L24146; DQ790163; DQ790199. *Maburea trinervis* Maas, Guyana, *R. Zagt s.n.*; 4256; DQ790114; DQ790165; DQ790201. *Malania oleifera* Chun & Lee, China, *Caoming 340*; 4158; DQ790115; DQ790151; DQ790188. *Minquartia guianensis* Aubl., Costa Rica, *D. Nickrent 2758*; 2758; L24396; DQ790148; DQ790185. *Ochanostachys amentacea* Mast., Java (at Bogor), *M. Chase 1329*; 4167; DQ790116; DQ790146; DQ790183 . *Octoknema* sp., Equatorial Guinea, *B. Senterre SO 291*; 4560; DQ790117; DQ790139; DQ790176. *Olax benthamiana* Miquel, Australia, *M. Chase 2176*; 4168; DQ790118; DQ790135; AY042620. *Olax emirnensis* Baker, Madagascar, *G. Schatz et al. 3620*; 4035; DQ790119; DQ790136; DQ790173. *Ongokea gore* (Hua) Pierre, Gabon, *E. Breteler et al. 14888*; 4184; DQ790120; DQ790140; DQ790177. *Phanerodiscus capuronii* Malécot, Schatz & J. Bosser, Madagascar, *G. Schatz et al. 3439*; 4204; DQ790122; DQ790143; DQ790180. *Ptychopetalum petiolatum* Oliver, Gabon, *E. Breteler 14745*; 4212; DQ790121; DQ790138; DQ790175. *Scorodocarpus borneensis* (Baillon) Becc., Malaysia, *S. P. Teo s.n.*; 3028; U59934; DQ790159; DQ790195. *Strombosia grandifolia* Hooker f., Gabon, *E. Breteler 15457*; 4268; DQ790123; DQ790156; DQ790192. *Strombosia philippinensis* (Baill.) Rolfe, Philippines, *S. Medbury s.n.*; 2831; AF039079; DQ790157; DQ790193. *Strombosia pustulata* Oliver, Gabon, *J. Wieringa 2781*; 4054; DQ790124; DQ790158; DQ790194. *Strombosiopsis tetrandra* Engl., Gabon, *J. Wieringa 3300*; 4055; DQ790125; DQ790155; DQ790191. *Tetrastylidium peruvianum* Sleumer, Peru, *H. van der Werff & R. Vasquez 13875*; 4205; DQ790126; DQ790154; DQ790190. *Ximenia americana* L., Bahamas, *D. Nickrent 2601*; 2601; L24428; DQ790149; DQ790186.