

## A Molecular Phylogeny of Santalaceae (Santalales)

Joshua P. Der<sup>1</sup> and Daniel L. Nickrent

Department of Plant Biology, Southern Illinois University, Carbondale, Illinois 62901-6509 U.S.A.

<sup>1</sup>Author for correspondence, present address: Department of Biology, Utah State University, 5305 Old Main Hill, Logan, Utah 84322 U.S.A. (jpd@biology.usu.edu)

Communicating Editor: Mark P. Simmons

**Abstract**—Santalaceae sensu stricto, the type family for the sandalwood order (Santalales), include approximately 40 genera and over 550 species distributed worldwide. Because the family possesses plesiomorphic and generalized traits that occur throughout the order, this diverse assemblage of hemiparasitic plants has been difficult to characterize and differentiate from related families. We present phylogenetic analyses of all genera of Santalaceae, as well as Viscaceae and selected Opiliaceae, using DNA sequences from nuclear small-subunit ribosomal DNA as well as the chloroplast genes *matK* and *rbcl*. The concatenated data set, analyzed with parsimony, likelihood, and Bayesian inference, gave congruent results, with the majority of clades fully resolved. Our results reveal that the family is polyphyletic and that the genera of Santalaceae, as traditionally classified, occur in nine well-supported clades. The South American herbaceous perennial genera *Arjona* and *Quinchamalium* are sister to *Schoepfia* (Schoepfiaceae). The Australian genus *Anthobolus* emerges as a member of Opiliaceae. Viscaceae remain intact and are well supported as monophyletic. The remaining genera included in Santalaceae occur in six well supported clades, but the relationships among these clades are not fully resolved. These clades are, based on a component generic name, *Comandra*, *Thesium*, *Cervantesia*, *Nanodea*, *Santalum* and *Amphorogyne*. Morphological features diagnostic of these clades are discussed with the intention that these results will serve as the foundation for a revised classification.

**Keywords**—Eremolepidaceae, mistletoe, parasitic plant, sandalwood, Viscaceae.

Santalales include approximately 160 genera and 2200 species distributed worldwide in tropical and temperate regions (Kuijt 1969; Nickrent 1997 - onward). The order is well supported as monophyletic; however, its placement within the global angiosperm phylogeny is unresolved (Soltis et al. 2000; Hilu et al. 2003; Soltis et al. 2003), residing in a polytomy with Berberidopsidales, Caryophyllids, Saxifragales, Rosids, and Asterids (Judd and Olmstead 2004). The Angiosperm Phylogeny Group (APG 2003) classification recognized five families within Santalales: Olacaceae, Loranthaceae, Misodendraceae, Opiliaceae, and Santalaceae sensu lato (including Viscaceae and Eremolepidaceae). Additionally, phylogenetic analyses using nuclear and mitochondrial data indicate that Balanophoraceae are included in Santalales, although the exact phylogenetic placement within the order was not determined (Nickrent et al. 2005). Synapomorphies of Santalales include pendulous ovules on a free-central placenta and the presence of triglycerides with 18-carbon polyunsaturated fatty acids (e.g. ximeninic or santalbic acid). Parasitism likely evolved once in the order, in Olacaceae (Malécot and Nickrent 2008), and this event represents one of 12 independent origins of haustorial parasitism in flowering plants (Nickrent 1997 - onward; Nickrent et al. 1998). Nutritional modes in Santalales vary broadly, ranging from autotrophs to hemiparasites and holoparasites (Balanophoraceae, in the latter case).

Santalaceae sensu stricto include ca. 40 genera and over 550 species as traditionally classified. Previous work showed that Santalaceae sensu stricto are paraphyletic (Nickrent and Duff 1996; Nickrent and Malécot 2001). To maintain monophyly, the Angiosperm Phylogeny Group (2003) considered Santalaceae sensu lato to also include the seven genera and ca. 540 species of Viscaceae, thus making Santalaceae sensu lato the largest family within the order with respect to number of species and the second largest (behind Loranthaceae) with respect to genera. Understanding evolutionary relationships within this group is important because within it three of the five lineages that evolved aerial parasitism occur: "Eremolepidaceae," tribe Amphorogyneae (Santalaceae), and Viscaceae (Nickrent 2002). Aerial parasitism is considered

highly derived and is associated with numerous specialized life history traits. A phylogenetic investigation of this group will allow us to discern the number of transitions to aerial parasitism thus providing insights into the evolution of this fascinating habit. This phylogeny will also provide empirical data for a future revision of the classification of these taxa.

**Characteristics of Santalaceae**—Santalaceae are distributed world wide, with about half the genera in dry or temperate areas and the other half in humid tropical biomes. Most genera are restricted to either the New World or the Old World, but a few exceptions exist, such as *Pyrrularia* and *Buckleya* in Asia and eastern North America (Li et al. 2001), *Comandra* in North America and Europe (Ram 1957), and *Thesium* with species occurring throughout the Old World and in Brazil (Kuijt 1969). Defined primarily by plesiomorphic and absent characters, Santalaceae have no clear synapomorphies and are difficult to distinguish from other families in Santalales (particularly Opiliaceae). Most species are small woody shrubs or herbaceous perennials, but a few are trees (notably *Santalum* and *Okoubaka*). Leaves are simple and usually entire, but may be reduced to scales or spines (Kuijt 1969). Tropical and subtropical species often have thick leathery and persistent leaves while thin deciduous leaves are common in temperate representatives. Some species have dimorphic branches, which may consist of short and long shoots (*Acanthosyris*) or an alternating series of squamate and foliate branches (*Dendromyza* and *Exocarpos*).

Flower size in Santalaceae varies considerably, from small and inconspicuous (e.g. tribe Amphorogyneae) to more showy flowers of moderate size (e.g. *Santalum* and *Quinchamalium*). Generally, a single perianth whorl is present (flowers are thus monochlamydous), but disagreement exists as to the homology of the perianth lobes. They have been interpreted as the calyx where the corolla is lost (Smith and Smith 1943), but fusion of stamens to this floral whorl presents difficulties for this concept. Others have used descriptors such as tepal and perigone (Dawson 1944; Hiepko 2000), but such terms are noncommittal. We interpret the perianth lobes as the corolla, as was done for *Phoradendron* (Kuijt 2003) and Eremolepidaceae (Kuijt 1988). The calyx is reduced and fused

to the wall of the inferior ovary, but in some cases a small remnant (calyculus) is present, such as in *Mida* (Bhatnagar 1960), *Nanodea*, and *Myoschilos* (Dawson 1944). It is most parsimonious to infer that this structure is homologous with the calyculus seen in Loranthaceae, Schoepfiaceae, and Opiliaceae (Stauffer 1961a). Finally, two sets of foliar organs occur at the apex of the female flower of *Buckleya distichophylla* (Nutt.) Torr. Although the outer, longer ones (deciduous upon fruiting) have been interpreted as bracts (Carvell and Eshbaugh 1982), it is more reasonable to consider these sepals and the inner ones (deciduous at pollination) petals. In most genera the corolla lobes are free or only with a very short floral cup. In other genera, such as *Nestronia*, *Santalum*, *Jodina*, and *Comandra*, the corolla forms a cup-shaped tube. *Arjona* and *Quinchamalium* differ from all other genera in having very long corolla tubes.

Approximately half the genera have strictly bisexual flowers whereas the remaining ones have unisexual flowers in various sexual systems that include dioecy, monoecy, andro-dioecy, and trioecy. In bisexual and male flowers, stamens are equal in number to, and inserted opposite, each corolla lobe. A tuft of hair is commonly found on the corolla immediately behind the point of stamen insertion. Flowers have a single style at the apex of an inferior, half-inferior or rarely superior ovary. One of the most consistent features of Santalaceae flowers is the presence of a conspicuous lobed floral disc at the base of the style, which often produces copious amounts of nectar (Macklin and Parnell 2002). Ovaries are unilocular, but the chamber may be partially divided at the base, with a short free-central placental stalk bearing one to five pendulous ovules (Smith and Smith 1943), only one of which develops into a seed.

Hemiparasitism has been documented for all but five genera of Santalaceae (Kuijt 1969; Der 2005). Santalaceae are generally not host specific (although there are exceptions) and a single individual may simultaneously parasitize hosts in numerous families, themselves (i.e. autoparasitism) or other individuals of the same species (Rao 1942a; Leopold and Muller 1983; Fineran 1991; Lepschi 1999). Tribe Amphorogyneae includes the whole suite of parasitic habits ranging from obligate root parasites to species variably root and/or stem parasitic (a trophic condition we term *amphiphagous*), twining stem parasites with secondary haustoria (i.e. dendroparasites), and strictly aerially parasitic shrubs (mistletoes).

Eremolepidaceae comprise a group of New World mistletoes that include three genera and 12 species (Kuijt 1988). The rank and systematic placement of this group has been controversial; it has been variously allied with Olacaceae via *Opilia* (Opiliaceae) (Kuijt 1968), Loranthaceae (Kuijt 1988), Santalaceae (Barlow and Wiens 1977), and Viscaceae (Barlow 1964; Bhandari and Vohra 1983). Molecular phylogenetic work placed Eremolepidaceae within Santalaceae (Nickrent and Duff 1996; Nickrent et al. 1998; Nickrent and Malécot 2001).

The mistletoes of Viscaceae have been classified both as a subfamily of Loranthaceae and as a distinct family in their own right. Current phylogenetic hypotheses place a monophyletic Viscaceae in a derived position along a paraphyletic grade of santalaceous genera (Nickrent and Duff 1996; Nickrent et al. 1998; Nickrent and Malécot 2001), which motivated the Angiosperm Phylogeny Group (2003) to include it within Santalaceae.

**Classification History of Santalaceae**—Santalaceae were first described in Robert Brown's (1810) *Prodromus Florae Novae Hollandiae*. Brown based his descriptions upon specimens he collected in Australia from 1802–1805 as the naturalist on Matthew Flinder's circumcontinental voyage (Stearn 1960). The next treatment of Santalaceae was that of Hieronymus (1889) who recognized 26 genera which he placed within three tribes: Anthoboleae, Osyrideae, and Thesieae. Hieronymus' classification was adopted by Rendle (1925) and was updated and revised by Pilger (1935), who retained the three tribes but rearranged the generic taxonomy in the family.

Since Pilger's work, subsequent authors have made significant contributions to our understanding of the floristics and generic and tribal relationships within Santalaceae. To date, Pilger's work still represents the most recent generic-level systematic and taxonomic treatment for the family worldwide. Following his revision of *Phacellaria*, Danser (1939) began work on the complex mistletoe genus *Henslowia* Blume. This genus was split into *Dendromyza*, *Cladomyza*, and *Hylomyza*, and the remaining species were synonymized with *Dendrotrophe* (Danser 1940, 1955). Stauffer revised Anthoboleae to include *Omphacomeria* (1959) and erected a fourth tribe, Amphorogyneae (1969) containing three genera segregated from Santalaceae (*Choretum*, *Leptomeria*, and *Phacellaria*). Amphorogyneae also included *Spirogardnera*, *Daenikera*, *Amphorogyne*, and the Indomalayan dendroparasites and mistletoes, including the taxa Danser split from *Henslowia* (Stauffer 1969; Stearn 1972). The original tribal designation for Amphorogyneae lacked a Latin diagnosis, but was later validly published (Stearn 1972). Recent taxonomic work on the Santalaceae of Thailand resulted in *Hylomyza* being synonymized with *Dufrenoya* and *Cladomyza* being lumped into *Dendromyza* (Macklin 2000; Macklin and Parnell 2000, 2002). The classification of Santalaceae and related families based on Pilger (1935) and subsequent workers is given in Table 1. Additionally, two new monotypic genera have been discovered in Madagascar and were described and named as *Staufferia* and *Pilgerina* (Rogers et al. in press).

The botanical community was deprived of a comprehensive monograph of Santalaceae by the premature death of Hans U. Stauffer in 1965. This group deserves further examination because of the numerous taxonomic additions and changes (above) and because preliminary molecular phylogenetic studies indicate the family is polyphyletic. Here we present the first phylogenetic analysis of Santalaceae and related taxa with comprehensive generic-level sampling. This phylogeny allows us to test family delimitations and to define major subclades to help clarify some of the morphological diversity in this heterogeneous group.

#### MATERIALS AND METHODS

**Taxon and DNA Sequence Sampling**—Representatives of all 40 genera in Santalaceae were sampled, including both new genera from Madagascar (Rogers et al. in press). Additionally, all seven genera in Viscaceae and the three genera classified as "Eremolepidaceae" were included. At least one species from each genus was sampled, but a second species was included for six of the more speciose genera to test their monophyly. Seven of the ten genera in Opiliaceae, *Schoepfia* (Schoepfiaceae), *Misodendrum* (Misodendraceae), and *Nuytsia* (Loranthaceae) were included as outgroup taxa for a total of 57 genera and 63 santalalean species. Voucher information and GenBank accession numbers are given in Appendix 1. In five genera, data for different genes were obtained from two different specimens and combined for phylogenetic analyses (Appendix 1).

Genomic DNA was isolated from herbarium, silica dried, or fresh-frozen plant tissue using a modified CTAB method (Nickrent 1994).

TABLE 1. Comparison of traditional and molecular classifications for Santalaceae and related families. The traditional classification is based on Pilger (1935) with modifications and additions by Danser (1955), Stauffer and Hürlimann (1957), Stauffer (1959, 1968, 1969), Stearn (1972), Hewson and George (1984), Macklin (2000) and Macklin and Parnell (2002). The classification of Viscaceae is after Barlow (1964), Eremolepidaceae follows Kuijt (1988), and Opiliaceae follows Hiepko (1979, 1982, 1985, 1987).

Traditional classification	Molecular classification (Fig. 1)
<b>Santalaceae R. Br.</b>	
Tribe Anthoboleae (Dumort.) Spach	
<i>Anthobolus</i> R. Br.	Opilia clade
<i>Exocarpos</i> Labill. (syn. <i>Elaphanthera</i> N. Hallé)	Santalum clade
<i>Omphacomeria</i> (Endl.) A. DC.	Santalum clade
Tribe Amphorogyneae Stauffer ex Stearn	
<i>Amphorogyne</i> Stauffer & Hürl.	Amphorogyne clade
<i>Choretrum</i> R. Br.	Amphorogyne clade
<i>Daenikera</i> Hürl. & Stauffer	Amphorogyne clade
<i>Dendromyza</i> Danser (syn. <i>Cladomyza</i> Danser)	Amphorogyne clade
<i>Dendrotrophe</i> Miq. (syn. <i>Henslowia</i> Blume)	Amphorogyne clade
<i>Dufrenoya</i> Chatin (syn. <i>Hylomyza</i> Danser)	Amphorogyne clade
<i>Leptomeria</i> R. Br.	Amphorogyne clade
<i>Phacellaria</i> Benth.	Amphorogyne clade
<i>Spirogardnera</i> Stauffer	Amphorogyne clade
Tribe Santaleae A, DC. (syn. Osyrideae Rchb.)	
<i>Acanthosyris</i> (Eichl.) Grieseb.	Cervantesia clade
<i>Buckleya</i> Torr.	Thesium clade
<i>Cervantesia</i> Ruiz & Pav.	Cervantesia clade
<i>Colpoon</i> P. J. Bergius (syn. <i>Fusanus</i> L., in part)	Santalum clade
<i>Comandra</i> Nutt.	Comandra clade
<i>Geocaulon</i> Fernald	Comandra clade
<i>Jodina</i> Hook. & Arn. ex Meissn.	Cervantesia clade
<i>Kunkeliella</i> Stearn	Thesium clade
<i>Mida</i> A. Cunn. ex Endl.	Nanodea clade
<i>Myoschilos</i> Ruiz & Pav.	Santalum clade
<i>Nanodea</i> Banks ex C. F. Gaertn.	Nanodea clade
<i>Nestronia</i> Raf. (syn. <i>Darbya</i> A. Gray)	Santalum clade
<i>Okoubaka</i> Pellegr. & Normand	Cervantesia clade
<i>Osyris</i> L.	Santalum clade
<i>Pyrrularia</i> Michx.	Cervantesia clade
<i>Rhoiacarpus</i> A. DC.	Santalum clade
<i>Santalum</i> L. (syn. <i>Eukarya</i> T. L. Mitch. and <i>Fusanus</i> R. Br., in part)	Santalum clade
<i>Scleropyrum</i> Arn. (syn. <i>Scleromelum</i> K. Schum. & Lauterb.)	Cervantesia clade
Tribe Thesieae Rchb.	
<i>Arjona</i> Cav.	Schoepfia clade
<i>Osyridocarpus</i> A. DC.	Thesium clade
<i>Quinchamalium</i> Molina	Schoepfia clade
<i>Thesidium</i> Sonder	Thesium clade
<i>Thesium</i> L. (syn. <i>Austroamericium</i> Hendrych)	Thesium clade
<b>Eremolepidaceae Tiegh. ex Kuijt</b>	
<i>Antidaphne</i> Poepp. & Endl.	Santalum clade
<i>Eubranchion</i> Hook.	Santalum clade
<i>Lepidoceras</i> Hook.	Santalum clade
<b>Viscaceae Miers.</b>	
<i>Arceuthobium</i> M. Bieb	Viscum clade
<i>Dendrophthora</i> Eichl.	Viscum clade
<i>Ginalloa</i> Korth.	Viscum clade
<i>Korthalsella</i> Tiegh.	Viscum clade
<i>Notothixos</i> Oliv.	Viscum clade
<i>Phoradendron</i> Nutt.	Viscum clade
<i>Viscum</i> L.	Viscum clade
<b>Opiliaceae Valetton</b>	
<i>Agonandra</i> Miers ex Benth.	Opilia clade
<i>Cansjera</i> Juss.	Opilia clade
<i>Champereia</i> Griffith	Opilia clade
<i>Gjellerupia</i> Lauterb.	Opilia clade
<i>Lepionurus</i> Blume.	Opilia clade
<i>Melianttha</i> Pierre	Opilia clade
<i>Opilia</i> Roxb.	Opilia clade
<i>Pentarrhopalopilina</i> Hiepko.	Opilia clade
<i>Rhopalopilina</i> Pierre	Opilia clade
<i>Urobotrya</i> Stapf.	Opilia clade

Nuclear small-subunit (SSU) rDNA and chloroplast *rbcl* and *matK* genes were PCR-amplified and sequenced using the primers and protocols reported in Rogers et al. (in press). Sequence data were not obtained from three samples for the SSU rDNA, one sample for *rbcl*, and three samples for *matK* (see Appendix 1).

**Phylogenetic Analysis**—Sequences were manually aligned using Se-AL v2.0a11 (Rambaut 2004). Alignment was straightforward for the SSU rDNA and *rbcl* genes, requiring few gaps (indels) for SSU rDNA and none for *rbcl*. In contrast, *matK* was much more variable and alignment required the introduction of many gaps. The *matK* alignment was informed by also examining the sequences translated into amino acids. All of the aligned nucleotide sites were included in phylogenetic analyses. Aligned data matrices are available in TreeBASE (study number S1840).

Datasets for all three genes were analyzed separately and concatenated using maximum parsimony (MP), maximum likelihood (ML; Felsenstein 1973), and Bayesian inference (BI; Yang and Rannala 1997). All trees were rooted specifying *Nuytsia* as the outgroup and incongruence among genes and optimality criteria was assessed with cut-off values of 75% bootstrap support for MP and ML analyses and 0.95 posterior probability for BI analyses. All heuristic MP searches were performed in PAUP\* version 4.0b10 (Swofford 2002) coding gaps as “missing” data, using starting trees generated from 100 random addition sequence replicates holding one tree at each taxon addition step, and tree-bisection-reconnection (TBR) branch swapping. All of the most parsimonious trees were saved and the strict consensus was computed. MP bootstrap (MPBS) analysis was performed on all datasets (10,000 BS replicates using TBR branch swapping on starting trees generated by ten random addition sequence replicates, holding one tree at each step) to assess clade stability in trees recovered in the heuristic MP searches. BS support values are reported for clades found in greater than 50% of the BS replicates. A “MaxTrees” limit of 1000 was imposed to limit the computational run time for the SSU rDNA dataset.

Models of DNA sequence evolution used in ML and BI analyses were evaluated for each gene, codon positions in *matK* and *rbcl* and for the nonpartitioned concatenated dataset using the second order Akaike Information Criterion (AICc; Akaike 1974) and the Bayesian Information Criterion (BIC; Schwarz 1978) implemented in Modeltest version 3.6 (Posada and Crandall 1998) using maximum likelihood scores estimated from one of the most parsimonious trees found in the heuristic parsimony searches. The total number of alignment sites in each data partition was used as the sample size for AICc and BIC calculations (Posada and Buckley 2004). In choosing a best-fit model of molecular evolution for each partition, the AICc and BIC weights for each model were examined and evidence ratios were calculated with the next-best model (Burnham and Anderson 2002). ML analyses were performed in PHYML version 2.4.4 (Guindon and Gascuel 2003), estimating the best-fit model parameters in the analysis. ML bootstrap (MLBS) analyses were implemented with 1,000 replicates.

Bayesian phylogenetic analyses were performed using the parallel Metropolis-coupled Markov chain Monte Carlo, or “p(MC)<sup>3</sup>,” algorithm in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003; Altekar et al. 2004). Codon positions were partitioned in *rbcl* and *matK* and the full dataset was separately analyzed partitioned by gene and partitioned by both gene and codon. Model parameters for each data partition were estimated independently as part of the analyses while tree topology and branch length were linked across all data partitions in each analysis. An uninformative flat Dirichlet prior was implemented for all parameters except the nucleotide state frequencies for the second codon position of *rbcl*, for which the prior was fixed and equal to implement the Jukes-Cantor model. Two simultaneous independent analyses were run, each with six p(MC)<sup>3</sup> chains distributed across 12 CPU cores on three nodes of a Linux cluster using the message-passing interface (MPI). Ten million p(MC)<sup>3</sup> generations were run in each analysis, with trees and parameter estimates sampled every 1,000 generations and printed to a file. To ensure that the two runs in each analysis had reached convergence and achieved a good sample of the posterior distribution, the average standard deviation of split frequencies was calculated and in all cases had dropped below 0.002 after discarding the first 25% of the sampled generations (2.5 million generations) as burn-in. As the posterior distribution of topologies from each run becomes more similar, this value approaches zero. The Potential Scale Reduction Factor (PSRF) was also examined for model parameters as an additional guide to convergence between the two runs in each analysis. BI posterior probabilities (PP) of clades (i.e. clade credibility values) and tree probabilities were calculated after 2.5 million generations were discarded as burn-in.

## RESULTS

Aligned datasets for each gene included 1,829, 1,429, and 1,284 nucleotide sites for SSU rDNA, *rbcl* and *matK*, respectively (of which 240, 303, and 608 of those sites were parsimony informative, respectively). Parsimony analysis resulted in 5,419 most parsimonious trees of length 1,238 for SSU rDNA, eight trees of length 1,235 for *rbcl*, and 60 trees of length 2,813 for *matK*. Strict consensus trees from these analyses are largely congruent, but differ in their level of resolution. The MP strict consensus trees are not shown, but are available in TreeBASE. MPBS values revealed no strongly conflicting clades among the three genes.

For each of the three genes, the concatenated dataset and the first codon position of *rbcl*, the best-fit model chosen was the general time reversible model (GTR; Tavaré 1986) with a proportion of invariant sites (I) and a gamma distributed rate parameter ( $\Gamma$ ; Yang 1993). The best-fit model for the second codon position of *rbcl* was the Jukes-Cantor (JC; Jukes and Cantor 1969) model with I +  $\Gamma$ , while the GTR +  $\Gamma$  was the best-fit model for each of the remaining data partitions. For all of these partitions, examination of the AICc and BIC evidence ratios revealed that the best-fit model had AICc and/or BIC weights that were at least an order of magnitude greater than that of the next-best model implemented in MrBayes.

Log likelihood scores of the ML tree found for each gene were -9357.932, -9224.844, and -15614.733 for SSU rDNA, *rbcl* and *matK*, respectively. Again, MLBS support values and Bayesian posterior probabilities (PP) revealed no strongly conflicting clades among the three genes. ML and Bayesian consensus trees for each gene are not shown but are available via TreeBASE. Analysis of the concatenated three-gene data set resulted in no strongly conflicting clades among the MP, ML or BI trees. The MP heuristic search found four most parsimonious trees length 5423 and the log likelihood of the optimal ML tree was -35730.410.

The BI phylogram with maximum parsimony bootstrap (MPBS), maximum likelihood bootstrap (MLBS), and Bayesian posterior probability (PP) values is shown in Fig. 1. The clades will be referenced here based upon a genus that occurs in that clade. In all analyses of the concatenated data set, the genera *Arjona* and *Quinchamalium* are strongly supported as sister to the outgroup taxon *Schoepfia* (MPBS and MLBS = 100, PP = 1.0). *Anthobolus*, another taxon previously considered part of Santalaceae, is placed in the *Opilia* clade (Opiliaceae), which is strongly supported here as monophyletic (MPBS and MLBS = 100, PP = 1.0). The remaining santalaceous taxa (i.e. members of Santalaceae, Viscaceae, and Eremolepidaceae) are monophyletic (MPBS = 95, MLBS = 87, PP = 1.0) with respect to the outgroup taxa. These taxa form seven strongly supported clades (Fig. 1), all of which have maximum support values (MPBS and MLBS = 100, PP = 1.0). While these clades are well supported and internal nodes within these clades are well resolved, they are separated by relatively short branch lengths and phylogenetic relationships among them are not well supported.

## DISCUSSION

Santalaceae are a morphologically, anatomically and embryologically diverse assemblage of hemiparasites that were previously ambiguously defined and were thus difficult to distinguish from other families in Santalales. Smith (1937, p.

14) noted this heterogeneity and suggested the family "perhaps should be divided." Since Pilger's 1935 classification, nine new genera have been newly discovered or newly circumscribed from existing genera: *Amphorogyne*, *Daenikera*, *Dendromyza*, *Dufrenoya*, *Kunkeliella*, *Okoubaka*, *Pilgerina*, *Spirogardnera*, and *Staufferia*. The authors of these genera attempted to incorporate these new taxa within the existing tribal classification, but this sometimes resulted in a violation of tribal trait boundaries. The creation of a fourth tribe by Stauffer (Amphorogyneae; 1969) significantly improved upon Pilger's (1935) classification by moving four genera from Santaleae, but this latter tribe remained extremely heterogeneous. Tribe Amphorogyneae is well supported as monophyletic using molecular data (Fig. 1, Table 1). In contrast, Santaleae and Thesieae are polyphyletic (Table 1). Below we discuss the santalaceous genera in the context of the nine major clades resolved using the concatenated three-gene dataset.

**Schoepfia Clade**—Previous molecular work has shown that *Schoepfia* is more closely related to Misodendraceae and Loranthaceae than to Olacaceae (Nickrent and Duff 1996; Nickrent et al. 1998; Malécot and Nickrent 2008). Thus we concur that this taxon should be classified in its own family, Schoepfiaceae Blume following van Tieghem (1896) and Judd et al. (2002). One of the surprising results of the present study was the placement of *Arjona* and *Quinchamalium* in a clade with *Schoepfia*, apart from other Santalaceae. This position is supported by several morphological synapomorphies. For example, floral bracts are fused into a cup that surrounds the base of the flower in both *Quinchamalium* and *Schoepfia*. Although they are widely distributed among Santalaceae taxa, poststaminal hairs are present on the corollas of *Arjona*, *Quinchamalium*, and *Schoepfia*. van Tieghem (1896) stated that the origin of these hairs in *Arjona* and *Quinchamalium*, as in *Schoepfia*, are epidermal, in contrast with Santalaceae where the hairs are hypodermal in origin. Johri and Bhatnagar (1960), however, disagree with this interpretation and consider the hairs epidermal in all taxa. All three genera have an inferior ovary with three locules in the lower portion. Moreover, the three genera are distylous. The woody habit of *Schoepfia* links to section *Xylarjona* Scottsberg of *Arjona* that contains small shrubs whose woody shoots form a xylopodium at the base (Pilger 1935). These synapomorphies, as well as strong support from molecular data, provide evidence that this clade of three genera should be classified together. Van Tieghem recognized the affinity of these genera when he stated (1896, p. 577; translated from French): "All things considered, it is thus not far from Santalaceae and very close to Arjonaceae that the family Schoepfiaceae arrives to take a seat in the group which, at this moment, we work to establish. This place is precisely, as mentioned above, that which Blume already assigned to them nearly one half-century ago."

**Opilia Clade**—Another surprising result of this study was the position of *Anthobolus* within Opiliaceae. This position is seen in analyses of all three gene partitions separately and of the concatenated data set, strongly supporting this association. In most traditional classifications of Santalaceae, *Anthobolus* was considered a part of this family and allied with *Exocarpos*. Indeed, tribe Anthoboleae was erected by Stauffer (1959) to accommodate these two genera as well as *Omphacomeria*. As shown below, *Exocarpos* and *Omphacomeria* were resolved as part of the *Santalum* clade (Fig. 1, Table 1), thus



1959), similar to the distribution of *Santalum* occurring throughout India, Australia, and the Pacific Islands (Harbaugh and Baldwin 2006, 2007). An apparent synapomorphy for Opiliaceae is the presence of cystoliths in the leaf epidermis (Hiepko 2000), but this anatomical feature was not observed in *Anthobolus leptomerioides* (Nickrent, pers. obs.). All species in the genus should be examined to confirm the absence of this character, which could then be interpreted as an evolutionary loss in the genus.

**Comandra Clade**—*Comandra* and *Geocaulon* are strongly supported as sister and form a clade among the basal Santalaceae polytomy. *Geocaulon* is restricted to north temperate and arctic North America and *Comandra* has a disjunct distribution in North America and Europe. Both genera are monotypic and quite similar in habit, with short (up to 30 cm) herbaceous upright flowering stalks that arise from a creeping rhizome. *Geocaulon* is distinguished from *Comandra* by having monoecious inflorescences (versus bisexual flowers in *Comandra*) and a thin herbaceous rhizome (versus a thick woody rhizome). *Comandra* was described in 1818 by Nuttall, from which *C. lividum* was subsequently segregated as *Geocaulon lividum* (Fernald 1928). Johri and Bhatnagar (1960) suggested that *Comandra* was distinctive enough from other groups in Santalaceae that it should be recognized at the tribal level (Comandreae) based on embryology and details of the ovary and placenta, which corroborated a tribal designation proposed by Van Tieghem (1896).

**Thesium Clade**—*Buckleya*, *Kunkeliella*, *Osyridocarpus*, *Thesidium*, and *Thesium* form a well supported clade with fully resolved generic relationships. *Buckleya*, which is sister to the remaining taxa in this clade, is a small tree with a fleshy fruit. *Osyridocarpus* and *Kunkeliella* are small shrubs while the remaining two genera (*Thesidium* and *Thesium*) show a trend toward the herbaceous habit with many subshrubs, herbaceous perennials, and annuals. The *Thesium* clade is primarily African in distribution, with *Thesidium* endemic to South Africa. The four species of *Kunkeliella* are rare and endangered endemics of the Canary Islands (Francisco-Ortega et al. 2000). The inclusion of *Kunkeliella* in this group with the majority of tribe Thesieae is not surprising considering that Stearn (1972) noted its remarkable similarity with *Osyridocarpus* and *Thesium*. Stearn even suggested that this new taxon might represent a new section of *Thesium* given that it has a fleshy fruit that is rare in Thesieae and is more characteristic of tribe Santaleae. *Osyridocarpus* is found in tropical and southern Africa and *Thesium* reaches its peak diversity in southern Africa, but extends throughout the Old World and Australia with a few species in Brazil. *Buckleya* has a distribution unlike all the other members of this clade, and is found disjunct between North America and China and Japan. The basal position of *Buckleya* in this clade has interesting implications for the biogeography of this group because its four species form two pairs – China with eastern North America and China with Japan (Li et al. 2001).

**Cervantesia Clade**—This clade includes six closely allied genera that have traditionally been classified in tribe Santaleae (*Scleropyrum*, *Okoubaka*, *Pyrrularia*, *Cervantesia*, *Jodina*, and *Acanthosyris*), as well as two newly named genera from Madagascar, *Staufferia* and *Pilgerina* (Rogers et al. in press). Within this clade there are two well supported subclades. The first (*Cervantesia* subclade) includes *Acanthosyris*, *Cervantesia*, and *Jodina*, and is well supported as monophyletic (MPBS and MLBS = 100, PP = 1.0). However, the pattern of

divergence among these three genera is not clear. This subclade is strictly South American in distribution. The second (*Pyrrularia*) subclade contains the remaining genera and is also well supported (MPBS and MLBS = 100, PP = 1.0), but in this case is fully resolved. Most genera in the *Pyrrularia* subclade are distributed in the Old World tropics whereas *Pyrrularia* has one species in eastern North America and two in Asia (similar to the distribution of *Buckleya*). Characteristics of the *Cervantesia* clade are that its members are small to large trees that have large drupaceous fruits with a stony pit. The close affinities of the genera in each of these subclades were recognized by Stauffer (1957, 1961b) in his *Santalales Studien* series.

**Nanodea Clade**—*Mida* and *Nanodea* form a robust clade (MPBS and MLBS = 100, PP = 1.0) in an intermediate position among the other santalaceous clades. Both genera are monotypic and have historically been classified in tribe Santaleae. These genera are both woody, but *Mida* is a tree to 8 m high, while *Nanodea* is a diminutive subshrub with a much branched creeping and cushion-like growth form in cold temperate Patagonia, Tierra del Fuego and the Islas Malvinas. *Mida* has a disjunct distribution between New Zealand (*M. salicifolia*) and the Juan Fernandez Islands (*M. fernandeziana*). Pilger (1935) showed *Nanodea* and *Mida* to be distantly related within Santaleae. Pilger allied *Mida* with the genera *Fusanus* and *Eucarya*, two genera that have more recently been included within *Santalum*. These molecular results show that *Mida* is not closely related to the sandalwoods, as asserted by Skottsberg (1930).

**Santalum Clade**—Tribe Santaleae (syn. tribe Osyrideae in Pilger 1935) is the most heterogeneous group within the traditional Santalaceae. Stauffer and Hürlimann (1957) alluded to this and stated that it was not a natural assemblage and was comprised of several distinct generic groupings within the tribe. Stauffer (1969) began a formal subdivision when he established tribe Amphorogyneae. This heterogeneity has been the source of much taxonomic confusion and many authors have reorganized the taxa in this traditional tribe in different ways (Van Tieghem 1896; Pilger 1935; Rao 1942b; Smith and Smith 1943; Johri and Bhatnagar 1960). The molecular analyses presented in this study further corroborate the polyphyletic nature of Santaleae (Table 1).

The *Santalum* clade includes 11 genera that represent parts of tribe Santaleae (including the type genus, *Santalum*), the mistletoes of Eremolepidaceae (*Antidaphne*, *Eubrachion*, and *Lepidoceras*), and *Exocarpos* and *Omphacomeria*, formerly of the now defunct tribe Anthoboleae. This clade is strongly supported but relationships among its various subclades are sometimes poorly resolved. Basalmost is the clade containing *Exocarpos* and *Omphacomeria*. As discussed above, *Omphacomeria* is an Australian endemic whereas *Exocarpos* is much more widespread throughout the Pacific and Indomalaya. The presence of *Exocarpos* at the base of this clade has significance with regard to the evolution of aerial parasitism. Although most species of *Exocarpos* are root parasitic shrubs and trees, some such as *E. pullei* of New Guinea can parasitize stems or roots (Lam 1945). It is hypothesized that the gene complexes necessary for the evolution of aerial parasitism may already be in place in such amphiphagous taxa (i.e. some degree of preadaptation). The three eremolepidaceous genera are all stem parasitic mistletoes and their monophyly suggests that the full manifestation of stem parasitism evolved in their common ancestor. Although not strongly

supported, the topology of the tree suggests these New World tropical mistletoes may share a most recent common ancestor with *Myoschilos* of South America.

The next well supported clade includes the basal-most *Santalum* lineage sister to a clade with three mainly African genera (*Colpoon*, *Osyris*, *Rhoiacarpus*) and the North American endemic *Nestronia*. These four genera have a very similar overall vegetative and floral morphology, being small shrubs with leathery leaves and polygamous/dioecious flowers arranged in small axillary cymes. Determining how *Nestronia* arrived in North America from Africa would make a fascinating biogeographic study.

*Santalum*, the type genus of Santalaceae, includes 15 extant species, 14 varieties, and one recently extinct species distributed from Australia to India and the Pacific (Harbaugh and Baldwin 2007). *Santalum* species are small trees or shrubs with opposite leathery or fleshy leaves, bisexual 4–5-merous flowers and spherical drupaceous fruits with a thin exocarp. *Santalum album* is well known for its fragrant heartwood oil and has been cultivated throughout its range. Molecular studies indicate the genus has a complex history of hybridization, allopolyploidy, and long-distance dispersal that have influenced its biogeographic pattern of genetic variation (Harbaugh and Baldwin 2006). The genus apparently originated in Australia followed by several long-distance dispersal and colonization events to and among Pacific islands, including Hawaii (Harbaugh and Baldwin 2007).

**Amphorogyne Clade**—This well supported clade is equivalent to tribe Amphorogyneae. Stauffer (1969) recognized the relative distinctiveness of these taxa by the presence of unusual anthers and placentae. The anthers have anterior and posterior locules of unequal size that are born on short and stout or nearly absent filaments. Each thecum dehisces transversely independently, in contrast to other Santalaceae where anther dehiscence is along a single longitudinal slit common to the two thecal locules. Additionally, the placenta is short to nearly absent and more strongly associated with the ovary tissue in this clade. The ovules are borne in (typically five) pockets at the base of the ovarian locule, in contrast to the stalked placental column with or without basal ovarian pockets in other Santalaceae.

Relationships within the *Amphorogyne* clade are fully resolved and three main lineages present an interesting combination of trophic modes. The first clade, sister to the remaining genera, contains *Amphorogyne* and *Daenikera*, both New Caledonian endemics. *Amphorogyne* is a root parasitic tree whereas *Daenikera* is amphiphagous and has undergone extreme reduction in habit (Stauffer 1969). The next clade is composed of *Choretrum*, *Leptomeria*, and *Spirogardnera*, all of which are root parasites endemic to Australia. The remaining genera in the third clade show a variety of trophic modes. Some species of *Dendrotrophe* are root parasitic lianas whose aerial stems clamber through the vegetation (but form no aerial haustoria), whereas other species are mistletoes. Further work is needed to determine whether some species are amphiphagous. *Dendromyza* species are adapted to aerial parasitism (as evidenced by their seed morphology) and after initial attachment, form secondary haustorial connections to the host from their twining branches. The growth form of these plants is referred to as dendroparasitic and is reminiscent of that of *Cassytha* (Lauraceae) or dodder (*Cuscuta*). Finally, the genera *Dufrenoya* and *Phacellaria* are true mistletoes that have primary haustorial connections to the host but do

not form epicortical roots (as in Loranthaceae) or haustorial twining stems (as in *Dendromyza*). Indeed, *Phacellaria* is a highly advanced squamate mistletoe that is an obligate epiparasite on other mistletoes in Loranthaceae and Santalaceae.

The Amphorogyne clade is curiously fractal with regard to trophic mode when compared to the Santalales as a whole or Santalaceae in particular. One can observe the following trend as having evolved more than once (with all or only some of the stages present in extant species): a) root parasite b) amphiphagous parasite, c) dendroparasite, d) leafy mistletoe, and e) squamate mistletoe. For example, in the Amphorogyne clade, the stages are represented by a) *Amphorogyne*, *Choretrum*, etc.; b) *Daenikera* and possibly *Dendrotrophe*; c) *Dendromyza*; d) *Dufrenoya* and e) *Phacellaria*. In the *Santalum* clade, these stages can be seen as a) *Myoschilos*, d) *Antidaphne* and *Lepidoceras*, and e) *Eubracion*. The trend toward the replacement of foliar leaves with scale leaves (the squamate habit) is also accompanied by decreasing photosynthetic ability, a trend that appears to have happened twice in the *Amphorogyne* clade – *Daenikera* compared with *Amphorogyne* and *Phacellaria* compared with *Dufrenoya*. Although derived parasites such as *Daenikera* (Hürlimann and Stauffer 1957; Stauffer 1969) and *Phacellaria* (Danser 1939) approach holoparasitism, it appears that all have retained some photosynthetic ability. As suggested in Nickrent et al. (1998), holoparasitism may not be compatible with the aerially parasitic habit, thus adding constraint upon this evolutionary direction.

**Viscum Clade**—This well supported clade is equivalent to the family Viscaceae and has long been recognized as a distinct monophyletic group of mistletoes based on morphological, embryological, cytological, anatomical, and molecular data (Barlow 1964; Kuijt 1968, 1969; Barlow and Wiens 1971; Barlow 1983; Bhandari and Vohra 1983; Nickrent et al. 1998; Kuijt 2003). This clade also includes a number of economically important forest pathogenic species (*Arceuthobium* spp.) and well-known commercial species, specifically the Christmas mistletoes (mainly *Viscum album* in Europe and *Phoradendron serotinum* in the U.S.).

The clades recovered within Viscaceae in this study do not conflict with previous MP results that sampled the same taxa but fewer genes (Nickrent et al. 1998; Nickrent and Malécot 2001). DNA sequences for Viscaceae are highly divergent from those in Santalaceae (especially *matK*), presenting challenges to alignment and contributing to longer branches in this group. Three clades are consistently seen (in single gene and combined analyses), *Viscum* and *Notothixos*, *Ginallia* and *Korthalsella*, and *Phoradendron* and *Dendrophthora*, while *Arceuthobium* has assumed different positions in the various analyses, but never with strong support. The position of *Korthalsella* in the three gene BI analysis is anomalous. In our analysis the *matK* sequence for *Korthalsella* was highly divergent (an average of 60% amino acid similarity with other Viscaceae) and contributed significantly to destabilizing clades within Viscaceae.

Following earlier molecular phylogenetic studies with incomplete taxon sampling in Santalaceae, the notion was still viable that Viscaceae shared a most recent common ancestor with the mistletoes of the Amphorogyne clade. That scenario would suggest one, not two, origins of the mistletoe habit in that portion of the phylogenetic tree. The results from the current phylogenetic investigation clearly support the concept that these mistletoes evolved independently. It is less

parsimonious to assume that the ancestor of Viscaceae and the Amphorogyne clade was a mistletoe because this would require several reversions to root parasitism, e.g. in the *Choretrum* and *Dendrotrophe* clades. Because the mistletoe habit is accompanied by many morphological specializations, this reversal seems unlikely. A more reasonable reconstruction for the common ancestor of the Viscaceae and the *Amphorogyne* clades might be an amphiphagous hemiparasite. Such plasticity may have provided this parasite with opportunities to adapt to a variety of environmental situations by facultatively parasitizing both roots and stems.

With comprehensive generic-level sampling in Santalaceae and relatives, and with DNA sequences for three genes, strong patterns of evolutionary relationships have emerged. The well supported clades found in this study represent several groups that (in most cases) are easier to diagnose and differentiate than the previous Santalaceae sensu lato. These results provide a much clearer concept of phylogenetic relationships and patterns of morphological divergence in the family and will greatly aid a future taxonomic revision.

**ACKNOWLEDGMENTS.** The authors wish to acknowledge all of the collectors of plant material used in this study (see appendix). Some of the sequences used in this study were generated by V. Malécot, M. García García, M. Martín Esteban, E. Nicholson, and R. Vidal-Russell. Thanks is also due to S. Sipes for allowing use of her ABI 377 DNA sequencer; A. Anderson and S. Kelchner for guidance in phylogenetic analysis and model selection methods; P. Wolf, K. Watrous, M. Simmons, and two anonymous reviewers provided comments which improved this manuscript. Computer time from the Center for High Performance Computing at Utah State University is gratefully acknowledged. The computational resource, the Uinta cluster supercomputer, was provided through the National Science Foundation under Grant No. CTS-0321170 with matching funds provided by Utah State University. Funding for DNA sequencing was provided by a grant to DLN from the NSF.

#### LITERATURE CITED

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions of Automatic Control* 19: 716–723.
- Altekar, G., S. Dwarkadas, J. Huelsenbeck, and F. Ronquist. 2004. Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. *Bioinformatics (Oxford, England)* 20: 407–415.
- Angiosperm Phylogeny Group. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141: 339–436.
- Barlow, B. A. 1964. Classification of the Loranthaceae and Viscaceae. *Proceedings of the Linnean Society of New South Wales* 89: 268–272.
- Barlow, B. A. 1983. Biogeography of Loranthaceae and Viscaceae. Pp. 19–45 in *The biology of mistletoes*, ed. D. M. Calder and P. Bernhardt. New York: Academic Press.
- Barlow, B. A. and D. Wiens. 1971. The cytogeography of the loranthaceous mistletoes. *Taxon* 20: 291–312.
- Barlow, B. A. and D. Wiens. 1977. Host-parasite resemblance in Australian mistletoes: the case for cryptic mimicry. *Evolution* 31: 69–84.
- Bhandari, N. N. and S. C. A. Vohra. 1983. Embryology and affinities of Viscaceae. Pp. 69–86 in *The biology of mistletoes*, ed. D. M. Calder and P. Bernhardt. New York: Academic Press.
- Bhatnagar, S. P. 1960. Morphological and embryological studies in the family Santalaceae. IV. *Mida salicifolia* A. Cunn. *Phytomorphology* 10: 198–207.
- Brown, R. 1810. *Prodromus Florae Novae Hollandiae et Insulae Van-Diemen*. London: Johnson.
- Burnham, K. P. and D. R. Anderson. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. New York: Springer-Verlag.
- Carvell, W. N. and W. H. Eshbaugh. 1982. A systematic study of the genus *Buckleya* (Santalaceae). *Castanea* 47: 17–37.
- Danser, B. H. 1939. A revision of the genus *Phacellaria* (Santalaceae). *Blumea* 3: 212–235.
- Danser, B. H. 1940. On some genera of Santalaceae Osyrideae from the Malay Archipelago, mainly from New Guinea. *Nova Guinea, New Series* 4: 133–149.
- Danser, B. H. 1955. Supplementary notes on the Santalaceae genera *Dendromyza* and *Cladomyza*. *Nova Guinea, New Series* 6: 261–277.
- Dawson, G. 1944. Las Santaláceas Argentinas. *Extracto de la Revista del Museo de La Plata (Nueva Serie). Sección Botánica* 6: 5–80.
- Der, J. P. 2005. *Molecular phylogenetics and classification of Santalaceae*. M.S. thesis. Carbondale, IL: Southern Illinois University.
- Fernald, M. L. 1928. Contributions from the Gray Herbarium of Harvard University, No. LXXIX. I. *Geocaulon*, a new genus of the Santalaceae. *Rhodora* 30: 21–24.
- Felsenstein, J. 1973. Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Systematic Zoology* 22: 240–249.
- Fineran, B. A. 1991. Root hemi-parasitism in the Santalales. *Botanische Jahrbücher* 113: 277–308.
- Francisco-Ortega, J., A. Santos-Guerra, S.-C. Kim, and D. J. Crawford. 2000. Plant genetic diversity in the Canary Islands: a conservation perspective. *American Journal of Botany* 87: 909–919.
- Guindon, S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696–704.
- Harbaugh, D. T. and B. G. Baldwin. 2006. Unraveling the complex history of sandalwoods (*Santalum*, Santalaceae). Abstract 114. *Botany 2006*. Chico, California: Botanical Society of America. <http://www.2006.botanyconference.org/engine/search/index.php?func=detail&aid=114>
- Harbaugh, D. T. and B. G. Baldwin. 2007. Phylogeny and biogeography of the sandalwoods (*Santalum*, Santalaceae): repeated dispersals throughout the Pacific. *American Journal of Botany* 94: 1030–1042.
- Hendrych, R. 1972. The natural history and systematics of the genus *Thesium* L. *Acta Universitatis Carolinae Biologica* 1970: 293–358.
- Hewson, H. J. and A. S. George. 1984. Santalaceae. Pp. 29–67 in *Flora of Australia*, ed. A. S. George. Canberra: Australian Government Publishing Service.
- Hiepko, P. 1979. A revision of Opiliaceae I. Genera of the eastern Old World, excluding *Opilia*. *Willdenowia* 9: 13–56.
- Hiepko, P. 1982. A revision of Opiliaceae II. *Opilia* Roxb. *Willdenowia* 12: 161–182.
- Hiepko, P. 1985. A revision of Opiliaceae III. *Urobotrya* Stapf. *Botanische Jahrbücher* 107: 137–152.
- Hiepko, P. 1987. A revision of Opiliaceae IV. *Rhopalopilium* Pierre and *Pentarthropalopilium* (Engler) Hiepko gen. nov. *Botanische Jahrbücher* 108: 271–291.
- Hiepko, P. 2000. Opiliaceae. *Flora Neotropica Monograph* 82: 1–53.
- Hieronymus, G. 1889. Santalaceae. Pp. 202–227 in *Die Natürlichen Pflanzenfamilien*, eds. A. Engler and K. Prantl. Leipzig: W. Engelmann.
- Hilu, K. W., T. Borsch, K. Müller, D. E. Soltis, P. S. Soltis, V. Savolainen, M. W. Chase, M. P. Powell, L. A. Alice, R. Evans, H. Sauquet, C. Neinhuis, T. A. B. Slotta, J. G. Rohwer, C. S. Campbell, and L. W. Chatrou. 2003. Angiosperm phylogeny based on *matK* sequence information. *American Journal of Botany* 90: 1758–1776.
- Hürlimann, H. and H. U. Stauffer. 1957. Santalales-Studien II. *Daenikera*, eine neue Santalaceen-Gattung. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* 102: 332–336.
- Johri, B. M. and S. P. Bhatnagar. 1960. Embryology and taxonomy of the Santalales I. *Proceedings of the National Institute of Science, India, Part 26B, Biological Sciences* 26: 199–220.
- Judd, W. S. and R. G. Olmstead. 2004. A survey of tricolpate (eudicot) phylogenetic relationships. *American Journal of Botany* 91: 1627–1644.
- Judd, W. S., C. S. Campbell, E. A. Kellogg, P. F. Stevens, and M. J. Donoghue. 2002. *Plant systematics: a phylogenetic approach*, Ed. 2. Sunderland: Sinauer Associates.
- Jukes, T. H. and C. R. Cantor. 1969. Evolution of protein molecules. Pp. 21–132 in *Mammalian protein metabolism* vol. 3, ed. H. N. Munro. New York, Academic Press.
- 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.
- Kuijt, J. 1988. Monograph of Eremolepidaceae. *Systematic Botany Monographs* 18: 1–60.
- Kuijt, J. 2003. Monograph of *Phoradendron* (Viscaceae). *Systematic Botany Monographs* 66: 1–643.
- Lam, H. J. 1945. Fragmenta Papuana. *Sargentia* 5: 1–196.

- Leopold, D. J. and R. N. Muller. 1983. Hosts of *Pyrularia pubera* Michx. (Santalaceae) in the field and in culture. *Castanea* 48: 138–145.
- Lepschi, B. J. 1999. Taxonomic revision of *Leptomeria* (Santalaceae). *Australian Systematic Botany* 12: 55–100.
- Li, J., D. E. Boufford, and M. J. Donoghue. 2001. Phylogenetics of *Buckleya* (Santalaceae) based on its sequences of nuclear ribosomal DNA. *Rhodora* 103: 137–150.
- Macklin, J. 2000. *A systematic revision of the Santalaceae R. Br. of Southeast Asia*. Ph.D. dissertation. Dublin, Ireland: Trinity College.
- Macklin, J. and J. Parnell. 2000. An introduction to the Santalaceae R. Br. of Thailand. *Thailand Forestry Bulletin* 28: 112–122.
- Macklin, J. and J. Parnell. 2002. An account of the Santalaceae of Thailand. *Thailand Forestry Bulletin* 30: 75–115.
- Malécot, V. and D. L. Nickrent. 2008. Molecular phylogenetic relationships of Olacaceae and related Santalales. *Systematic Botany* 33: in press.
- Nickrent, D. L. 1994. From field to film: rapid sequencing methods for field collected plant species. *BioTechniques* 16: 470–475.
- Nickrent, D. L. 1997 - onward. The parasitic plant connection. <http://www.parasiticplants.siu.edu/>
- Nickrent, D. L. 2002. Mistletoe phylogenetics: current relationships gained from analysis of DNA sequences. Pp. 48–57 in *Proceedings of the forty-eighth western international forest disease work conference*, ed. P. Angwin. Redding, California: USDA Forest Service.
- 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, Spain: Junta de Andalucía, Dirección General de Investigación Agraria.
- Nickrent, D. L. and V. Malécot. 2001. A molecular phylogeny of Santalales. Pp. 69–74 in *Proceedings of the 7th international parasitic weed symposium*, eds. A. Fer, P. Thalouarn, D. M. Joel, L. J. Musselman, C. Parker, and J. A. C. Verkleij. Nantes, France: Faculté des Sciences, Université de Nantes.
- Nickrent, D. L., J. Der, and F. Anderson. 2005. Discovery of the photosynthetic relatives of the “Maltese mushroom” *Cynomorium*. *BMC Evolutionary Biology* 5: 38.
- 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, MA: Kluwer Academic Publishers.
- Pilger, R. 1935. Santalaceae. Pp. 52–91 in *Die natürlichen pflanzenfamilien*. Ed. 2, vol. 16b, eds. A. Engler and K. Prantl. Leipzig: W. Engelmann.
- Posada, D. and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.
- Posada, D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics (Oxford, England)* 14: 817–818.
- Ram, M. 1957. Morphological and embryological studies in the family Santalaceae. I. *Comandra umbellata* (L.) Nutt. *Phytomorphology* 7: 24–35.
- Rambaut, A. 2004. Se-AL: sequence alignment editor v2.0a11. Oxford: Department of Zoology, University of Oxford. <http://evolve.zoo.ox.ac.uk>
- Rao, L. N. 1942a. Parasitism in the Santalaceae. *Annals of Botany* 6: 131–150.
- Rao, L. N. 1942b. Studies in the Santalaceae. *Annals of Botany* 6: 151–177.
- Rendle, A. B. 1925. *The classification of flowering plants* vol. 2. *Dicotyledons*. London: Cambridge University Press.
- Rogers, Z. S., D. L. Nickrent, and V. Malécot. New arborescent Santalaceae from Madagascar. *Annals of the Missouri Botanical Garden* (In press).
- Ronquist, F. and J. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* 19: 1572–1574.
- Schwarz, G. 1978. Estimating the dimension of a model. *Annals of Statistics* 6: 461–464.
- Skottsberg, C. 1930. The geographic distribution of the sandal-woods and its significance. Pp. 435–442 in *Proceedings of the fourth Pacific science congress* vol. 3, ed. O. de Vries, H.J. Lam and H.J.T. Bijlmer. Batavia and Bandoeng, Java, Indonesia: Pacific Science Association.
- Smith, A. C. 1937. A Colombian species of *Cervantesia* R. & P. *Tropical Woods* 51: 12–14.
- Smith, F. H. and E. C. Smith. 1943. Floral anatomy of the Santalaceae and related forms. *Oregon State Monographs, Studies in Botany* 5: 1–93.
- Soltis, D. E., A. E. Sinters, M. Zanis, S. Kim, J. D. Thompson, P. S. Soltis, L. P. Rosne De Craene, 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.
- 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.
- Stauffer, H. U. 1957. Santalales-Studien I. Zur Stellung der Gattung *Okoubaka* Pellegrin et Normand. *Berliner Schweize Botanische Gesellschaft* 67: 422–427.
- Stauffer, H. U. 1959. Santalales-Studien IV. Revisio Anthobolearum; eine Morphologische Studie mit Einschluss der Geographie, Phylogenie und Taxonomie. *Mitteilungen aus dem Botanischen Museum der Universität Zürich* 213: 1–260.
- Stauffer, H. U. 1961a. Beiträge zum Blütendiagramm der Santalales. *Verhandlungen der Schweizerische Naturforschenden Gesellschaft* 141: 123–125.
- Stauffer, H. U. 1961b. Santalales-Studien VII. Sudamerikanische Santalaceae I. *Acanthosyris*, *Cervantesia* und *Jodina*. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* 106: 406–412.
- Stauffer, H. U. 1968. Santalales-Studien IX. *Spirogardnera*, eine neue Santalaceen-Gattung aus West-Australien. *Mitteilungen aus dem Botanischen Museum der Universität Zürich* 113: 305–309.
- Stauffer, H. U. 1969. Santalales-Studien X. Amphorogyneae eine neue tribus der Santalaceae. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* 114: 49–76.
- Stauffer, H. U. and H. Hürlimann. 1957. Santalales-Studien III. *Amphorogyne*, eine weitere Santalaceen-Gattung aus Neukaledonien. *Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich* 102: 337–349.
- Stearn, W. T. 1960. An introduction to Robert Brown's *Prodromus Florae Novae Hollandiae*. Pp. v–lii in *Historiae Naturalis Classica, Tomus VI: Prodromus Florae Novae Hollandiae et Insulae Van-Diemen (1810) Supplementum Primum (1830) by Robert Brown*, eds. J. Cramer and H. K. Swann. New York: Hafner Publishing Co.
- Stearn, W. T. 1972. *Kunkeliella*, a new genus of Santalaceae in the Canary Islands. *Cuaderno Botanica Canariensis* 16: 11–26.
- Swofford, D. L. 2002. PAUP\*: phylogenetic analysis using parsimony (\*and other methods). Version 4.0 beta 10. Sunderland: Sinauer Associates.
- Tavaré, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* 17: 57–86.
- van Tieghem, P. 1896. Sur les Phanerogames a ovule sans nucelle, formant le group des Innucellees ou Santalinee. *Bulletin de la Société Botanique de France* 43: 543–577.
- Yang, Z. 1993. Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* 10: 1396–1401.
- Yang, Z. and B. Rannala. 1997. Bayesian phylogenetic inference using DNA sequences: a Markov Chain Monte Carlo method. *Molecular Biology and Evolution* 14: 717–724.

APPENDIX 1. Voucher information for taxa used in this study. Voucher information is listed as follows: taxon name, collection locality, collector(s) name and number (herbarium), Nickrent voucher accession number, Genbank accession numbers for the three loci: nuclear small-subunit ribosomal DNA, *rbcl*, *matK*; sequences not obtained indicated by N/A. Sequences for different genes were obtained from different specimens and concatenated in three gene analyses for *Dendrotrophe*, *Phacellaria*, *Schoepfia*, *Misodendrum*, and *Viscum*. Asterisk (\*) indicates a new sequence published in this study.

**Ingroup—Santalaceae.** *Acanthosyris asipapote* M. Nee, Bolivia, *M. Nee & I. Vargas* 45009 (NY), 4051, DQ329163, DQ329171, DQ329193. *Acanthosyris falcata* Griseb., Bolivia, *M. Nee* 46690 (NY), 4053, DQ329164, DQ329172, DQ329183. *Amphorogyne celastroides* Stauffer & Hurl., New Caledonia, *McPherson* 18051 (MO), 4564, EF584571\*, N/A, EF584614\*. *Anthobolus leptomerioides* F. Muell., Australia, *B. Lepschi & Craven* 4352 (PERTH), 4311, EF584572\*, EF584589\*, EF584615\*. *Antidaphne viscoidea* Poeppig & Endlicher, Costa Rica, *S. Sargent* S.N. (SIU), 2730, L24080, L26068, EF464500. *Arjona tuberosa* Cav., Argentina, *J. Puntieri* S.N. (SIU), 4566, EF464468, EF464532, EF464513. *Buckleya distichophylla* Torrey, U.S.A., *L. J. Musselman*

S.N. (SIU), 2735, X16598, DQ329180, DQ329191. *Cervantesia tomentosa* Ruiz & Pavon, Bolivia, L. J. Dorr & L. C. Barnett 6941 (MO), 4273, DQ329165, DQ329173, DQ329184. *Choretum pauciflorum* A. DC., Australia, B. Lepschi, T. R. Lally & B. R. Murray 4237 (PERTH), 4222, EF584573\*, EF464522, EF464503. *Colpoon compressum* Berg., South Africa, D. L. Nickrent, K. Steiner & A. Wolfe 4084 (SIU), 4084, EF584574\*, EF584590\*, EF584616\*. *Comandra umbellata* (L.) Nutt., U.S.A, G. Tonkovitch S.N. (SIU), 2739, DQ329170, DQ329181, DQ329192. *Daenikera corallina* Hürlimann & Stauffer, New Caledonia, J. Munzinger 2054 (MO), 4876, EF464462, EF464523, EF464504. *Dendromyza* sp. (Pilger) Stauffer, Papua New Guinea, D. L. Nickrent & R. Banka 4466 (LAE), 4466, EF464463, EF464524, EF464505. *Dendromyza* sp. (Pilger) Stauffer, Papua New Guinea, D. L. Nickrent & R. Banka 4483 (LAE), 4483, N/A, EF584591\*, N/A. *Dendrotrophe varians* (Blume) Miq., Malaysia, D. L. Nickrent & C. Calvin 4014 (SIU), 4014, N/A, N/A, EF464501. *Dendrotrophe varians* (Blume) Miq., Australia, D. L. Nickrent 2827 (SIU), 2827, L24087, EF464520, N/A. *Dufrenoya sphaerocarpa* (Danser) Stauffer, Indonesia, G. G. Hambali S.N. (no voucher), 2754, AF039071, EF584592\*, EF584617\*. *Eubranchion ambiguuum* (Hooker & Arnot) Engler, Puerto Rico, D. L. Nickrent, D. Clark & P. Clark 2699 (SIU), 2699, L24141, L26071, EF464498. *Exocarpos aphyllus* R. Br., Australia, A. Markey S.N. (SIU), 3094, EF584575\*, EF584593\*, EF584618\*. *Exocarpos bidwillii* Hook., New Zealand, B. Molloy S.N. (SIU), 2745, L24142, EF584594\*, EF584619\*. *Geocaldon lividum* (Rich.) Fern., U.S.A, J. Fetzner S.N. (SIU), 3047, AF039072, EF584595\*, EF584620\*. *Jodina rhombifolia* (Hook. & Arn.) Reiss., Bolivia, M. Nee 46673 (NY), 4052, DQ329166, DQ329174, DQ329185. *Kunkeliella subsucculenta* S. Guerra, Canary Islands, A. S. Guerra S.N. (SIU), 4374, EF584576\*, EF584596\*, EF584621\*. *Lepidoceras chilense* (Molina) Kuijt, Chile, C. Marticorena & R. Rodríguez 10043 (CONC), 4065, EF464459, EF464519, EF464499. *Leptomeria aphylla* R. Br., Australia, B. Lepschi & Whalen 4875 (PERTH), 4609, N/A, EF584597\*, EF584622\*. *Leptomeria spinosa* (Miq.) A. DC., Australia, A. Markey S.N. (SIU), 3081, EF464460, EF464521, EF464502. *Mida salicifolia* A. Cunn., New Zealand, C. C. Ogle 3413 (CANB), 4233, EF584577\*, EF584598\*, EF584623\*. *Myoschilos oblongatum* Ruiz & Pavón, Argentina, R. Vidal-Russell S.N. (SIU), 4504, EF584578\*, EF584599\*, EF584624\*. *Nanodea muscosa* Banks ex C.F. Gaertn., Argentina, L. Collado S.N. (no voucher), 4893, EF584579\*, EF584600\*, EF584625\*. *Nestronia umbellula* Raf., U.S.A, L. J. Musselman S.N. (SIU), 2736, L24399, EF584601\*, EF584626\*. *Okoubaka aubrevillei* Pellegr. & Normand, Cameroon, M. Cheek 6007 (K), 4173, N/A, DQ329175, DQ329186. *Omphacomeria acerba* (R. Br.) A. DC., Australia, B. Lepschi & B.R. Murray 4213 (PERTH), 4221, EF584580\*, EF584602\*, EF584627\*. *Osyridocarpus schimperianus* (Hochst.) A. DC., South Africa, D. L. Nickrent 4110 (SIU), 4110, EF584581\*, EF584603\*, EF584628\*. *Osyris alba* L., Spain, D. L. Nickrent, A. Aparicio & I. Sanchez García 4062 (SIU), 4062, EF584582\*, EF584604\*, AY042623. *Phacellaria compressa* Benth., Thailand, J. F. Maxwell 91-242 (HU), 4911, N/A, N/A, EF584629\*. *Phacellaria rigidula* Benth., China, Yulong Ding S.N. (SIU), 5042, EF584583\*, EF584605\*, N/A. *Pilgerina madagascariensis* Z.S. Rogers, Nickrent & Malécot, Madagascar, R. Rabevohitra, J. Rabenantoandro & R. Razakamalala 4485 (MO), 4954, DQ329169, DQ329178, DQ329189. *Pyrularia pubera* Michx., U.S.A, L. J.

Musselman S.N. (SIU), 2737, L24415, DQ329179, EF464507. *Quinchamalium chilense* Lam., Argentina, R. Vidal-Russell S.N. (SIU), 4503, EF464469, EF464533, EF464514. *Rhoiacarpos capensis* A. DC., South Africa, D. L. Nickrent & G. Marx 4117 (SIU), 4117, EF584584\*, EF584606\*, EF584630\*. *Santalum album* L., India, R. Narayana S.N. (no voucher), 2734, L24416, L26077, AY957453. *Santalum macgregorii* F. Muell., Papua New Guinea, D. L. Nickrent & J. Beko 4499 (LAE), 4499, EF584585\*, EF584607\*, EF584631\*. *Scleropyrum pentandrum* (Dennst.) Mabblerley, Thailand, S. Suddee, A. Patton, T. Jonganurak, & V. Chamchurnroon 1007 (DBN), 4347, DQ329167, DQ329176, DQ329187. *Spirogardnera rubescens* Stauffer, Australia, S. Patrick S.N. (SIU), 4996, EF464458, EF464518, EF464497. *Staufferia capuronii* Z.S. Rogers, Nickrent & Malécot, Madagascar, R. Randrianaivo, A. Ratodimanana, T. Razafindrabeaza, H. Rajanatsoa, P. Rakotondramanza & O. Rabozanahary 825 (MO), 4956, DQ329168, DQ329177, DQ329188. *Thesidium fragile* Sond., South Africa, D. L. Nickrent & A. Wolfe 4102 (SIU), 4102, EF584586\*, EF584608\*, EF584632\*. *Thesium fruticosum* Hill, South Africa, D. L. Nickrent & E. Brink 4115 (SIU), 4115, EF584587\*, EF584633\*. *Thesium impeditum* Hill, South Africa, K. Steiner S.N. (no voucher), 2845, L24423, EF584610\*, EF584634\*. **Viscaceae.** *Arceuthobium verticilliflorum* Engelm., Mexico, D. L. Nickrent & A. Flores 2065 (SIU), 2065, L24042, L26067, N/A. *Dendrophthora clavata* (Benth.) Urb., Colombia, M. Melampy S.N. (SIU), 2182, L24086, L26069, EF584636\*. *Ginalloa arnottiana* Korth., Malaysia, Yii et al. S.52260 (SAR), 2965, L24144, L26070, EF584637\*. *Korthalsella lindsayi* (D. Oliv.) Engl., New Zealand, B. Molloy S.N. (SIU), 2740, L24150, L26073, EF584638\*. *Notothoxos leiophyllum* Schumann, Australia, D. L. Nickrent 2785 (SIU), 2785, L24402, EF584612\*, N/A. *Phoradendron californicum* Nutt., U.S.A, J. Paxton S.N. (SIU), 2689, AF039070, EF584613\*, EF584639\*. *Viscum articulatum* Burman, Australia, D. L. Nickrent 2812 (SIU), 2812, L24427, EF464517, N/A. *Viscum articulatum* Burman, Australia, D. L. Nickrent 2782 (SIU), 2782, N/A, N/A, EF464496.

**Outgroup—Schoepfiaceae.** *Schoepfia schreberi* Gmel., Bahamas, D. L. Nickrent 2599 (SIU), 2599, L24418, L11205, N/A. *Schoepfia schreberi* Gmel., Costa Rica, R. Vidal-Russell S.N. (SIU), 4915, N/A, N/A, DQ787447. **Misodendraceae.** *Misodendrum linearifolium* A. DC, Argentina, D. E. Bran S.N. (SIU), 2829, L24397, L26074, N/A. *Misodendrum linearifolium* A. DC, Chile, G. Amico S.N. (SIU), 4591, N/A, N/A, DQ787438. **Loranthaceae.** *Nuytsia floribunda* (Labill.) R. Br. ex G. Don, Australia, B. Lamont S.N. (SIU), 2747, DQ790103, DQ790134, DQ787446. **Opiliaceae.** *Agonandra macrocarpa* L. O. Williams, Costa Rica, D. L. Nickrent & E. Olson 2764 (SIU), 2764, L24079, DQ790130, DQ790169. *Cansjera leptostachya* Benth., Australia, D. L. Nickrent 2815 (SIU), 2815, L24084, DQ790128, DQ790167. *Champeria manillana* Bl., S. E. Asia, W. Forstreuter S.N. (SIU), 3014, L24746, DQ790129, DQ790168. *Lepionurus sylvestris* Bl., Indonesia, G. Hambali S.N. (no voucher), 2879, DQ790101, DQ790131, DQ790170. *Opilia amentacea* Roxb., Australia, D. L. Nickrent 2816 (SIU), 2816, L24407, L2076, AY042621. *Pentarhopalopilium marquesii* (Engl.) Hiepko, Gabon, J. J. F. E. deWilde & R. W. deWilde-Bakhuizen 11212 (MO), 4180, DQ790102, DQ790127, DQ790166. *Urobotrya siamensis* Hiepko, Thailand, Geesink, Hiepko & Maxwell 7807 (BHU), 4369, EF584588\*, EF584611\*, EF584635\*.