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Inflorescence evolution in Santalales: integrating morphological characters and molecular phylogenetics

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PREMISE OF THE STUDY: The sandalwood order (Santalales) includes members that present a diverse array of inflorescence types, some of which are unique among angiosperms. This diversity presents not only interpretational challenges but also opportunities to test fundamental concepts in plant morphology. Here we used modern phylogenetic approaches to address the evolution of inflorescences in the sandalwood order.

METHODS: Phylogenetic analyses of two nuclear and three chloroplast genes were conducted on representatives of 146 of the 163 genera in the order. A matrix was constructed that scored nine characters dealing with inflorescences. One character, "trios", that encompasses any grouping of three flowers (i.e., both dichasia and triads) was optimized on samples of the posterior distribution of trees from the Bayesian analysis using BayesTraits. Three nodes were examined: the most recent common ancestors of (A) all ingroup members, (B) Loranthaceae, and (C) Opiliaceae, Santalaceae s.l., and Viscaceae.

KEY RESULTS: The phylogenetic analysis resulted in many fully resolved nodes across Santalales with strong support for 18 clades previously named as families. The trios character was not supported for nodes A and C, whereas it was supported for node B where this partial inflorescence type is best described as a triad.

CONCLUSIONS: Essentially every major inflorescence type can be found in Santalales; however, the dichasium, a type of partial inflorescence, is rarely seen and is not plesiomorphic for the order. In the family Erythropalaceae, inflorescences are mostly in small, axillary fascicles or cymes. Successive families show both cymose and racemose types and compound systems (e.g., thyrses). Inflorescences in Amphorogynaceae and Viscaceae are not dichasial and in general are difficult to compare to "standard" inflorescences.

KEY WORDS dichasium; Loranthaceae; mistletoe; parasitic plant; sandalwood order; Santalaceae; triad; Viscaceae.

For many angiosperm groups, well-resolved molecular phylogenies have provided the topological framework useful in addressing hypotheses concerning the evolution of morphological characters (Soltis et al., 2018). Although obtaining DNA sequences and generating phylogenetic trees has become relatively straightforward, the interpretation of morphological characters remains nettlesome, often requiring developmental and anatomical investigation. Moreover, assessing homology and properly defining the characters and character states in a matrix can be subjective and ambiguous. Homology assessment is especially difficult with inflorescences because they are complex in development, structure, and function (Kirchoff and Claßen-Bockhoff, 2013; Landrein and Prenner, 2013), and understanding their ontology and morphology is prerequisite to placing them in the proper evolutionary context.

Descriptive terminology is central to understanding inflorescences; however, the wide diversity of types seen across angiosperms results in either the same term being applied differently by different workers or different terms being applied to the same structure. Moreover, as discussed by Endress (2010), inflorescence classifications can be either descriptive or typological, and each approach has limitations, particularly for unusual or reduced inflorescences. In general, greater progress has been made in understanding inflorescence evolution among closely related taxa (i.e., at the species or genus level), whereas comparisons among higher taxonomic

ranks (e.g., families, orders) are more problematic owing to difficulties in recognizing homologous characters and character states. Terminology must of necessity be based on accurate information, and attempts must be made to relate apparently novel conditions to the existing corpus of knowledge.

As in other angiosperm orders, species within Santalales display a vast diversity of inflorescence types. Although much has been written on various families within the order, no broadly sampled comparative study of inflorescences exists. A detailed study of general inflorescence morphology was made by Stauffer (1963), but for Santalales, only *Santalum* and *Thesium* were examined. Despite the lack of broad sampling, the general discussion touched upon important issues such as branching patterns, flowering sequence, synflorescences, partial inflorescences vs. paraclades, positional phenomena, etc. Inflorescence architecture was used, to a large extent, in the reassessment of familial relationships within Santalales (Kuijt, 1968), but did not include a survey of all genera in the order. The cladistic study of morphological and anatomical characters in Santalales (focused mainly on Olacaceae) by Malécot et al. (2004) only used the presence/absence of inflorescence bracts and trichomes; thus, no attempt was made to score inflorescence types across the entire order. The most detailed descriptions of inflorescences in Santalales have appeared in works dealing with mistletoes. The mistletoe habit (aerial parasitism) has evolved five times independently in Santalales (Nickrent et al., 2010), and two of these events (Loranthaceae and Viscaceae) are the most speciose families in the order. The morphology of inflorescences in Loranthaceae was reviewed by Kuijt (1981), but in contrast, no comparative morphological study encompassing all seven genera in Viscaceae has been published; such information must be sought in works dealing with individual genera (see Rutishauser, 1937; Danser, 1941; Kuijt, 1959, 1961, 1970, 2003; Barlow, 1984a; Kirkup et al., 2000).

Recently, Suaza-Gaviria et al. (2017) studied some mistletoe species from the Andean region and concluded that dichasia are plesiomorphic for the order generally. A large proportion of that paper deals with the inflorescence structure in one family, Viscaceae, and more specifically with two genera *Phoradendron* and *Dendrophthora* (tribe Phoradendreae). Because that study had limited taxon sampling, did not utilize modern methodologies to address morphological character evolution, and contained numerous factual errors, the present study approached this issue by conducting a comprehensive analysis of inflorescence morphology and evolution in Santalales. Here we report a highly resolved molecular phylogeny for which nearly all genera in the order were sampled, summarize all available literature dealing with Santalales inflorescence morphology, provide a matrix for inflorescence morphological characters for all genera in the order, and utilize Bayesian ancestral state reconstruction methods to examine inflorescence evolution on the molecular tree.

MATERIALS AND METHODS

Molecular methods

All 18 photosynthetic families and 146 of the 163 genera in Santalales were included in the molecular analysis of five genes as shown in Appendix S1. Of the 17 missing genera, all but three are from Loranthaceae. Santalales is sister to the superasterid clade (Soltis et al., 2018). The superrosids are sister to the superasterids; thus, the vast diversity in this clade presents difficulties in choosing a reasonable outgroup. For this reason, no outgroup outside of Santalales was used. Six genera outside Santalales were used as outgroups in a previous study (Su et al., 2015), which showed Erythropalaceae and Strombosiaceae to be sister to the remaining families in the order. The trees produced in the present study were rooted based on this information. The holoparasitic families Balanophoraceae and Mystropetalaceae were also not included because they lack (or have highly altered) chloroplast genes.

In this study, 386 of the 596 sequences were previously published and obtained from NCBI GenBank. Of these 386, 12 were corrected with updated sequence information. The remaining 210 sequences were newly generated using a genome skimming approach (Dodsworth, 2015). For newly sequenced genera, DNA was extracted from silica gel-dried tissue using the silica column method outlined by Neubig et al. (2014). DNA concentration was determined using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) using the manufacturer's protocol. DNA quality was determined using gel electrophoresis. Samples were equilibrated to approximately equal concentration and differentially sheared according to DNA quality determined by electrophoresis. Libraries were prepared with Illumina adapters and a unique 8-nucleotide barcode for separation after sequencing. Samples were sequenced on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA, USA) with 64 DNA samples multiplexed per lane giving reads of 100 bp. The resulting fastq files were processed using Geneious version 7.1.9 (Kearse et al., 2012) where paired ends were matched and the ends trimmed using default settings. The complete ribosomal cistron of *Spondias tuberosa* (KX522674) was first used as a reference to assemble the *Coula edulis* (Coulaceae) cistron. The *Coula* cistron was then used as reference to assemble the majority of the remaining Santalales using 10–20% sensitivity with 10–25 iterations. For some taxa, such as members of Viscaceae, with high substitution rates that differed markedly from *Coula*, de novo assemblies of the HiSeq sequence data, trimmed according to default settings, were performed using Velvet (Zerbino and Birney, 2008) as implemented in Geneious, with a *k*-mer of 71. These sequences were then sorted by length, then, starting with the longest sequences, those with high coverage (depth) were examined with BLAST to locate the rDNA cistron. Those incomplete cistrons were then used as a reference in Geneious with varying sensitivities and 25 or more iterations to assemble the entire cistron. Other fast-rate genera were assembled by using closely related genera as references.

Individual alignments were manually produced for nuclear small-subunit rDNA (SSU), large-subunit rDNA (LSU), chloroplast *rbcL*, *matK*, and *accD* using Se-Al version 2.0 a11 (Rambaut, 2007). All gaps were treated as missing data. Because the chloroplast genes are protein-coding, the DNA sequences were translated into amino acid sequences, and indels were introduced while maintaining sequence frame. In some genera, the *matK* sequences contained premature stop codons; thus, if no posttranscriptional modification occurs, these truncated sequences could represent pseudogenes. The sequences in Se-Al were exported as NEXUS files and concatenated using Mesquite version 3.5 (Maddison and Maddison, 2018).

The concatenated data matrix (Appendix S2) was partitioned by gene and codon position, resulting in 11 data subsets (SSU, LSU, and first, second, and third codon positions for *accD*, *matK*, and *rbcL*). The best-fitting data partitioning scheme and set of substitution models were inferred with PartitionFinder version 2.1.1 (Lanfear et al., 2016) using a greedy search and the Bayesian

information criterion. For downstream analyses, two sets of substitution models were analyzed in PartitionFinder: (1) GTR with and without gamma, using RAxML version 8.0 (Stamatakis, 2006) and (2) the 24 standard models available in MrBayes version 3.2.6 (Ronquist et al., 2012), using PhyML (Guindon et al., 2010). A partitioned maximum likelihood (ML) analysis was conducted using RAxML-HPC version 8.2.10 (Stamatakis, 2006, 2014) with 1000 rapid bootstrap replicates and a subsequent ML search for the best-known-likelihood topology (-f a option). Also, a partitioned Bayesian inference (BI) analysis was conducted using MrBayes version 3.2.6 with four independent runs (each with one cold and three heated chains) for 100 million generations, sampling once every thousand generations, with branch lengths linked across data subsets. The average standard deviation of split frequencies and potential scale reduction factors (PSRFs) were used to assess convergence—when the average standard deviation of split frequencies dropped below 0.01 (after removal of a relative burn-in fraction of 0.25) and PSRFs for all parameters were all 1.000, the runs were assumed to have converged. All ML and BI analyses were conducted on CIPRES (Miller et al., 2010).

Maximum parsimony (MP) analyses were conducted using PAUP* version 4.0 (Swofford, 2002). Three MP bootstrap (BS) analyses were conducted: (1) the nuclear ribosomal genes (SSU + LSU), (2) the chloroplast genes (*rbcL* + *matK* + *accD*), and (3) the concatenated 5-gene data set. All characters received equal weight (of type "unord"), and gaps were treated as missing data. Maximum parsimony bootstrap (MPBS) heuristic searches used 1000 random stepwise addition replicates with tree bisection–reconnection branch swapping, holding 10 trees of length ≥1 at each step.

Morphological methods

Information on inflorescence and reproductive features for all 163 genera of Santalales was assembled from the literature (94 sources) and original observations as summarized in Appendix S3. The descriptive information was organized as follows: inflorescence position, overall inflorescence features, bracts and bracteoles, plant sexuality, flower sex, dichasia presence, and triad presence. More details about how the characters were scored can be found in Appendix S4. Morphological information was used to produce a matrix in Mesquite with 10 characters, each with 2–8 discrete states, for 146 genera (Table 1) The Nexus file of this matrix can be obtained in Appendix S5. Three-flowered partial inflorescences have been variously described as dichasia, triads, etc. (see below). For this reason, one character ("trios") was constructed by merging dichasia and triads, thus allowing a very liberal scoring for ancestral state reconstruction. This merging does not mean that we consider dichasia and triads to by synonymous (see Appendix S10 for definitions) but was only done given uncertainty as to which triads represent dichasia and which do not. Our goal was to not bias the analysis testing the hypothesis that dichasia are plesiomorphic in Santalales. The distribution of character states on the Bayesian tree was also examined using the function "Trace Character History" in Mesquite.

BayesTraits version 3.01 (Pagel et al., 2004) was used to compare hypotheses of alternative character states at key nodes while accounting for phylogenetic uncertainty. First, after removing burn-in, trees resulting from the four independent MrBayes analyses were pooled. Three sets of 1000 randomly sampled trees from the pooled MrBayes trees were then generated. Using these three sets of trees, BayesTraits analyses were performed in which the alternative states for the "trios" character (absent or present) were "fossilized" (fixed) for three nodes of interest—(A) the ingroup root node (comprising all taxa in the phylogeny; Fig. 1), (B) the node representing the most recent common ancestor (MRCA) of Loranthaceae and (C) the node representing the MRCA of Opiliaceae, Santalaceae s.l., and Viscaceae. To assess convergence, we ran three independent BayesTraits analyses for each of the three sets of randomly chosen trees, resulting in a total of nine BayesTraits analyses for each of the two-character states at each node. Each run consisted of 10 million generations with a 1 million generation (10%) burn-in, sampled every 1000 generations. After several preliminary analyses, uniform priors (0–200) were determined to be appropriate for the rates of gain (q01) and loss (q10) of the "trios" character. Marginal log likelihoods were estimated for each run using stepping stone sampling with 100 stones, each run for 1000 iterations. Marginal log likelihoods were compared for each state at each node by calculating Bayes factors, which are equal to twice the difference between the marginal log likelihoods calculated for each character state.

RESULTS

Phylogenetic analyses

The 5 genes × 146 taxa matrix contains 730 cells, of which 596 (82%) were filled in this study. Nearly all genera had SSU and *matK* sequences whereas *accD* had the most missing data (66 genera). The final concatenated matrix contained 10,428 characters of which 3499 were parsimony informative. Although the lengths of the nuclear and chloroplast matrices were comparable (5316 and 5112, respectively), the number of parsimony-informative characters in the chloroplast matrix was nearly double that of the nuclear matrix (2300 vs. 1203). Examination of the MPBS consensus trees resulting from the nuclear and chloroplast matrices shows that analysis of the latter resulted in a more highly resolved tree than that of the former. Through inspection, no significant topological differences were seen, thus justifying concatenation of all five genes for a total evidence analysis. The MPBS trees resulting from the nuclear, chloroplast, and 5-gene analyses can be found in Appendix S6.

The partitions and models chosen for RaxML and BI are provided in Appendix S7. The MrBayes analyses ran for 12,465,000 generations before automatically stopping based on the topological convergence diagnostic. The BI tree with posterior probability values (BIPP) added to all nodes is shown in Fig. 1. Sixty percent of the nodes on the Bayesian consensus tree (Fig. 1) received posterior probabilities of 1.0, and this percentage increased to 81% for nodes 0.9 or greater. All families as circumscribed by Nickrent et al. (2010) received high support (BIPP 0.95 or greater). The RAxML tree is highly congruent with the BI tree in topology and nodal support values (Appendix S8). *Brachynema* was not included in the analyses of Nickrent et al. (2010) but here is shown to be a member of Erythropalaceae, strongly supported as sister to *Maburea*.

BayesTraits analysis

The BI tree (Fig. 1) was rooted with the four genera of Erythropalaceae. In a small fraction (0.4%) of the MrBayes trees, the outgroup was not monophyletic owing to *Erythropalum* being sister to Strombosiaceae. In those cases, the trees were rooted

TABLE 1. The 10 inflorescence/floral characters scored for 146 genera of Santalales.[a]

		Characters									
Taxon	**Family**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Acanthosyris	Cervantesiaceae	0	0	(2 3)	1	0	0	1	1	0	0
Actinanthella, *Bakerella*, *Berhautia*, *Emelianthe*, *Englerina*, *Globimetula*, *Oliverella*, *Oncocalyx*, *Phragmanthera*, *Septulina*, *Spragueanella*, *Tapinanthus*, *Vanwykia*	Loranthaceae	0	0	5	0	0	0	1	0	0	0
Aetanthus	Loranthaceae	0	0	(3 5)	0	0	0	1	0	0	0
Agelanthus	Loranthaceae	0	(0 1)	(5 6)	0	0	0	1	0	0	0
Agonandra	Opiliaceae	1	(0 1)	(1 3)	1	0	1	1	0	2	1
Alepis	Loranthaceae	0	0	1	0	0	0	1	0	0	0
Amphorogyne	Amphorogynaceae	0	0	(2 4)	0	0	0	1	1	0	0
Amyema	Loranthaceae	1	0	(5 6 8)	0	0	1	1	0	0	0
Amylotheca, *Decaisnina*	Loranthaceae	1	0	(1 3)	1	0	1	1	0	0	0
Anacolosa	Aptandraceae	0	0	(0 3)	0	0	0	1	0	0	0
Anthobolus	Opiliaceae	0	0	(1 5)	0	0	0	1	0	2	1
Antidaphne	Santalaceae	0	0	(1 2)	0	0	0	(0 1)	1	(1 2)	1
Aptandra	Aptandraceae	0	(0 1)	4	0	0	0	1	1	(0 2)	(0 1)
Arceuthobium	Viscaceae	0	(0 1)	2	0	0	0	1	0	2	1
Arjona	Schoepfiaceae	0	1	2	0	0	0	1	0	0	0
Atkinsonia	Loranthaceae	0	0	1	0	0	0	1	0	0	0
Baratranthus	Loranthaceae	0	0	6	0	0	0	1	0	(0 2)	(0 1)
Brachynema	Erythropalaceae	0	0	0	0	0	0	0	0	0	0
Buckleya	Thesiaceae	1	(0 1)	(3 5 8)	0	1	0	1	0	2	1
Cansjera	Opiliaceae	0	0	2	0	0	0	1	0	0	0
Cathedra	Aptandraceae	0	0	0	0	0	0	0	2	0	0
Cecarria, *Benthamina*	Loranthaceae	0	0	5	0	0	0	1	0	0	0
Cervantesia	Cervantesiaceae	0	(0 1)	(1 3)	1	0	0	1	0	0	0
Champereia	Opiliaceae	0	0	4	0	0	0	(0 1)	0	3	(0 1)
Chaunochiton	Aptandraceae	0	(0 1)	4	0	0	0	1	0	0	0
Choretrum	Amphorogynaceae	0	0	(3 8)	0	0	0	1	0	0	0
Cladocolea	Loranthaceae	1	(0 1)	(1 2 6)	1	0	1	1	0	(0 2)	(0 1)
Colpoon	Santalaceae	0	(0 1)	4	0	0	0	1	(1 2)	0	0
Comandra	Comandraceae	1	1	4	0	1	0	1	0	0	0
Coula, Minquartia, Ochanostachys	Coulaceae	0	0	1	0	0	0	1	0	0	0
Curupira	Ximeniaceae	0	0	5	0	0	0	1	1	0	0
Dactyliophora	Loranthaceae	1	0	5	0	0	1	1	0	0	0
Daenikera	Amphorogynaceae	0	1	4	0	0	0	1	0	0	0
Dendromyza	Amphorogynaceae	0	0	(7 8)	0	0	0	1	0	(1 2)	1
Dendropemon	Loranthaceae	0	0	(1 2 5)	0	0	0	1	0	0	0
Dendrophthoe, *Helixanthera*	Loranthaceae	0	0	(1 2)	0	0	0	1	0	0	0
Dendrophthora, *Phoradendron*, *Viscum*	Viscaceae	0	(0 1)	2	0	0	0	1	0	(1 2)	1
Dendrotrophe	Amphorogynaceae	0	(0 1)	(1 4 5 8)	0	0	0	1	1	(0 1 2)	(0 1)
Desmaria	Loranthaceae	1	1	(3 5)	1	0	1	1	0	0	0
Diogoa	Strombosiaceae	0	0	(0 1)	0	0	0	1	0	0	0
Diplatia	Loranthaceae	1	0	6	0	0	1	1	0	0	0
Dufrenoya	Amphorogynaceae	0	0	5	0	0	0	1	0	(2 3)	1
Dulacia, *Olax*	Olacaceae	0	0	(1 4)	0	0	0	1	1	0	0

(Continued)

TABLE 1. (Continued)

Taxon	Family	Characters									
		1	2	3	4	5	6	7	8	9	10
Elytranthe	Loranthaceae	0	0	1	0	0	0	1	0	0	0
Engomegoma	Strombosiaceae	0	0	0	0	0	0	1	0	0	0
Erianthemum	Loranthaceae	0	(0 1)	(1 2 6)	0	0	0	1	0	0	0
Erythropalum	Erythropalaceae	1	0	3	0	1	0	1	0	0	(0 1)
Eubrachion	Santalaceae	0	0	2	0	0	0	1	(0 1)	1	1
Exocarpos	Santalaceae	0	(0 1)	(1 2 8)	0	0	0	1	0	(0 3)	(0 1)
Gaiadendron	Loranthaceae	1	0	(1 3)	1	0	1	(0 1)	0	0	0
Geocaulon	Comandraceae	0	0	3	0	0	0	1	1	3	(0 1)
Ginalloa	Viscaceae	0	(0 1)	2	0	0	0	1	0	1	1
Harmandia	Aptandraceae	0	0	1	0	0	0	1	0	1	1
Heisteria	Erythropalaceae	0	0	0	0	0	0	(0 1)	0	0	0
Helicanthes, Sogerianthe	Loranthaceae	0	0	8	0	0	0	1	0	0	0
Hondurodendron	Aptandraceae	0	0	(1 3)	1	0	0	1	0	2	1
Ileostylus, Muellerina	Loranthaceae	1	0	(1 3)	1	0	1	1	0	0	0
Jodina	Cervantesiaceae	0	0	7	0	0	0	1	0	0	0
Korthalsella	Viscaceae	0	0	2	0	0	0	1	0	1	1
Lepeostegeres	Loranthaceae	1	0	6	1	0	1	1	0	0	0
Lepidaria	Loranthaceae	0	0	6	0	0	0	1	0	0	0
Lepidoceras	Santalaceae	0	0	(1 2 8)	0	0	0	1	0	(1 2)	1
Lepionurus	Opiliaceae	?	0	(1 3)	1	0	?	1	1	0	0
Leptomeria	Amphorogynaceae	0	(0 1)	1	0	0	0	1	1	0	0
Ligaria	Loranthaceae	0	0	8	0	0	0	1	0	0	0
Loranthus	Loranthaceae	0	0	2	0	0	0	1	0	(0 2)	(0 1)
Loxanthera	Loranthaceae	1	0	1	1	0	1	1	0	0	0
Lysiana	Loranthaceae	0	0	5	0	0	0	1	0	0	0
Maburea	Erythropalaceae	0	0	0	0	0	0	1	0	0	0
Macrosolen	Loranthaceae	0	0	(1 2)	0	0	0	1	0	0	0
Malania	Ximeniaceae	0	0	5	0	0	0	?	?	0	0
Melientha	Opiliaceae	0	0	4	0	0	0	1	0	2	0
Mida	Nanodeaceae	0	0	1	0	0	0	1	1	2	1
Misodendrum	Misodendraceae	0	(0 1)	(1 2 7)	0	0	0	1	0	(1 2)	1
Moquiniella	Loranthaceae	0	(0 1)	5	0	0	0	1	0	0	0
Myoschilos	Santalaceae	0	0	2	0	0	0	1	0	0	0
Nanodea	Nanodeaceae	0	0	5	0	0	0	1	0	0	0
Nestronia	Santalaceae	0	0	(5 8)	0	0	0	1	0	2	(0 1)
Notanthera	Loranthaceae	1	(0 1)	(1 3)	1	0	1	1	0	0	0
Notothixos	Viscaceae	0	1	(2 3)	0	0	0	1	0	1	1
Nuytsia	Loranthaceae	1	1	(1 3)	1	0	1	1	0	3	(0 1)
Octoknema	Octoknemaceae	0	0	(1 2)	0	0	0	1	0	2	1
Oedina	Loranthaceae	0	0	(1 2)	0	0	0	1	0	0	0
Okoubaka	Cervantesiaceae	?	0	4	0	?	0	1	0	(0 1)	(0 1)
Omphacomeria	Santalaceae	0	(0 1)	(2 8)	0	0	0	1	0	2	1
Oncella	Loranthaceae	0	0	1	0	0	0	1	0	0	0
Ongokea	Aptandraceae	0	0	4	0	0	0	1	0	0	0
Opilia	Opiliaceae	0	0	1	1	0	0	1	1	0	0
Oryctanthus	Loranthaceae	0	(0 1)	2	0	0	0	1	0	0	0
Osyridicarpos	Thesiaceae	0	0	(1 3)	0	0	0	1	1	0	0
Osyris	Santalaceae	0	0	3	0	0	0	1	1	(2 3)	(0 1)
Passovia	Loranthaceae	1	(0 1)	(1 2)	1	0	1	1	0	(0 2)	(0 1)
Pentarhopalopilia	Opiliaceae	0	0	5	0	0	0	1	0	0	0
Peraxilla	Loranthaceae	0	(0 1)	2	0	0	0	1	1	0	0
Phacellaria	Amphorogynaceae	0	(0 1)	(2 8)	0	0	0	(0 1)	0	(0 1 2 3)	(0 1)
Phanerodiscus	Aptandraceae	0	0	(0 7)	0	0	0	1	0	0	0
Pilgerina	Cervantesiaceae	0	(0 1)	1	0	0	0	1	1	0	0
Plicosepalus	Loranthaceae	0	(0 1)	5	0	0	0	1	0	0	0
Psittacanthus	Loranthaceae	1	(0 1)	(1 3 5)	1	0	1	1	0	0	0
Ptychopetalum	Olacaceae	0	0	1	0	0	0	1	1	0	0
Pyrularia	Cervantesiaceae	0	(0 1)	(1 3)	1	0	0	1	0	(2 3)	(0 1)

(Continued)

TABLE 1. (Continued)

		Characters									
Taxon	**Family**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Quinchamalium	Schoepfiaceae	0	1	(2 6)	0	0	0	1	0	0	0
Rhoiacarpos	Santalaceae	0	(0 1)	(3 4)	0	0	0	1	0	0	0
Rhopalopilia	Opiliaceae	0	0	(1 5)	0	0	0	1	0	0	0
Santalum	Santalaceae	0	(0 1)	4	0	0	0	1	0	0	0
Schoepfia	Schoepfiaceae	0	0	(1 2)	0	0	0	1	0	0	0
Scleropyrum	Cervantesiaceae	0	0	2	0	0	0	1	1	(2 3)	(0 1)
Scorodocarpus	Strombosiaceae	0	0	(1 3)	1	0	0	1	1	0	0
Scurrula	Loranthaceae	0	(0 1)	(1 5)	0	0	0	1	0	0	0
Socratina	Loranthaceae	1	(0 1)	5	0	0	1	1	0	0	0
Spirogardnera	Amphorogynaceae	0	1	(2 7)	0	0	0	1	1	0	0
Staufferia	Cervantesiaceae	0	0	(3 4)	1	0	0	1	1	2	(0 1)
Strombosia	Strombosiaceae	0	0	(0 3)	0	0	0	1	0	0	0
Strombosiopsis	Strombosiaceae	0	0	(0 1 2)	0	0	0	1	0	0	0
Struthanthus	Loranthaceae	1	0	(1 2)	1	0	1	1	(0 1)	2	1
Taxillus	Loranthaceae	0	0	(1 5)	0	0	0	1	0	0	0
Tetrastylidium	Strombosiaceae	0	0	0	0	0	0	1	0	0	0
Thesium	Thesiaceae	1	(0 1)	(1 3)	1	(0 1)	0	1	0	(0 2)	(0 1)
Tolypanthus	Loranthaceae	0	0	6	0	0	0	1	0	0	0
Tripodanthus	Loranthaceae	1	1	(1 3)	1	0	1	1	0	0	0
Tristerix	Loranthaceae	0	(0 1)	1	0	0	0	1	0	0	0
Tupeia	Loranthaceae	1	1	(1 3)	1	0	1	0	?	2	1
Urobotrya	Opiliaceae	0	0	(1 3)	1	0	0	(0 1)	0	0	0
Ximenia	Ximeniaceae	0	(0 1)	5	0	0	0	(0 1)	0	(0 2)	(0 1)

[a]The characters scores represent all species in the genus (see Appendices S4 and S5). Characters and character states (missing or unknown = ?) are **1** Trios: 0 absent, 1 present; **2** Inflorescence position: 0 axillary, 1 terminal; **3** inflorescence form: 1 fascicles, 2 racemes, 3 spikes, 4 cymes, 5 panicles, 6 umbels, 7 capitula, 8 glomerules, 9 unifloral; **4** Conflorescence: 0 absent, 1 present; **5** Dichasia: 0 absent, 1 present; **6** Triads: 0 absent, 1 present; **7** Bract/bracteole presence: 0 absent, 1 present; **8** Bract/bracteole persistence: 0 persistent, 1 caducous, 2 accrescent; **9** Plant sexuality: 0 synoecious, 1 monoecious, 2 dioecious, 3 polygamous; **10** Flower sex: 0 bisexual, 1 unisexual.

with the other three Erythropalaceae genera. Three independent BayesTraits analyses for three random samples of Bayesian trees for each character state were conducted, resulting in nine Bayes factors for each character state fossilization. Marginal log likelihoods (and thus Bayes factors) were very consistent across tree samples and runs, so we report only the lowest Bayes factor calculated across all runs and tree samples for each state/node (Appendix S9). For node A (Fig. 1) and node C, Bayes factors supported state 0 (trio absent) (Bayes factors = 3.96426 for node A and 4.07061 for node C). Following Kass and Raftery (1995), these Bayes factors constitute positive evidence in favor of the ancestors represented by these nodes lacking a trio. By contrast, state 1 (trio present) was favored for the node B representing the MRCA of Loranthaceae (Bayes factor = 4.53829, indicating positive evidence for state 1 at this node).

Inflorescences in Santalales

Looking at overall inflorescence architecture across the order (Table 1; Appendices S3 and S11), the general form for Erythropalaceae is an axillary, fasciculate, or cymose inflorescence with bisexual flowers bearing persistent bracts. In the Strombosiaceae clade, additional variation in inflorescence architecture evolved that includes axillary spikes and racemes of bracteate, bisexual flowers. The small family Coulaceae has relatively uniform inflorescence morphology with racemes of bisexual flowers. In the Ximeniaceae, Olacaceae, and Aptandraceae clades, panicles, umbels, and "glomerules" evolved among the dozen genera and unisexual flowers become more common. The enigmatic dioecious genus *Octoknema* occurs unresolved along the backbone of the Santalales tree, likely owing to missing data. *Octoknema* has spikes and racemes as seen in some Strombosiaceae and all Coulaceae.

The remaining members of Santalales occur in two well-supported clades (unnamed here). One is composed of the families Misodendraceae, Schoepfiaceae, and Loranthaceae and the other of eight families: Opiliaceae, Comandraceae, Thesiaceae, Cervantesiaceae, Santalaceae, Nanodeaceae, Amphorogynaceae, and Viscaceae (Fig. 1). The clades containing the two small families Misodendraceae and Schoepfiaceae are sister, and that clade is sister to the largest family in the order, Loranthaceae. Interpreting inflorescence type in Misodendraceae has proven difficult, but they were here scored as having spikes, racemes, and "glomerules". *Misodendrum* exhibits a number of morphological modifications that are also seen in the other four mistletoe clades such as scale leaves, monoecy/dioecy, and diminutive flowers. Inflorescences in Schoepfiaceae are generally spikes and racemes with the first evolution of a capitulum taking place in *Quinchamalium*.

Seven of the nine inflorescence states used in Character 3 are seen in Loranthaceae. Conflorescences (generally thyrses, Character 4) are frequent in the tribes Nuytsieae, Gaiadendreae, Elytrantheae, and Psittacantheae but absent in most subtribes of Lorantheae (the exception being subtribe Ileostylinae). Umbels are seen throughout the family; however, this inflorescence type is most frequent in the African subtribes. Although much variation exists for the "trios" character within Loranthaceae, ancestral state reconstruction supports its presence at node B. These partial inflorescences are best described as triads (Character 6), a term introduced by Eichler (1868) that has been used in nearly all morphological and systematic work on Loranthaceae

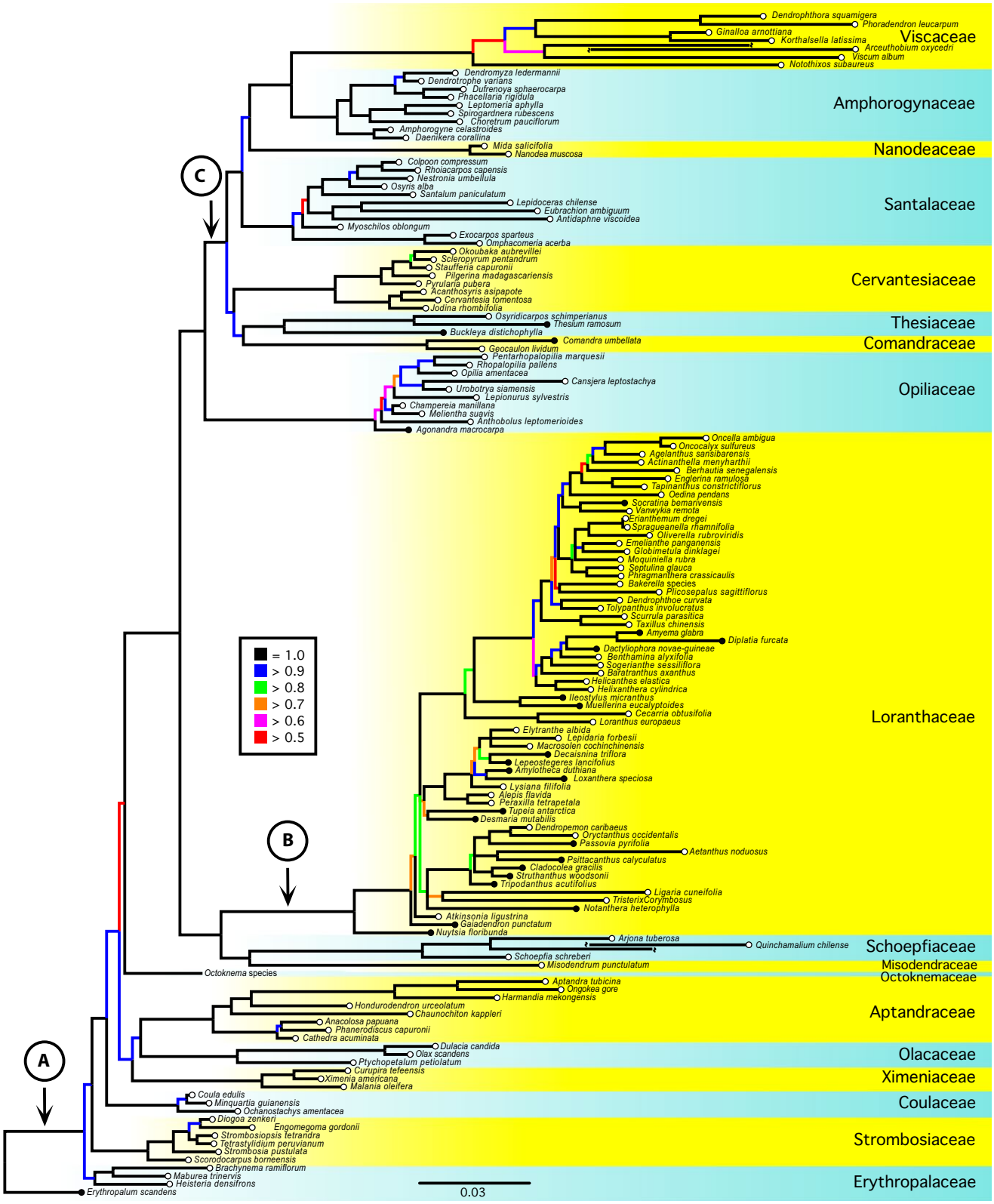

FIGURE 1. Bayesian inference phylogram for 146 genera of Santalales. Branch lengths are proportional to the amount of sequence evolution for that taxon or clade. The six colors in the legend inset indicate posterior probability values. Family names for the clades follow the classification of Nickrent et al. (2010). Capital letters represent nodes "fossilized" in the BayesTraits analyses for the most recent common ancestors of (A) all ingroup taxa, (B) Loranthaceae, and (C) Opiliaceae, Santalaceae s.l. and Viscaceae. Circles at branch termini indicate states for character 1 ("trios"): absent (open) and present (filled). The bar equals the number of substitutions per site.

from the neotropics (Kuijt, 1981), Malesia (Danser, 1931), and Australia (Barlow, 1984b). Curiously, triads (and dyads) are almost completely absent in African Loranthaceae where umbels predominate. Recaulescence of bracts and bracteoles is frequent in Loranthaceae. Rarely, all three flowers of a triad are sessile on the inflorescence axis, e.g., in *Peristethium* (Kuijt, 2012: figs. 11, 17) but even then the prophylls or their scars are usually recognizable.

State 0 (trio absent) was supported at node C (Opiliaceae to Viscaceae) in Bayesian reconstructions, and character optimization with parsimony supports the presence of a raceme in that common ancestor (Appendix S11). Indeed, the raceme is supported as the plesiomorphic state for Opiliaceae that also includes members with pedicel length variants such as spikes and umbels (as well as paniculate patterns). As mentioned above, Comandraceae and Thesiaceae have members with true dichasia, but interestingly the common ancestor was reconstructed (with parsimony) as equivocal for Character 1 (trios) but cymose for Character 3 (inflorescence form). Both cymose and racemose inflorescences are seen in Cervantesiaceae, and because of this the ancestor is reconstructed as equivocal. For Santalaceae, the plesiomorphic state is a spike; however, panicles are frequently seen in the clade composed of *Santalum* to *Colpoon*. Inflorescences in Nanodeaceae and Amphorogynaceae are extremely diverse, showing eight of the nine types scored here. This diversity of types may stem partially from varying interpretations by different workers (see Notes in Appendix S2). For *Choretrum*, *Daenikera*, and others, vegetative and reproductive shoots cannot be readily differentiated based on pherophyll (bract) size; thus, the entire branching system is reproductive. For the mistletoe *Phacellaria*, flowers form in the axils of bracts (or not), that are at first spirally arranged. Later, through intercalary growth, the stem axis can stretch displacing bracts and flowers and allowing for the addition of other (adventitious) flowers (Danser, 1939). The inflorescences of Viscaceae present even more examples of the lines being blurred between vegetative and reproductive shoots. Here, all members of Viscaceae were scored as spicate (for lack of a better term), but see below.

DISCUSSION

The results shown in Fig. 1 represents the most resolved phylogenetic tree obtained to date for Santalales, likely deriving from both increased taxon and gene sampling. Genome skimming produced high quality, complete DNA sequences for each of the five genes used, and their inclusion strongly affects the degree of support for the clades, including those along the "backbone" that were sometimes weakly supported in previous analyses. For example, relationships among the families Opiliaceae and Santalaceae sensu APG are fully resolved. In contrast, the intergeneric relationships in Viscaceae are not fully resolved, a result observed in previous studies (Der and Nickrent, 2008; Su et al., 2015; Maul et al., 2018), despite the fact that nearly all the sequences were derived from skimming. As evidenced by their long branches, genera in Viscaceae show high substitution rates, comparable to those seen in the holoparasitic family Mystropetalaceae (Su et al., 2015). Increased rates of sequence evolution were also seen in the Andean herb *Quinchamalium* but interestingly not in its closely related sister genus *Arjona*. Optimization of character states for key nodes on the highly resolved BI tree provide new insights into the evolution of inflorescences in Santalales.

Dichasia and triads in Santalales

The concepts dichasium and cyme (or cyme-like structures) present issues that have not been resolved and continue to cause confusion. This ambiguity can be appreciated by perusing definitions of dichasium, cyme, and thyrse from various authors (Appendix S10). Endress (2010) limited the application of the term cyme (or cymose branching) to partial inflorescences (i.e., lateral branches of the main inflorescence axis), and we agree with this interpretation. In contrast, Engler and Krause (1935), Fernald (1950), Gleason (1958), Troll (1964), Bailey and Bailey (1976), and Rickett (1955) regarded the cyme as a full inflorescence. Parallel to definitional problems with cymes are related issues with dichasia. A dichasium was seen by Lawrence (1951) and Simpson (2006) as an entire inflorescence, but as a "cymose partial inflorescence" by Weberling (1989).

As shown in their illustrations below their fig. 7, Suaza-Gaviria et al. (2017) allowed for a number of modifications of a dichasium such as loss of the terminal flower of the first order axis, loss of both lateral axes (and their flowers), and the loss of floral pedicels. In some cases, their scoring designated full inflorescences rather than partial ones, which contradicts Endress (2010). These interpretations introduce the question as to what constitutes a dichasium. Endress (2010) included the dichasium within the cymose (vs. racemose) branching pattern where no more than two lateral branches can occur on each axis. He viewed features other than ramification pattern, such as the presence of a flower on the first order axis, the relative ages of the terminal and lateral flowers, lateral axes positioned opposite each other on the first order axis, the presence of bracteoles, and the presence of petioles, all as developmentally unstable elements, even though these features are commonly seen in many dichasia. This definition is less constrained than ones proffered by other authors. For example, for the positions of lateral axes, Weberling (1989), Gleason and Cronquist (1991), and Endress (2010) allowed for laterals to be subopposite, whereas Lawrence (1951), Kiger and Porter (2001), and Simpson (2006) specified that the laterals be opposite. The terminal flower on the first order axis of *Fagus* is missing, but the dichasial branching pattern can be seen in second and third order ramifications (Fey and Endress, 1983). In that example, the cymose nature of the inflorescence is reinforced by patterns seen in relatives (*Castanea*, *Quercus*) where different reductions and elaborations occur.

The presence of dichasia (based on our definition) across all Santalales clades was examined (see Character 5, Table 1, Appendix S5). This character can also be viewed where the morphological characters were optimized using parsimony on the Bayesian consensus tree (Appendix S11). A dichasium can be seen in Comandraceae and Thesiaceae and is present only rarely elsewhere. One interesting outlier is the monospecific genus *Erythropalum* (Erythropalaceae) whose inflorescence has been described as "a compound dichasium" (Takhtajan, 1997) or composed of "repeatedly dichotomous many-flowered cymes" (Sleumer, 1984). Although *Erythropalum* was excluded from Santalales by Kuijt (2015, p. 128), it is strongly supported as a member of the order in molecular analyses.

Other groupings of three flowers occur in Santalales that may not have all five of the elements of a strict dichasium. The

term "triad" has been widely adopted by a number of authors, particularly for the mistletoe family Loranthaceae. A triad is a partial inflorescence, i.e., a structure lateral to the first-order inflorescence axis. A triad is a morphologically noncommittal term; thus, in some cases, it may be homologous with dichasium. Examination of the optimization of Character 6 (triads) on the Bayesian tree (Appendix S11) shows that they are mainly present in Loranthaceae. Some genera in Opiliaceae have conflorescences (thyrses) where the partial inflorescences are dyads (*Gjellerupia*, *Opilia*) or dichasia/triads (*Agonandra*, *Gjellerupia*).

Given the above, four fundamental questions can be asked: (1) What is a dichasium? (2) What types of modifications of dichasia are allowed such that they can still be called dichasia? (3) Do dichasia (and/or their modifications) exist in Santalales? (4) Are dichasia plesiomorphic for the order? According to our definition, a dichasium is a partial inflorescence with a cymose branching pattern where all branching orders have only two lateral branches (Appendix S10). Modifications include unequal ages of the lateral branches bearing flowers and the loss of the terminal flower on the first-order axis. Because of losses (and reductions), the distinction between racemose and cymose patterns cannot always be made unless evidence exists on phylogenetically related taxa that have less reduced inflorescences (Fey and Endress, 1983; Endress, 2010). For the third question, it appears that dichasia are rare in Santalales but do occur scattered across the phylogenetic tree. Unreduced dichasia (one terminal, two lateral flowers, prophyllar bracteoles often present) can be seen in *Erythropalum*, Thesiaceae, and Comandraceae (Appendix S11). When the term triad is included, which is interpreted by some as a dichasium (e.g., Barlow, 1993, 1997), this inflorescence type is frequent among genera of Loranthaceae (Appendix S11). For Viscaceae, the term dichasium or "cymule" has been used to describe inflorescences in *Ginalloa* (Barlow, 1997), *Notothixos* (Barlow, 1983), and *Viscum* (Polhill and Wiens, 1998). We view the presence of dichasia in the family as equivocal and controversial because (1) the distinction between vegetative and reproductive axes is often nebulous, (2) prophyllar bracteoles may or may not be present, (3) the number of flowers in the partial inflorescences may vary, and (4) adventive flowers can be added to a floral "group" in collateral and serial positions. For the fourth question, BayesTraits strongly supported the absence of the "trios" character at node A that encompasses all ingroup taxa; thus, dichasia do not appear to be plesiomorphic for the order Santalales. This result contrasts with the position of Suaza-Gaviria et al. (2017, p. 32) who stated, "The predominant pattern of partial inflorescence architecture that can be traced back to the common ancestor of Loranthaceae, Santalaceae, and Viscaceae and related families consists of dichasia or dichasia-derived cymes (Fig. 7)."

Inflorescences in Viscaceae

Viscaceae are the most derived family within the order and, as such, display markedly increased substitution rates as exemplified by branch lengths on the phylogenetic tree (Fig. 1). Such increased rates of molecular evolution likely underlie large amounts of phenotypic change over evolutionary time. In addition to the reduction and loss of morphological features, the distinction between vegetative and reproductive structures is blurred, possibly beginning in the common ancestor to Santalaceae, Amphorogynaceae, and Viscaceae. For example, in some species of *Santalum*, pherophylls within inflorescences can be bracts or leafy, and inflorescences can even return to the vegetative condition (Stauffer, 1963).

We contend that the triad, a partial inflorescence, is absent in Viscaceae where most members have either crowded, sessile flowers or very short, 1-internode inflorescences—rarely compound, as in *Notothixos* (Kuijt, 1969: figs. 2–17a). In *Arceuthobium* and *Korthalsella*, when three sessile flowers are subtended by a bract, we do not equate this with a triad, mainly because the lateral flowers lack subtending prophylls. *Viscum* has very short, determinate, 1–3-flowered inflorescences that have been variously interpreted: as triads (Danser, 1941; Barlow, 1984a, 1996; Sanjai and Balakrishnan, 2006; Suaza-Gaviria et al., 2017) as well as dichasia or cymes (Polhill and Wiens, 1998; Kirkup et al., 2000).

The inflorescence type present in the closely related genera *Phoradendron* and *Dendrophthora* (Phoradendreae) is a clear synapomorphy that is unique not only in Santalales but also perhaps in angiosperms generally. Suaza-Gaviria et al. (2017) introduced a new term and concept called the "floral row", which was used to describe a horizontal grouping of an odd number (3, 5, 7, or 9) of flowers. They equate the floral row with a dichasium, drawing evidence from the fact that in triseriate and biseriate inflorescence types (Fig. 2; Appendix S10), an older apical flower occurs above two younger laterals. We do not endorse this interpretation because, for the biseriate inflorescence type, once the top three flowers are assigned to a "floral row", only two flowers remain in each of the lower rows. In addition, uniseriate and multiseriate types cannot be accommodated with this concept because here floral rows are impossible to delimit. Suaza-Gaviria et al. (2017) also attempted to confirm the existence of dichasia using vascular trace information in the fertile internodes, but the data neither support this idea nor the concept that the fertile internodes are coenosomes. In general, one would expect that the morphology of a dichasium would be reflected in its vascular structure. Specifically, one would expect a clearly separate vasculature to lead to the point where the two lateral flowers are attached; here, three separate sets of bundles would exist to supply the central and two lateral flowers. Furthermore, since the two lateral flowers are axillary to the two prophylls that flank the central flower, one might expect to see some vascular traces leading to each of those two prophylls (note there are no signs of prophylls in Phoradendreae inflorescences). None of the features just described are present from the anatomical work on the triseriate species *Phoradendron bolleanum* (Kuijt, 1959: fig. 10e as *P. pauciflorum*). Here the vascular bundles supplying the flowers lead straight to the intercalary meristem (see below) in the axil of the fertile bract, and there is no connection between the three flowers of a "floral row".

One of the unique features of the Phoradendreae inflorescence is the intercalary meristem (Evert, 2006) that produces flowers at its base resulting in an elongating internode with the oldest flowers at the distal end. Suaza-Gaviria et al. (2017) accepted the presence of an intercalary meristem but believed that floral rows (dichasia) are produced there, not individual flowers. We maintain that this distinction is specious; for indeed, their fig. 2G clearly shows the basal origin of an individual flower from an intercalary meristem, with two younger flowers being initiated subsequently. The lack of anatomical evidence, the variations in seriation types (Fig. 2), and the lack of subtending prophylls (floral bracteoles) are strong evidence that together do not support the existence of a "floral row" or that it is a remnant of a triad or dichasium.

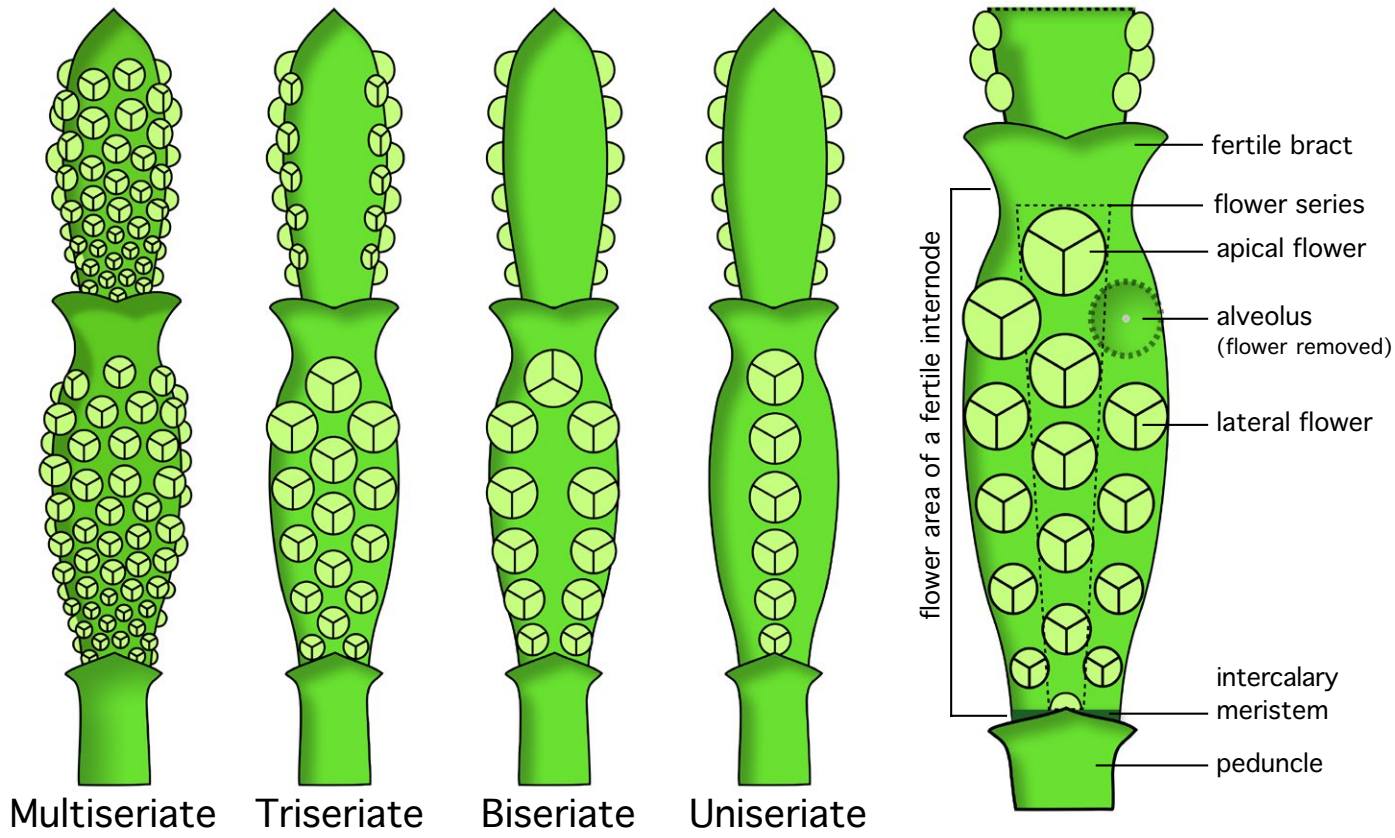

FIGURE 2. Diagrammatic representation of inflorescence morphology in Phoradendreae (*Phoradendron* and *Dendrophthora*, Viscaceae). The four left-hand figures represent inflorescence types with different flower seriation. The right-hand figure shows a fertile internode of a triseriate inflorescence with the component parts labeled.

Evolution and development of inflorescences

Comparative morphology has provided the "raw material" necessary to assess primary homology of plant structures. But as pointed out by Hufford and McMahon (2003), translating these data into hypotheses useful for phylogenetic inference has not been straightforward, mainly because of difficulty ascertaining morphoclines (i.e., transformation series of character states). Specifically within Santalales, inflorescence evolution in Loranthaceae was surveyed independently by Barlow (1966, 1992) and Kuijt (1981). Barlow primarily utilized reduction trends, whereas Kuijt used elaboration. Holoparasitic plants in particular are renowned for reductions and losses of morphological organs; hence, it is tempting to assume that this trend is pervasive in all parasites, hemiparasites included. As shown in the parsimony reconstructions of characters on the Santalales molecular tree (Appendix S11, Character 3), both elaboration and reduction trends in inflorescences have occurred among the clades.

Progress has been made in understanding the developmental, genetic, and evolutionary processes underlying inflorescence morphology (Coen and Nugent, 1994; Prusinkiewicz et al., 2007). The interrelationship among cymes, racemes, and panicles was made with the introduction of the transient model that can accommodate all of these in a single framework (Coen and Nugent, 1994). Here the degree of "vegetativeness" is variable and transient and together with maturation kinetics affects the branching structure of the inflorescence. Although these ideas were developed using model plants, how the expression of such genes explains varied inflorescence phenotypes within clades of angiosperms in general has not been addressed. Recently, orthologues of *TFL1* (terminal flower), *LFY* (leafy), and *AP1* (apetala) have been identified in *Cornus* and their expression patterns examined in developing inflorescences using in situ hybridization and quantitative PCR (Ma et al., 2016). Looking at six groups within *Cornus*, each with different types of inflorescences, a clear correlation was seen between TFL1 and AP1 expression and inflorescence branch number. We propose that studies such as those conducted with *Cornus* are required to understand the complex array of inflorescence types seen in Santalales. Three species of *Santalum* with different degrees of "vegetativeness" in their inflorescences were illustrated by Stauffer (1963), which invites study of the expression levels of LFY across a genus with a well-resolved species phylogeny (Harbaugh and Baldwin, 2007). Questions about ovular reduction in Santalales were addressed by Brown, Nickrent, and Gasser (2010) where expression patterns for orthologs of *ANT* and *BELL1* suggested a fusion between integuments and nucellus, not the loss of integuments in unitegmic and ategmic species. Similar evo-devo approaches have great potential in ascertaining homology between unmodified and highly modified phenotypes.

CONCLUSIONS

A well-resolved phylogenetic tree incorporating nearly all genera of Santalales provides important insights into the evolutionary development of partial and compound inflorescences. Among the three key nodes on this tree, a grouping of three flowers (the trios character) was supported only for Loranthaceae where this partial inflorescence is best described as a triad. In contrast to the conclusion reached by Suaza-Gaviria et al. (2017), character optimization using parsimony does not support the dichasium as being plesiomorphic for the order. Our data suggest that the plesiomorphic partial inflorescence in Santalales was an axillary fascicle or cyme, with bisexual flowers bearing persistent bracts, features found in the family Erythropalaceae. A trend beginning with Santalaceae and ending with Viscaceae is the phenomenon where vegetative and reproductive structures sometimes become less distinct, making comparison to "standard" inflorescence types difficult. Assessing primary homology for inflorescences in Santalales in the context of a phylogeny is the first step that will hopefully encourage future research. Detailed morphological and developmental studies that will certainly advance our knowledge of these amazing parasites.

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AUTHOR CONTRIBUTIONS

D.L.N., J.K., and F.E.A. all wrote portions of the manuscript. DNA sequences were assembled and aligned by D.L.N. Phylogenetic analyses were performed by D.L.N. and F.E.A.

DATA ACCESSIBILITY

All sequences generated in this study are deposited in GenBank (Appendix S1). The alignments used in this study are available from the MorphoBank (http://morphobank.org/permalink/?P3212) and Appendix S2. The NEXUS file that contains morphological characters relating to inflorescences and plant sexuality for all 163 genera in Santalales is available in Appendix S5 and is also archived at the MorphoBank link above. Photographs and other information for all members of Santalales can be found on the Parasitic Plant Connection website (https://parasiticplants.siu.edu/).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Voucher and gene accession numbers for 146 genera across the five genes used in this study.

APPENDIX S2. NEXUS file of the concatenated 5-gene multiple sequence alignment of nuclear SSU rDNA and LSU rDNA and chloroplast *rbcL*, *matK*, and *accD*. The matrix is 146 taxa (genera) by 10,428 characters (bases). Data are archived at MorphoBank (https://morphobank.org/).

APPENDIX S3. Summary of inflorescence and floral features across the 163 genera of Santalales (two entries for *Helixanthera*, African and Asian) derived from various primary literature sources.

APPENDIX S4. Notes on how the morphological characters and character states were interpreted and scored for the Santalales taxa used in this study.

APPENDIX S5. NEXUS file of 10 morphological characters relating to inflorescences and plant sexuality for all 163 genera in Santalales. Data archived on MorphoBank (https://morphobank.org/).

APPENDIX S6. Maximum parsimony bootstrap consensus trees for (1) concatenated nuclear SSU and LSU rDNA; (2) concatenated chloroplast *rbcL*, *matK*, and *accD*; and (3) the concatenated 5-gene matrix. Bootstrap numbers at the nodes were obtained from 1000 replications.

APPENDIX S7. The partitions and models chosen for RaxML and BI using PartitionFinder v. 2.1.1 (Lanfear et al., 2016).

APPENDIX S8. Maximum likelihood phylogram obtained from RAxML-HPC v. 8.2.10 (Stamatakis, 2006, 2014), with 1000 rapid bootstrap replicates and a subsequent ML search for the best-known-likelihood topology (-f a option).

APPENDIX S9. The lowest marginal log likelihoods (and thus Bayes factors) calculated across all runs and tree samples for each state at the three selected nodes, A, B, and C.

APPENDIX S10. Definitions of dichasium, cyme, and thyrse present in the literature.

APPENDIX S11. The Bayesian inference (BI) tree with the 10 morphological characters (NEXUS file, Appendix S5) optimized using maximum parsimony as implemented in Mesquite.

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