

CLARIFICATION OF THE RELATIONSHIP BETWEEN APIACEAE AND ARALIACEAE BASED ON *MATK* AND *RBCL* SEQUENCE DATA¹

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Apiaceae and Araliaceae (Apiales) represent a particularly troublesome example of the difficulty in understanding evolutionary relationships between tropical-temperate family pairs. Previous studies based on *rbcL* sequence data provided insights at higher levels, but were unable to resolve fully the family-pair relationship. In this study, sequence data from a more rapidly evolving gene, *matK*, was employed to provide greater resolution. In Apiales, *matK* sequences evolve an average of about two times faster than *rbcL* sequences. Results of phylogenetic analysis of *matK* sequences were first compared to those obtained previously from *rbcL* data; the two data sets were then combined and analyzed together. Molecular analyses confirm the polyphyly of apiaceous subfamily Hydrocotyloideae and suggest that some members of this subfamily are more closely related to Araliaceae than to other Apiaceae. The remainder of Apiaceae forms a monophyletic group with well-defined subclades corresponding to subfamilies Apioideae and Saniculoideae. Both the *matK* and the combined *rbcL-matK* analyses suggest that most Araliaceae form a monophyletic group, including all araliads sampled except *Delaribrea* and *Mackinlaya*. The unusual combination of morphological characters found in these two genera and the distribution of *matK* and *rbcL* indels suggest that these taxa may be the remnants of an ancient group of pro-araliads that gave rise to both Apiaceae and Araliaceae. Molecular data indicate that the evolutionary history of the two families is more complex than simple derivation of Apiaceae from within Araliaceae. Rather, the present study suggests that there are two well-defined “families,” both of which may have been derived from a lineage (or lineages) or pro-araliads that may still have extant taxa.

Key words: Apiaceae (Umbelliferae); Apiales; Araliaceae; family pairs; *matK*; phylogenetics; *rbcL*.

Apiaceae and Araliaceae are a particularly troublesome example of the difficulty in resolving relationships between closely related pairs of plant families. This “family-pair dilemma” also characterizes a number of other dicot groups, including Bombacaceae-Malvaceae, Caparaceae-Brassicaceae, Moraceae-Urticaceae, Bignoniaceae-Scrophulariaceae, Verbenaceae-Lamiaceae, and Apocynaceae-Asclepiadaceae (see Judd, Sanders, and Donoghue, 1994). In each of these pairs, one family is mostly woody and tropical, whereas its counterpart is largely herbaceous and temperate. With Araliaceae and Apiaceae, the difficulties at the interfamilial level are further compounded by problems at almost every other taxonomic level as well. For example, the placement of these two families among the other orders of dicots has been the subject of widespread disagreement. Virtually all systems unite Apiaceae and Araliaceae into a single order (Apiales or Araliales) that is traditionally placed near Cornaceae in subclass Rosidae (Takhtajan, 1987; Thorne,

1992). Cronquist (1988) maintained the rosid alliance, but suggested that Apiales were more closely related to Sapindales than Cornaceae. Dahlgren (1980), stressing biochemical characters, suggested that Araliales were most closely related to Pittosporaceae, and united these two groups in his superorder Araliiflorae, somewhat near Asteriflorae. In addition to ordinal-level uncertainties, patterns of variation within each family overlap, blurring familial boundaries and obscuring interfamilial relationships. Within Apiaceae, most workers use the treatment of Drude (1898), in which the family is divided into three subfamilies, Apioideae, Saniculoideae, and Hydrocotyloideae, and thence into 12 tribes, but this system is often regarded as highly unnatural (reviewed in Plunkett, Soltis, and Soltis, in press). Within Araliaceae, there are no widely accepted subgroups, and none of the various treatments of the family are considered to reflect evolutionary relationships. Eyde and Tseng (1971) proposed dividing the family into two main groups, based on patterns of floral vasculature and leaf morphology (palmately vs. pinnately compound leaves), but this proposal has never been widely applied or tested.

The most persistent problem presented by Apiales, however, has been the relationship of Apiaceae to Araliaceae. Traditionally, the two families have been envisioned as separate lineages that diverged from a common ancestor, sometimes referred to as the “pro-araliad” (see Rodríguez, 1971). Alternatively, a number of taxa in Apiales (e.g., *Myodocarpus*, *Hydrocotyle*, *Klotzschia*, and *Mackinlaya*) have been proposed as “bridging genera” between the two families (see Baumann, 1946; Philipson,

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1951; Shoup and Tseng, 1977), leading some authors to argue that Apiaceae are merely a large, specialized group derived from within Araliaceae (e.g., Walters, 1961; Thorne, 1973; Judd, Sanders, and Donoghue, 1994). Studies of relationships between these families based on traditional sources of information have been plagued by pervasive parallel evolution found within this order (reviewed in Plunkett, Soltis, and Soltis, 1996). In a recent molecular-cladistic analysis, Plunkett, Soltis, and Soltis (1996) used DNA sequence variation in the chloroplast gene *rbcL* to examine relationships involving Apiaceae and Araliaceae. That study provided many insights into the evolutionary history of Apiales, suggesting, for example, that (1) Apiales should be included within an expanded subclass Asteridae (see also Downie and Palmer, 1992; Chase et al., 1993; Olmstead et al., 1992, 1993); (2) Pittosporaceae are the sister group to Apiales; (3) Apiales and Pittosporaceae form the core of an "Apialean Alliance" along with four enigmatic genera, *Aralidium*, *Melanophylla*, *Toricellia*, and *Griselinia*; (4) the apiaceous subfamily Hydrocotyloideae is polyphyletic; and (5) the remainder of Apiaceae (i.e., subfamilies Apioideae, Saniculoideae, and parts of Hydrocotyloideae) is monophyletic. The slow substitution rate and extensive database of sequences make *rbcL* sequence data well suited for phylogenetic studies at a variety of higher taxonomic levels, from interfamilial to subclass (see *Annals of the Missouri Botanical Garden* [1993] vol. 80, no. 3, for numerous examples). However, despite the demonstrated utility of *rbcL* sequences in resolving relationships at higher levels in Apiales, its slow rate of sequence evolution has proven to be a limitation in resolving the relationship between Araliaceae and Apiaceae. Specifically, the *rbcL* topology suggested that Apiaceae were derived from within a paraphyletic Araliaceae, but the nodes defining this paraphyly were among the weakest regions of the *rbcL* tree (as estimated by bootstrap values [$\leq 25\%$] and decay values [$=1$]; Fig. 1). Moreover, an alternative hypothesis of two monophyletic families was only two steps longer than the most parsimonious trees. Thus, despite the important information provided by the study of *rbcL* in Apiaceae and Araliaceae, additional characters are clearly needed to resolve the relationship between the two families.

Recently, a number of investigators studying angiosperm phylogeny have used several alternative DNA sequences, including the nuclear 18S rDNA (nrDNA) (Nickrent and Soltis, 1995), the internal transcribed spacer (ITS) region of nrDNA (Baldwin et al., 1995), and the chloroplast genes *matK* (Johnson and Soltis, 1994; Steele and Vilgalys, 1994), and *ndhF* (e.g., Olmstead and Reeves, 1995). Among these, ITS variability is generally too great to be useful at interfamilial levels (a finding confirmed by studies in Apiaceae by Soltis and Kuzoff [1993] and Downie and Katz-Downie [1996]). Conversely, variability of 18S rDNA sequences is generally too low, providing fewer characters than *rbcL* (see Nickrent and Soltis, 1995). Studies of *matK* and *ndhF* have suggested that both of these genes evolve at rates appropriate for studies at the inter- and infrafamilial levels (e.g., Johnson and Soltis, 1994, 1995; Steele and Vilgalys, 1994; Clark, Zhang, and Wendel, 1995; Olmstead and Reeves, 1995). The single-copy gene *matK*, generally lo-

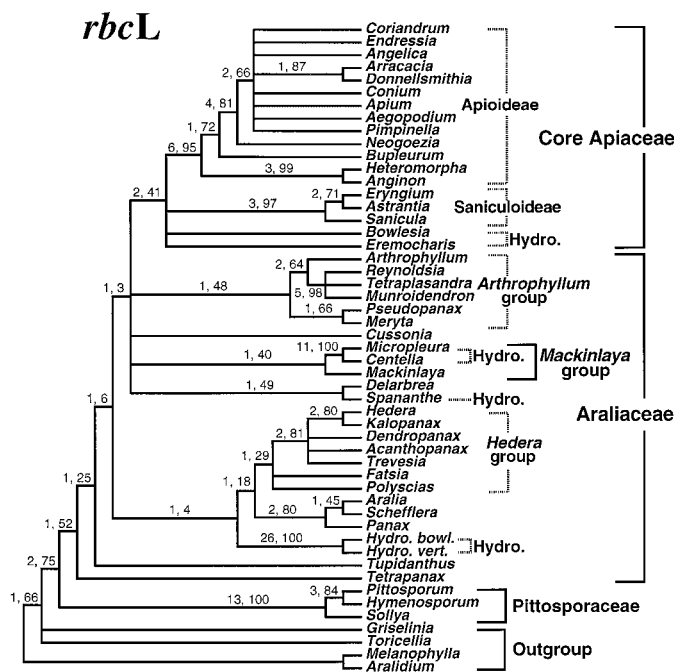


Fig. 1. Strict consensus of the 5736 most parsimonious trees based on *rbcL* sequence data from 51 taxa (redrawn from Plunkett, Soltis, and Soltis, 1996). Decay values (first) and bootstrap percentages (second) are mapped along each branch. Major groups discussed in the text are bracketed and labeled (subfamily Hydrocotyloideae is abbreviated "Hydro.>").

cated within a class II intron in the transfer RNA gene for lysine (*trnK*) (Neuhaus and Link, 1987), encodes a maturase involved in the splicing of introns in plastid genes (Liere and Link, 1995). Sequence data from *matK* have been applied successfully to phylogenetic analyses of relationships within two unrelated dicot families, Polemoniaceae (Steele and Vilgalys, 1994; Johnson and Soltis, 1995) and Saxifragaceae (Johnson and Soltis, 1994, 1995; Johnson et al., 1996). In comparisons of *rbcL* and *matK* data sets from Saxifragaceae sensu stricto, Johnson and Soltis (1994) found that phylogenetic relationships from the two analyses were highly concordant. However, the substitution rate of *matK* in Saxifragaceae is approximately three times faster than that of *rbcL*, and the results of comparative *matK* sequencing permitted fine-scale resolution of relationships comparable to that achieved using chloroplast DNA restriction site analysis. Early trials among several taxa of Apiales suggested that the substitution rate of *matK* was also appropriate for inter- and infrafamilial study in this order. Herein, we explore further the relationship of Apiaceae to Araliaceae by analysis of *matK* sequences. These sequences were derived from a nearly identical set of taxa as used in the earlier *rbcL* study (Plunkett, Soltis, and Soltis, 1996) and can thus provide an alternative phylogenetic hypothesis directly comparable to the *rbcL* topology. Further, given the differences in substitution rates between the two genes, a combination of the two data sets may provide more resolution than either one separately (see Hillis, 1987; Huelsenbeck et al., 1994; de Queiroz, Donoghue, and Kim, 1995). After examination

for congruence, therefore, the two data sets were combined for additional analysis.

MATERIALS AND METHODS

We obtained *matK* sequences from 47 of the 51 taxa used in the *rbcL* interfamilial study (Plunkett, Soltis, and Soltis, 1996). The genera sampled included: *Aegopodium*, *Angelica*, *Anginon*, *Apium*, *Arracacia*, *Bupleurum*, *Coriandrum*, *Domnellsmithia*, *Endressia*, *Heteromorpha*, *Neogozia*, *Pimpinella*, *Astrantia*, *Eryngium*, *Sanicula*, *Centella*, *Eremocharis*, *Hydrocotyle*, *Micropleura*, *Acanthopanax*, *Aralia*, *Arthropodium*, *Cussonia*, *Delarbraea*, *Dendropanax*, *Fatsia*, *Hedera*, *Kalopanax*, *Mackinlaya*, *Meryta*, *Munroidendron*, *Panax*, *Polyscias*, *Pseudopanax*, *Reynoldsia*, *Schefflera*, *Tetrapanax*, *Tetraplasandra*, *Trevesia*, *Tupidanthus*, *Hymenosporum*, *Pittosporum*, *Sollya*, *Griselinia*, *Aralidium*, and *Melanophylla*. Sequences from two additional araliads not included in the *rbcL* study, *Cheirodendron* (both *matK* and *rbcL*) and *Gastonia* (*matK* only), were also derived. Table 1 provides information on sources, voucher specimens, and GenBank accession numbers for all taxa and sequences used in this study. The original *rbcL* data set included sequences from four additional genera (*Bowlesia*, *Conium*, *Spananthe*, and *Toricellia*) from which we were unable to obtain *matK* sequences, presumably due to divergence at the PCR primer sites.

Total DNA was extracted from leaf tissue using the CTAB method of Doyle and Doyle (1987) as modified by Soltis et al. (1991) for fresh or frozen samples, or by Cullings (1992) for small amounts of dry samples taken from herbarium sheets or dried directly in silica gel. Double-stranded copies of *matK* were amplified from total DNA using the polymerase chain reaction (PCR). Initially, double-stranded products were produced using the *trnK*-3914F and *trnK*-2R primers described in Johnson and Soltis (1994). Due to presumed primer divergence, these primers failed to produce usable double-stranded product in a number of taxa, and one or both of the following primers were used as alternatives: *matK*-710F and *matK*-2000R (Table 2). Single-stranded copies were amplified by PCR using one of the primers singly, with the double-stranded product as template (Kreitman and Landweber, 1989). The single-stranded DNA products were purified by PEG-precipitation (Morgan and Soltis, 1993) to permit direct sequencing. Sequences were obtained by Sequenase-mediated dideoxynucleotide reactions (U.S. Biochemical Corp., Cleveland, OH). Three primers were typically used for sequencing: *matK*-1168R, *matK*-1470R, and *matK*-1412F (Table 2). Where these primers proved ineffective, one or more of the following primers were also used: *matK*-710F, *matK*-1176F, and *matK*-1412R (Table 2). Approximately two-thirds (1 140 bp) of *matK* from each of 49 taxa were used for phylogenetic analysis.

A series of separate phylogenetic analyses was performed to examine relationships between Apiaceae and Araliaceae. The first series involved all of the 49 *matK* sequences generated herein, including 20 sequences from Apiaceae, 23 from Araliaceae, and three from Pittosporaceae. Sequences from *Melanophylla*, *Aralidium*, and *Griselinia* were included as outgroups on the basis of the previous *rbcL* study (Plunkett, Soltis, and Soltis, 1996). Because the *matK* sequences in Apiales contain a number of indels (see below) that possess potentially informative characters, these regions were included in the phylogenetic analysis and alignment gaps were treated as missing data. An additional analysis in which the indels were scored as binary characters and added to the data matrix was also performed. Sequences were analyzed using the Heuristic Search option of PAUP* (Version 4.0; D. Swofford, in prep., with permission), with MULPARS, tree-bisection-reconnection (TBR) branch swapping, simple addition, and ACCTRAN optimization. Attempts to find all most parsimonious trees using multiple replicates and random addition proved problematic because early trials yielded over 45 000 trees, after which computer memory was exhausted. A maxtree limit was then set at 30 000 in order to balance the desire to examine the largest number of trees with the practical limitations of time and computer memory. To search for the additional most parsimonious topol-

ogies, the strict consensus of these 30 000 trees was loaded as a topological constraint for a subsequent analysis (which also had a maxtree limit of 30 000). No additional minimum length trees were found during this second search, suggesting that the strict tree from the first analysis adequately summarizes relationships in all most parsimonious trees. Both the resulting tree topology and pairwise distance measurements of all taxa indicated that the large number of trees resulted from poor resolution of relationships among a group of taxa from Araliaceae. A series of additional analyses in which one, two, three, and finally four of these araliads were removed yielded dramatic decreases in the number of resulting trees (e.g., the analysis of 45 *matK* sequences resulted in only 740 most parsimonious trees). The strict consensus trees resulting from each of the smaller analyses were identical to the strict tree from the original analysis of 49 *matK* sequences (with the exception of relationships involving the deleted taxa). For these reasons, the strict tree from the original analysis will be used throughout. Confidence in specific clades of the resulting topology was estimated by bootstrap analysis (1 000 replicates, maxtree limit of 100 per replicate) (Felsenstein, 1985), and decay analysis (Bremer, 1988; Donoghue et al., 1992) using the "converse constraint" method described in Baum, Sytsma, and Hoch (1994).

A second series of analyses involved a data set that combined 48 of the *matK* sequences from the present study with the available *rbcL* sequences from the same 48 taxa. The resulting combined data set contained a total of 2 544 characters, of which 2 514 were used for phylogenetic analysis (bp 1–30 from the *rbcL* sequences are excluded because they are derived from the internal 5' PCR primer, Z1). Using the PAUP* options described above, two versions of the combined data set (with and without *matK* indels coded as binary characters) were analyzed phylogenetically. To search for multiple islands of most parsimonious trees (Maddison, 1991), the analysis was repeated 100 times with random addition. Bootstrap (100 replicates, maxtree limit of 20 000 per replicate) and decay analyses (as above) were performed to estimate confidence in the clades of the resulting topologies.

Forty-seven taxa are common to the present study of *matK* sequences and the original *rbcL* study (Plunkett, Soltis, and Soltis, 1996). A third series of analyses based on sequences from these 47 taxa was performed to make comparisons among the separate and combined data sets, and among the respective topologies resulting from phylogenetic analysis of each of these. The level of nonrandom structure present in all three data sets was estimated by skewness (Hillis, 1991; Huelsenbeck, 1991; Hillis and Huelsenbeck, 1992) and randomization tests (Archie, 1989; Faith and Cranston, 1991; Källersjö et al., 1992). In the skewness test, the distribution of 10 000 random trees was examined using the Random Tree option of PAUP*, yielding the g_1 statistic. Data sets that are considerably skewed to the left are considered to contain a significant amount of hierarchical structure (Hillis and Huelsenbeck, 1992; but see also Källersjö et al., 1992). The randomization tests were performed using the Permutation option of PAUP* with 1 000 replicates and heuristic searches (maxtree limits set at 100). This test produces the permutation tail probability (PTP) statistic, and data sets yielding values of PTP ≤ 0.05 (or 0.01) are considered to differ significantly from randomized data. Two calculations were used to estimate rates of sequence divergence between *matK* and *rbcL* in Apiales. The first measure is a simple comparison of the percentage of variable sites over the length of the each gene (or gene portion) for the two separate data sets. For the second estimate, the *matK* distance values of 30 pairs of taxa were compared to the *rbcL* distance values of the same 30 pairs.

Phylogenetic analysis (following the procedures outlined above) was performed on each of the three data sets of 47 taxa. Two trees were saved from each analysis: the strict consensus and the majority rule tree with compatible groupings (which is identical to one of the most parsimonious trees; hereafter referred to as the "MR tree"). Codon position and transition to transversion ratios were estimated for the *rbcL* and *matK* data sets with the MR tree resulting from each analysis, using the computer program MacClade (version 3.01; Maddison and Maddison,

TABLE 1. Sources and voucher specimen information for the 49 species for which *matK* sequences were obtained for this study. GenBank accession numbers for all *rbcL* and *matK* sequences used in this study are also provided. GenBank numbers followed by one or two asterisks were taken from previously published papers (Olmstead et al., 1992, and Xiang et al., 1993, respectively). Abbreviations used for botanical gardens: HoBG: Honolulu; HuBG: Huntington; KBG: Kew; MoBG: Missouri; NYBG: New York; UCBG: University of California at Berkeley. Accession numbers from these gardens follow the abbreviations (when available).

Species	Voucher	Source	<i>rbcL</i> GenBank accession number	<i>matK</i> GenBank accession number
Apiaceae: Apioideae				
<i>Aegopodium podagraria</i> L.	G. Plunkett 1332, WS	cultivated, Pullman, WA	U50220	U58542
<i>Angelica lucida</i> L.	no voucher	cultivated, Pullman, WA	U50221	U58545
<i>Anginon rugosum</i> Thunb.	no voucher	L. Constance, personal collection, C-2399	U50222	U58546
<i>Apium graveolens</i> L.	G. Plunkett 1334, WS	cultivated, Pullman, WA	L11165	U58548
<i>Arracacia aegopodioides</i> (Kunth) Coult. & Rose	G. Plunkett 1268, WS	Oaxaca State, Mexico	U50223	U58549
<i>Bupleurum falcatum</i> L.	G. Plunkett 1325, WS	NYBG	U50224	U58552
<i>Coriandrum sativum</i> L.	G. Plunkett 1337, WS	cultivated, Pullman, WA	L11676*	U58557
<i>Donnellsmithia cordata</i> (Coult. & Rose) Math. & Const.	G. Plunkett 1270, WS	Oaxaca State, Mexico	U50225	U58560
<i>Endressia castellana</i> Coincy	no voucher	L. Constance, personal collection, C-2184	U50226	U58562
<i>Heteromorpha trifoliata</i> (Wendl.) Eckl. & Zeyner	G. Plunkett 1345, WS	L. Constance, personal collection, C-2038	U50227	U58565
<i>Neogoezia minor</i> Hemsl.	G. Plunkett 1272, WS	Oaxaca State, Mexico	U50228	U58570
<i>Pimpinella saxifraga</i> L.	G. Plunkett 1324, WS	cultivated, Pullman, WA	U50229	U58576
Apiaceae: Saniculoideae				
<i>Astrantia</i> × <i>rosensimfonie</i>	G. Plunkett 1327, WS	NYBG	U50230	U58585
<i>Eryngium bourgattii</i> Gouan.	G. Plunkett 1321, WS	NYBG	U50231	U58586
<i>Sanicula gregari</i> Bickn.	D. Ware 9898, WILLI	James City Co., VA	L11170	U58589
Apiaceae: Hydrocotyloideae				
<i>Centella erecta</i> (L. f.) Fer.	D. Ware s.n., WILLI	Dare Co., NC	U50233	U58599
<i>Eremocharis fruticosa</i> Phil.	Dillon & Teillier 5082, UC	L. Constance, personal collection, C-2382	U50234	U58590
<i>Hydrocotyle bowlesiodes</i> Math. & Const.	G. Plunkett 1373, WS	L. Constance, personal collection, C-222	U50235	U58600
<i>Hydrocotyle verticillata</i> Thunb.	D. Ware 10036, WILLI	James City Co., VA	U50236	U58601
<i>Micropleura renifolia</i> Lag.	G. Plunkett 1273, WS	Oaxaca State, Mexico	U50237	U58602
Araliaceae				
<i>Acanthopanax trifoliatum</i> (L.) Mer.	G. Plunkett 1318, WS	NYBG, 68124	U50239	U58603
<i>Aralia spinosa</i> L.	G. Plunkett 1371, WS	cultivated, Pullman, WA	L11166	U58604
<i>Arthropphyllum pinnatum</i> Clarke	G. Plunkett 1357, WS	HoBG, F-2255	U50240	U58605
<i>Cheirodendron trigynum</i> (Gaud.) A. Heller	L. Johnson 92.110, WS	Kauai Co., HA	U50241	U58606
<i>Cussonia spicata</i> Thunb.	G. Plunkett 1341, WS	MoBG, 760020-1	U50242	U58607
<i>Delarbrea michieana</i> (F. v. Muell.) F. v. Muell.	G. Plunkett 1366, WS	HuBG, 51837	U50243	U58608
<i>Dendropanax arboreus</i> (L.) Dence. & Planche	G. Plunkett 1352, WS	UCBG, 90.3027	U50244	U58609
<i>Fatsia japonica</i> Decne. & Planche	G. Plunkett 1368, WS	NYBG	U50245	U58610
<i>Gastonia rodriguesiana</i>	no voucher	KBG, 662-86.06150	n/a	U58611
<i>Hedera helix</i> L.	G. Plunkett 1368, WS	cultivated, Pullman, WA	L01924*	U58612
<i>Kalopanax pictum</i> Nakai	G. Plunkett 1328, WS	NYBG, 89648	U50246	U58591
<i>Mackinalaya macroscladia</i> (F. Muell.) F. Muell.	G. Plunkett 1365, WS	HuBG, 61733	U50247	U58613
<i>Meryta sinclairii</i> Seem.	G. Plunkett 1367, WS	HuBG, 13566	U50248	U58614
<i>Munroidendron racemosum</i> (C.N. Forbes) Sherff	G. Plunkett 1342, WS	MoBG, 850020	U50249	U58592
<i>Panax quinquefolium</i> L.	D. Ware 10046, WILLI	James City Co., VA	U50250	U58615
<i>Polyscias guilfoylei</i> Bailey	G. Plunkett 1322, WS	NYBG	U50251	U58616
<i>Pseudopanax arboreus</i> (Murr.) Philipson	G. Plunkett 1353, WS	UCBG, 80.0655	U50252	U58617
<i>Reynoldsia sandwicensis</i> A. Gray	G. Plunkett 1359, WS	HoBG, 70.0188	U50253	U58618
<i>Schefflera arboricola</i> Hayata	G. Plunkett 1317, WS	NYBG	U50255	U58619
<i>Tetrapanax papyriferus</i> Koch	G. Plunkett 1344, WS	MoBG, 863189	U50256	U58620
<i>Tetraplasandra hawaiiensis</i> A. Gray	G. Plunkett 1378, WS	HoBG, 67.1227	U50257	U58593
<i>Trevesia palmata</i> Vis.	G. Plunkett 1329, WS	NYBG	U50258	U58621
<i>Tupidanthus calypratus</i> J.D. Hook. & Thomps.	G. Plunkett 1351, WS	NYBG	U50259	U58622
Others				
<i>Aralidium pinnatifidum</i> (Jungh. & de Vriese) Miq.	K. Saleh, s.n., KEP	Selaegor, Malaysia	Q. Xiang, unpublished	U58627
<i>Griselinia lucida</i> G. Forst.	Cameron s.n., AKU	New Zealand	L11225**	U58628
<i>Hymenosporum flavum</i> E.J. Muell	G. Plunkett 1364, WS	HuBG, 44368	U50260	U58623
<i>Melanophylla alnifolia</i> Baker	G. Schatz 3552, MO	Prov. Antananarivo, Madagascar	U50254	U58626
<i>Pittosporum tobira</i> Ait.	G. Plunkett 1388, WS	cultivated, Pullman, WA	U50261	U58624
<i>Sollya heterophylla</i> Lindl.	G. Plunkett 1361, WS	HuBG, 31833	U50262	U58625

TABLE 2. Sequences and sources of primers used for PCR amplification and sequencing. Asterisks indicate modifications of published primer sequences. Nucleotides follow IUPAC abbreviations.

Primer	5' sequence 3'	Published source
<i>trnK</i> -3914F	GGG GTT GCT AAC TCA ACG G	Johnson and Soltis, 1994*
<i>matK</i> -710F	GTA TCG CAC TAT GTW TCA TTT GA	Plunkett et al., in press
<i>matK</i> -1168R	ATT GAA TGA ATT GAT CGT A	Johnson and Soltis, 1995
<i>matK</i> -1176F	CAA TTC ATT CAM TAT TTC CTT	Plunkett et al., in press
<i>matK</i> -1412F	ATA TAA TTC TTA TGT ATG TG	Johnson and Soltis, 1994
<i>matK</i> -1412R	CAC ATA SAT AAG AAT TAT AT	Plunkett et al., in press
<i>matK</i> -1470R	AAG ATG TTG ATY GTA AAT GA	Johnson and Soltis, 1994
<i>matK</i> -2000R	ATT TCT GCA TAT GCG CAC AAA TC	Plunkett et al., in press
<i>trnK</i> -2R	AAC TAG TCG GAT GGA GTA G	Steele and Vilgalys, 1994*

1992). To examine levels of incongruence among the data sets, two measures were estimated: I_{MF} (the incongruence test of Mickevich and Farris [1981]) and I_M (the incongruence test described by Swofford [1991]). In both measures, the amount of incongruence (measured as homoplasy) found within each separate data set ("within-data-set components") is partitioned from the amount of homoplasy found between the two data sets ("between-data-sets component"), and these values are compared to the total amount of homoplasy ("total incongruence") from both data sets (Swofford, 1991). The two tests differ, however, in the estimation of total incongruence. For I_{MF} , total incongruence is estimated by measuring the amount of homoplasy resulting from a combined analysis, and this is then divided into the value of the between-data-sets component. In I_M , the data sets do not need to be combined, alleviating concerns about possible disparities in the number of characters between data sets. Instead, total incongruence is measured by comparing the length of the most parsimonious topology resulting from one analysis against the data set of the other, and vice versa. From the sum of these two values, the combined incongruence of the two within-data-set components is subtracted, and this difference is divided by the total incongruence (see Swofford, 1991). However, the I_M test requires that each of the most parsimonious trees resulting from the separate analyses be examined against the other data set, and that the best-fitting tree be used in the calculations. Given the large number of most par-

simonious trees resulting from the present analyses, this was clearly impractical. Instead, we chose the MR tree from each analysis as an exemplar of all the most parsimonious trees. Because it is unlikely that the MR tree is also the best-fitting tree when examined against the other data set, the I_M estimated from these values is likely to be slightly inflated, a conservative approach when testing for congruence. In addition to these tests, the strict consensus trees resulting from the *rbcL* and *matK* analyses were used to generate "total strict" and "total semi-strict" consensus trees (see Swofford, 1991; see also de Queiroz, 1993).

RESULTS

Variability comparisons—Sequence variation and divergence rates in *matK* and *rbcL*—The aligned portion of the *matK* sequences from members of Apiales was 1 116 bp long, although some taxa were missing a 30- to 60-bp fragment in the region between two of the sequencing primers (*matK*-1412F and *matK*-1470R). Of the 1 116 characters, 265 (=23.7%) were parsimony-informative among 47 taxa (Table 3). Sequence length was highly conserved among all taxa examined with the exception of nine insertions or deletions (indels), all of which occurred in triplets (three, six, or nine bp), as would be expected of a protein-coding region. Because visual alignment was straightforward, no computer aligning programs were needed. Six of the indels (labeled *a-f* in Table 4) were potentially phylogenetically informative. Indel *a*, an insertion of six bp, was found in both species of *Hydrocotyle*. Indel *b*, also an insertion of six bp, was found in all taxa except *Griselinia*, *Aralidium*, *Melanophylla*, *Delarabrea*, *Mackinlaya*, *Centella*, *Micropleura*, and the three genera of Pittosporaceae. Two indels were found in all or most of the representatives of Apioideae: indel *c* (a deletion of three bp) characterizes all apioids and indel *f* (an insertion of six bp) is found in all apioids except *Bupleurum*, *Heteromorpha*, and *Anginon*; indel *f* is also found in *Aralidium*. Indel *d*, an insertion of six bp, was found in all taxa except *Melanophylla*, *Griselinia*, *Aralidium*, *Panax*, and *Aralia*. Indel *e*, a deletion of nine bp, was found in all taxa except *Griselinia*, *Aralidium*, and *Melanophylla*.

The previously published length of *rbcL* in Apiales was 1 428 bp in all but six taxa (viz., *Delarabrea*, *Mackinlaya*, *Centella*, *Micropleura*, *Panax*, and *Aralia*). Length variation in all cases was due to insertions at the extreme 3' terminus of the gene, in the usual position of the stop codon (bp positions 1 426–1 428) (Table 5; and Plunkett, Soltis, and Soltis, 1996). Among the 47 *rbcL* sequences re-evaluated in the present study, 168 charac-

TABLE 3. Comparison of the three data sets based on sequences derived from the same 47 species. CI and RI are the consistency and retention indices, respectively; g_1 is the skewness statistic; PTP is the permutation tail probability. Transition to transversion ratios, codon position ratios, and the length of alternative separate topologies were estimated using the majority-rule tree with compatible groupings (MR tree), which is identical to one of the most parsimonious trees.

Measurement	Data set		
	<i>rbcL</i>	<i>matK</i>	Combined
Total number of characters	1 398	1 116	2 514
Number of informative characters	168 (=12.0%)	265 (=23.7%)	433 (=17.2%)
Length of most parsimonious trees	575	796	1 393
Sum of minimum possible lengths	353	563	916
Codon position ratio (1:2:3)	1.56:1:3.49	1.20:1:1.68	n/a
Transition : transversion	1.18:1	1.13:1	n/a
CI (excluding uninformative characters)	0.53	0.61	0.56
RI	0.72	0.84	0.79
g_1	-0.593	-0.506	-0.560
PTP	0.001	0.001	0.001
Length of topology from alternative separate data set against this data set	622	855	n/a

TABLE 4. Portions of *matK* sequences with potentially phylogenetically informative insertions and deletions (indels) from representatives of Apiales. Letters (a-f) correspond to those discussed in the text. Base-pair positions correspond to those of the published sequences of mustard and tobacco (Neuhaus and Link, 1987). Straight dashes represent alignment gaps; crooked dashes represent deletions; bases in boldface and italics represent insertions; question marks represent missing data.

Taxon	Nucleotide sequence					
	a	b	c	d	e	f
<i>Aralidium</i>	8 9 0	9 0 0	9 5 0	9 9 0	1 3 7 0	1 5 3 0
<i>Griselinia</i>	GATCATGGTTTA	-----AATA	TATGACATATAAATCC	CG? ?TG-----TAT	AGTCTTATTAATCACTCCAAA	GAAAACTTTGTACTTT
<i>Melanophylla</i>	GATCATGGTTTA	-----AATA	TATGACAAATAAATCC	CGAATG-----TAT	AGTCTTATTAATCACTCCAAA	GAACAT-----CCTT
<i>Pitiosporum</i>	GATCATGGTTTA	-----AATA	TATGACAAATAAATCC	C?AATG-----TAT	AGTCTTATTAATCACTCCAAA	GAACAT-----CCTT
<i>Sollya</i>	GATCATGGTTTA	-----AATA	TATGACAAATAAATCC	CAAATG TATCAGTAT	ACT-----CCAAA	GAACAT-----CCTT
<i>Hymenosporum</i>	GATCATGGTTTA	-----AATA	TATGACAAATAAATCC	CAAATG TATCAGTAT	ACT-----CCAAA	GAACAT-----CCTT
<i>Delabrea</i>	GATCATGGTTTA	-----AATA	TATGACAAATAAATCC	CAAATG TATCAGTAT	ACT-----CCAAA	GAACAT-----CCTT
<i>Mackinlaya</i>	GATCATGGTTTA	-----AATA	TATGACAAATAAATCC	CGAA?G TATCAGTAT	ACT-----CCAAA	GAACAT-----CCTT
<i>Centella</i>	GATCATGGTTTA	-----AATA	TATGACAAATAAATCC	CGAATG TATCAGTAT	ACT-----TCAAA	GAACAT-----CCTT
<i>Micropleura</i>	GATCATGGTTTA	-----AATA	TATGACAAATAAATCC	CGAATG TATCAGTAT	ACT-----CCAAA	GAACAT-----CCTT
<i>Hydro. bowl.</i>	GATCATGGTTTA	AATAGAAATA	TATGACAAATAAATTC	CGAATG TATCAGTAT	ACC-----CCAAA	AACTAT-----CCTT
<i>Hydro. vert.</i>	GATCATGGTTTA	AATAGAAATA	TATGACAAATAAATTC	CGAATG TATCAGTAT	ACC-----CCAAA	AACTAT-----CCTT
<i>Aralia</i>	GATCATGGTTTA	AATAGAAATA	TATGACAAATAAATTC	CGAA?G-----TAT	ACT-----CCAAA	AAATAT-----CCTT
<i>Panax</i>	GATCATGGTTTA	AATAGAAATA	TATGACAAATAAATTC	CGAATG TATCAGTAT	ACT-----CCAAA	AAATAT-----CCTT
<i>Dendropanax</i>	GATCATGGTTTA	AATAGAAATA	TATGACAAATAAATTC	CGAATG TATCAGTAT	ACT-----CCAAA	AAATAT-----CCTT
<i>Reynoldsia</i>	GATCATGGTTTA	AATAGAAATA	TATGACAAATAAATTC	CGAATG TATCAGTAT	ACT-----CCAAA	AAATAT-----CCTT
<i>Coriandrum</i>	GATCATGGTTTA	AATAGAAATA	TATGACAAATAAATTC	CGAATG TATCAGTAT	ACT-----CCAAA	AAATAT-----CCTT
<i>Petroselinum</i>	GATCATGGTTTA	AATAGAAATA	TCT~--AATAAATTC	CGAATG TATCAGTAT	ACT-----CCAAA	GAAC?CTTTGTACTTT

TABLE 5. Insertions in the region of the stop codon (in boldface and italics) in the *rbcL* sequences from representatives of the Apialian Alliance. The stop codon TAA is found at codon position 476 (bp positions 1426–1428) in all taxa sampled except *Mackinlaya*, *Delarbraea*, *Micropleura*, *Centella*, *Panax*, and *Aralia*, which have insertions in this region (in minuscules). Dashes represent alignment gaps.

Taxon	Codon position									
	470	471	472	473	474	475	---	---	---	476
Typical	NNN	NNN	NNN	NNN	NNN	NNN	---	---	---	TAA
<i>Melanophylla</i>	CAA	GCA	ATG	GAT	ACT	TTG	---	---	---	TAA
<i>Sollya</i>	CAA	GCA	ATG	GAT	ACT	TTG	---	---	---	TAA
<i>Kalopanax</i>	GCC	GCA	GTG	GAT	GTT	TTG	---	---	---	TAA
<i>Apium</i>	CAA	GCA	ATG	GAT	ACN	TTG	---	---	---	TAA
<i>Sanicula</i>	GCC	GCA	ATG	GAT	ACN	TTG	---	---	---	TAA
<i>Hydrocotyle</i>	CAA	GCA	GTG	GAT	ACT	ATT	---	---	---	TAA
<i>Mackinlaya</i>	GAA	GCA	ATG	GAT	ACT	TTG	gac	---	aaa	TAA
<i>Delarbraea</i>	GAA	GCA	ATG	GAT	ACT	TTG	gat	---	aaa	TAA
<i>Micropleura</i>	GAA	GCA	ATG	GAT	ACT	TTG	gac	gta	aaa	TAA
<i>Centella</i>	GCC	GCA	GTC	GAT	ACT	TTG	gac	aaa	aaa	TAA
<i>Panax</i>	GAA	GCA	GTG	GAT	ATT	TTG	gat	ggt	gtg	TAA
<i>Aralia</i>	GAA	GCA	ATG	GAT	ATN	TTG	gat	---	---	TAA

ters (=12.0% of the 1398 characters used in phylogenetic analysis) were potentially informative (Table 3). Comparison of sequence divergence rates based on percentage of informative characters indicates that in Apiales, *matK* evolves 1.98 times faster than *rbcL*. An average value of 1.98 (range of 0.4–3.7) was also estimated on the basis of distance comparisons of 30 pairs of taxa from both data sets. These values indicate that *matK* evolves more slowly in Apiales than in Saxifragaceae sensu stricto, where *matK* evolves 3.1 times faster than *rbcL* (Johnson and Soltis, 1995). The range of distance values resulting from pairwise comparisons of all 47 taxa was 1.4–6.0% for the *rbcL* data set, and 0–14.0% for the *matK* data set. Although *matK* generally evolves faster than *rbcL*, among a number of taxa from Araliaceae, rates are nearly equal (e.g., between *Meryta* and *Polyscias*, *matK* evolves only 1.2 times faster than *rbcL*), and in some cases, *matK* evolves more slowly than *rbcL* (e.g., between *Kalopanax* and *Reynoldsia*, *rbcL* evolves 1.4 times faster than *matK*). Such “reversals” in substitution rates were not found in comparisons involving any taxa outside Araliaceae.

Other comparisons among the data sets—For direct comparison of a number of variability and phylogenetic measures, data sets using the same 47 taxa were analyzed phylogenetically. For each of the three data sets (*rbcL*, *matK*, and combined), the following measurements were calculated using PAUP* and MacClade: the length of the most parsimonious trees, the sum of minimum possible lengths, the consistency index (CI, excluding uninformative characters), the retention index (RI), the number of potentially informative characters, and the *g*₁ and PTP statistics (Table 3). Codon position and transition to transversion ratios were also estimated for each of the separate data sets using the resulting MR trees. The most parsimonious trees from the analysis of 47 *rbcL* sequences were 575 steps long, of which 148 steps were first-position substitutions, 95 were second-position changes, and 332 were third-position changes (1.56:1:3.49). The short-

est trees resulting from the *matK* analysis were 796 steps, of which 247 were first-position substitutions, 205 were second-position changes, and 344 were third-position changes (1.20:1:1.68). Despite the disparity in the ratios of third-position substitutions, the absolute value of these changes in both the *rbcL* and *matK* data sets is quite similar. This suggests that the increased variability in *matK* is due largely to a relaxation of selection pressures on first- and second-codon positions in that gene. Transition to transversion ratios between the data sets were more similar: 1.18:1 for *rbcL* and 1.13:1 for *matK*. CI (and RI) values were similar for the three analyses (Table 3), and in all cases, they were considerably higher than the values observed by Sanderson and Donoghue (1989) for data sets of this size (their regression equation for 47 taxa produces an expected CI of 0.337). Skewness tests for each data set suggest that all three contain significant amounts of nonrandom structure. The g_1 statistic was -0.59 for the *rbcL* data set, -0.51 for the *matK* data set, and -0.56 for the combined data set; all g_1 values are significant at $P < 0.01$ (Hillis and Huelsenbeck, 1992). Permutation tests yielded a value of 0.001 for all three data sets, indicating that the data are significantly different from randomized data above the 99% confidence level.

Phylogenetic reconstructions—*Phylogeny estimation based on the matK data set*—Phylogenetic analysis of nucleotide substitutions from 49 *matK* sequences yielded over 30 000 most parsimonious trees (see above), each of 805 steps. The strict consensus of these trees (Fig. 2) fails to resolve a dichotomy between Pittosporaceae and Apiales, and depicts a basal polytomy of four clades instead. The first clade comprises all representatives from Pittosporaceae and is supported by a bootstrap value (BS) of 100% and decay index value (DI) of 21. A second clade contains only the araliad *Delarbraea*. A third clade unites the two species of *Hydrocotyle* (from Apiaceae subfamily Hydrocotyloideae) and all but two araliads (viz., *Delarbraea* and *Mackinlaya*), supported by a BS of 97% and a DI of 8. Within this third clade, the araliads form a single subclade (BS = 80%, DI = 4), hereafter referred to as “core Araliaceae.” The large number of most parsimonious trees resulting from this analysis was due in large part to the lack of resolution within this subclade. The fourth major clade in the strict tree comprises most members of Apiaceae (excepting only *Hydrocotyle*) and the araliad *Mackinlaya*. Although confidence levels for the fourth clade are rather low (BS = 42%, DI = 1), support for its two constituent subclades is much stronger. One subclade unites the hydrocotyloid genus *Eremocharis* and all taxa from apiaceous subfamilies Apioideae and Saniculoideae (BS = 100%, DI = 6), hereafter referred to as “core Apiaceae.” The second subclade includes the two remaining hydrocotyloids, *Centella* and *Micropleura*, with the araliad *Mackinlaya* (BS = 98%, DI = 7) and is labeled the “*Mackinlaya* group” in Fig. 2. The analysis of the *matK* data set in which the six potentially informative indels were coded as binary characters yielded a strict tree nearly identical to that of the analysis of nucleotide substitutions alone; the only major difference was the collapse of the sister group relationship between core Apiaceae and the *Mackinlaya* group, leaving five

(instead of four) major clades in the basal polytomy (arrow in Fig. 2).

Phylogeny estimation based on the combined matK-rbcL data set—Phylogenetic analysis of the combined data set of *matK* and *rbcL* sequences from 48 representative members of Apiales yielded 1 512 most parsimonious trees, each of 1 408 steps, distributed on a single island (sensu Maddison, 1991). The strict consensus of these trees (Fig. 3) reveals a polytomy of three major clades, and again fails to resolve the Pittosporaceae-Apiales dichotomy (although Pittosporaceae are sister group to Apiales in 50% of the most parsimonious trees). The Pittosporaceae clade is supported by a BS of 100% and a DI of 35. A second clade corresponds exactly to the core Apiaceae clade from the *matK* analysis (BS = 100%, DI = 8). The identical *Mackinlaya* group is also found in the combined tree, but it does not form the sister group to core Apiaceae as in the *matK* topology. Instead, it is found within the third major clade, which also includes core Araliaceae, the two species of *Hydrocotyle*, and *Delarbraea*. Support for the branch uniting all of these taxa into a single clade, however, is low (BS = 34%, DI = 1). Within this clade, there are two subclades: (1) the core Araliaceae plus the two species of *Hydrocotyle* (BS = 99%, DI = 9); and (2) the *Mackinlaya* group (BS = 100%, DI = 12) united to *Delarbraea* (BS = 41%, DI = 1). Adding indels as binary characters to the combined data set further resolved only one terminal trichotomy (Fig. 3).

Incongruence among the data sets—Levels of incongruence among the data sets were relatively low. Calculation of I_{MF} requires two values from each of the three analyses: the minimum possible length and the length of the most parsimonious trees (Table 3). For the three Apiales data sets, I_{MF} was determined to be 4.6%. Table 3 also provides the additional values needed to calculate I_M (i.e., the length of the *rbcL* topology when examined against the *matK* data set, and the length of the *matK* topology when examined against the *rbcL* data set). Due to practical limitations (see above), the MR tree resulting from each of the separate analyses was used to estimate these lengths. Calculation of I_M yielded an estimate of 18.9% incongruence.

The strict consensus trees resulting from the *rbcL* and *matK* analyses of the same 47 taxa were used to generate “total” strict and semistrict consensus trees (Fig. 4). In the total strict tree, only one major clade, core Apiaceae, is well resolved. Several other smaller clades (e.g., the *Mackinlaya* group, Pittosporaceae) are also resolved, but basal relationships are completely obscured. The semistrict tree (=“combinable-component consensus,” see Swofford, 1991) resolves groups even if they are not fully dichotomous in some trees, as long as they are not contradicted in any of the trees. Although this approach is less conservative, it does permit greater resolution to help identify areas of possible congruence. The topology of the semistrict tree shows five major clades: Pittosporaceae, *Delarbraea*, the *Mackinlaya* group, *Hydrocotyle*-core Araliaceae, and core Apiaceae.

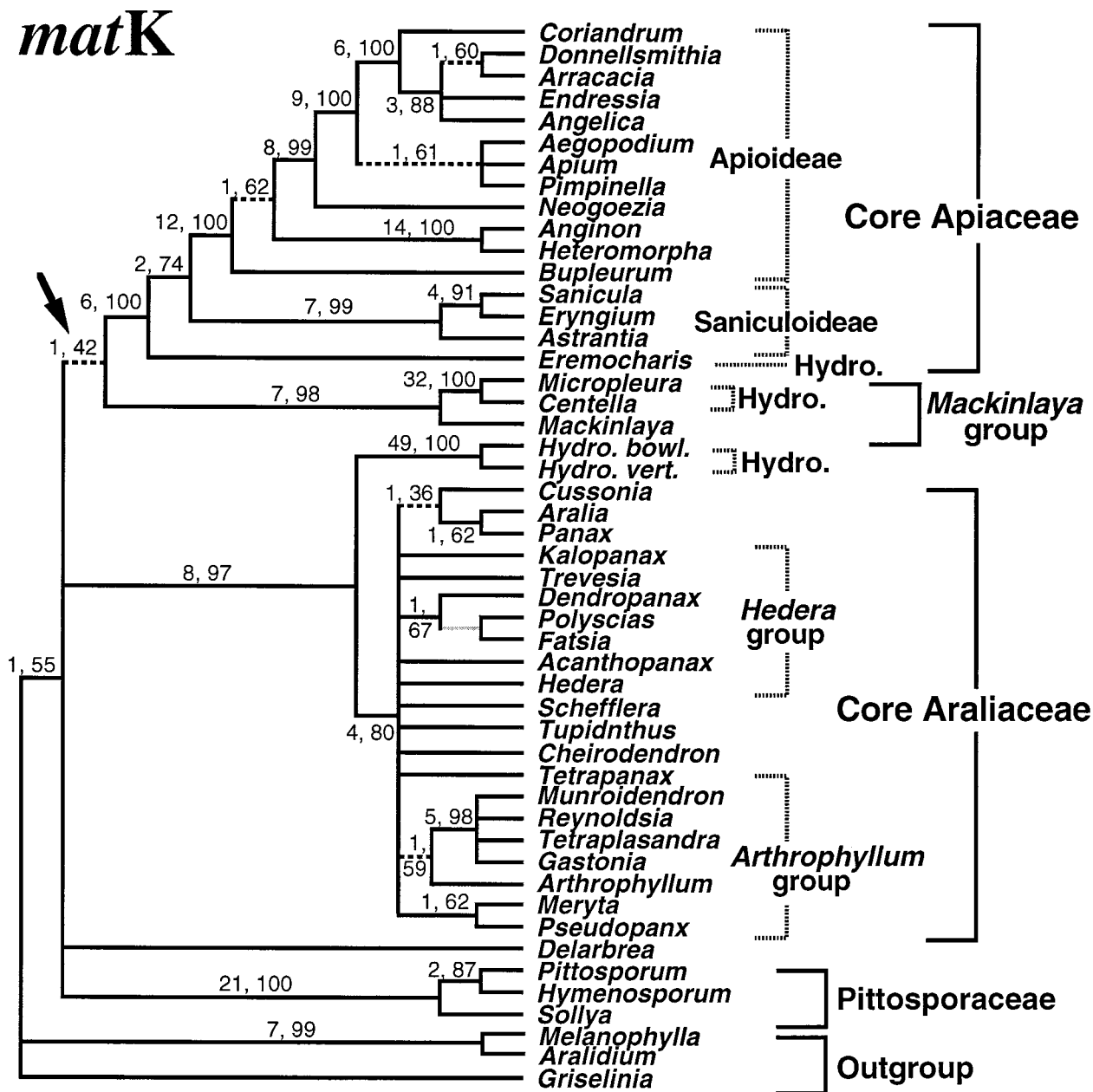


Fig. 2. Strict consensus of 30000 of the most parsimonious trees based on phylogenetic analysis of *matK* nucleotide substitutions from 49 taxa. Decay values (first) and bootstrap percentages (second) are mapped along each branch. Major groups discussed in the text are bracketed and labeled (subfamily Hydrocotyloideae is abbreviated "Hydro.>"). The dashed branches represent clades that collapse when indels were coded as binary characters and added to the data set; the arrow points to the collapse of the clade uniting the *Mackinlaya* group and core Apiaceae. The grey branch (leading to *Polyscias* and *Fatsia*) is the only clade to collapse when indel characters were not included.

DISCUSSION

Comparison of cladograms I: *matK* vs. *rbcL* analyses—The topologies of the *matK* and *rbcL* cladograms agree on several major points. Both trees show that apiaceous subfamilies Apioideae and Saniculoideae form distinct clades and that, with the hydrocotyloid *Eremocharis*, these form a monophyletic group that includes only members of Apiaceae (the core Apiaceae). Both trees also show two clades of hydrocotyloids outside of core Apiaceae and allied instead to araliaceous taxa (*Centella* and

Micropleura to *Mackinlaya*, and the two species of *Hydrocotyle* to either core Araliaceae or a subset of these araliads). These will be referred to as "araliaceous hydrocotyloids" to differentiate them from those found within core Apiaceae. Both *matK* and *rbcL* data, therefore, suggest that subfamily Hydrocotyloideae is polyphyletic, a result that is not surprising given the paucity of unique morphological characters defining this group (see Plunkett, Soltis, and Soltis, 1996). Among the araliads are several clades that are supported by both the *matK* and *rbcL* cladograms, including the alliance of (1)

Combined

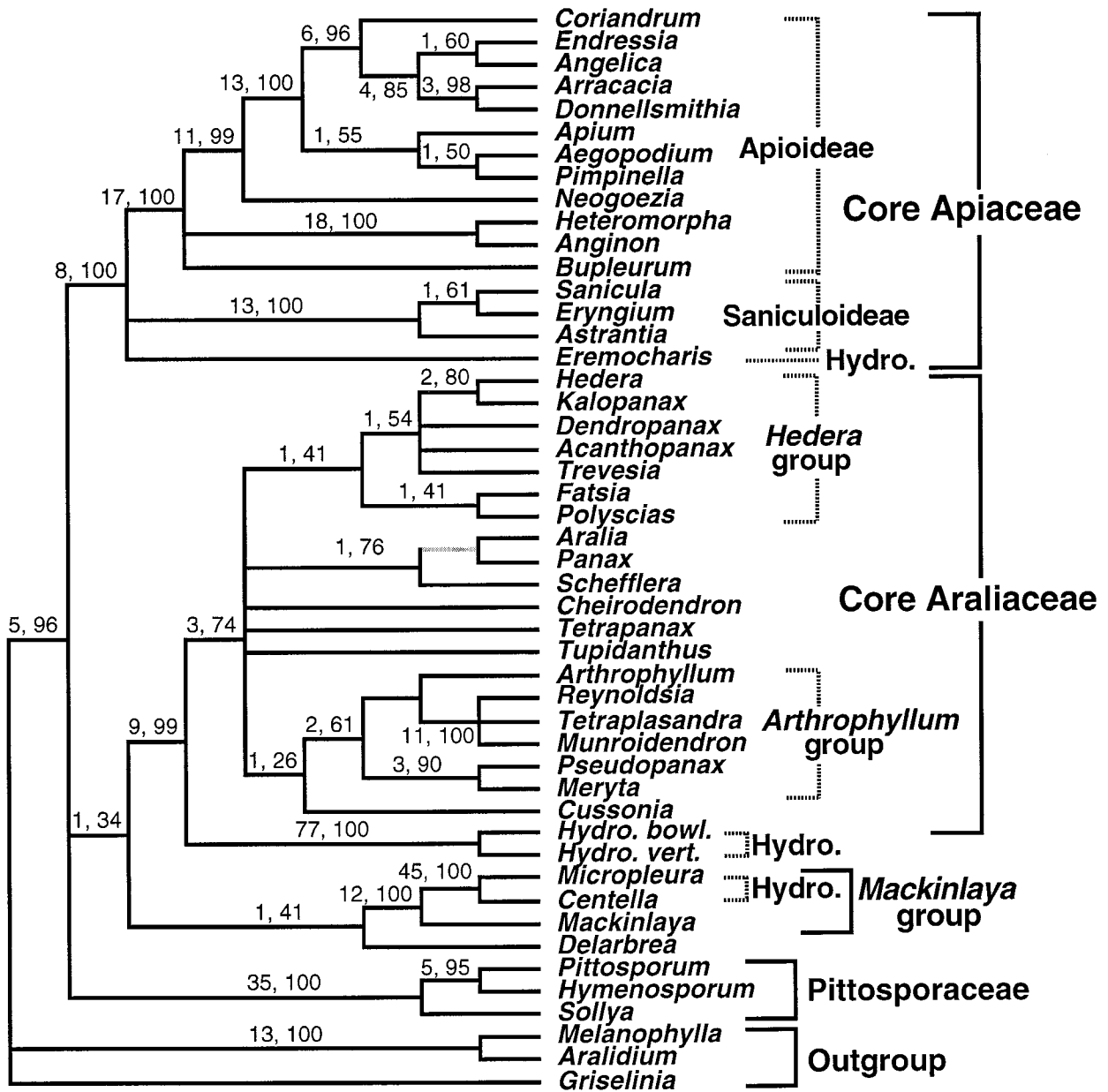


Fig. 3. Strict consensus of the 1512 most parsimonious trees based on the combined data set of nucleotide substitutions from *rbcL* and *matK* sequences from 48 taxa. Decay values (first) and bootstrap percentages (second) are mapped along each branch. Major groups discussed in the text are bracketed and labeled (subfamily Hydrocotyloideae is abbreviated "Hydro."). The grey branch (leading to *Aralia* and *Panax*) represents the only additional clade to be resolved when *matK* indels were included in the data set as binary characters.

Munroidendron, *Reynoldsia*, *Tetraplasandra*, and *Arthrophyllum*; (2) *Aralia* and *Panax*; and (3) *Meryta* and *Pseudopanax*.

The only area of substantial incongruence between the two analyses is found among the members of Araliaceae. In both cladograms, core Apiaceae forms a monophyletic group, but in the *rbcL* cladogram, it forms part of a pentachotomy (the other four of which are all araliads). Sister to this polytomy is a sixth clade (again of araliads). This topology suggests that Apiaceae are derived from within

a paraphyletic Araliaceae. In the *matK* cladogram, however, all araliads, with the exception of *Mackinlaya* and *Delarbrea*, form a single clade (core Araliaceae). As discussed previously (above, and in Plunkett, Soltis, and Soltis, 1996), support for the nodes in the *rbcL* tree that portray a paraphyletic Araliaceae is very weak, and trees supporting a monophyletic Araliaceae are only two steps longer than the most parsimonious *rbcL* trees. Further, when the *rbcL* data set is weighted according to codon position (which is strongly biased in *rbcL*), all members

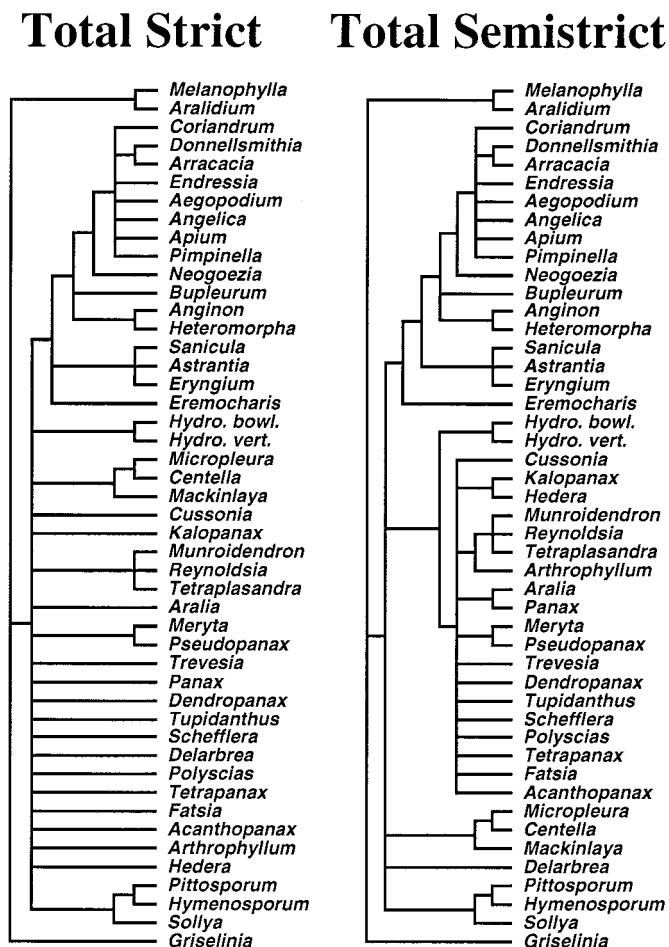


Fig. 4. "Total" strict and semistrict trees resulting from the consensus of strict trees from the *matK* and *rbcL* analyses of 47 taxa.

of Araliaceae (except *Mackinlaya*) form a single clade (tree not shown). Given these facts, and the strong support for the monophyletic core Araliaceae-*Hydrocotyle* group in the *matK* tree (BS = 97%, DI = 8), incongruence between the two data sets is more likely due to a lack of sufficient characters in this region of the *rbcL* cladogram.

Comparison of cladograms II: combined vs. separate analyses—After the separate analyses, the two data sets were combined and analyzed together. Combining data sets is considered controversial and potentially misleading if there is nonindependence of the data sets (e.g., through genome exchange or lateral transfer of genes), or if the data are from diverse sources and/or produce heterogeneous results when analyzed separately (see Bull et al., 1993; de Queiroz, 1993; Huelsenbeck et al., 1994; de Queiroz, Donoghue, and Kim, 1995; Miyamoto and Fitch, 1995; but see also Kluge, 1989; Chippindale and Wiens, 1994). These issues are not problematic for *matK* and *rbcL* data sets from Apiales because both genes are from the chloroplast genome, in which recombination is unknown, and because natural hybridization is virtually unknown in Apiales (see Bell, 1971; Heywood, 1978). Together, these features make both genome exchange

("chloroplast capture") and lateral transfer of genes extremely unlikely. Moreover, as Bull et al. (1993) point out, data sets can be combined as long as the null hypothesis of homogeneity is not rejected, allowing for low or even modest amounts of heterogeneity. Statistical measurements of homogeneity remain elusive, but in the present study, incongruence values ($I_{MF} = 4.6\%$; $I_M = 18.9\%$) and visual inspection of the total strict and semistrict trees suggest that the *rbcL* and *matK* data sets are not overly heterogeneous. Therefore, the concerns of Bull et al. (1993) and Huelsenbeck et al. (1994) that the different data sets may represent "different evolutionary histories" is a condition that is unlikely for two chloroplast genes, especially in this order and at this level of phylogeny.

Concern has also been raised regarding differential rates of character evolution between data sets (see Bull et al., 1993; Chippindale and Wiens, 1994). This is a problem, however, only where the rate of the more quickly evolving characters is inappropriate for the taxonomic level of inquiry; such characters could produce large amounts of unreliable data that would swamp out the more slowly evolving (and hence more reliable) characters when combined (Huelsenbeck et al., 1994). This concern also does not apply to the *rbcL* and *matK* data sets in Apiales. Although the rate of sequence divergence of *matK* is approximately two times faster than that of *rbcL*, *matK* is still highly conserved (14% divergence between the most divergent genera). This fact, coupled with the congruence between the two data sets in most regions of the trees, indicates that the results from the analysis of the *matK* data set are not spurious. Again, the only major area of discordance between the two data sets centers on the weakly supported result of a paraphyletic Araliaceae in the *rbcL* cladogram, and (as discussed above) this is likely due to insufficient characters in this region of the *rbcL* tree. This suggests that *matK* is not evolving too rapidly for this level of study, but rather that *rbcL* is providing too few characters. Thus, the differential rates of evolution between *rbcL* and *matK* are not problematic in Apiales, and the two data sets should be suitable for combination.

The results from the combined analysis largely agree with those of the separate analyses. For example, as was observed in the *rbcL* and *matK* trees, the combined cladogram (Fig. 3) reveals a well-supported, monophyletic core Apiaceae (Apioideae, Saniculoideae, and *Eremocharis*), a polyphyletic Hydrocotyloideae (with two of the three clades allied with araliads), and a number of similar araliaceous clades (e.g., *Munroidendron-Reynoldsia-Tetraplasandra-Arthropphyllum*, *Aralia-Panax*, and *Meryta-Pseudopanax*). The relationship between core Apiaceae and core Araliaceae is nearly identical to that found in the *matK* cladogram, showing a monophyletic core Araliaceae allied with *Hydrocotyle*, but excluding the araliads *Delarbrea* and *Mackinlaya*. Within core Araliaceae, there are also two clades that correspond largely to clades found in the *rbcL* tree (labeled "Arthropphyllum group" and "Hedera group" in Figs. 1, 3); these groups were only partially resolved in the *matK* cladogram.

Unlike either of the separate analyses, however, the cladogram from the combined analysis shows a single clade that unites all members of Araliaceae and the ar-

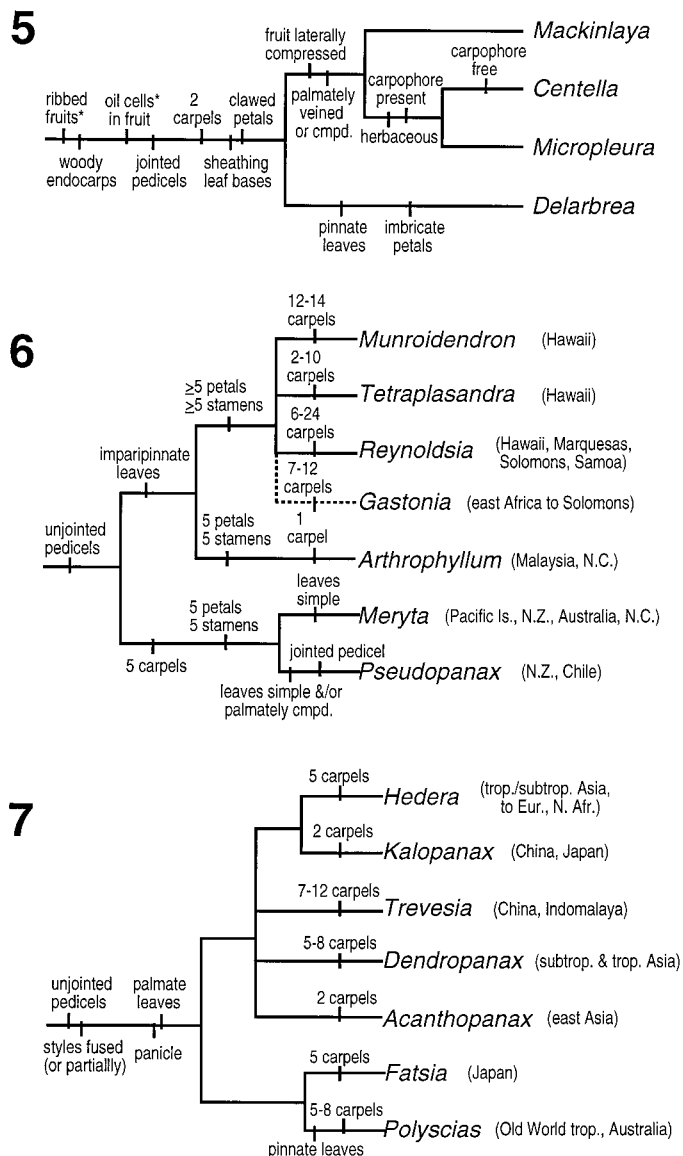
aliaceous hydrocotyloids. This clade comprises two subclades (Fig. 3): the *Hydrocotyle*-core Araliaceae clade, and a clade uniting *Delaribrea* and the *Mackinlaya* group (see below for discussion). The three analyses, therefore, present three different treatments of Araliaceae: (1) the *rbcL* cladogram suggests that Araliaceae are paraphyletic, and Apiaceae are derived from within Araliaceae; (2) the *matK* cladograms suggest that core Araliaceae are monophyletic, but leaves the relationship between Araliaceae and Apiaceae unresolved; and (3) the combined cladogram suggests that all araliads (plus the araliaceous hydrocotyloids) form a monophyletic group. Huelsenbeck et al. (1994) described a hypothetical scenario (similar to the result in Apiales) in which a combined data set could produce a topology not found in either of the individual data sets. In their example, both of the separate data sets may contain phylogenetic signal that reflects the actual relationships of the organisms as well as conflicting signals that do not. When the data sets are combined, the “real” signals from each data set are additive, but the conflicting noise may be canceled out. Olmstead and Sweere’s (1994) study of three molecular data sets (cpDNA restriction site data, and data from *rbcL* and *ndhF* sequences) from Solanaceae supports this scenario. In that study, the three possible pairwise combinations of the data sets were analyzed. In all cases, the topologies resulting from these analyses produced clades not found in the trees resulting from the separate analyses of the two data sets involved. These unique clades were found, however, in the topologies resulting from the separate analysis of the third data set, and from the combined analysis of all three data sets. In Apiales, both the *matK* and combined cladograms show the core Araliaceae-*Hydrocotyle* clade and the near-basal positions of *Delaribrea* and the *Mackinlaya* group; because the *rbcL* data set was weakly supported in this region of the tree (discussed above), we consider the results of the *matK* and combined analyses more robust.

Interfamilial relationships—The addition of *matK* sequence data to the previously existing *rbcL* data set has helped to resolve two large clades within Apiales (core Apiaceae and core Araliaceae) that correspond largely to the traditional familial circumscriptions. Thus, the vast majority of Araliaceae are monophyletic with regard to Apiaceae. Aside from the clearly polyphyletic hydrocotyloids, *Delaribrea* and the *Mackinlaya* group represent the only exceptions to the two-family system. Moreover, these two lineages represent the least stable regions in the three cladograms (cf. Figs. 1–3), suggesting that their precise placement within the order may be very important in understanding the early evolution of Apiales. A closer examination of a variety of characters may be useful in inferring the relationships involving these genera. For example, the distribution of insertions and deletions (indels) among these taxa provides a picture of relationships not entirely clear from analysis of nucleotide substitutions alone. Because the treatment of indels in phylogenetic analysis remains controversial, we took conservative approaches in analyzing these characters (see above). However, indels are relatively rare events in *matK* and *rbcL* sequences. Thus, even when added to the data matrix as binary characters, they had only equal weight to the nu-

cleotide substitutions, perhaps an underweighting of such relatively rare characters. An a posteriori examination of indel distributions may provide some additional insights into basal relationships. The outgroup taxa (*Melanophylla*, *Griselinia*, and *Aralidium*) all share three identical *matK* indels (labeled *b*, *d*, and *e* in Table 4). These indels can be interpreted as plesiomorphic because they are also found in *matK* sequences from Cornales (Q. Xiang, Ohio State University, personal communication), which are sister to the Apiallean Alliance-Asteridae (sensu stricto) clade in the “expanded Asteridae” of Olmstead et al. (1992, 1993). One of these indels (*b*) is also found in *Delaribrea*, *Mackinlaya*, *Centella*, and *Micropleura*, and the three genera of Pittosporaceae. Thus, the distribution of plesiomorphic *matK* indels suggests that *Delaribrea* and the taxa of the *Mackinlaya* group represent early lineages within Apiales.

Indels in the *rbcL* sequences of *Delaribrea*, *Mackinlaya*, *Centella*, and *Micropleura* also suggest a close relationship among these four taxa. The stop codon for *rbcL* is usually found at codon position 476 (bp positions 1426–1428), but in *Delaribrea*, *Mackinlaya*, *Centella*, and *Micropleura*, there are insertions of six or nine bp at this position (Table 5). *Mackinlaya* and *Delaribrea* share a six-bp insertion of similar sequence (GAC/T AAA), and *Centella* and *Micropleura* appear to have each gained an additional triplet (AAA and GTA, respectively). The terminal 20–50 bp of the previously published sequences from three of the outgroup taxa (*Toricellia*, *Aralidium*, *Griselinia*) are unknown, but the stop codon was in the normal position in *Melanophylla* and the three genera of Pittosporaceae, suggesting that the *rbcL* insertions are synapomorphic. Only two other genera within Apiales, *Aralia* and *Panax*, have additional codons at this position, but these insertions lack the triplet AAA (Table 5) that is characteristic of the insertions of *Mackinlaya*, *Delaribrea*, *Centella*, and *Micropleura*, suggesting that the *rbcL* insertions in *Panax* and *Aralia* represent an independent event. Thus, despite the fact that *Delaribrea* and the *Mackinlaya* group represent the least stable branches in the cladograms, the molecular data taken as a whole (including indels) suggest that *Delaribrea*, *Mackinlaya*, *Centella*, and *Micropleura* are closely related and may represent the remnants of an ancestral lineage within Apiales. Moreover, moving these taxa to a basal position in the combined cladogram produces a tree only two steps longer than the most parsimonious trees.

The potentially pivotal role of *Delaribrea* and the *Mackinlaya* group in understanding the early evolution of Apiales is reinforced when the unusual suite of morphological characters found in these taxa is examined (Fig. 5). Both genera share a number of features that are unusual in Araliaceae as traditionally defined, but are characteristic of the hydrocotyloids *Centella*, *Micropleura*, and the rest of Apiaceae. These include bicarpellate gynoecia, clawed petals, and sheathing leaf bases (Philipson, 1951; Lowry, 1986). In *Mackinlaya*, the fruit exhibits strong lateral compression (Philipson, 1951). The fruits of *Delaribrea*, and of its close relatives *Myodocarpus* and *Pseudosciadium* (not analyzed herein), are characterized by ribs and large secretory oil ducts (Lowry, 1986). Although the drupes of *Delaribrea* are only slightly compressed (Smith and Stone, 1968), *Myodocarpus*



Figs. 5–7. Individual clades from the strict consensus tree resulting from the combined analysis along which morphological and geographical characters of the genera are mapped. 5. The “*Mackinlaya* group” and *Delarbraea*; asterisks indicate character states unknown in *Mackinlaya*. 6. The “*Arthrophyllum* group;” *Gastonia* (dashed branch) has been included on the basis of *matK* data alone. 7. The “*Hedera* group.”

produces schizocarps that are strongly flattened and have free carpophores (Baumann, 1946). Together with the molecular characters, the mixture of araliaceous vegetative features with typically apiaceous floral and fruit morphology strongly suggests that the *Mackinlaya* group and *Delarbraea* may represent a basal lineage (or lineages) within Apiales.

That the *Mackinlaya* group and *Delarbraea* occupy basal branches within Apiales also agrees with the revised concepts of ancestral vs. derived character states in Apiales proposed by Plunkett, Soltis, and Soltis (1996). By examining trends in morphological evolution based on phylogenetic analysis of *rbcL* sequences, it was evident that all or most of the close relatives of Apiales were

bicarpellate, woody, paleotropical, and polygamous. All of these features also characterize *Mackinlaya* and *Delarbraea*. Additionally, laterally compressed fruits may also be ancestral. Baumann (1946) considered the compressed fruits found in *Myodocarpus* and *Hydrocotyle* to represent the ancestral state within Apiales, but Tseng (1967) dismissed this proposal because he felt that *Hydrocotyle* was highly derived. Furthermore, he found it hard to envision the terete fruits found in both Apiaceae and Araliaceae as evolving from compressed ones. Although laterally compressed fruits are not common in the relatives of Apiales, they are found in some members of Pittosporaceae, and their presence in *Mackinlaya*, *Myodocarpus*, and to some degree in *Delarbraea*, as well as several other araliads (e.g., *Seemannaralia* [Burt and Dickison, 1975] *Tieghemopanax*, *Nothopanax*, *Acanthopanax*, *Astrotricha*, and *Stilbocarpa* [Tseng, 1967]), and in numerous genera of Apiaceae, indicates that Baumann may have been correct.

Simple leaves were also considered ancestral by Plunkett, Soltis, and Soltis (1996), and this feature provides a classic example of how morphological parallelisms have plagued inferences of phylogeny in Apiales. Simple leaves are found among all the relatives of Apiales (i.e., Pittosporaceae, *Griselinia*, *Aralidium*, and *Melanophylla*), and even among the basally branching clades of Apiaceae (e.g., *Bupleurum* and *Heteromorpha*; Figs. 1–3). However, because leaf shape is highly variable among the genera of Araliaceae, and is often variable even within species (Philipson, 1951, 1970a), it is thus difficult to establish both homology and polarity. For example, *Delarbraea* is characterized by imparipinnate (oddly pinnate) leaves (Lowry, 1986), but the species of its close relative *Myodocarpus* show a progression in leaf shape from simple to pinnately lobed, to pinnately compound (G. M. Plunkett, personal observation). Similarly, *Mackinlaya* is usually described as having palmately compound leaves, but the leaves of some species are simple or merely lobed (although Philipson [1951] interpreted these as “reduced”). Among other species of *Mackinlaya*, Philipson (1951) noted that the central leaf lobes may in turn become compound, forming what he termed “pseudo-pinnate” leaves, a condition that approaches the imparipinnate leaves found in *Delarbraea* and its relatives. Future phylogenetic work involving additional species of *Mackinlaya* and other related araliads will be needed to understand fully the ancestral state of araliaceous leaves. The progression from simple to lobed to fully divided leaves within two potentially ancient lineages, however, suggests a possible transition from the simple leaves of the relatives of Apiales to the typically palmate leaves of *Mackinlaya*, to the imparipinnate leaves of *Delarbraea* and its relatives.

New hypotheses of relationships within Apiales derived from the analysis of the molecular data provide an opportunity to re-examine some of the seemingly conflicting claims of older studies regarding interfamilial relationships. Baumann (1946) proposed that *Myodocarpus* provided a link to Apiaceae through Hydrocotyloideae based on fruit similarities (bicarpellate, flattened schizocarp with free carpophore). In contrast, Rodríguez (1957), in his study of wood anatomy, concluded that the wood of *Myodocarpus* was very “primitive” and that the genus

represented a poor bridge to Apiaceae. *Myodocarpus* could not be included in the present study (despite several attempts to extract DNA from herbarium material of this New Caledonian endemic), but a growing body of evidence confirms that *Delarbraea* and the monotypic *Pseudosciadium* are its closest relatives (e.g., Lowry, 1986; Oskolski and Lowry, unpublished data). If these genera are indeed closely related, then the present study suggests that *Myodocarpus* (along with *Delarbraea*) occupies a basal branch within Apiales rather than a “bridge” between Apiaceae and Araliaceae. Baumann’s theory of progression from *Myodocarpus* into the Hydrocotyloideae does, however, make sense given the placement of the hydrocotyloids *Micropleura* and *Centella* near *Delarbraea* in the combined cladogram. His assertion that *Myodocarpus* represents an intermediate between Apiaceae and Araliaceae was doubtlessly influenced by his assumption that Hydrocotyloideae were a monophyletic group within Apiaceae, an assumption not supported by molecular evidence. Rodríguez (1957), rejecting *Myodocarpus* as a link, proposed that the araliaceous tribe Mackinlayineae (*Mackinlaya* and the segregate genus *Anomopanax*) represented the best example of an extant intermediate group between the two families. The placement of *Mackinlaya* and *Delarbraea* within the molecular cladograms, however, suggests that neither should be viewed as intermediates along a phyletic progression from Araliaceae (ancestral) to Apiaceae (derived). Rather, molecular and morphological characters support the idea that these genera may represent the remnants of an ancient group from which both families evolved. This agrees with Lowry’s (1986) phylogeographic assessment of *Delarbraea*, *Myodocarpus*, and *Pseudosciadium*, in which he describes these genera as relicts of an ancient lineage of araliads that survived in the “equable climates” of places like New Caledonia but not in the changing climates of Australia. Indeed, *Mackinlaya* and *Delarbraea* may be descendants of the hypothetical “pro-araliads” that both Baumann (1946) and Rodríguez (1957, 1971) described as the stock that gave rise to both Araliaceae and Apiaceae.

Other relationships in Apiales—In addition to resolving the interfamilial relationships within Apiales, the molecular cladograms also provide insights into monophyletic groups within each family. In the core Apiaceae, for example, all three data sets support the close relationship of Apioideae to Saniculoideae. Furthermore, two hydrocotyloids (*Eremocharis* and *Bowlesia*) form a lineage at the base of core Apiaceae. In fact, with the exception of the araliaceous hydrocotyloids (*Hydrocotyle*, *Centella*, *Micropleura*, and *Spananthe*), the molecular topologies resemble the traditional subfamilial treatment of Apiaceae by Drude (1898). In contrast, the topology within subfamily Apioideae seriously contradicts Drude’s tribal system. Although the sample of taxa from within Apioideae in the present study is relatively small (12 out of ≈250 genera), studies based on larger samples [based on ITS (Downie and Katz-Downie, 1996) and additional *matK* sequence data (Plunkett, Soltis, and Soltis, in press)] confirm the polyphyly of nearly all of Drude’s tribes (see Plunkett, Soltis, and Soltis, in press, for a com-

parison of relationships in Apiaceae based on *rbcL*, *matK*, and ITS data).

Within core Araliaceae, relationships among many of the taxa were not well resolved, but several genera were consistently allied. For example, the *rbcL* and *matK* data sets both show a close relationship between *Aralia* and *Panax*, even though the specific relationship of these taxa differs among the trees. In the *rbcL* tree, *Schefflera* and *Aralia* form a clade to which *Panax* is the sister group. In the *matK* tree, *Aralia* and *Panax* form a clade to which *Cussonia* is sister; the placement of *Schefflera* is unresolved. In the combined tree, *Aralia*, *Panax*, and *Schefflera* form a trichotomy. Indel distributions also support a relationship between *Panax* and *Aralia*. They both share an insertion at codon 476 (the normal stop codon position; Table 5) in their *rbcL* sequences, and lack an insertion (indel *d*; Table 4) found in the *matK* sequences of all other members of Apiales and Pittosporaceae (but not the outgroup taxa).

The *Arthropphyllum* group and the *Hedera* group (Figs. 1–3) provide good examples of the ability to interpret trends in morphological evolution with the use of phylogenetic trees, especially trees that are based on independent data sets (e.g., molecular characters). All three analyses reveal at least part of the *Arthropphyllum* group (*Pseudopanax*, *Meryta*, *Arthropphyllum*, *Reynoldsia*, *Tetraplasandra*, *Munroidendron*, and in the *matK* tree, *Gastonia*; see Fig. 6). In the *matK* tree, however, the relationship of *Meryta*-*Pseudopanax* to the other taxa is not resolved. Philipson (1970b) considered *Reynoldsia*, *Tetraplasandra*, *Munroidendron*, and *Gastonia* to be closely related based on the shared presence of two character states: unjointed pedicels and imparipinnate leaves. *Arthropphyllum* also shares these states, although the leaves in this genus are sometimes bipinnate or the upper leaves may become trifoliate or simple. The *rbcL* and combined analyses (see Figs. 1, 3) suggest that *Meryta* and *Pseudopanax* form the sister group to this clade. *Meryta* has unjointed pedicels, but its leaves are simple (Fig. 6). *Pseudopanax* differs in both characters, having jointed pedicels and palmately compound and/or simple leaves. The presence of simple leaves in *Arthropphyllum*, *Meryta*, and *Pseudopanax* suggests that simple leaves may have given rise to both imparipinnate leaves (in *Munroidendron*, *Reynoldsia*, and *Tetraplasandra*) and to palmate leaves (in *Pseudopanax*). The flowers of *Meryta* and *Pseudopanax* are pentamerous in all whorls, but among the other members of the *Arthropphyllum* group the flowers become more highly modified. The perianth and androecium of *Arthropphyllum* are pentamerous, but there is a reduction in carpel number to one. In the *Munroidendron*-*Tetraplasandra*-*Reynoldsia*-*Gastonia* clade, there are increases in the numbers of petals and stamens, and great variation in the number of carpels (see Fig. 6, and Philipson, 1970b). Phylogeographic distributions of these genera can also be traced along the cladogram. *Munroidendron* and *Tetraplasandra* are restricted to the Hawaiian Islands, and *Reynoldsia* is found from Hawaii south to the Marquesas, the Society Islands, and Samoa (Philipson, 1970b). *Gastonia* has a wider distribution, from eastern Africa east to the Solomon Islands. The distribution of *Arthropphyllum* extends from the Nicobar Islands (west of the Malay Peninsula) to New Caledonia.

Meryta is found from Australia, New Zealand, and New Caledonia east to the Pacific Islands, and *Pseudopanax* can be found from New Zealand to South America. Thus, the molecular cladograms confirm Philipson's (1970b) alliance of *Munroidendron*, *Reynoldsia*, *Tetraplasandra*, and *Gastonia*, and suggest that *Arthrophyllum*, and possibly *Meryta* and *Pseudopanax*, should be added to this group.

The members of the *Hedera* group (*Hedera*, *Kalopanax*, *Trevesia*, *Dendropanax*, *Acanthopanax*, *Fatsia*, and *Polyscias*) form nearly identical clades in both the *rbcL* and combined trees (Figs. 1, 3), but this clade is only partially supported by the *matK* tree (only *Dendropanax*, *Polyscias*, and *Fatsia* form a clade; Fig. 2). The genera of the *Hedera* group are united by the presence of four morphological characters (Fig. 7): (1) palmately lobed or compound leaves (except in *Polyscias*), (2) unjointed pedicels, (3) panicles, and (4) fused or partially fused styles (except in *Fatsia*). Phytogeographic patterns also support the alliance of these genera, all of which are native to subtropical Asia (especially China). Some taxa are more broadly distributed, such as *Polyscias*, which is found throughout the Old World tropics and Australia, and *Hedera*, which is found from tropical and subtropical Asia west to Europe and northern Africa. Others are more narrow in their distributions, such as *Fatsia*, which is restricted to Japan. However, the phytogeographic and morphological characters that help to unite the members of the *Hedera* group may also be found among other araliads. For example, unjointed pedicels are found not only in members of the *Hedera* group, but also in the *Arthrophyllum* group. Additionally, many genera outside the *Hedera* group have palmate leaves or a subtropical-Asian distribution. Thus, these clades provide a further example of the utility of molecular data in helping to interpret traditional sources of taxonomic information in Apiales.

The present study has provided new insights into the phylogenetic relationships within Apiales, suggesting that: (1) Hydrocotyloideae are polyphyletic, (2) *Delarbraea* and *Mackinlaya* may be the descendants of a very ancient lineage of pro-araliads that may have given rise to both Apiaceae and Araliaceae, (3) apart from *Delarbraea*, *Mackinlaya*, and the araliaceous hydrocotyloids (*Hydrocotyle*, *Centella*, and *Micropleura*) there are two major monophyletic groups within Apiales (core Apiaceae and core Araliaceae), which correspond to the traditional circumscriptions of the two families, (4) within core Apiaceae, Saniculoideae and part of Hydrocotyloideae are sister groups of subfamily Apioideae, and (5) older concepts of ancestral character states (e.g., carpel number, leaf shape, fruit shape) may need to be significantly revised. This study has also provided a broad conceptual framework that can be used as a starting point for future studies of some of the many questions left unaddressed here. For example, more intensive sampling from the relatives of *Delarbraea*, from among the hydrocotyloids, and from the Apioideae-Saniculoideae clade, coupled with morphological studies of these groups, will provide additional insights into the relationship between Apiaceae and Araliaceae.

Lastly, the present study sheds light on the ongoing controversy surrounding the taxonomic status of Api-

aceae and Araliaceae. Although the move to unite Apiaceae and Araliaceae into a single family (Thorne, 1973; Judd, Sanders, and Donoghue, 1994) does indeed reflect the evolutionary history of these groups, that common history is already reflected in the widely accepted ordinal circumscription (sensu Cronquist). In their cladistic study of morphological and anatomical characters from five members of Araliaceae and six of Apiaceae, Judd, Sanders, and Donoghue (1994) concluded that Apiaceae were derived from within Araliaceae, and combined the two groups into a single family, Apiaceae (sensu lato). This formal taxonomic change has recently been incorporated into various papers and texts (most notably Zomlefer, 1994), and it is, therefore, appropriate to evaluate their treatment in light of the data presented herein. Relationships among three of the five araliads in their cladogram (*Schefflera*, *Dendropanax*, and *Oreopanax*) are unresolved, and the derivation of Apiaceae from within Araliaceae results largely from the placement of a single taxon, *Aralia*, as basal to all taxa in Apiales. Moreover, this result is supported by only a single character, petal aestivation. Like Baumann (1946), they place a heavy emphasis on the link between Apiaceae and Araliaceae represented by *Myodocarpus*, which falls out as sister group to Apioideae-Saniculoideae in their cladogram. This relationship is heavily influenced by interpretation of bicarpelly and vittae (oil canals in the fruit not associated with vascular tissue) as derived characters within Apiales, and upon the homology of the schizocarpic fruits of *Myodocarpus* and Apiaceae. Evidence from a number of lines, however, suggests that these assumptions should be seriously questioned. Molecular evidence, for example, suggests that bicarpelly was ancestral within Apiales, not derived. Further, the schizocarps and carpophores of *Myodocarpus* appear to be structurally quite different from those of Apiaceae (G. M. Plunkett, personal observation; see also plates and illustrations in Baumann, 1946) and schizocarps with carpophores or carpophore-like structures are also found in a number of other bicarpellate genera in Araliaceae (e.g., *Harmsiopanax*, *Stilbocarpa*, *Astrotricha*, and *Mackinlaya*) and in the araliaceous hydrocotyloids. This suggests that the establishment of homology for schizocarpic fruits is not straightforward. Moreover, the fruits of *Myodocarpus* are very similar to those of *Delarbraea*, both of which are bicarpellate and possess identical oil vesicles (see Lowry, 1986), suggesting that *Myodocarpus*, like *Delarbraea*, is part of a basally branching clade in Apiales, not a bridge.

Finally, although the *rbcL* results weakly confirm the treatment of Judd, Sanders, and Donoghue, the *matK* and combined *matK-rbcL* analyses do not. Molecular data indicate that the evolutionary history of the two families is more complex than simple derivation of Apiaceae from within Araliaceae. Rather, the present study suggests that there are two well-defined "families" (core Apiaceae and core Araliaceae), both of which may have been derived from a lineage (or lineages) of pro-araliads that may still have extant taxa (e.g., the *Mackinlaya* group, *Delarbraea*). Re-alignment of these basally branching taxa within Apiales remains problematic, but the current system more accurately reflects the topology suggested by the *matK* and combined analyses. The alternative, lumping the families together, merely moves the difficulties in this

order from the interfamilial level to the infrafamilial level. This approach hides rather than resolves the difficulties in Apiales, and this seems contrary to the goals of phylogenetic systematics. If a natural and meaningful system of classification is to be achieved, phylogenetic relationships must be clarified, rather than obscured.

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