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Phylogeny of Labiatae and Verbenaceae Inferred from *rbcL* Sequences

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ABSTRACT. Parsimony analysis of *rbcL* sequences supports monophyly of the Labiatae s.l., which includes the Labiatae, subfamilies Caryopteridoideae, Chloanthoideae, and Viticoideae of the Verbenaceae, and Symphoremataceae. Representatives of subfamily Verbenoideae (Verbenaceae s. str.) do not form a monophyletic group with the Labiatae s.l. *Avicennia* (Avicenniaceae), *Cyclocheilon* (Nesogenaceae), and *Euthystachys* and *Retzia* (Stilbaceae), included in the Verbenaceae by many authors, are distinct from the Labiatae s.l. and Verbenaceae s. str. The inferred phylogeny also provides a framework to interpret character evolution. Results suggest that uniovulate locules have evolved in at least two lineages of Lamiales s.l. and that a gynoeceum with four locules by the development of false partitions apparently has evolved independently in the Labiatae s.l. and the Verbenaceae s. str. Whereas a dry fruit is plesiomorphic in the Lamiales s.l., a fleshy fruit is plesiomorphic in the Labiatae s.l. with possible reversal in four lineages.

A close relationship between the Labiatae and Verbenaceae has long been recognized (Cronquist 1981). The two families share opposite leaves, zygomorphic flowers and a bicarpellate gynoeceum that by the formation of false partitions develops into four uniovulate locules. The Labiatae generally are distinguished from the Verbenaceae by a deeply four-lobed ovary with a gynobasic style, whereas most Verbenaceae have an unlobed ovary with a terminal style. However, taxa with an intermediate morphology exist in both families, and the boundary between the two families is somewhat arbitrary (Cronquist 1981). This view is also supported by the phylogenetic study of Cantino (1992) which suggests that Labiatae as circumscribed by Bentham (1876) and Briquet (1895–1897) are polyphyletic, with at least four independent lineages of Labiatae having arisen within a paraphyletic Verbenaceae (Cantino 1992).

In an effort to circumscribe monophyletic groups, Cantino et al. (1992) proposed a classification of Labiatae that differs substantially from the previous schemes of Bentham (1876) and Briquet (1895–1897), but is very similar to a classification proposed by Junell (1934). Cantino et al. (1992) include the Labiatae sensu Briquet (1895–1897), plus subfamilies Caryopteridoideae, Chloanthoideae, Viticoideae and tribe Monochileae (subfamily Verbenoideae) of the Verbenaceae in a broadly circumscribed Labiatae s.l. (see Table 1). Subfamily Verbenoideae, minus tribe Monochileae, form a

narrowly circumscribed Verbenaceae s. str. The changes proposed by Cantino et al. (1992) and Junell (1934) were adopted by Thorne (1992) who also segregated Avicenniaceae, Nesogenaceae, Phrymaceae, Stilbaceae (including *Retzia*) and Symphoremataceae from the Verbenaceae (Table 1). The terms Verbenaceae s. str. and Labiatae s.l. as used herein refer specifically to the taxa as delineated in the first column of Table 1.

Thorne (1992) places the Labiate s.l. and Verbenaceae s. str., along with four small families, Avicenniaceae, Nesogenaceae, Phrymataceae, and Symphoremataceae, distinguished by gynoeceal traits described above, in suborder Lamiineae in the large order Scrophulariales (Table 1). Thorne's Scrophulariales are characterized by zygomorphic flowers with 2 or 4 (rarely 5) didynamous stamens, the presence of "placentoid" in the anthers (also in Solanales), cellular endosperm (helobial in Acanthaceae), terminal haustoria (except *Hippuris*), embryogeny of onagrad type, occurrence of protein crystals in the nuclei, stachyose and other oligosaccharides as a storage substance, decarboxylated iridoids (although decarboxylated iridoids are lacking in Gesneriaceae and some Labiatae, and both decarboxylated iridoids and secoiridoids are present in the Oleaceae), anthraquinones derived from shikimic acid rather than acetate, and verbascosides (Cronquist 1981; Mølgaard and Ravn 1988; Dahlgren 1991; Jensen 1992; Wagenitz 1992). Given the distribution of some of these traits, it is not yet

TABLE 1. Comparison of classifications of Labiatae and Verbenaceae.

Thorne (1992) and Cantino et al. (1992)	Briquet (1895–1897)	Bentham (1876)
LAMIALES s.l.		
Scrophulariineae		
Stilbaceae ¹		
Lamiineae		
Nesogenaceae		
Phrymaceae	Phrymaceae	
	Verbenaceae	Verbenaceae
		Phrymeae
	Stilboideae ¹	Stilbeae ¹
Symphoremataceae	Symphoremoidae	Symphoremeae
Avicenniaceae	Avicennioideae	Avicenniae
Verbenaceae		
Verbenoideae	Verbenoideae	Verbeneae
Labiatae		
Viticoideae	Viticoideae	Viticeae
	Caryopteridoideae	Caryopterideae
Chloanthoideae	Chloanthoideae	Chloanthae
	Labiatae	Labiatae
Prostantherioideae		Prostanthereae
Ajugoideae	Ajugoideae	Ajugeae
Teucroideae ²		
(including Monochileae)		
Scutellarioideae	Scutellarioideae	
Lamioideae	Lamioideae	Lamiae
	Prasioideae	Prasiae
Pogostemonoideae	Pogostemonoideae	
Nepetoideae	Nepetoideae	Nepeteae
		Salviae
		Mentheae
	Ocimoideae	Ocimeae
	Lavanduloideae	
	Catoferioideae	

¹Thorne (1992) includes *Retzia* in Stilbaceae and places the family in suborder Scrophulariineae, whereas Briquet (1895–97) and Bentham (1876) recognize subfamily Stilboideae and tribe Stilbeae, respectively, within the Verbenaceae. ²Tribe Monochileae was included in Verbenoideae by Briquet (1895–97) and Verbeneae by Bentham (1876), it is included in subfamily Teucroideae by Thorne (1992).

possible to devise explicit hypotheses of synapomorphies for Thorne’s Scrophulariales. However, monophyly of a clade that corresponds closely to the Scrophulariales sensu Thorne (1992) is supported by *rbcL* sequence data (Olmstead et al. 1992, 1993) and cpDNA restriction site data (Downie and Palmer 1992); this clade is called the Lamiales s.l. by those authors. Whereas interfamilial relationships within this large clade are not clearly resolved by molecular data, there is no indication that the two suborders recognized by Thorne (Scrophulariineae and Lamiineae) are monophyletic. Judd et al. (1994) suggest the Labiatae s.l. and Verbenaceae s. str.

[suborder Lamiineae sensu Thorne (1992)] together comprise a monophyletic group, but molecular studies (Olmstead et al. 1993; Olmstead and Reeves 1995) disagree with this conclusion.

This study was undertaken to provide greater resolution of phylogenetic relationships within the Lamiales s.l. It complements the morphological analysis of Cantino (1992) with molecular data and increases the sample of Labiatae s.l. and Verbenaceae s. str. that were included in the analyses of Downie and Palmer (1992) and Olmstead et al. (1992, 1993). The following specific questions are addressed: 1) Is Thorne’s (1992) suborder Lamiineae monophyletic? 2) Is the phylogeny inferred from *rbcL* sequences consistent with the phylogeny inferred from morphology (Cantino 1992)? 3) Do *rbcL* sequence data support monophyly of the taxonomic groups proposed by Cantino et al. (1992) and Thorne (1992)? 4) What trends in gynoecial character evolution are inferred?

MATERIALS AND METHODS

The study group includes representatives of 24 angiosperm families recognized by Thorne (1992) with more extensive sampling of Labiatae s.l. and Verbenaceae s. str. The sample of Labiatae s.l. encompasses all of the major clades identified in Cantino’s (1992) phylogenetic analysis with at least one representative from each of the eight subfamilies of Labiatae recognized by Cantino et al. (1992). The sample of Verbenaceae s. str. includes at least one representative of each tribe of Verbenoideae recognized by Moldenke (1971), with the exception of tribe Monochileae [its two genera are included in the Labiatae by Cantino et al. (1992)]. Representatives from the Asterales, Boraginales, Ericales, Gentianales, and Solanales are included as outgroups. Vouchers and Genbank accession numbers are listed in Appendix 1. The complete data set is available upon request from the authors and is deposited in TreeBASE (<http://herbaria.harvard.edu/treebase>).

Total DNA was extracted following the CTAB method of Doyle and Doyle (1987) and further purified by CsCl density centrifugation. The gene *rbcL* was amplified by Polymerase Chain Reaction (PCR) in two steps to obtain single-stranded DNA (Kaltenbock et al. 1992) using primers described by Olmstead et al. (1992). Prior to sequencing, excess primers and salts were removed from the ssDNA by spin dialysis (Centricon-100, Amicon) or alcohol

precipitation. Both the forward and reverse strands were sequenced using a set of 10 internal sequencing primers provided by G. Zurawski (DNAX, Palo Alto).

With two exceptions, a total of 1,402 nucleotide positions were analyzed. These corresponded to positions 27 to 1,428 of the *rbcL* reading frame (in *Tetrachondra* and *Congea* positions 27 to 1,351 were sequenced). The first 26 nucleotide positions are the 5' PCR primer binding site. Insertions and deletions (indels) downstream from position 1,428 made sequence alignments problematical among distantly related taxa, so the sequence in this region was excluded from the analysis. Two percent of the data cells included in the analysis were missing values.

Parsimony analysis was performed using PAUP version 3.1 (Swofford 1993). The presence of multiple islands (Maddison 1991) of equally parsimonious trees was assessed using a strategy similar to that of Olmstead et al. (1993): an initial search was conducted using RANDOM addition sequence with 10,000 replicates, NNI branch swapping and the MULPARS option off. Beginning with each of the trees saved after the initial search, a more rigorous analysis then was conducted using TBR branch-swapping and the MULPARS option on. The equally parsimonious trees obtained from each of these more rigorous analyses then were compared using the CONDENSE option to determine whether they represented a single island. Support for inferred clades is given by the number of synapomorphies and bootstrap analysis (100 bootstrap replications, CLOSEST addition sequence, NNI branch swapping with MULPARS on) (Felsenstein 1985).

Number of locules, number of ovules in each locule and fruit type are used to distinguish major groups of Lamiales s.l. The evolution of these characters was explored using MacClade vers. 3.2 (Maddison and Maddison 1992). Because the selection of outgroups is diverse (representing four orders), we assigned multiple states to reflect the range of variation for the trait within a clade. Hence *Nicotiana* (Solanaceae, Solanales), which has a dry dehiscent fruit, was given the two character states, dry dehiscent and fleshy, to reflect the occurrence of both states in the Solanaceae. Character state assignments were based upon descriptions in Bentham (1876), Junell (1934), and Cronquist (1981) and are summarized in Table 2.

RESULTS

New sequences of *rbcL* for 26 species of Labiatae and Verbenaceae were analyzed in combination with 35 previously published sequences (Appendix 1). Of the 1,402 nucleotide positions compared, 913 were constant, 194 had variation unique to single taxa and 295 were informative, varying among two or more taxa. The coding region of *rbcL* varied in length from 1,428 to 1,464 bases translating proteins with 475–487 amino acid residues. Point mutations, insertions and/or deletions (indels) that disrupt the first recognizable stop codon account for all the observed length variation in the coding region of *rbcL*.

The global search with 10,000 random addition replicates produced three equally parsimonious trees of 1,538 steps. Starting with each of the above trees, more rigorous searches yielded three distinct islands. The first island was composed of 1,368 trees of 1,538 steps, the second 4,680 trees of 1,537 steps and the third 576 trees of 1,536 steps. A strict consensus of island 576 (length = 1,536) is shown in Fig. 1. Most of the character changes occurred in the third codon position (minimum-maximum values = 1006–1009 third codon changes for the trees in island 576); there were fewer changes in the first (368–370) and second codon positions (159–160). Transitions occurred approximately 1.7 times more frequently than transversions (proportions range from 981:555 to 943:593 as inferred from trees in island 536).

The Lamiales s.l. emerge as a clade in all three islands. In a representative tree, the majority rule consensus tree of island 576 (Fig. 2), 17 characters change along the branch leading to Lamiales s.l. (using the ACCTRAN optimization in PAUP) and the lineage occurred in 85% of the bootstrap replications (Fig. 1). *Tetrachondra* (Tetrachondraceae) emerges at the base and is the sister group to all other members of the clade (Figs. 1, 2). The Gesneriaceae and Oleaceae diverge early and form an unresolved trichotomy with a clade that includes all other Lamiales s.l. Relationships among the remaining families of Lamiales s.l. remain poorly resolved.

Labiatae s.l. [e.g. Cantino et al. (1992)] plus *Congea* (Symphoremataceae sensu Thorne 1992) also emerge as a clade in all three islands. This lineage is supported by eight synapomorphies in the tree in Fig. 2 and occurs in 92% of bootstrap replications (Fig. 1). Monophyletic groups within

TABLE 2. Character state assignment for Figs. 3–5. Taxa for which more than one character state is presented were scored as polymorphic (e.g. *Nicotiana*, Solanaceae). Classification follows Thorne (1992).

Taxon	Number of locules	Ovules in each locule	Fruit
ASTERALES			
Asteraceae			
<i>Achillea</i>	1 & 2 & 4 & >4	1 & numerous	dry dehiscent & indehiscent & fleshy
ERICALES			
Ericaceae			
<i>Erica</i>	>4 (rarely 1)	numerous (rarely 1)	dry dehiscent & fleshy
GENTIANALES			
Gentianaceae			
<i>Gentiana</i>	1 (rarely 2)	numerous	dry dehiscent (rarely fleshy)
SOLANALES			
Boraginaceae			
<i>Borago</i>	2 & 4	1 & 2	dry indehiscent & fleshy
Solanaceae			
<i>Nicotiana</i>	2 (rarely 4)	numerous	dry dehiscent & fleshy
Tetrachondraceae			
<i>Tetrachondra</i>	4	1	dry indehiscent
SCROPHULARIALES			
Acanthaceae			
<i>Justicia</i>	2 (rarely 1)	2 & numerous	dry dehiscent
<i>Nelsonia</i>	2 (rarely 1)	2 & numerous	dry dehiscent
<i>Ruellia</i>	2 (rarely 1)	2 & numerous	dry dehiscent
Avicenniaceae			
<i>Avicennia</i>	1	4	dry dehiscent
Bignoniaceae			
<i>Catalpa</i>	1 & 2 (rarely 4)	numerous	dry dehiscent & fleshy
<i>Tabebuia</i>	1 & 2 (rarely 4)	numerous	dry dehiscent & fleshy
Callitrichaceae			
<i>Callitriche</i>	4	1	dry indehiscent
Gesneriaceae			
<i>Nematanthus</i>	1	numerous	dry dehiscent (rarely fleshy)
<i>Streptocarpus</i>	1	numerous	dry dehiscent (rarely fleshy)
Labiatae			
<i>Ajuga</i>	4	1	dry indehiscent
<i>Callicarpa</i>	4	1	fleshy
<i>Caryopteris</i>	4	1	dry dehiscent & indehiscent
<i>Clerodendrum</i>	4	1	fleshy
<i>Elsholtzia</i>	4	1	dry indehiscent
<i>Glechoma</i>	4	1	dry indehiscent
<i>Gmelina</i>	4	1	fleshy
<i>Holmskioldia</i>	4	1	fleshy
<i>Lamium</i>	4	1	dry indehiscent
<i>Marrubium</i>	4	1	dry indehiscent
<i>Mentha</i>	4	1	dry indehiscent
<i>Oncinocalyx</i>	4	1	dry indehiscent
<i>Petitia</i>	4	1	dry indehiscent
<i>Physostegia</i>	4	1	dry indehiscent
<i>Plectranthus</i>	4	1	dry indehiscent
<i>Pogostemon</i>	4	1	dry indehiscent
<i>Prasium</i>	4	1	fleshy
<i>Premna</i>	4	1	fleshy
<i>Prostanthera</i>	4	1	dry indehiscent
<i>Salvia</i>	4	1	dry indehiscent
<i>Scutellaria</i>	4	1	dry indehiscent
<i>Tectona</i>	4	1	dry indehiscent

TABLE 2. Continued.

Taxon	Number of locules	Ovules in each locule	Fruit
Labiatae Continued			
<i>Teucrium</i>	4	1	dry indehiscent
<i>Tinnea</i>	4	1	dry indehiscent
<i>Trichostema</i>	4	1	dry indehiscent
Martyniaceae			
<i>Proboscidea</i>	1 & 2	1 & numerous	dry dehiscent
Myoporaceae			
<i>Myoporum</i>	2 (rarely 4–10)	2 (rarely 1–8)	fleshy
Nesogenaceae			
<i>Cyclocheilon</i>	1 & 2	1	dry indehiscent
Oleaceae			
<i>Jasminum</i>	2	2 (rarely 1–4)	dry dehiscent & indehiscent & fleshy
<i>Ligustrum</i>	2	2 (rarely 1–4)	dry dehiscent & indehiscent & fleshy
<i>Nyctanthes</i>	2	2 (rarely 1–4)	dry dehiscent & indehiscent & fleshy
Pedaliaceae			
<i>Sesamum</i>	2	numerous	dry dehiscent
Phrymaceae			
<i>Phryma</i>	1	1	dry indehiscent
Scrophulariaceae			
<i>Antirrhinum</i>	2	numerous (rarely 2)	dry dehiscent
<i>Digitalis</i>	2	numerous (rarely 2)	dry dehiscent
<i>Paulownia</i>	2	numerous	dry dehiscent
Stilbaceae			
<i>Euthystachys</i>	2	1	dry dehiscent & indehiscent
<i>Retzia</i>	1 & 2	2	dry dehiscent
Symphoremataceae			
<i>Congea</i>	1	4	fleshy
Lentibulariaceae			
<i>Utricularia</i>	1	numerous	dry dehiscent
Verbenaceae			
<i>Bouchea</i>	4	1	dry indehiscent
<i>Petrea racemosa</i>	2	1	fleshy
<i>Petrea volubilis</i>	2	1	fleshy
<i>Rhaphithamnus</i>	4	1	dry indehiscent
<i>Stachytarpheta</i>	4	1	dry indehiscent
<i>Verbena</i>	4	1	dry indehiscent

the labiate clade (Figs. 1, 2) correspond to subfamilies Lamioideae (six synapomorphies; 75% bootstrap value), Nepetoideae (19; 100%), Scutellarioideae (nine; 71%) and Teucroideae plus *Ajuga*, (five; 55%).

Two major lineages are identified in Labiatae s.l. (Figs. 1, 2) The first consists primarily of subfamily Nepetoideae. The second consists of the three other clades identified above: subfamilies Lamioideae, Scutellarioideae, and Teucroideae (plus *Ajuga*). The remaining taxa, mostly members of subfamilies Viticoideae and Chloanthoideae sensu Cantino et al. (1992) along with *Congea* and *Pogostemon*, are basal members of the family or of each of the major lineages within the family (Figs. 1, 2).

Verbenaceae s. str. form two independent clades

(Figs. 1, 2). *Petrea racemosa* and *P. volubilis* (Tribe Petreeae) form a distinct clade (10 synapomorphies; 100% bootstrap values) that emerges as the sister group to Bignoniaceae. The remaining Verbenaceae s. str. form a second clade that is supported by seven synapomorphies and includes two groups; *Rhaphithamnus* and *Verbena* (tribes Citharexyleae and Verbeneae) form a clade (8; 71%) that is the sister group to a clade comprising *Stachytarpheta* and *Bouchea* (tribe Lantaneae) (10; 99%).

DISCUSSION

The analysis of *rbcL* sequences suggests that Lamiales s.l. are composed of many distinct lineages, among which relationships are poorly

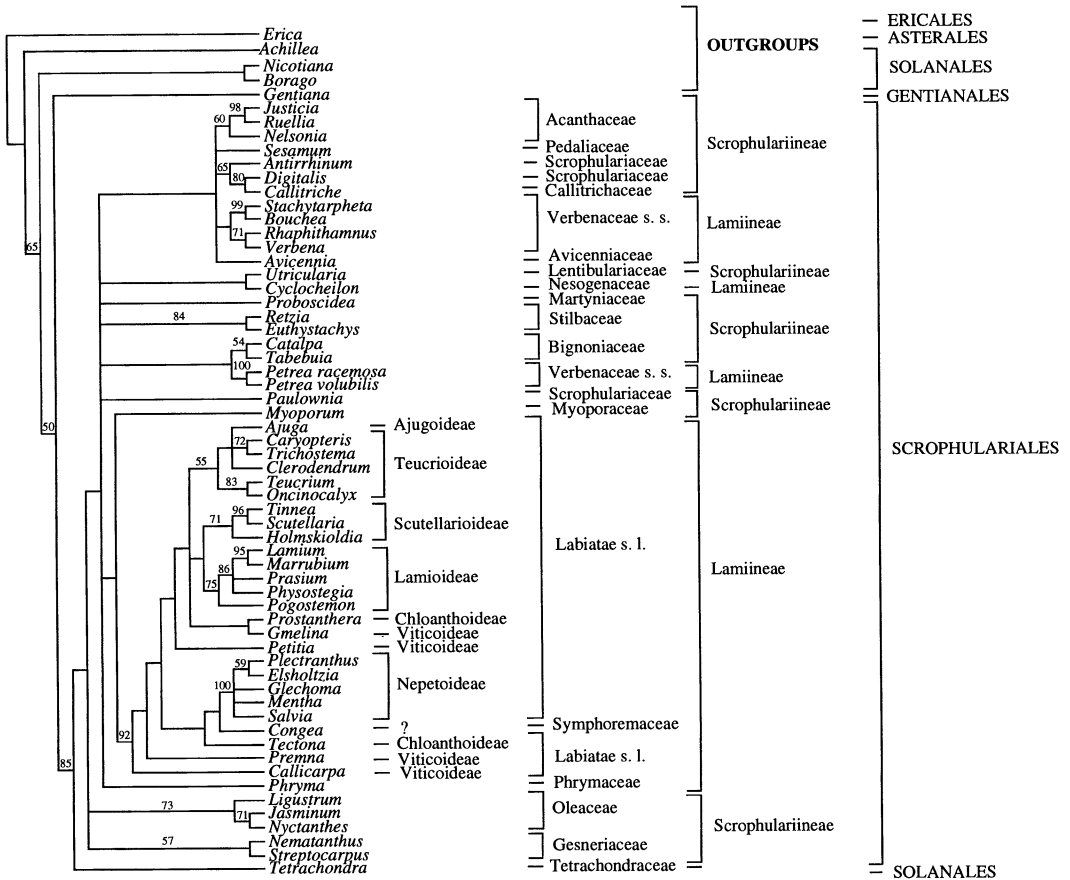


FIG. 1. Strict consensus tree of 576 trees of 1,536 steps. Inferred relationships are compared to classifications of Labiatae and Verbenaceae proposed by Thorne (1992) and Cantino et al. (1992). Bootstrap values >50% are indicated.

resolved (Figs. 1, 2; Table 1). Three of the internal branches near the base of Lamiales s.l. are supported only by a single synapomorphy in the majority rule consensus tree (Fig. 2). Similar results were obtained by Olmstead et al. (1992, 1993) and Downie and Palmer (1992). These findings have notable systematic implications and provide a framework to study character evolution.

Systematic Implications. The data do not support monophyly of Thorne's (1992) suborders Scrophulariineae and Lamiineae (Figs. 1, 2; also see Table 1). An analysis in which suborder Lamiineae was constrained to form a monophyletic group resulted in trees of 1,545 steps, nine steps longer than the shortest trees without the constraint. The monophyly of Labiatae s.l. and Verbenaceae s. str., as suggested by Judd et al. (1994) also is not supported by *rbcL* sequences. *Avicennia*, *Cyclocheilon*, *Phryma* [included in Verbenaceae by Bentham

(1876) and Briquet (1895–1897)] and *Tetrachondra* [included in Labiatae by Cronquist (1981)] emerge in positions distinct from the Labiatae s.l. or Verbenaceae s. str.

An analysis that constrained Labiatae s.l. to monophyly (excluding *Congea*) found trees of 1441 steps; five steps longer than the shortest trees. In this case *Congea* emerged as sister group to a monophyletic Labiatae s.l.

At the base of Labiatae s.l. and of each of its two major lineages are representatives of subfamilies Viticoideae and Chloanthoideae (Figs. 1, 2). Cantino et al. (1992) recognized that Viticoideae probably were paraphyletic and might be basal in the family. Chloanthoideae are a primarily Australian group circumscribed by Cantino et al. (1992) to include members of Prostanthereae (traditionally in Labiatae s. str.) and Chloanthoideae, plus *Tectona* (traditionally in Verbenaceae s.l.) *Tectona*, the native teak

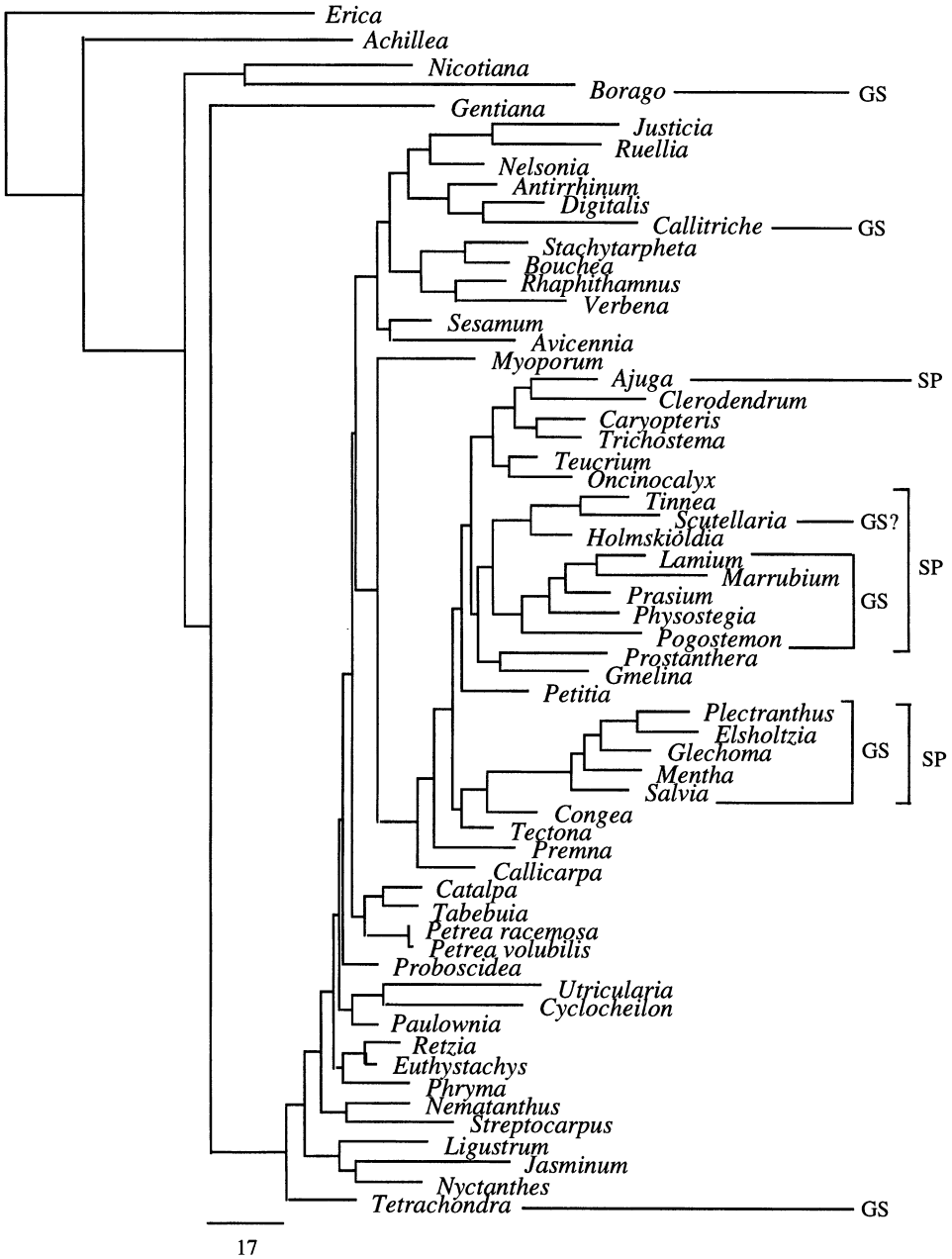


FIG. 2. Majority rule consensus of island 576, which is also one of the most parsimonious trees (length = 1,536 steps, Consistency Index = 0.421, Retention Index = 0.465). Branch length is indicated by the scale bar. Taxa having a gynobasic style (GS) and suprareticulate pollen (SP) are indicated.

of southeast Asia, and *Petitia*, of the West Indies, are the basal taxa in the two major lineages identified here; previously these have been grouped together in tribe Tectoneae (sensu Moldenke 1971). The *rbcL* analysis recognizes two major clades of

Labiatae s.l. (Figs. 1, 2). The monophyletic subfamily Nepetoideae dominates one of these lineages. Subfamily Nepetoideae is one of the largest and most distinctive groups of Labiatae and is characterized by hexacolpate pollen, exalbuminous seeds

and an investing embryo (Cantino 1992). This subfamily contains most of the aromatic herbs commonly associated with the "mint" family (Labiatae). Nominal sampling of this large clade was justified by the strong evidence for its monophyly (Cantino 1992; Kaufmann and Wink 1994; Wagstaff et al. 1995). The second major clade of Labiatae s.l. (Fig. 1, 2) contains representatives of seven subfamilies. Within this lineage, three monophyletic groups emerge: Lamioideae plus *Pogostemon* (Pogostemonoideae), Scutellarioideae, and Teucrioideae plus *Ajuga* (Ajugoideae). Lamioideae and Pogostemonoideae traditionally are assigned to the Labiatae. The other two monophyletic groups (Scutellarioideae, Teucrioideae plus *Ajuga*) each consist of a combination of taxa previously assigned to the Verbenaceae and Labiatae s. str. (Cantino 1992).

Three of these four large clades of Labiatae s.l., corresponding to subfamilies Nepetoideae, Scutellarioideae, and Lamioideae, are supported by *rbcl* sequences and morphology. However, two large clades identified by Cantino (1992), one consisting of taxa characterized by supracretate pollen and the other consisting of taxa characterized by a gynobasic style, are not supported by the *rbcl* data (Fig. 2). The *rbcl* data suggest that supracretate pollen has evolved independently in three lineages of Labiatae: 1) in a clade composed of subfamilies Scutellarioideae, Pogostemonoideae and Lamioideae; 2) in a clade composed of subfamily Nepetoideae, and 3) in a clade represented in this analysis by *Ajuga* (Figs. 1, 2). Constraining the supracretate pollen clade of Cantino (1992) to monophyly resulted in trees 4 steps longer than the optimal trees.

Members of Lamioideae and Pogostemonoideae share a gynobasic style with subfamily Nepetoideae and are grouped together in the morphology-based analysis of Cantino (1992). However, the *rbcl* results suggest that a gynobasic style has evolved independently in at least two lineages. This finding is congruent with the results from restriction site data (Wagstaff et al. 1995). Constraining taxa with a gynobasic style to monophyly resulted in trees two steps longer than the shortest trees. A structure analogous to the gynobasic style of Labiatae has evolved independently in Boraginaceae and Tetradachnaceae (Cronquist 1981, Skottsberg 1913).

Two clades of Verbenaceae are nested in distinct positions in Lamiales s.l. (Figs. 1, 2). *Petrea racemosa* and *P. volubilis* (tribe Petreeae, sensu Moldenke 1971) form a clade that is the sister group to

Bignoniaceae. A second clade is represented by *Stachytarpheta* and *Bouchea* (Lantaneae), *Rhaphithamnus* (Citharexyleae), and *Verbena* (Verbeneae). The genus *Petrea* includes 14 species that are widely distributed in the Neotropics (Rueda 1994). The genus is distinct from other American members of tribe Petreeae by a crest on the calyx and asperous leaves. *Petrea* is further distinguished from other Verbenaceae by fleshy fruits with two, one-celled and one-seeded pyrenes (Briquet 1895–1897; Rueda 1994). Constraining Verbenaceae s. str. to monophyly found trees one step longer than the shortest trees, suggesting that the relationship of *Petrea* to Bignoniaceae requires further examination. A close relationship between tribes Citharexyleae and Verbeneae is indicated by this analysis and may be further supported by the presence of distinctive intranuclear inclusions of the lamellar-type that are lacking in other Verbenaceae s. str. These inclusions are proteinaceous in nature, lack a membrane and are often associated with the nucleolus (Bigazzi 1984, 1989). Monophyly of tribes Citharexyleae and Verbeneae is further supported by colporate or colpate pollen with a distinctive thickening of the exine immediately adjacent to the colpi or pores (Raj 1983; Chadwell et al. 1992). However, this feature apparently has evolved independently in *Avicennia* and in *Petrea*, if the relationship of *Petrea* to Bignoniaceae holds up in further study.

Junell (1934) recognized that the gynoecial structure of *Avicennia* (Avicenniaceae) and *Congea* (Symphoremataceae) was distinct from other Labiatae and Verbenaceae, but suggested that these taxa were derived from a viticoid ancestor. The *rbcl* data include *Congea* within Labiatae s.l. (Figs. 1, 2). However, *Avicennia* emerges outside Labiatae s.l. and within a clade that includes Verbenaceae s. str. (excluding *Petrea*), Scrophulariaceae, Callitrichaceae, Pedaliaceae and Acanthaceae (Figs. 1, 2). *Avicennia* variously has been included in Verbenaceae (Bentham 1876; Briquet 1895) or recognized as the distinct family Avicenniaceae (Duke 1991; Thorne 1992). Constraining *Avicennia* to form a clade with Verbenaceae s. str. found trees 5 steps longer than the shortest tree. The results presented here identify *Avicennia* as a distinct lineage and support continued recognition of a distinct family Avicenniaceae (Figs. 1, 2). Avicenniaceae include eight species of mangrove found in sheltered tropical coastlines around the world (Duke 1991).

Some authors (e.g., Bentham 1876; Cronquist 1981) include *Cyclocheilon*, *Euthystachys*, and *Phryma* in Verbenaceae s. str. In the *rbcl* analysis these taxa

are nested within Lamiales s.l., but emerge as lineages distinct from other Labiatae s.l. and Verbenaceae s. str. (Figs. 1, 2). *Cyclocheilon* and *Euthystachys* were included in tribe Stilbeae by Bentham (1876) and subfamily Stilboideae by Briquet (1895). However Thorne (1992) places *Cyclocheilon* in Nesogenaceae and *Euthystachys* in Stilbaceae. *Cyclocheilon* was segregated from Nesogenaceae and recognized as a distinct family by Marais (1981). In the *rbcL* analysis (Figs. 1, 3) *Cyclocheilon* emerges as a distinct lineage that is the sister group to *Utricularia* (Lentibulariaceae).

Monophyly of Stilbaceae sensu Thorne (1992) and Bremer et al. (1994), including *Euthystachys* and *Retzia*, is well-supported by *rbcL* sequences (Figs. 1, 2). The occurrence of the iridoids stilbericoside and unedoside are synapomorphies of the family (Jensen 1992). This small distinctive lineage includes 13 species that are endemic to South Africa.

Phryma was included in Verbenaceae by Bentham (1876), but recognized as the monotypic family Phrymataceae by Briquet (1895) and Thorne (1992). In this analysis, *Phryma* emerges as a distinct lineage, not clearly associated with any other group within Lamiales s.l. (Figs. 1, 2). The lineage is composed of a single species with a disjunct distribution in deciduous forests of eastern Asia and eastern North America.

Tetrachondra (Tetrachondraceae) emerges at the base of Lamiales s.l. and is the sister group to a clade composed of all other members of the order (Figs. 1, 2). Tetrachondraceae include 2 species of diminutive herbs with a disjunct distribution in Patagonia and New Zealand. The family is characterized by minute tetramerous flowers having a bicarpellate gynoecium composed of four locules with one ovule in each locule. The gynoecium is deeply four parted with a gynobasic style. Thorne (1992) places the enigmatic Tetrachondraceae in Solanales. However, *Tetrachondra* has been placed variously in Boraginaceae, Scrophulariaceae, and Labiatae (Skottsberg 1913).

Character Evolution. Traditional classifications of the Lamiales s.l. (e.g., Thorne 1992) have relied heavily on gynoecial morphology, so it is of interest to examine the evolution of gynoecial traits on a tree not based on reproductive morphology. The radiation of the Lamiales s.l. involved diverse changes in gynoecial structure and the number of propagules produced by each flower. The gynoecium of Lamiales s.l. always is derived from two carpels, but has from one to four locules (rarely more). Each locule contains from one to numerous

ovules. Placentation is variously axile, basal, apical, free-central, or parietal. The mature fruit is dehiscent or indehiscent and generally a capsule, drupe, or schizocarp (Cronquist 1981). We explore the evolution of ovule number, locule number and fruit type in Lamiales s.l. (Table 2; Figs. 3–5) not so much to provide rigorous inference concerning character evolution throughout the order (sampling is too limited in many groups) as to explore the appropriateness of using gynoecial traits in classification in this clade.

Two groups of Lamiales s.l. traditionally are recognized (e.g., Takhtajan 1987; Thorne 1992). Lamiineae sensu Thorne (1992) are distinguished from Scrophulariineae by a gynoecium that forms four uniovulate locules by the intrusion of false partitions from the carpellary midribs (Cronquist 1981; Thorne 1992; Judd et al. 1994); the fruit (with exceptions) develops into indehiscent, half-carpellary, one-seeded drupes or nutlets. By comparison most Scrophulariineae have a bilocular gynoecium with two-many ovules and a dehiscent capsular fruit (Cronquist 1981). The evolution of one-seeded propagules with a fleshy or hardened pericarp from numerous naked seeds dispersed from a dry dehiscent capsule is a fundamental shift in mode of reproduction within Lamiales s.l. Inference from *rbcL* sequences suggests that monophyletic groups based on the distinction in gynoecial morphology (e. g., Scrophulariineae and Lamiineae) cannot be made (Figs. 3–5).

The *rbcL* data are unable to resolve the plesiomorphic state for ovule number in Lamiales s.l. (Fig. 3). The conventional view (e. g., Cronquist 1981) that the reduced ovule number found in the Lamiineae (sensu Thorne 1992) is derived is consistent with these results, but with the caveat that the reduction must have occurred several times. Tetrachondraceae have four ovules attached at the base to axile placenta. Oleaceae (represented by *Jasminum*, *Ligustrum*, *Nyctanthes*) typically have four ovules that are attached to an axile placenta, but occasionally have from one to many ovules (Cronquist 1981). Gesneriaceae (*Nematanthus*, *Streptocarpus*) typically have numerous ovules that are attached to two parietal placentae. Scrophulariaceae (*Antirrhinum*, *Digitalis*), and Bignoniaceae (*Catalpa*, *Tabebuia*) (Cronquist 1981) generally have numerous ovules attached to axile placentae. The evolution of four ovules on a free-central placenta (Junell 1934; Cronquist 1981) has occurred independently in

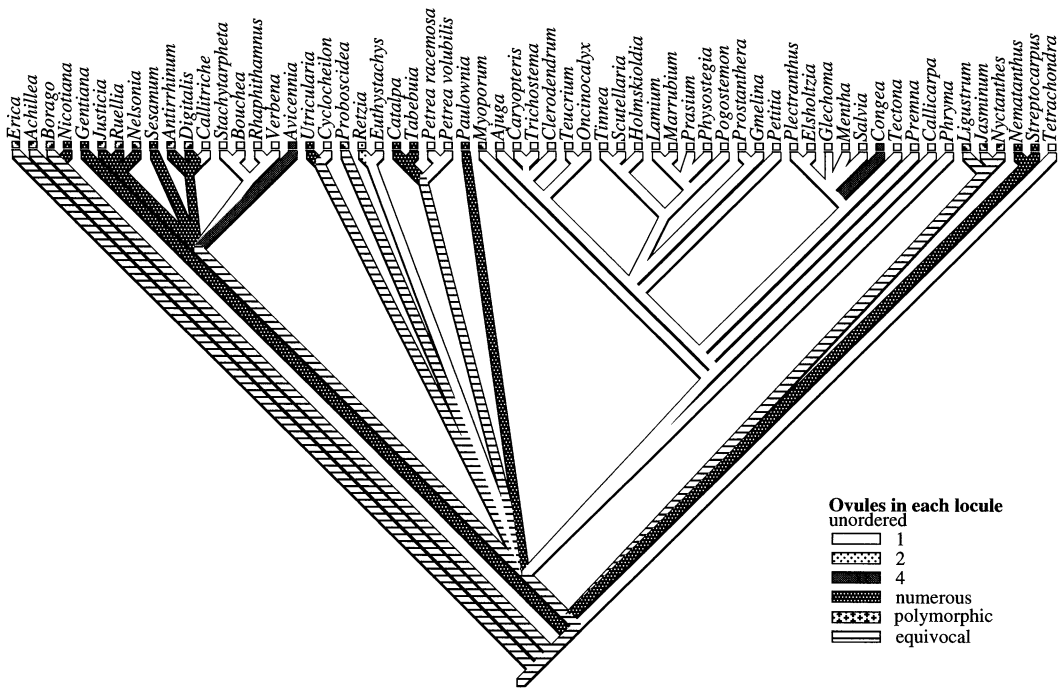


FIG. 3. Strict consensus tree with the character “number of ovules in each locule” optimized onto it using MacClade. See legend at lower right for interpretation of character states.

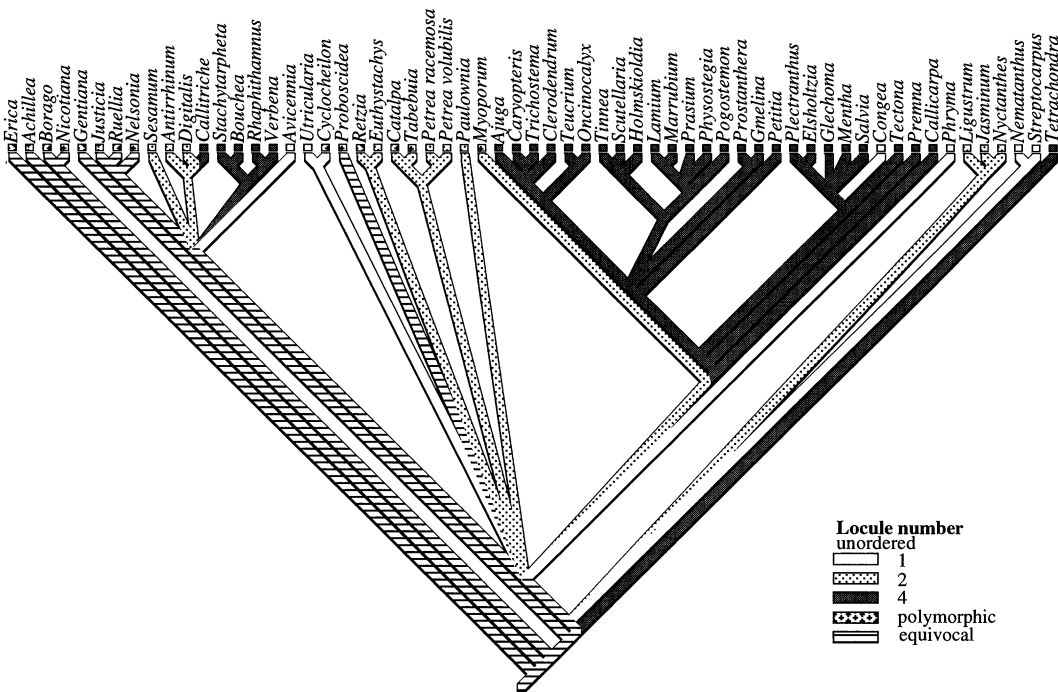


FIG. 4. Strict consensus tree with the character “number of locules” optimized onto it using MacClade. See legend at lower right for interpretation of character states.

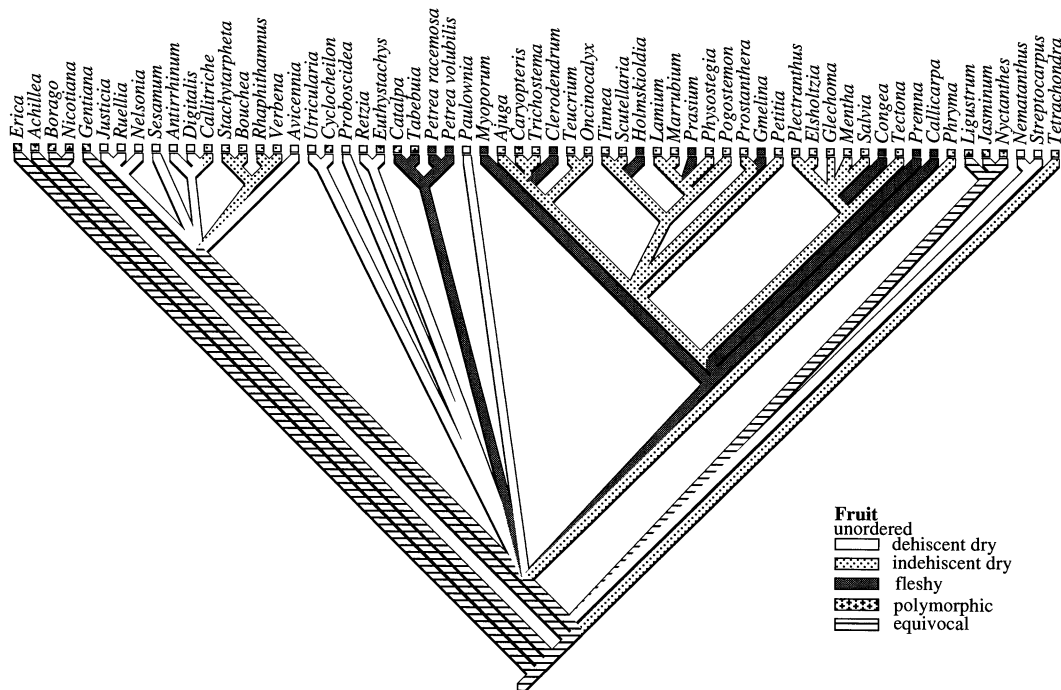


FIG. 5. Strict consensus tree with the character "fruit type" optimized onto it using MacClade. See legend at lower right for interpretation of character states.

Avicenniaceae (*Avicennia*) and Symphoremataceae (*Congea*) (Fig. 3). Two ovules in each locule are characteristic of most Acanthaceae (e. g., *Justicia*, *Ruellia*, although there are numerous ovules in *Nelsonia*, representing Nelsonioideae, which are basal in the family; Scotland et al. 1995), which typically have axile placentae. Myoporaceae (*Myoporium*), which have pendulous ovules (Cronquist 1981), Callitrichaceae (*Callitriche*), Labiatae s.l., and Verbenaceae s. str. also have two ovules per locule. The most reduced condition occurs in *Phryma*, which has a single ovule with basal placentation.

Locule number is equivocal at the base of the Lamiales s.l., being either bilocular as in most Scrophulariineae or unilocular as in *Avicennia* (Avicenniaceae), *Phryma* (Phrymataceae), Gesneriaceae and some Stilbaceae, (Fig. 4) The gynoecium of *Retzia* (Stilbaceae) is unilocular at the base (Cronquist 1981), whereas that of *Euthystachys* is bilocular. An apomorphic increase in number of locules has occurred in at least four lineages of Lamiales s.l. (Fig. 4). Callitrichaceae, most Labiatae s.l., Tetrachondraceae, and Verbenaceae s. str. are characterized by four uniovulate chambers (Fig. 4), which develop by intrusion of false partitions from the carpellary midribs (Cronquist 1981). The absence of

these partitions, presumed to be lost secondarily (Cronquist 1981), results in a bilocular gynoecium with two ovules in each locule in some members of subfamily Chloanthoideae (Labiatae s.l.). Reduction in locule number to a greater extent is inferred in *Congea* (Symphoremataceae), which has a unilocular gynoecium with four ovules (Fig. 4) (Junell 1934; Cronquist 1981).

The *rbcl* data similarly suggest that fruit type is equivocal at the base of Lamiales s.l., and in most instances a clear transition between a dry dehiscent, dry indehiscent and fleshy fruit cannot be made (Fig. 5). In Labiatae s.l., however, the fleshy drupaceous fruit is inferred to be plesiomorphic with transition to a dry indehiscent fruit early in the diversification of the family followed by reversal to fleshy fruits in at least four lineages (Fig. 5). The four pyrenes of *Callicarpa* and *Premna* are enclosed in a single unlobed fleshy fruit, whereas in *Holmskioldia* (only slightly fleshy), *Prasium*, and in some species of *Clerodendrum* the gynoecium develops into four fleshy drupaceous fruits each with a single pyrene (Junell 1934; Cronquist 1981). A schizocarp that breaks into four dry, indehiscent, one-seeded mericarps is the derived state found in most Labiatae s.l. (Fig. 5). Perhaps the shift from a

fleshy drupaceous fruit to a dry schizocarp followed colonization of regions with a seasonal climate, and the hardened schizocarp is a developmentally arrested fleshy fruit. With the notable exception of *Prasium* (found in the mediterranean region), most labiates with fleshy fruits are restricted to tropical regions. Selection for seed dispersal is likely an important factor.

Much work remains before the preliminary revisions of Cantino et al. (1992) and Thorne (1992) should be fully embraced. Monophyly of the Lamiales s.l. and several included lineages is supported by *rbcL* sequences. The inclusion of *Congea* in an expanded Labiatae s.l. should be tested with additional evidence. Further work is necessary to circumscribe lineages of Viticoideae and Chloanthoideae (Labiatae s.l.). Monophyly of Verbenaceae s. str. is weakly supported, and relationships should be investigated further, with expanded sampling. Some researchers have placed morphologically distinct taxa such as *Phryma*, *Cylocheilon*, *Euthystachys* and *Avicennia* in pre-existing families, whereas the *rbcL* data suggest these are small lineages that are distinct from these families. It does not seem surprising that the lamialean radiation resulted in several large clearly defined lineages as well as several smaller, but equally distinct ones. Morphological studies in the order have focused largely upon mature plants, yet the developmental processes that lead to the gynoeceal diversity within Lamiales s.l. are not well understood. In Labiatae s.l. assessing homologies of the pericarp wall might provide a greater understanding of the evolutionary transition from fleshy to dry fruits in the family.

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APPENDIX 1. Taxa included in the *rbcL* survey. These are arranged according to the classification of Thorne (1992). Citations are given for the source of previously published sequences.

Taxon	Source of DNA/voucher	Genbank accession number
ASTERALES		
Asteraceae		
<i>Achillea millefolium</i> L.	Kim et al. (1992)	
ERICALES		
Ericaceae		
<i>Erica australis</i> L.	Kron and Chase (1993)	
GENTIANALES		
Gentianaceae		
<i>Gentiana procera</i> Holm.	Olmstead et al. (1993)	
SOLANALES		
Boraginaceae		
<i>Borago officinalis</i> L.	Olmstead et al. (1992)	
Solanceae		
<i>Nicotiana tobacum</i> L.	Lin et al. (1986)	
Tetrachondraceae		
<i>Tetrachondra hamiltoni</i> Petrie ex D. Oliver	NEW ZEALAND, Lake te Anau. <i>P. N. Johnson</i> 236/92 Lincoln, New Zealand	U28885
SCROPHULARIALES		
Acanthaceae		
<i>Justicia odora</i> (Forssk.) Lam	Olmstead et al. (1993)	
<i>Nelsonia canescens</i> (Lam.) Nees	Olmstead et al. (1993)	
<i>Ruellia graecizans</i> Backer	Chase et al. (1993)	
Avicenniaceae		
<i>Avicennia nitida</i> Jacq.	UNITED KINGDOM, Cultivated Royal Botanic Gardens, Kew, 255.86.0243	U28868
Bignoniaceae		
<i>Catalpa</i> sp.	Olmstead et al. (1992)	
<i>Tabebuia heterophylla</i> (A. de Candolle) Britton	Olmstead and Reeves (1995)	
Callitricaceae		
<i>Callitriche heterophylla</i> Pursh. emend Darby	Olmstead et al. (1992)	
Gesneriaceae		
<i>Streptocarpus holstii</i> Engl.	Olmstead et al. (1993)	
<i>Nematanthus hirsutus</i> (Mart.) Wiehler	Olmstead and Reeves (1995)	
Labiatae		
¹ <i>Ajuga reptans</i> L.	Wagstaff et al. (1995)	
<i>Callicarpa dichotoma</i> (Lour.) K. Koch	Olmstead et al. (1993)	
<i>Caryopteris incana</i> Thunb. Miq.	U.S.A., Cultivated, Ohio University, Athens, Co., <i>P. D. Cantino</i> 1387 BHO.	U28869
<i>Clerodendrum fragrans</i> Hort. ex Vent.	Olmstead et al. (1992)	
¹ <i>Elsholtzia stauntonii</i> Benth.	Wagstaff et al. (1995)	U28872
¹ <i>Glechoma hederacea</i> L.	Wagstaff et al. (1995)	
<i>Gmelina hystrix</i> Schultes ex Kurz	UNITED KINGDOM, Cultivated, Royal Botanic Gardens, Kew, 381.74.02999, ver. <i>D. R. Hunt</i> 19.8.75.	U28873
<i>Holmskioldia sanguinea</i> Retz.	U.S.A., Athen, Ohio, Cultivated, Ohio University <i>P. D. Cantino</i> 1361 BHO.	U28874
¹ <i>Lamium purpureum</i> L.	Wagstaff et al. (1995)	
¹ <i>Marrubium vulgare</i> L.	Wagstaff et al. (1995)	U28875
¹ <i>Mentha rotundifolia</i> (L.) Huds.	Wagstaff et al. (1995)	U28876
<i>Oncinocalyx betchei</i> F. Muell.	AUSTRALIA, Cultivated, Royal Botanical Garden, Sydney <i>A. Hay</i> 842447.	U31458
<i>Petitia domingensis</i> Jacq.	GERMANY, Freiburg, Cultivated, Albert Lud- wigs Universität, <i>U. Falk and H. Rimpler</i> 2494.	U28878

APPENDIX 1. Continued.

Taxon	Source of DNA/voucher	Genbank accession number
<i>Physostegia virginiana</i> (L.) Benth.	Olmstead et al. (1992)	
¹ <i>Plectranthus barbatus</i> Andr.	Wagstaff et al. (1995)	U28882
<i>Pogostemon cablin</i> (Blanco) Benth.	Olmstead et al. (1993)	
¹ <i>Prasium majus</i> L.	Wagstaff et al. (1995)	U31459
<i>Premna japonica</i> Miq.	U.S.A., Ohio, Athens Co., Cultivated, Ohio University <i>P. D. Cantino 1394</i> BHO.	U28883
<i>Prostanthera rotundifolia</i> R. Br.	Olmstead et al. (1993)	
<i>Salvia divinorum</i> Epling and Játiva	Olmstead et al. (1993)	
<i>Scutellaria bolanderi</i> A. Gray	Olmstead et al. (1993)	
<i>Tectona grandis</i> L. f.	U.S.A., Hawaii, Cultivated, Waimea Botanical Garden, <i>S. J. Wagstaff and R. G. Olmstead 92–299</i> COLO.	U28884
<i>Teucrium fruticans</i> L.	Olmstead et al. (1993)	
<i>Tinnea zambesiaca</i> Baker	UNITED KINGDOM, Cultivated, Royal Botanic Gardens, Kew 461.56.46104.	U28886
<i>Trichostema dichotomum</i> L.	U.S.A., Ohio, Athens, Co., <i>P. D. Cantino 1368</i> BHO.	U28887
Lentibulariaceae		
<i>Utricularia biflora</i> Roxb.	Chase et al. (1993)	
Martyniaceae		
<i>Sesamum indicum</i> L.	Olmstead et al. (1993)	
Myoporaceae		
<i>Myoporum mauritianum</i> A. de Cand.	Olmstead and Reeves (1995)	
<i>Cyclocheilon somaliense</i> Engl.	YEMEN, Hadramaut, <i>Mats Thulin 8364</i> Uppsala.	U28871
Oleaceae		
<i>Ligustrum vulgare</i> L.	Olmstead et al. (1992)	
<i>Jasminum suavisissimum</i> Lindl.	Olmstead et al. (1993)	
<i>Nyctanthes arbor-tristis</i> L.	UNITED KINGDOM, Cultivated, Royal Botanic Gardens, Kew, 099.86.00993, ver. <i>S. Atkins 23.11.1987</i> .	U28877
Pedaliaceae		
<i>Proboscidea louisianica</i> Thell.	Chase et al. (1993)	
Phrymaceae		
<i>Phryma leptostachya</i> L.	U.S.A., Ohio, Athens, Co., <i>P. D. Cantino 1376</i> BHO.	U28881
Scrophulariaceae		
<i>Antirrhinum majus</i> L.	Olmstead et al. (1992)	
<i>Digitalis purpurea</i> L.	Olmstead et al. (1993)	
<i>Paulownia tomentosa</i> (Thunb.) Steudel	Olmstead and Reeves (1995)	
Stilbaceae		
<i>Retzia capensis</i> Thunb.	Bremer et al. (1994)	
<i>Euthystachys abbreviata</i> (E. Mey.) A. DC.	Bremer et al. (1994)	
Symphoremataceae		
<i>Congea tomentosa</i> Roxb.	U.S.A. Florida, Cultivated, Fairchild Botanical Garden, 59259.	U28870
Verbenaceae		
<i>Bouchea fluminensis</i> (Vell.) Moldenke	Olmstead and Reeves (1995)	
<i>Petrea racemosa</i> Kunth	UNITED KINGDOM, Cultivated, Royal Botanic Gardens, Kew, 000.73.17818	U28879
<i>Petrea volubilis</i> L.	U.S.A., Cultivated, Matthaei Botanic Garden, University of Michigan 760280.	U28880
<i>Rhaphithamnus spinosus</i> (A. L. Juss.) Moldenke	Olmstead and Reeves (1995)	
<i>Stachytarpheta dichotoma</i> (Ruiz et. Pav.) Vahl.	Olmstead and Reeves (1995)	
<i>Verbena bonariensis</i> L.	Olmstead et al. (1993)	

¹Voucher specimens are cited in Wagstaff et al. (1995).