Disintegration of the Scrophulariaceae[1]

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A molecular systematic study of Scrophulariaceae sensu lato using DNA sequences of three plastid genes (*rbcL, ndhF,* and *rps2*) revealed at least five distinct monophyletic groups. Thirty-nine genera representing 24 tribes of the Scrophulariaceae s.l. (sensu lato) were analyzed along with representatives of 15 other families of Lamiales. The Scrophulariaceae s.s. (sensu stricto) include part or all of tribes Aptosimeae, Hemimerideae, Leucophylleae, Manuleae, Selagineae, and Verbasceae (= Scrophularieae) and the conventional families Buddlejaceae and Myoporaceae. Veronicaceae includes all or part of tribes Angelonieae, Antirrhineae, Cheloneae, Digitaleae, and Gratioleae and the conventional families Callitrichaceae, Globulariaceae, Hippuridaceae, and Plantaginaceae. The Orobanchaceae include tribes Buchnereae, Rhinantheae, and the conventional Orobanchaceae. All sampled members of Orobanchaceae are parasitic, except *Lindenbergia,* which is sister to the rest of the family. Family Calceolariaceae Olmstead is newly erected herein to recognize the phylogenetic distinctiveness of tribe Calceolarieae. The Calceolariaceae are close to the base of the Lamiales. The Stilbaceae are expanded by the inclusion of *Halleria. Mimulus* does not belong in any of these five groups.

Key words: Calceolariaceae; Lamiales; *ndhF*; Orobanchaceae; *rbcL*; *rps2*; Scrophulariaceae; Stilbaceae; Veronicaceae.

The angiosperm order Lamiales (sensu Olmstead et al., 1993; Angiosperm Phylogeny Group, 1998) contains several large and well-known families with both tropical and temperate distributions. As traditionally circumscribed (e.g., von Wettstein, 1891), the Scrophulariaceae are the largest of these families and have a worldwide distribution. This family can be distinguished from related families with relative ease. However, it is through the absence of traits characteristic of related families that plants usually are assigned membership to Scrophulariaceae. The characteristics of a typical scroph, including flowers that are bilaterally symmetric and often tubular, ovaries with axile placentation and numerous ovules, capsular fruits, and seeds with endosperm, each are shared with one or several related families. This interpretation has been corroborated by a series of molecular phylogenetic studies of families in the Lamiales (Acanthaceae—Hedren, Chase, and Olmstead, 1995; Scotland et al., 1995; McDade and Moody, 1999; Gesneriaceae—Smith et al., 1997; Lamiaceae—Wagstaff and Olmstead, 1997; Wagstaff et al., 1998; Buddlejaceae—Oxelman, Backlund, and Bremer, 1999; Bignoniaceae—Spangler and Olmstead, 1999), which have determined that many of the related families are monophyletic, thereby suggesting that their distinguishing traits are synapomorphies for those families.

The absence of uniquely defining traits raises the possibility that the Scrophulariaceae are not monophyletic. This was examined by Olmstead and Reeves (1995), who identified two distinct clades composed of members of the family and suggested that the Scrophulariaceae are polyphyletic. Subsequently, a third clade was identified consisting of the parasitic members of the Scrophulariaceae and Orobanchaceae (dePamphilis, Young, and Wolfe, 1997; Wolfe and dePamphilis, 1998; Young, Steiner, and dePamphilis, 1999). Overlap in sampling between these two studies for taxa in Scrophulariaceae and other families in Lamiales was minimal, leaving open the possibility that these three clades may not all be distinct from each other (e.g., Wolfe and dePamphilis, 1997). Furthermore, the limited sampling of Scrophulariaceae s.l. (sensu lato) in these studies also left open the possibility that there could be additional distinct clades within the traditionally circumscribed family.

Like most large families, the history of the classification of Scrophulariaceae includes many treatments differing in circumscription of the family (see Olmstead and Reeves, 1995) and of suprageneric groupings within the family (see Thieret, 1967; Barringer, 1993). Most contemporary classifications of Scrophulariaceae are derived directly from Bentham's treatments (1846, 1876). He recognized three subfamilies, Pseudosolaneae, Antirrhinoideae, and Rhinanthoideae. Pseudosolaneae were defined on the basis of traits representing a supposed connecting link with the Solanaceae (nearly regular floral symmetry, alternate phyllotaxis, and presence of a fifth stamen, as in *Verbascum*). The other two subfamilies were distinguished on the basis of floral aestivation: posterior corolla lobes external to lateral lobes in bud in Antirrhinoideae and vice versa in Rhinanthoideae (Pseudosolaneae was similar to Antirrhinoideae in this respect). Pennell (1935), citing work by Robertson (1891) and Robyns (1931) proposed that the supposed similarities to Solanaceae were actually derived within Scrophulariaceae. Therefore he eliminated subfamily Pseudosolaneae and assigned its genera to Antirrhinoideae (see also Thieret, 1967; Armstrong and Douglas, 1989). Subse-

quent to Bentham's work, the families Orobanchaceae, Globulariaceae, Selaginaceae, Plantaginaceae, and Lentibulariaceae all have been considered by some authors to be part of the Scrophulariaceae (e.g., Hallier, 1903; Bellini, 1907; Melchior, 1964; Barringer, 1993). In Bentham's initial circumscription (Bentham, 1846), Buddlejaceae and the zygomorphic members of the Solanaceae were included. Recent molecular studies provide evidence that Myoporaceae and the aquatic families Callitrichaceae and Hippuridaceae belong to clades dominated by scrophs, and they provide further evidence that Buddlejaceae, Globulariaceae, Plantaginaceae, and Selaginaceae also belong in such clades (Olmstead and Reeves, 1995; Reeves and Olmstead, 1998; Wolfe and dePamphilis, 1998; Oxelman, Backlund, and Bremer, 1999).

Our present concern is with the identification of major lineages of plants conventionally assigned to the Scrophulariaceae and their relationship to other lineages of Lamiales. The study reported here represents the integration of two research programs addressing phylogenetic relationships in the Scrophulariaceae and Lamiales. In one, *rbcL* and *rps2* sequences were applied to questions of the evolution of parasitism in the Scrophulariaceae and Orobanchaceae (dePamphilis, Young, and Wolfe, 1997; Wolfe and dePamphilis, 1998; Nickrent et al., 1998). In the other, *rbcL* and *ndhF* sequences were applied to questions about the circumscription and phylogenetic placement of Scrophulariaceae in the Lamiales (Olmstead and Reeves, 1995). By expanding the efforts of each group to include data from all three genes for representatives of previously defined clades in Lamiales and suprageneric groups in the conventional Scrophulariaceae, this study attempts to address questions of clade circumscription.

All taxa in our resulting classification represent groups hypothesized to be monophyletic based on the evidence presented here. We provide phylogenetic definitions (deQueiroz and Gauthier, 1992, 1994; Cantino, Olmstead, and Wagstaff, 1997) for those clades that are derived primarily from portions of the traditional Scrophulariaceae.

MATERIALS AND METHODS

A total of 65 taxa was included in the study (Appendix). The primary emphasis in sampling was aimed at including as diverse an array of lineages of Scrophulariaceae s.l. as possible. The basis for sampling came from treatments of Scrophulariaceae infrafamilial classification (e.g., Bentham, 1876; Wettstein, 1891; Hallier, 1903; Thieret, 1967; Barringer, 1993). Thirty-nine genera (including two representatives of the genus *Orobanche*) representing ~24 tribes commonly assigned to the Scrophulariaceae s.l. were included. Evidence from prior studies (e.g., Olmstead and Reeves, 1995; Young, Steiner, and dePamphilis, 1999) suggested that for the identification of distinct lineages of Scrophulariaceae s.l., representative sampling throughout the Lamiales would be needed. Relying on previously published molecular systematic studies of the Lamiales (Olmstead et al., 1993; Olmstead and Reeves, 1995; Scotland et al., 1995; Smith et al., 1997; Wagstaff and Olmstead, 1997; Wagstaff et al., 1998; Spangler and Olmstead, 1999), we were able to select representatives of all clearly defined clades for inclusion. Sampling of non-scroph members of Lamiales added another 22 genera representing 15 families. Efforts were made to sample two or more representatives, which span the root node of each clade for all except very small clades. This strategy should represent the non-scroph clades as fully as possible, while keeping the number of taxa to a modest size. For a few genera, sequences for one of the genes came from a different species than for the others (Appendix). However, in all of these cases there is little doubt that the species sampled were much more closely related to each other than either was to any other species in the study.

Outgroups include representatives of each of the other three closely related orders, Boraginales, Gentianales, and Solanales.

New sequences for all three genes were obtained by direct sequencing of PCR (polymerase chain reaction) products either by manual or automated sequencing as previously described (*rbcL*—Olmstead et al., 1993; Olmstead and Reeves, 1995; Wolfe and dePamphilis, 1997; *ndhF*—Olmstead and Sweere, 1994; Olmstead and Reeves, 1995; *rps2*—dePamphilis, Young, and Wolfe, 1997; Young, Steiner, and dePamphilis, 1999). Sequence alignments were done by eye.

The three genes included in this study form a single linkage group as part of the chloroplast genome, so conflicts that might arise between data partitions from different sources subject to different evolutionary histories should not exist. However, differences in rates of evolution also have been suggested to lead to incongruence among characters from different genes (e.g., Bull et al., 1993), so we conducted the incongruence length difference (ILD) test (Farris et al., 1994), as implemented in PAUP* version 4.0b2 (Swofford, 1999), which tests whether the predefined partitions in the data differ significantly from random partitions of the combined data set. The analysis was conducted with 100 replicates, using a heuristic search strategy with a single random-order-entry starting tree, TBR (tree bisection-reconnection) branch swapping, and saving up to ten trees for each replicate.

Parsimony analyses of the data were conducted for each gene independently (results not shown) and in combination using PAUP*. All nucleotide substitutions were weighted equally (Olmstead, Reeves, and Yen, 1998); gaps were scored as missing and were not coded as characters (see below). Two heuristic search strategies were followed. In one, ten random-order-entry replicate searches were conducted using TBR branch swapping with MULPARS on. In the other (used only on the combined data set), 500 random order entry replicate searches were conducted, but saving only two trees per replicate. The second search was designed to search for shorter trees or islands of equally parsimonious trees not found by the first search. The likelihood score for each of the equally parsimonious trees obtained then was calculated using the following parameters: transition: transversion ratio of 2.0, base frequencies estimated from the data, and a discrete gamma rate distribution with four categories and an $\alpha = 0.4$ (estimated from the most parsimonious trees).

Bootstrap analysis was conducted with 1000 replicates using TBR swapping, but with MULPARS off (DeBry and Olmstead, 2000). Decay analysis was performed using AutoDecay vers. 4.0 (Eriksson, 1998). Constrained searches were conducted to examine the cost in parsimony of consolidating some groups of traditional scroph taxa. A test for significant difference from the maximum parsimony result then was conducted using the Kishino-Hasegawa/Templeton's test (Templeton, 1983; Kishino and Hasegawa, 1989), as implemented in PAUP* with the parsimony criterion.

RESULTS

Sequences of *rbcL* were 1402 nucleotides (nt) in length (positions 27–1428 in tobacco) and required seven gaps in the alignment, all of which were in the pseudogene sequences for *Orobanche ramosa* and *Boschniakia strobilacea* (previously described in Wolfe and dePamphilis, 1997, 1998), each of which had one or more frameshift deletions (four in *O. ramosa*; one in *B. strobilacea*). Missing data account for 4.1% of the *rbcL* matrix. Most of the missing data fall in a region at the 5′ end of *rbcL,* upstream from the conserved EcoRV restriction site used in cloning some sequences (Olmstead et al., 1992), and in a region downstream from position 1325, the location of a PCR primer site used to amplify some sequences. Only 0.3% of the *rbcL* matrix was missing otherwise. The *rbcL* matrix provides 292 parsimony informative sites to the combined data set (25% of the total), along with 227 variable, but uninformative, sites and 883 invariant sites.

Aligned sequences of *ndhF* were 2197 nt in length (positions 24–2109 in tobacco). Sequences of *ndhF* were the most length variable of the three genes studied, with 35 gaps (none

of which required shifts in reading frame) inserted into the sequences to produce the alignment. The *ndhF* partition of the data set includes the greatest number of missing taxa (*Alectra, Boschniakia, Buchnera, Castilleja, Harveya, Melampyrum, Orobanche corymbosa, Orobanche ramosa, Striga, Tozzia*). The inability to obtain *ndhF* sequences for any of these taxa may imply a missing or divergent *ndhF* gene in these taxa. Supporting this possibility is the observation of very light or missing hybridization signals in all taxa except *Castilleja* when Southern blots are probed with the plastid *ndhF* gene (C. dePamphilis, unpublished data). At least one member of Orobanchaceae, *Epifagus virginiana,* is confirmed to be missing *rbcL* and *ndhF* from the plastid genome (dePamphilis and Palmer, 1990; Wolfe, Morden, and Palmer, 1992). Missing data, not counting the ten missing taxa, account for 3.4% of the total matrix. The missing data are more unevenly distributed than in the *rbcL* matrix, with several sequences missing substantial portions (e.g., *Melasma*—1033 nt missing; *Callitriche*—753; *Hippuris*—279; *Barleria* and *Elytraria*—276; *Callicarpa*—174). Apart from the missing portions from these six taxa, only 1.2% of matrix cells are missing. The *ndhF* matrix provides 745 parsimony informative sites to the combined data set (63% of the total), along with 365 variable, but uninformative, sites.

The aligned sequences for *rps2* were 611 nt in length (positions 48–656 in tobacco) and include a single 3-nt insertion in *Buchnera* as the only alignment gap. Seven taxa, including two of the outgroups and five representatives of non-scroph families, were not represented in the data set for *rps2* (*Borago, Callicarpa, Elytraria, Gentiana, Nematanthus, Retzia,* and *Streptocarpus*). With the fewest informative characters, *rps2* sequences were included primarily to resolve relationships among the parasitic scrophs, where *ndhF* was missing for some taxa, and among other clusters of scroph taxa, so the absence of *rps2* data from outgroups and related families (e.g., Acanthaceae, Gesneriaceae, Lamiaceae) should not be problematic. Missing data, not counting the seven missing taxa, account for 0.2% of the matrix. Thus, *rps2* provides 142 parsimony-informative sites to the combined data set (12% of the total), along with 105 variable, but uninformative, sites.

The combined data set consists of 4210 nt of aligned sequence, including 1179 parsimony-informative characters. Data for one or another of the three genes are missing for 17 taxa, but no taxon is missing more than one sequence and *rbcL* is present for all. Of the 43 gaps inserted into the sequences to obtain the alignment, only eight (six insertions and two deletions) are shared among two or more taxa. The gaps were not used as characters, but the following inferences were made. Five gaps (three insertions and two deletions) provide synapomorphies for groups that are supported by 99–100% bootstrap support based on nt substitutions alone (Figs. 1,2). The other three gaps (all insertions representing duplications of adjacent sequence) are shared by two or, in one case, three taxa that are distantly related with one or more splits in the tree having bootstrap values of 94% or greater (usually 100%) occurring between the taxa sharing the insertions.

The ILD test results revealed no significant difference ($P = 0.11$) between the partitions defined by the three genes and random partitions of the same size as the genes, but drawn from the combined data set.

The analysis of the combined data set yielded 240 most parsimonious trees of 6610 steps (consistency index, CI, including all variable characters = 0.44, excluding uninforma-

tive characters = 0.36; retention index, RI, = 0.41). One of those trees (the one with the greatest likelihood score) is illustrated in Fig. 1, and the strict consensus of all trees is shown in Fig. 2. Results of the individual gene parsimony analyses (trees not shown) are summarized as follows. The *rbcL* analysis yielded 630 most parsimonious trees of length 1568 (CI = 0.45/0.34; RI = 0.41). The length of the *rbcL* data optimized onto the 240 trees obtained from the combined data analysis ranged from 34 to 44 steps longer (2.2–2.8%). The *ndhF* analysis yielded 44 trees of length 4385 (CI = 0.43/0.36; RI = 0.40). The length of the *ndhF* data on the combined data trees ranged from 3 to 13 steps longer (0.1–0.3%). The *rps2* analysis yielded 28 trees of length 609 (CI = 0.56/0.46; RI = 0.60). The length of the *rps2* data on the combined data trees ranged from 15 to 17 steps longer (2.5–2.8%).

The strict consensus tree of the 240 most parsimonious trees (Fig. 2) has only six trichotomous nodes within the ingroup and only six additional nodes with bootstrap values below 50%. While the poorly resolved portions of the tree include segments of the main stem from which the major family-level clades diverge, many of the branches that diverge are well supported. Branches that represent well supported non-scroph lineages include the Lamiaceae (99% bootstrap support), Acanthaceae (91%), Bignoniaceae (100%), Verbenaceae (73%), Gesneriaceae (94%), and a branch composed of all Lamiales except Oleaceae (100%), thereby confirming the placement of that family near the base of the order (Wagstaff and Olmstead, 1997; Oxelman, Backlund, and Bremer, 1999). Interspersed among these clades on the tree are several clades that are composed entirely, or in part, of taxa historically assigned to Scrophulariaceae. These include two clades identified by Olmstead and Reeves (1995) as "Scroph I" (Scrophulariaceae s.s.) and "Scroph II" (Veronicaceae). This study contains many more representatives for each of these two clades. The third major clade is one identified by dePamphilis, Young, and Wolfe (1997) and consists primarily of the parasitic members of the Scrophulariaceae s.l. (Orobanchaceae). This clade has two non-parasitic taxa, *Lindenbergia* and *Paulownia,* as progressively more distant sister groups. The small tribe Calceolarieae comprises a fourth clade and the Stilbaceae plus the scroph genus *Halleria* comprise a fifth clade. Additional taxa included in this study that often are assigned to the Scrophulariaceae, but which fall outside of these five groups, include *Mimulus* (most parsimoniously placed as either sister to the Orobanchaceae clade or the Lamiaceae) and *Schlegelia* (weakly supported in a clade with Bignoniaceae). Other taxa typically not assigned to the Scrophulariaceae are found nested within clades otherwise consisting primarily of scrophs. This includes Callitrichaceae, Globulariaceae (but see Barringer, 1993), Hippuridaceae, and Plantaginaceae within the Veronicaceae, and Buddlejaceae and Myoporaceae within the Scrophulariaceae s.s.

A series of constrained searches was implemented to explore whether the aggregation of two or more of the distinct scroph clades would result in substantially less parsimonious trees. Constraints combining the Veronicaceae, Orobanchaceae, Scrophulariaceae, and Stilbaceae/Halleria clades in various combinations resulted in trees 1–16 steps longer (Table 1). Among those comparisons uniting the three main scroph clades (Veronicaceae, Orobanchaceae, Scrophulariaceae), results typically were mixed, with some resulting trees significantly less parsimonious at the 5% level, while others are not, based on Kishino-Hasegawa/Templeton tests. When the Cal-
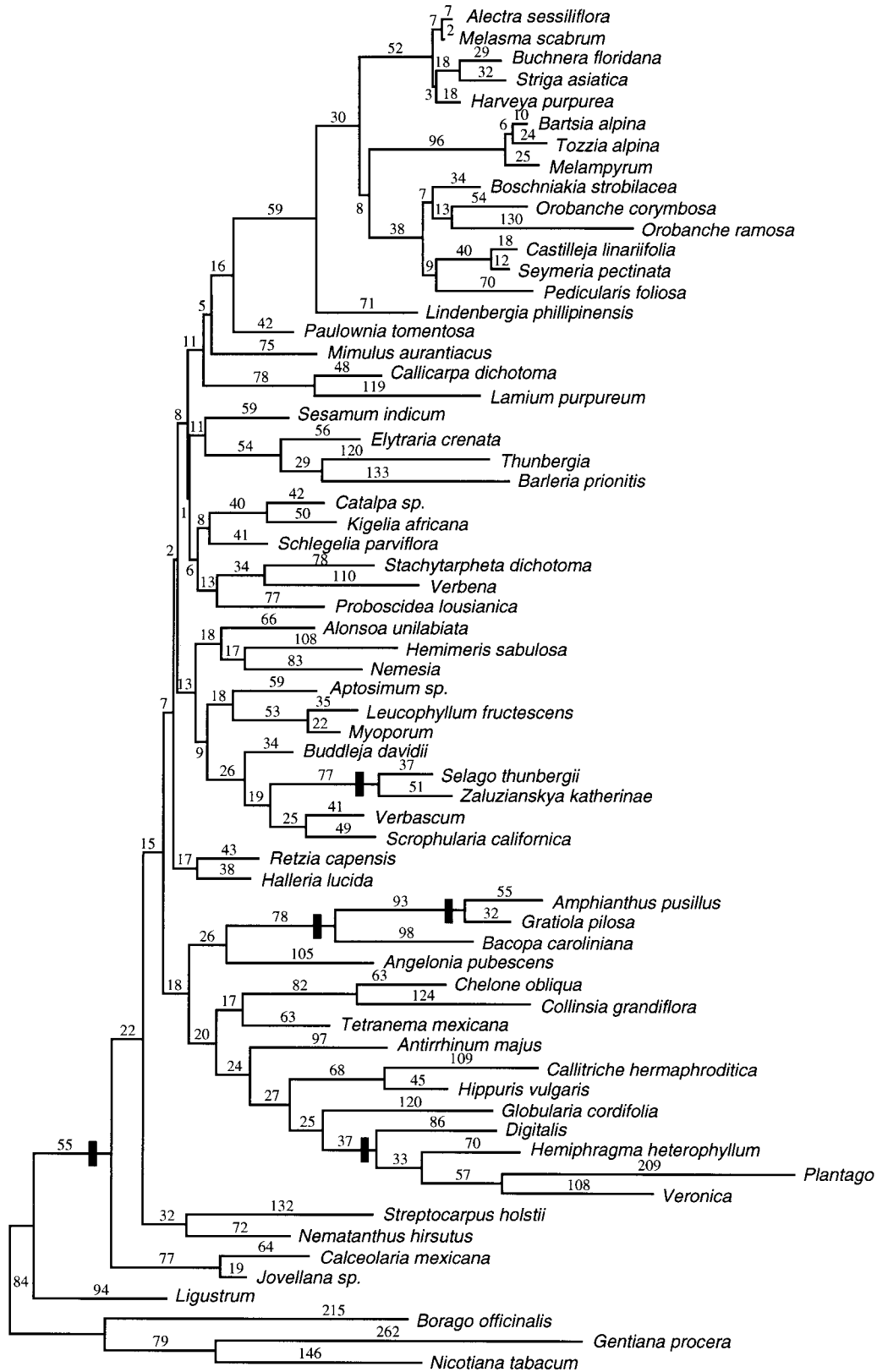
Fig. 1. One of the 240 most parsimonious trees (the one with the greatest likelihood score) showing branch lengths (ACCTRANS optimization). Bars indicate synapomorphic insertions or deletions in *ndhF* sequences.
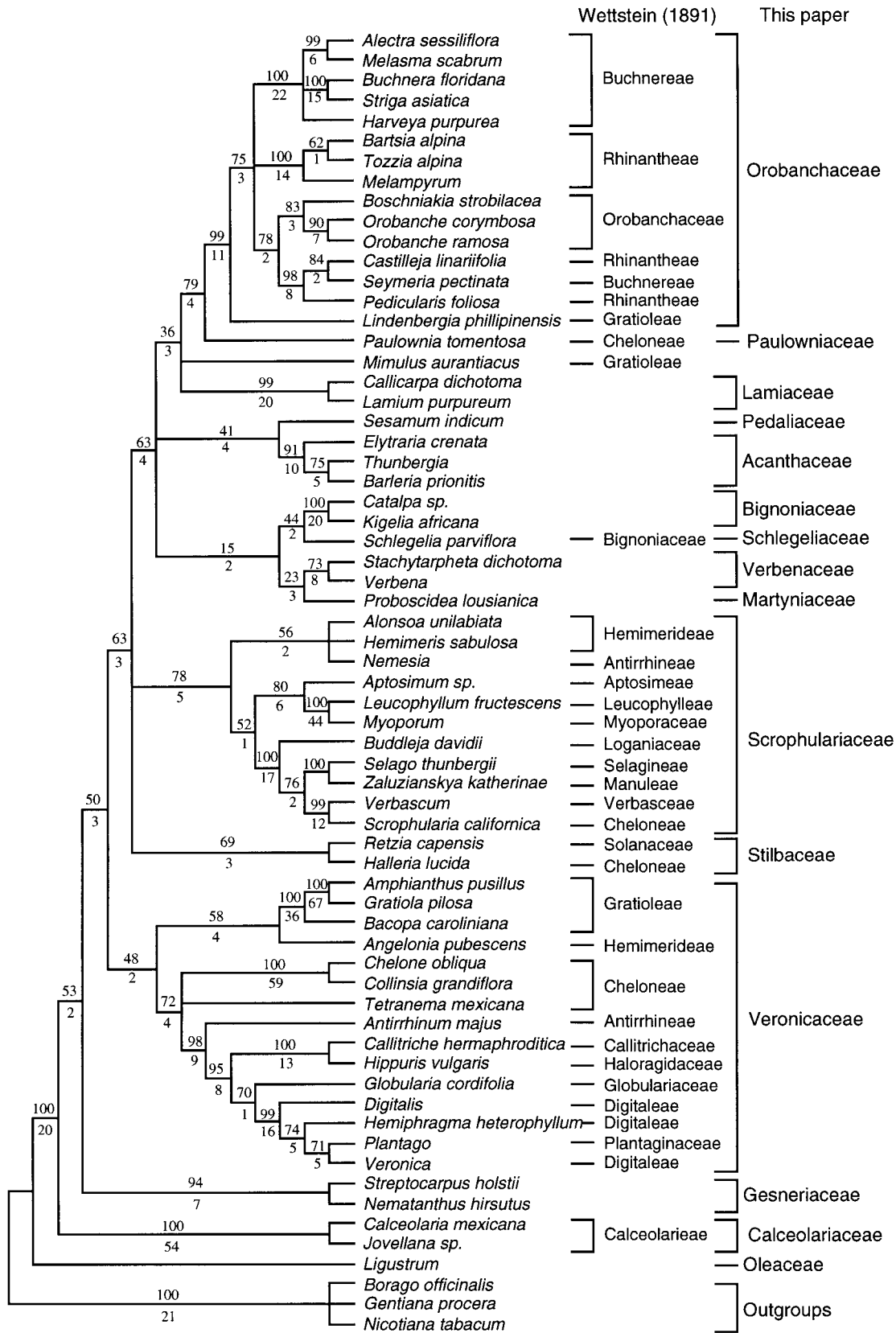
Fig. 2. Strict consensus of the 240 most parsimonious trees. For taxa in the Scrophulariaceae s.l., tribal classification (or familial classification for taxa included in the major scroph lineages that were recognized as distinct families) according to von Wettstein (1891), and other contributors to *Die Natürlichen Pflanzenfamilien* (Engler and Prantl, 1887–1915), is indicated immediately to their right. The family-level classification accepted by the authors is indicated farthest right. Numbers above the branches represent bootstrap proportions; numbers below the branches represent decay values.

TABLE 1.   Results of the constraint analyses and Kishino-Hasegawa/Templeton's (K-H/T) tests.

Constraint	Tree length	No. of trees	K-H/T test
None	6610	240	not applicable
Scrophulariaceae/Stilbaceae	6611	120	n.s.
Antirrhinaceae/Scrophulariaceae	6613	24	n.s.
Antirrhinaceae/Scrophulariaceae/Stilbaceae	6615	72	n.s.
Antirrhinaceae/Stilbaceae	6615	120	$P < 0.05$ for 2 of 120 trees
Antirrhinaceae/Orobanchaceae/Scrophulariaceae/Stilbaceae	6621	12	n.s.
Antirrhinaceae/Orobanchaceae/Scrophulariaceae	6623	66	$P < 0.05$ for 33 of 66 trees
Antirrhinaceae/Orobanchaceae/Scrophulariaceae/Stilbaceae, and *Schlegelia*	6626	12	$P < 0.05$ for 6 of 12 trees
Antirrhinaceae/Calceolariaceae/Orobanchaceae/Scrophulariaceae/Stilbaceae, and *Schlegelia*	6636	24	$P < 0.05$ for all trees
Scrophulariaceae sensu Wettstein (1891), including Orobanchaceae s.s.	6762	15	$P < 0.0001$ for all trees

ceolariaceae are included, resulting trees are 26 steps longer and all are significantly less parsimonious.

DISCUSSION

The three genes encode plastid proteins with diverse functions, including photosynthesis (*rbcL*), chlororespiration (*ndhF*), and translation (*rps2*). The first two genes, *rbcL* and *ndhF,* have been used widely in plant systematics, where they have been suggested to represent slowly and rapidly evolving sequences, respectively (Olmstead and Palmer, 1994). However, the greatest difference between the two genes in terms of their evolutionary rates exists primarily in the greater proportion of invariant sites in *rbcL* (63 vs. 49% for *rbcL* and *ndhF,* respectively, in this study). Analysis of rates of change in parsimony-informative sites in the Asteridae (including representatives of the Lamiales) indicates that there is no significant difference between the two genes (Olmstead, Reeves, and Yen, 1998). Therefore, the fact that *ndhF* is approximately one and one-half times the length of *rbcL* and has fewer invariant sites results in its contributing more than 2.5 times as many characters to this analysis (745 vs. 292). Statistical tests for evolutionary rates of parsimony informative sites in *rps2,* such as those reported for *rbcL* and *ndhF,* have not been done, but relative to its size, the numbers of invariant sites is intermediate between those two (60%), suggesting that it has similar rates as well.

While less frequently used than *rbcL* and *ndhF, rps2* may deserve greater attention by plant systematists (dePamphilis, Young, and Wolfe, 1997; Wolfe et al., 1997; Young, Steiner, and dePamphilis, 1999). Congruence among parsimony-informative characters in *rps2,* measured by CI or RI, is greater than for either *rbcL* or *ndhF,* both when considering the most parsimonious trees derived from *rps2* alone and when considering the trees derived from the combined three-gene data set. The fact that *rps2* is smaller (608 nt in most taxa) means that it provided fewer informative sites (142 sites, only 12% of the total).

Relationships among groups in the Lamiales have proven to be difficult to resolve with morphological and molecular approaches (Lu, 1990; Olmstead et al., 1993; Olmstead and Reeves, 1995). Assuming that the method of analysis is consistent for a particular study, increasing the number of characters will increase the probability that the phylogeny is reconstructed accurately (Huelsenbeck and Hillis, 1993; Cummings, Otto, and Wakely, 1995; Hillis, 1996). It is probably

for this reason that the trees derived from the *ndhF* data alone, which contribute 63% of the parsimony characters, are more similar to the combined data trees than are those derived from *rbcL* or *rps2*. Given the historical difficulty in resolving relationships in this group and the contrasting highly resolved tree presented here (Figs. 1–2), the effort to include three gene sequences and over 4000 nt of sequence seems well justified. These results are the most complete statement of phylogeny for the Scrophulariaceae and, by extension, the Lamiales yet made.

Systematic implications—These results imply that the traditional Scrophulariaceae are not monophyletic. Sampling of Scrophulariaceae s.l. was not comprehensive, but every effort was made for sampling to be representative of the traditional infrafamilial classification. For the several families of Lamiales that have been studied extensively and found to be monophyletic, our sampling included representatives that span the root node, or another very deep node, in each case and the analyses resolved those groups to be monophyletic (e.g., Bignoniaceae—Spangler and Olmstead, 1999; Acanthaceae—Scotland et al., 1995; Hedren, Chase, and Olmstead, 1995; Gesneriaceae—Smith et al., 1997); Lamiaceae—Wagstaff and Olmstead, 1997, Wagstaff et al., 1998). The finding that Pedaliaceae (sensu Cronquist, 1981), represented by *Sesamum* and *Proboscidea,* are not monophyletic is consistent with other molecular studies (Olmstead et al., 1993; Oxelman, Backlund, and Bremer, 1999) and morphological studies (e.g., Bretting and Nilsson, 1988) in separating the New World genera as the family Martyniaceae.

Although these results indicate that the Scrophulariaceae as traditionally defined are not monophyletic, the strength of the evidence is modest and we do not wish to overstate that conclusion. Tests comparing the results of the unconstrained parsimony analysis with the results of searches in which various combinations of traditional scroph groups are constrained to monophyly (Table 1) are unambiguous in rejecting a broadly defined Scrophulariaceae including Calceolarieae, but are equivocal for a more narrowly defined group in which the Scrophulariaceae s.s., Veronicaceae, and Orobanchaceae are constrained to monophyly, with or without *Schlegelia* (in each case half of the constrained trees are significantly worse than the unconstrained trees). Expanded studies of cpDNA sequences (*rps16* intron—B. Oxelman, personal communication, Uppsala University; *matK*—C. dePamphilis and N. Young, un-

published data) corroborate the studies presented here, whereas a study of ITS sequences suggests, albeit with weak support, a monophyletic Scrophulariaceae s.l. (A. Wolfe, unpublished data).

It seems evident that recognition of the traditional Scrophulariaceae is based on shared characteristics that are common throughout Lamiales. Whether this means that the traditional Scrophulariaceae are a paraphyletic, evolutionary grade and are ancestral to most of the rest of the order (except the bas-almost branches, including Oleaceae, Tetrachondraceae, and *Polypremum*; Wagstaff and Olmstead, 1997; Oxelman, Backlund, and Bremer, 1999) hinges on our ability to accurately and completely reconstruct the phylogeny of the group and then to reconstruct morphological character states for ancestral nodes. A polyphyletic group would be one in which their shared similarities include some characters that have converged and are not retained from a common ancestor. A par-aphyletic grade would be expected to retain the plesiomorphic states for characters in the clade to which they belong through-out the diversification of the clade.

While it might be tempting to consider the scrophs to be a grade, it is too speculative to do so at this time. The assignment of individual character states for family-level clades throughout the Lamiales is difficult, because many groups are polymorphic for important floral traits (e.g., Wagstaff and Olmstead, 1997). This problem persists even though identification of monophyletic groups in the Lamiales has progressed dramatically since a previous attempt at cladistic analysis of morphological traits in the Lamiales (Lu, 1990). Also, uncertainties in resolution of Lamiales phylogeny in this study, the absence of several small distinct lineages (e.g., Avicenniaceae, Phrymaceae, Lentibulariaceae), and the possibility of extinct lineages with critical character combinations make the assignment of accurate ancestral states intractable.

It probably is fair to conclude that some clades of the former Scrophulariaceae share plesiomorphic traits relative to their closest relatives in the Lamiales, but it is impossible to know whether convergence in other clades, or reversal to plesio-morphic states in one or more lineages may have occurred, thereby rendering a verdict of paraphyly for the traditional Scrophulariaceae invalid. It is apparent that some clades comprised largely of scroph taxa are paraphyletic with respect to other commonly recognized families, including Callitricha-ceae, Hippuridaceae, Globulariaceae, Plantaginaceae, and even Buddlejaceae and Myoporaceae. However, these are small families defined by apomorphic traits.

Rather than debating polyphyly vs. paraphyly, the primary conclusion from this cpDNA study is that Scrophulariaceae, as traditionally circumscribed, are not monophyletic. The more important contribution we hope to make is to identify clades from the dismembered Scrophulariaceae s.l. that merit recognition.

Below, the major clades of Lamiales that consist predominantly of plants previously assigned to the traditional Scrophulariaceae are discussed in turn. For each, support from the molecular analysis for the major clade as well as clades nested within them is presented, along with information on the distribution and taxonomic history of the groups. Some of the morphological and anatomical evidence corroborating these results is described where available. However, comprehensive morphological descriptions for each clade are not attempted and morphological synapomorphies for them are not known at present. Given the great diversity represented by some of these clades (e.g., Veronicaceae and Scrophulariaceae), morphological descriptions would necessarily be imprecise. Recognizing these clades as taxa at this time represents a major first step towards the understanding of these groups. Future study may identify morphological or anatomical synapomorphies for these clades and enable more useful morphological descriptions to be developed. The descriptions in Judd et al. (1999) provide a useful starting point for such studies.

Scrophulariaceae sensu stricto—This analysis confirms the identity of a distinct clade simply referred to as ''Scroph I'' by Olmstead and Reeves (1995). Support for this clade is moderately strong, with 7–13 substitutions (delayed and accelerated transformation optimizations, respectively), bootstrap support of 78% and a decay value of 5 (Fig. 2). Given the constraints of our sampling, this clade is circumscribed to include the three tribes assigned by Bentham (1876) to his subfamily Pseudosolaneae (Aptosimeae, Leucophylleae, Verbasceae), tribes Hemimerideae (not including *Angelonia*), Manuleae, and Selagineae, families Buddlejaceae and Myoporaceae, and the type genus *Scrophularia* (Cheloneae sensu Bentham, 1876).

The predominant concentration of genera in this clade is distributed in the southern hemisphere, particularly in Africa. Tribes Aptosimeae, Hemimerideae, Manuleae, and Selagineae occur almost exclusively in Africa and Myoporaceae are distributed predominantly in Australia. Buddlejaceae occur in South Africa, but their greatest distribution is in the neotropics and East Asia. *Scrophularia* and tribe Verbasceae are the only predominantly north temperate groups. *Leucophyllum* (with *Eremogeton* forming tribe Leucophylleae), which occurs in the deserts of North America, and *Scrophularia* are the only genera of Scrophulariaceae native to North America.

Based on seed and embryo characteristics (Schmid [1906] and Hartl [1959] summarized in Thieret [1967]) and the presence of idioblasts in leaves (Lersten and Curtis, 1997), a close relationship for *Scrophularia* with *Verbascum*, rather than with tribe Cheloneae, has been postulated. Our results strongly support this relationship. At the very least, these genera should be assigned to one tribe, Scrophularieae. *Selago* (Selagineae) and *Zaluzianskya* (Manuleae) form a strongly supported clade (100% bootstrap) restricted to South Africa and are sister to the *Verbascum*/*Scrophularia* clade. *Globularia*, which together with tribe Selagineae form Cronquist's (1981) Globulariaceae, does not belong in this clade. This close relationship between Manuleae and Selagineae and the distinctness of *Globularia* is consistent with evidence from pollen morphology (Argue, 1993). *Buddleja*, representing Buddlejaceae, forms a strongly supported clade with the above two clades (100%). Oxelman, Backlund, and Bremer (1999) sampled all genera of Buddlejaceae and found strong support for a monophyletic Buddlejaceae s.s. (*Buddleja, Emorya, Gomphostigma,* and *Nicodemia*) consistent in placement with the results presented here. *Aptosimum* (Aptosimeae) and *Leucophyllum* (Leucophylleae) form a well-supported clade with Myoporaceae (80%) and together are sister to the clade described above. The strongly supported relationship between *Leucophyllum* and Myoporaceae (100%) confirms conclusions derived from the study of pollen (Niezgoda and Tomb, 1975) and leaf anatomy (Karrfalt and Tomb, 1983; Lersten and Beaman, 1998). Niezgoda and Tomb (1975) recommended that Leucophylleae be made a subfamily of Myoporaceae, citing more similarities between them than between Leucophylleae and other scrophs. Empha-

sizing the similarities between Leucophylleae, Myoporaceae, and Scrophulariaceae, Henrickson and Flyr (1985), in their monograph of the Leucophylleae, questioned the validity of maintaining Myoporaceae distinct from Scrophulariaceae.

The Hemimerideae (sensu Bentham 1846, minus *Angelonia*), represented by *Hemimeris, Nemesia,* and *Alonsoa* form a moderately supported clade (56%) that is sister to the rest of Scrophulariaceae sensu stricto. Relationships among the three genera are unresolved in the strict consensus of the most parsimonious trees (Fig. 2). Without any discussion, Pennell (1919) segregated *Angelonia* into its own tribe. This is consistent with having a chromosome number of $2n = 20$, which is not found otherwise in the Hemimerideae (Steiner, 1996), and with its phylogenetic separation from the rest of the tribe. However, Barringer's (1993) segregation of *Alonsoa* as Alonsoeae is not supported by these results.

To maintain Buddlejaceae and Myoporaceae as distinct families would require expanding the Myoporaceae to include Leucophylleae and Aptosimeae, and elevating Hemimerideae to familial status. Not only would this would require additional splitting, it would require that groups with lower bootstrap support be recognized as family-level clades (smaller Scrophulariaceae—76%; Hemimerideae—56%). Therefore, we recommend establishing a revised Scrophulariaceae that will encompass this entire clade, including Buddlejaceae and Myoporaceae.

We provide the following clade definition. Scrophulariaceae are the least inclusive clade that contains *Hemimeris sabulosa, Myoporum mauritianum,* and *Scrophularia californica.*

Veronicaceae—This large clade was recognized by Olmstead and Reeves (1995) as "Scroph II" and contains most of the remaining nonparasitic scrophs. Support for this clade is weak, with 6–18 substitutions, bootstrap of 48%, and decay of two steps (Fig. 2). This clade includes part or all of Bentham's (1876) tribes Digitaleae, Antirrhineae, Cheloneae, and Gratioleae, the small tribe Angelonieae (segregated from Hemimerideae by Pennell [1919]), and the small families Callitrichaceae, Globulariaceae (excluding Selagineae), Hippuridaceae, and Plantaginaceae. The clade does not seem to have a geographic focus; several of the groups represented here have worldwide distributions.

The oldest valid family name associated with taxa included in this clade is Veronicaceae (published in 1782). However, Plantaginaceae (1789), Globulariaceae (1805), Callitrichaceae (1821), and Hippuridaceae (1821) all are conserved names and, therefore, have precedent over Veronicaceae (Plantaginaceae is used for this group in Judd et al. [1999]). Because those names all are associated with small and more or less clearly defined groups and the predominant membership in this clade comes from the traditional Scrophulariaceae, a name for this clade reflecting that association is desirable. In consideration of the fact that Veronicaceae was among the earliest published names, we have selected it as the name for this clade. Under the International Code of Botanical Nomenclature (ICBN), if this clade is recognized at the rank of family, it would be Plantaginaceae. However, we prefer to retain the name Plantaginaceae for the smaller clade with which it has long been associated.

Bentham's (1876) tribe Digitaleae (*Digitalis, Hemiphragma,* and *Veronica*) and Plantaginaceae form a strongly supported clade (99%, Fig. 2). Bentham's three subtribes of Digitaleae, which have been treated as tribes in subsequent treatments

(e.g., Rouy, 1909; Pennell, 1935), all are represented in this study: *Digitalis* (Digitaleae), *Hemiphragma* (Hemiphragmieae), and *Veronica* (Veroniceae). In Bentham's (1876) subfamilial classification, Digitaleae consisted of the nonparasitic members of subfamily Rhinanthoideae. The segregation of the Digitaleae from the Rhinanthoideae was anticipated by Bellini (1907), who included the Orobanchaceae with the remaining Rhinanthoideae on the basis of their shared parasitic habit (see also Armstrong and Douglas, 1989). Plantaginaceae have frequently been suggested to be close to Scrophulariaceae (and included in it by Hallier [1903]), but their placement has been obscure. Barringer (1993) placed *Globularia* and *Poskea* in tribe Globularieae, but suggested a close relationship with Manuleae and Selagineae, which, in this study, fall in the Scrophulariaceae s.s. Callitrichaceae and Hippuridaceae are two monogeneric families of aquatic plants with very reduced floral morphology, whose taxonomic placement also has been problematic (e.g., Cronquist, 1981). All three families now seem well resolved as members of Veronicaceae (Olmstead and Reeves, 1995; Reeves and Olmstead, 1998).

Tribe Cheloneae sensu Bentham (1876) is represented in this study by *Chelone, Collinsia, Halleria, Paulownia, Scrophularia,* and *Tetranema.* The artificial nature of Bentham's concept for this tribe, based on the presence of a cymose inflorescence, has been exposed in previous treatments (reviewed in Wolfe et al., 1997). As mentioned above, *Scrophularia* belongs with tribe Verbasceae (= Scrophularieae), *Paulownia* has been removed to its own family (Nakai, 1949; see discussion below), and *Halleria* belongs with *Retzia* (Stilbaceae). Cheloneae s.s. comprise New World genera only (Wolfe et al., 1997). Our results agree with Wolfe et al. (1997) in finding a close relationship between the core Cheloneae (*Chelone* and four other genera) and tribe Collinsieae (*Collinsia* and *Tonella*), also native to North America. *Tetranema,* from Mexico and Central America, was not placed close to the North American Cheloneae in the combined cpDNA RFLP data analysis of Wolfe et al. (1997). In contrast, *Tetranema* falls in the clade with Chelone and *Collinsia* in some of the most parsimonious trees in this study and is removed by only one node in the others (in agreement with some of the data partitions in Wolfe et al. [1997]). Cheloneae, Collinsieae, and the clade described in the preceding paragraph form a moderately well-supported group (71%, Fig. 2).

Three representatives of tribe Gratioleae (*Amphianthus, Bacopa,* and *Gratiola*) form a strongly supported group (100%) and together with *Angelonia* (Angelonieae) comprise a moderately supported clade (58%) that is sister group to the rest of Veronicaceae. Bentham (1876) divided Gratioleae into five subtribes, which have been considered tribes in various treatments. Of these, four are represented here. Gratiolieae (*Gratiola*), Herpestidineae (*Bacopa*), and Limoselleae (*Amphianthus*) belong together in this clade. The placement of Mimuleae (*Mimulus*) is uncertain, but it clearly does not belong to Veronicaceae. *Lindenbergia* (subtr. Stemodiinae), as discussed below, belongs elsewhere, as well. The Torrenieae were not sampled.

Support for the Veronicaceae is not strong. Support for the clade that includes *Antirrhinum* along with Callitrichaceae, Globulariaceae, Hippuridaceae, and Plantaginaceae is very strong (98%) and the rest of the Veronicaceae consists of three to four lineages of scrophs that diverge earlier. Adherence to a strict rule of support for named clades (e.g., ≥70% bootstrap) would require splitting this clade into three smaller

groups, with the two smaller clades composed entirely of traditional Scrophulariacae and the larger clade also dominated by scrophs. The clade Veronicaceae also was found by Oxelman (personal communication) in a study of chloroplast *ndhF* and *rps16* intron sequences. We anticipate that further evidence from cpDNA will only serve to strengthen support for the Veronicaceae. This group could benefit greatly from additional morphological and anatomical study to identify, if possible, synapomorphies uniting the Gratiolieae with the rest of the Veronicaceae.

We provide the following clade definition. Veronicaceae are the least inclusive clade that contains *Gratiola pilosa, Angelonia pubescens, Chelone obliqua,* and *Antirrhinum majus.*

Orobanchaceae—Bellini (1907) proposed a radically different basis for the primary division in the Scrophulariaceae relative to Bentham's (1876) use of floral aestivation. He divided the family on the basis of whether the plants were parasitic or not. His subfamily Scrophularioideae contained Bentham's (1876) Pseudosolaneae, Antirrhinoideae, and tribe Digitaleae (Rhinanthoideae sensu Bentham). His subfamily Rhinanthoideae contained Bentham's tribes Gerardieae (= Buchnereae) and Pedicularieae (= Rhinantheae) plus the Orobanchaceae. (Note: Both Buchnereae Benth. and Gerardieae Benth were first used in the same publication and have equal priority. The Buchnereae have been recognized more often in recent studies, because it is based on a currently accepted genus name.) Bellini's (1907) concept of these parasitic plants forming a natural group has received support from studies of anatomy and morphology (Boeshore, 1920; Kuijt, 1969; Armstrong and Douglas, 1989) and has been borne out by molecular phylogenetic studies (dePamphilis, Young, and Wolfe, 1997; Wolfe and dePamphilis, 1998; Young, Steiner, and dePamphilis, 1999).

The clade comprising the parasitic plants in our analysis is well supported, with 8–30 substitutions (this broad range results from differences in character optimization onto the tree when *ndhF* sequences are missing from several taxa; only four taxa in this clade have sequences for all three genes), 75% bootstrap, and a decay value of 3 (Fig. 2). This result is congruent with results from *rps2* alone (dePamphilis, Young, and Wolfe, 1997), *rbcL* alone and in combination with *rps2* (Nickrent et al., 1998; Wolfe and dePamphilis, 1998), and *matK* alone and in combination with *rps2* (Young, Steiner, and dePamphilis, 1999). Within this clade, Buchnereae and Rhinantheae are not monophyletic, while Orobanchaceae s.s. (represented by *Orobanche* and *Boschniakia*) are monophyletic. These results are consistent with studies cited above. Two species of *Orobanche* were included in this study, because prior evidence from the analysis of *rps2* and *matK* (Young, Steiner, and dePamphilis, 1999) indicated that the genus was biphyletic with *Boschniakia* more closely related to the clade containing *O. corymbosa.* Our results place the two *Orobanche* species together with 90% bootstrap support, but Young, Steiner, and dePamphilis included much greater sampling of related taxa and had more informative characters for the genes they sampled (*ndhF* could not be sequenced for *Orobanche* and *Boschniakia*), so our results should not be considered to be in strong disagreement with theirs. For further discussion of molecular systematic implications for the parasitic scrophs, see Young, Steiner, and dePamphilis (1999).

The closest relatives of this clade provide perhaps the most interesting challenge to taxonomic circumscriptions in the groups derived from the traditional Scrophulariaceae. A conveniently circumscribed Orobanchaceae could be restricted to the parasitic plants of the traditional Scrophulariaceae and Orobanchaceae. However, *Lindenbergia,* a nonparasitic genus, forms a clade with the parasites that is much more strongly supported than the parasite clade by itself (39–59 substitutions, 99% bootstrap, decay of 11; Figs. 1, 2). This confirms the similar placement found by Young, Steiner, and dePamphilis (1999), who included many fewer nonparasitic scrophs and outgroups. *Lindenbergia* has corolla aestivation similar to the other rhinanthoid taxa found in this clade (Brühl, 1920), even though it is traditionally assigned to tribe Gratioleae in subfamily Antirrhinoideae (Bentham, 1876). Several other genera assigned with *Lindenbergia* to subtribe Stemodiinae (Bentham, 1876) have floral aestivation typical of Antirrhinoideae (Brühl, 1920). For this reason *Lindenbergia* has been suggested to belong to the Rhinanthoideae (debate reviewed in Thieret, 1967).

Sister to the clade comprising the parasites and *Lindenbergia* is *Paulownia,* a genus of large trees native to East Asia. The inclusive clade is supported by 7–16 substitutions, bootstrap of 79% and decay of four steps (Fig. 2). *Paulownia* has been placed alternately in the Scrophulariaceae (tribe Cheloneae) and Bignoniaceae and has been assigned to a family of its own (Nakai, 1949). Its floral aestivation is antirrhinoid, unlike *Lindenbergia* and the parasitic members of this clade. Molecular phylogenetic studies that have attempted to test the placement of *Paulownia* in either the Scrophulariaceae (Olmstead and Reeves, 1995) or Bignoniaceae (Spangler and Olmstead, 1999) have failed, because neither study included any members of the Orobanchaceae. However, it was included in studies of the parasitic plants, where it was weakly resolved as either sister to the parasites (Wolfe and dePamphilis, 1998; *Lindenbergia* not sampled) or as part of an unresolved node with representatives of other families immediately below *Lindenbergia* and the parasites (Young, Steiner, and dePamphilis, 1999).

In some of the most parsimonious trees, *Mimulus* forms the next branch below *Lindenbergia* and *Paulownia.* However, in other trees, *Mimulus* forms a clade with the Lamiaceae. Bentham (1876) placed *Mimulus* in a subtribe Mimulinae of tribe Gratioleae. No other close relatives of *Mimulus* have been sampled here, but are the subject of ongoing research (Beardsley and Olmstead, unpublished data).

Deciding where to cut the tree to circumscribe families will not satisfy everyone's concepts. In this case we prefer to take a conservative approach and include *Lindenbergia* with the parasites in the revised family Orobanchaceae based on the strength of molecular evidence (Young, Steiner, and dePamphilis, 1999). This clade is unlikely to be contradicted with increasing evidence. This leaves *Paulownia* as a monogeneric family. *Paulownia* is unlike Orobanchaceae or *Lindenbergia* in habit and in the character of floral aestivation. The placement of *Mimulus* is unclear, but seems unlikely to be with any of the other segregate families of Scrophulariaceae s.l. and is equally parsimoniously placed with the Lamiaceae here. Therefore, we leave *Mimulus* unassigned to any more inclusive taxon within the Lamiales until further work is done.

We agree with the clade definition proposed by Young, Steiner, and dePamphilis (1999) as follows: Orobanchaceae are the least inclusive clade that contains *Orobanche uniflora, Schwalbea americana,* and *Lindenbergia phillipinensis.*

Calceolariaceae—*Calceolaria* is one of the largest genera in the traditional Scrophulariaceae with over 300 species distributed throughout the New World from Mexico to Chile. Two other small genera typically are assigned to the Calceolarieae (Molau, 1988): *Jovellana* with 4–5 species distributed in Chile and New Zealand and *Porodittia* with a single species in Peru. This group is distinctive for the combination of two stamens and a two-lobed corolla, unique in the traditional Scrophulariaceae. *Calceolaria* has been divided into three subgenera and 22 sections (Molau, 1988) with some of the major groupings defined by distinctive habits (shrubs, vines, and herbs). *Calceolaria* is one of only a handful of genera in Scrophulariaceae s.l. to have oil-producing glands (elaiophores) in the corolla. In this genus the elaiophore consists a dense patch of glandular trichomes within an infolded lobe of the lower corolla lip. The other genera with oil-producing flowers in this study are restricted to Scrophulariaceae sensu stricto (e.g., Hemimerideae). One species each of *Calceolaria* and *Jovellana* are included here. Our results indicate that this clade is one of the most distinct and isolated of all clades in Lamiales with a supporting branch of 77–79 substitutions, bootstrap value of 100%, and decay of 54 steps (Fig. 2).

This clade is recognized as Calceolariaceae and we provide the following clade definition and make the necessary nomenclatural change. Calceolariaceae are the least inclusive clade that contains *Calceolaria pinnata, Porodittia triandra,* and *Jovellana violacea.*

Rafinesque (1838) suggested a family rank for this group (as Calceolides), but his publication does not meet requirements for valid publication under the ICBN, thereby necessitating proper validation as follows.

Calceolariaceae (G. Don) Raf. ex Olmstead, fam. et stat. nov.

Basionym: Calceolarieae G. Don, Edinburgh New Philos. J. 19: 112. 1835.

Type: *Calceolaria pinnata* L.

Included genera: *Calceolaria, Jovellana, Porodittia*

Stilbaceae—Lastly, *Halleria* belongs to a clade with *Retzia* of the Stilbaceae (7–17 steps; 69% bootstrap; decay of three steps, Fig. 2). The traditional Stilbaceae comprise five small genera totaling ~12 species. *Retzia* was assigned to either the Loganiaceae, Buddlejaceae, or the monotypic Retziaceae, but has been shown by wood anatomy (Carlquist, 1986) and molecular studies to belong with the Stilbaceae (Bremer et al., 1994; Oxelman, Backlund, and Bremer, 1999). *Nuxia* (not sampled here), formerly assigned to Loganiaceae or Buddlejaceae, has been shown to belong to Stilbaceae (Oxelman, Backlund, and Bremer, 1999). *Halleria* was assigned to the tribe Hallerieae by Don (1838), Cheloneae by Bentham (1876), and Teedieae by Barringer (1993). Another small tribe Bowkerieae (four genera, eight species), segregated from Cheloneae by Barringer (1993) also is restricted to South Africa and may belong in this clade as well (A. Wolfe, unpublished data; B. Oxelman, personal communication). Unlike the traditional Stilbaceae, which have small flowers, *Retzia* and *Halleria* have long red tubular corollas adapted for bird pollination. *Retzia* has the ericoid habit characteristic of the traditional Stilbaceae, whereas *Halleria* has broad leaves. All of these groups have predominantly South African distributions, with only *Nuxia* extending north into tropical Africa and Arabia. Although aggregating this group with the Scrophulariaceae s.s. is only one step less parsimonious in our study, it

is unlikely that increased sampling is going to result in a well-supported group combining these two clades, or that this group will be found not to be monophyletic.

We provide the following clade definition. The Stilbaceae are the least inclusive clade that contains *Stilbe vestita, Retzia capensis,* and *Halleria lucida.*

Conclusion—We have shown that the traditional Scrophulariaceae are an unnatural assemblage of plants distributed throughout the phylogenetic tree of Lamiales. They are characterized by a suite of generalized traits, which may be plesiomorphic or commonly recurring in the Lamiales. The lack of distinguishing characters have precluded systematists from identifying clades within the traditional Scrophulariaceae that are comparable to other more well-defined clades in the Lamiales that are traditionally recognized as families. At the same time the presence of distinctive characteristics have defined some smaller clades that are derived from the Scrophulariaceae. In most cases these traits are associated with distinct life histories of the derivative groups, such as parasitism (Orobanchaceae), wind pollination (Plantaginaceae), and the aquatic habit (Callitrichaceae and Hippuridaceae). We have identified five distinct monophyletic groups composed primarily of taxa traditionally assigned to Scrophulariaceae. We recognize the following clades: Calceolariaceae, Orobanchaceae, Scrophulariaceae, Stilbaceae, and Veronicaceae. Additional segregate genera, including *Mimulus,* await further work to assess their taxonomic status.

LITERATURE CITED

ANGIOSPERM PHYLOGENY GROUP. 1998. An ordinal classification of the families of flowering plants. *Annals of the Missouri Botanical Garden* 85: 531–553.

ARGUE, C. L. 1993. Pollen morphology in the Selagineae, Manuleae (Scrophulariaceae), and selected Globulariaceae, and its taxonomic significance. *American Journal of Botany* 80: 723–733.

ARMSTRONG, J. E., AND A. W. DOUGLAS. 1989. The ontogenetic basis for corolla aestivation in Scrophulariaceae. *Bulletin of the Torrey Botanical Club* 116: 378–389.

BARRINGER, K. 1993. Five new tribes in the Scrophulariaceae. *Novon* 3: 15–17.

BELLINI, R. 1907. Criteri per una nuova classificazione delle Personatae (Scrophulariaceae et Rhinanthaceae) *Annali di Botanica* 6: 131–145.

BENTHAM, G. 1846. Scrophulariaceae. *In* A. DeCandolle [ed.], *Prodromus* 10: 180–586.

———. 1876. Scrophulariaceae. *In* G. Bentham and J. D. Hooker [eds.], *Genera plantarum*, vol. 2, 913–980. Reeve and Co., London, UK.

BOESHORE, I. 1920. The morphological continuity of Scrophulariaceae and Orobanchaceae. *Contributions of the Botanical Laboratory of the University of Pennsylvania* 5: 139–177.

BREMER, B., R. G. OLMSTEAD, L. STRUWE, AND J. A. SWEERE. 1994. *rbcL* sequences support exclusion of *Retzia, Desfontainia,* and *Nicodemia* (Buddlejaceae) from the Gentianales. *Plant Systematics and Evolution* 190: 213–230.

BRETTING, P. K., AND S. NILSSON. 1988. Pollen morphology of the Martyniaceae and its systematic implications. *Systematic Botany* 13: 51–59.

BRÜHL, P. 1920. On the systematic position of *Lindenbergia,* Lehmann. *Journal of the Department of Science, University of Calcutta* 2: 11–16.

BULL, J. J., J. P. HUELSENBECK, C. W. CUNNINGHAM, D. L. SWOFFORD, AND P. J. WADDELL. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42: 384–397.

CANTINO, P. D., R. G. OLMSTEAD, AND S. J. WAGSTAFF. 1997. A comparison of phylogenetic nomenclature with the current system: a botanical case study. *Systematic Biology* 46: 313–331.

CARLQUIST, S. 1986. Wood anatomy of Stilbaceae and Retziaceae: ecological and systematic implications. *Aliso* 11: 299–316.

CHASE, M. W., ET AL. 1993. Phylogenetics of seed plants: an analysis of

nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 528–580.

CRONQUIST, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, New York, USA.

CUMMINGS, M. P., S. P. OTTO, AND J. WAKELY. 1995. Sampling properties of DNA sequence data in phylogenetic analysis. *Molecular Biology and Evolution* 12: 814–822.

DEBRY, R. W., AND R. G. OLMSTEAD. 2000. A simulation study of reduced tree-search effort in bootstrap resampling analysis. *Systematic Biology* 49: 171–179.

DEPAMPHILIS, C. W., AND J. D. PALMER. 1990. Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. *Nature* 348: 337–339.

———, N. D. YOUNG, AND A. D. WOLFE. 1997. Evolution of plastid gene *rps2* in a lineage of hemiparasitic and holoparasitic plants: many losses of photosynthesis and complex patterns of rate variation. *Proceedings of the National Academy of Sciences, USA* 94: 7367–7372.

DE QUEIROZ, K., AND J. GAUTHIER. 1992. Phylogenetic taxonomy. *Annual Review of Ecology and Systematics* 23: 449–480.

———, AND ———. 1994. Toward a phylogenetic system of biological nomenclature. *Trends in Ecology and Evolution* 9: 27–31.

DON, G. 1838. Scrophularineae. *In* J. G. and F. Rivington et al. [eds.], A general history of the dichladymous plants, comprising complete descriptions of the different orders, vol. 4, 501–640. London, UK.

ENGLER, A., AND K. PRANTL. 1887–1915. Die naturlichen pflanzenfamilien. Wilhelm Engelmann, Leipzig, Germany.

ERIKSSON, T. 1998. AutoDecay vers. 4.0 (program distributed by author). Department of Botany, Stockholm University, Stockholm, Sweden.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

HALLIER, H. 1903. Ueber die abgrenzung und verwandtschaft der einzelnen sippen bei den Scrophulariaceen. *Bulletin de L'Herbier Boissier,* II 3: 181–207.

HARTL, D. 1959. Das alveolierte endosperm bei Scrophulariaceen, seine entstehung, anatomie und taxonomische bedeutung. *Beitrage zur Biologie der Pflanzen* 35: 95–110.

HEDREN, M., M. W. CHASE, AND R. G. OLMSTEAD. 1995. Relationships in the Acanthaceae and related families as revealed by cladistic analysis of *rbcL* nucleotide sequences. *Plant Systematics and Evolution* 194: 93–109.

HENRICKSON, J., AND L. D. FLYR. 1985. Systematics of *Leucophyllum* and *Eremogeton* (Scrophulariaceae). *Sida* 11: 107–172.

HILLIS, D. M. 1996. Inferring complex phylogenies. *Nature* 383: 130–131.

HUELSENBECK, J. P., AND D. M. HILLIS. 1993. Success of phylogenetic methods in the four-taxon case. *Systematic Biology* 42: 247–264.

JUDD, W. S., C. S. CAMPBELL, E. A. KELLOGG, AND P. F. STEVENS. 1999. Plant systematics: a phylogenetic approach. Sinauer, Sunderland, Massachusetts, USA.

KARRFALT, E. E., AND A. S. TOMB. 1983. Air spaces, secretory cavities, and the relationship between Leucophylleae (Scrophulariaceae) and Myoporaceae. *Systematic Botany* 8: 29–32.

KISHINO, H., AND M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* 29: 170–179.

KUIJT, J. 1969. The biology of parasitic flowering plants. University of California Press, Berkeley, California, USA.

LERSTEN, N. R., AND J. M. BEAMAN. 1998. First report of oil cavities in Scrophulariaceae and reinvestigation of air spaces in leaves of *Leucophyllum frutescens*. *American Journal of Botany* 85: 1646–1649.

———, AND J. D. CURTIS. 1997. Anatomy and distribution of foliar idioblasts in *Scrophularia* and *Verbascum* (Scrophulariaceae). *American Journal of Botany* 84: 1638–1645.

LU, A.-M. 1990. A preliminary cladistic study of the families of the superorder Lamiiflorae. *Botanical Journal of the Linnaean Society* 103: 39–57.

MCDADE, L. A., AND M. L. MOODY. 1999. Phylogenetic relationships among Acanthaceae: evidence from noncoding trnL-trnF chloroplast DNA sequences. *American Journal of Botany* 86: 70–80.

MELCHIOR, H. 1964. Syllabus der pflanzenfamilien II. Gebrüder Borntraeger, Berlin, Germany.

MOLAU, U. 1988. Scrophulariaceae—Part I. Calceolarieae. *Flora Neotropica Monograph* 47: 1–325.

NAKAI, T. 1949. Classes, ordinae, familiae, subfamilieae, tribus, genera nova quae attinent ad plantas Koreanas. *Journal of Japanese Botany* 24: 8–14.

NICKRENT, D. L., R. J. DUFF, A. E. COLWELL, A. D. WOLFE, N. D. YOUNG, K. E. STEINER, AND C. W. DEPAMPHILIS. 1998. Molecular phylogenetic and evolutionary studies of parasitic plants. *In* P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants II, DNA sequencing, 211–241. Kluwer, Boston, Massachusetts, USA.

NIEZGODA, C. J., AND A. S. TOMB. 1975. Systematic palynology of tribe Leucophylleae (Scrophulariaceae) and selected Myoporaceae. *Pollen et Spores* 17: 495–516.

OLMSTEAD, R. G., B. BREMER, K. SCOTT, AND J. D. PALMER. 1993. A parsimony analysis of the Asteridae sensu lato based on *rbcL* sequences. *Annals of the Missouri Botanical Garden* 80: 700–722.

———, H. J. MICHAELS, K. SCOTT, AND J. D. PALMER. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbcL*. *Annals of the Missouri Botanical Garden* 79: 249–265.

———, AND J. D. PALMER. 1994. Chloroplast DNA systematics: a review of methods and data analysis. *American Journal of Botany* 81: 1205–1224.

———, AND P. A. REEVES. 1995. Evidence for the polyphyly of the Scrophulariaceae based on chloroplast *rbcL* and *ndhF* sequences. *Annals of the Missouri Botanical Garden* 82: 176–193.

———, ———, AND A. C. YEN. 1998. Patterns of sequence evolution and implications for parsimony analysis of chloroplast DNA. *In* P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants II DNA sequencing, 164–187. Kluwer, Boston, Massachusetts, USA.

———, AND J. A. SWEERE. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Systematic Biology,* 43: 467–481.

OXELMAN, B., M. BACKLUND, AND B. BREMER. 1999. Relationships of the Buddlejaceae s.l. investigated using parsimony jackknife and branch support analysis of chloroplast *ndhF* and *rbcL* sequence data. *Systematic Botany* 24: 164–182.

PENNELL, F. W. 1919. Scrophulariaceae of the southeastern United States. *Proceedings of the Academy of Natural Sciences of Philadelphia* 71: 224–291.

———. 1935. The Scrophulariaceae of eastern temperate North America. *Academy of Natural Sciences of Philadelphia Monographs* 1: 1–650.

RAFINESQUE, C. S. 1838. Flora Telluriana 4: 57.

REEVES, P. A., AND R. G. OLMSTEAD. 1998. Evolution of novel morphological, ecological, and reproductive traits in a clade containing *Antirrhinum*. *American Journal of Botany* 85: 1047–1056.

ROBERTSON, C. 1891. Flowers and insects, Asclepiadaceae to Scrophulariaceae. *Transactions of the Academy of Science of St. Louis* 5: 569–598.

ROBYNS, W. 1931. L'organisation florale des Solanacées zygomorphes. *Memoires de l'academie royale des sciences, lettres et beaux-arts de Belgique. Classe des sciences [In octavo]* 11: 1–96.

ROUY, G. 1909. Conspectus des tribus et des genres de la famille des Scrophularicées. *Revue Generale de Botanique* 21: 194–207.

SCHMID, E. 1906. Beitrage zur entwicklungsgeschichte der Scrophulariaceen. *Beihefte zum Botanischen Centralblatt* 20: 175–299.

SCOTLAND, R. W., J. S. SWEERE, P. A. REEVES, AND R. G. OLMSTEAD. 1995. Higher level systematics of Acanthaceae determined by chloroplast DNA sequences. *American Journal of Botany* 82: 266–275.

SMITH, J. F., J. C. WOFLRAM, K. D. BROWN, C. L. CARROLL, AND D. S. DENTON. 1997. Tribal relationships in the Gesneriaceae: evidence from DNA sequences of the chloroplast gene *ndhF*. *Annals of the Missouri Botanical Garden* 84: 50–66.

SPANGLER, R. E., AND R. G. OLMSTEAD. 1999. Phylogenetic analysis of the Bignoniaceae and relatives based on cpDNA gene sequences of *rbcL* and *ndhF*. *Annals of the Missouri Botanical Garden* 86: 36–46.

STEINER, K. E. 1996. Chromosome numbers and relationships in tribe Hemimerideae (Scrophulariaceae). *Systematic Botany* 21: 63–76.

SWOFFORD, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4. Sinauer, Sunderland, Massachusetts USA.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the humans and apes. *Evolution* 37: 221–244.

THIERET, J. W. 1967. Supraspecific classification in the Scrophulariaceae: a review. *Sida* 3: 87–106.

VON WETTSTEIN, R. 1891. Scrophulariaceae. *In* A. Engler and K. Prantl [eds.], Die Natürlichen Pflanzenfamilien. vol. 4(3b), 39–107. Wilhelm Engelmann, Leipzig, Germany.

WAGSTAFF, S. J., AND R. G. OLMSTEAD. 1997. Phylogeny of the Labiatae and Verbenaceae inferred from *rbcL* sequences. *Systematic Botany* 22: 165–179.

———, P. A. REEVES, L. HICKERSON, R. E. SPANGLER, AND R. G. OLM-STEAD. 1998. Phylogeny of Labiatae s.l. inferred from cpDNA sequences. *Plant Systematics and Evolution* 209: 265–274.

WOLFE, A. D., AND C. W. DEPAMPHILIS. 1997. Alternate paths of evolution for the photosynthetic gene *rbcL* in four nonphotosynthetic species of *Orobanche*. *Plant Molecular Biology* 33: 965–977.

———, AND ———. 1998. The effect of relaxed functional constraints on the photosynthetic gene *rbcL* in photosynthetic and nonphotosynthetic parasitic plants. *Molecular Biology and Evolution* 15: 1243–1258.

———, W. J. ELISENS, L. E. WATSON, AND C. W. DEPAMPHILIS. 1997. Using restriction-site variation of PCR-amplified cpDNA genes for phylogenetic analysis of tribe Cheloneae (Scrophulariaceae). *American Journal of Botany* 84: 555–564.

WOLFE, K. H., C. W. MORDEN, AND J. D. PALMER. 1992. Function and evolution of a minimal plastid genome from a nonphotosynthetic parasitic plant. *Proceedings of the National Academy of Sciences, USA* 89: 10648–10652.

YOUNG, N. D., K. E. STEINER, AND C. W. DEPAMPHILIS. 1999. The evolution of parasitism in the Scrophulariaceae/Orobanchaceae: plastid gene sequences refute an evolutionary transition series. *Annals of the Missouri Botanical Garden* 86: 876–893.

APPENDIX. Taxa included in this study. Classification is as recognized by the authors. For the classification of von Wettstein (1891), see Fig. 2. Sequences not available for analysis are indicated by ''na.''

Taxon	DNA source or voucher[a]	GenBank accession number[b]		
		rbcL	ndhF	rps2
Lamiales				
Acanthaceae				
Barleria prionitis L.	Chase et al., 1993; Scotland et al., 1995	GBAN-L01886	GBAN-U12653	GBAN-AF248247
Elytraria crenata Vahl.	Scotland et al., 1995;	GBAN-AF188127	GBAN-U12657	na
Thunbergia alata Bojer	Scotland et al., 1995		GBAN-U12667	GBAN-AF248248
T. usumbarica Lindau	Chase et al., 1993	GBAN-L12596		
Bignoniaceae				
Catalpa sp.	Olmstead et al., 1992	GBAN-L11679	GBAN-L36397	GBAN-AF248256
Kigelia africana Benth.	Spangler et al., in press; Young, Steiner, and dePamphilis, 1999	GBAN-AFI0248	GBAN-AF102632	GBAN-U48764
Calceolariaceae				
Calceolaria mexicana Benth	*C. W. dePamphilis 90.68* (PAC)	GBAN-AF123669	GBAN-AF123679	GBAN-AF055162
Jovellana sp.	cultivated at U.C. Berkeley; no voucher	GBAN-AF123666	GBAN-AF123684	GBAN-AF248258
Gesneriaceae				
Nematanthus hirsutus (Mart.) Wiehler	Olmstead and Reeves, 1995	GBAN-L36446	GBAN-L36404	na
Streptocarpus holstii Engl.	Olmstead et al., 1993	GBAN-L14409	GBAN-L36415	na
Lamiaceae				
Callicarpa dichotoma (Lour.) K. Koch	Olmstead et al., 1993	GBAN-L14393	GBAN-L36395	na
Lamium purpureum L.	Olmstead et al., 1993; *C. W. dePamphilis 94.06* (PAC)	GBAN-U75702	GBAN-U78694	GBAN-AF248258
Martyniaceae				
Proboscidea louisianica (Mill.) Thell.	Chase et al., 1993	GBAN-L01946	GBAN-AF123690	GBAN-AF248260
Oleaceae				
Ligustrum japonicum Buch.-Ham. ex D. Don	Young, Steiner, and dePamphilis, 1999			GBAN-U48769
Ligustrum vulgare L.	Olmstead et al., 1992	GBAN-L11686	GBAN-AF130164	
Orobanchaceae				
Alectra sessiliflora Benth.	Young, Steiner, and dePamphilis, 1999	GBAN-AF026820	na	GBAN-U48742
Bartsia alpina L.	Young, Steiner, and dePamphilis, 1999	GBAN-AF190903	GBAN-AF123678	GBAN-U48751
Boschniakia strobilacea A. Gray	Young, Steiner, and dePamphilis, 1999	GBAN-AF26818	na	GBAN-U48758
Buchnera floridana Gand.	Young, Steiner, and dePamphilis, 1999	GBAN-AF026822	na	GBAN-U48744
Castilleja linariifolia Benth.	Young, Steiner, and dePamphilis, 1999	GBAN-AF026823	na	GBAN-U48739
Harveya purpurea Harv.	Young, Steiner, and dePamphilis, 1999	GBAN-AF026830	na	GBAN-U48749
Lindenbergia phillipinensis Benth.	Young, Steiner, and dePamphilis, 1999	GBAN-AF123664	GBAN-AF123686	GBAN-AF055151
Melampyrum lineare Lam.	Wolfe and dePamphilis, 1998	GBAN-AF026834	na	GBAN-AF055148
M. sylvaticum L.	Young, Steiner, and dePamphilis, 1999	GBAN-AF190904	na	GBAN-U48743
Melasma scabrum Berg.	*K. E. Steiner 2250* (PAC)	GBAN-U73969	GBAN-AF123687	GBAN-U48760
Orobanche corymbosa (Rydb.) Ferris	Young, Steiner, and dePamphilis, 1999	GBAN-U73971	na	GBAN-U48761
O. ramosa L.	Young, Steiner, and dePamphilis, 1999	GBAN-AF026836	na	GBAN-U48740
Pedicularis foliosa L.	Young, Steiner, and dePamphilis, 1999	GBAN-AF026837	GBAN-AF123689	GBAN-AF055141
Seymeria pectinata Pursh	Young, Steiner, and dePamphilis, 1999	GBAN-AF026838	GBAN-AF123691	GBAN-U48746
Striga asiatica (L.) Kuntze	Young, Steiner, and dePamphilis, 1999	GBAN-AF026843	na	GBAN-U48754
Tozzia alpina L.	Young, Steiner, and dePamphilis, 1999		na	
Paulowniaceae				
Paulownia tomentosa Steud.	Olmstead and Reeves, 1995	GBAN-L36447	GBAN-L36406	GBAN-AF055255
Pedaliaceae				
Sesamum indicum L.	Olmstead et al., 1993	GBAN-L14408	GBAN-L36413	GBAN-AF248261
Schlegeliaceae				
Schlegelia parviflora (Oerst.) Monach.	Olmstead and Reeves, 1995	GBAN-L36448	GBAN-L36410	GBAN-AF055152
Scrophulariaceae				
Alonsoa unilabiata (L.f.) Steud.	*K. E. Steiner 2388* (PAC)	GBAN-AF026821	GBAN-AF188184	GBAN-AF248262
Aptosimum sp.	no voucher	GBAN-AF123671	GBAN-AF123676	GBAN-AF248263

APPENDIX.   Continued.

Taxon	DNA source or voucher[a]	GenBank accession number[b] rbcL	ndhF	rps2
Scrophulariaceae				
Buddleja davidii Franchet	Olmstead et al., 1993	GBAN-L14392	GBAN-L36394	GBAN-AF248264
Hemimeris sabulosa L.	C. W. dePamphilis 93.52 (PAC) Young, Steiner, and dePamphilis, 1999	GBAN-AF123668	GBAN-AF123682	GBAN-U48765
Leucophyllum fructescens I.M. Johnston	Young, Steiner, and dePamphilis, 1999	GBAN-AF123665	GBAN-AF123685	GBAN-AF055156
Myoporum mauritianum A. DC.	Olmstead and Reeves, 1995	GBAN-L36445	GBAN-L36403	GBAN-AF055157
M. parvifolium R. Br.	Young, Steiner, and dePamphilis, 1999	GBAN-AF123663	GBAN-AF123688	GBAN-AF248265
Nemesia cheiranthus	K. E. Steiner s.n. (PAC)			GBAN-U48762
N. strumosa Benth.	Wolfe s.n. (OS)	GBAN-L36449	GBAN-L36411	
Scrophularia californica Cham. & Schltdl.	Olmstead and Reeves, 1995	GBAN-L36450	GBAN-L36412	GBAN-U48763
Selago thunbergii Choisy	Olmstead and Reeves, 1995	GBAN-L36452	GBAN-L36417	
Verbascum blattaria L.	Young, Steiner, and dePamphilis, 1999	GBAN-AF123662	GBAN-AF123693	GBAN-AF248266
V. thapsus L.	Olmstead and Reeves, 1995			
Zaluzianskya katherinae	C. W. dePamphilis 90.18 (PAC)			
Stilbaceae				
Halleria lucida L.	C.W. dePamphilis 90.23 (PAC)	GBAN-AF026828	GBAN-AF188185	GBAN-AF248267
Retzia capensis Thunb.	Bremer et al., 1994	GBAN-Z29669		na
Verbenaceae				
Stachytarpheta dichotoma Vahl.	R. Olmstead 951; WTU	GBAN-U32161		GBAN-AF248268
Verbena bonariensis L.	Olmstead et al., 1993	GBAN-L14412		GBAN-AF055153
V. bracteata Lag. & Rodr.	Olmstead and Reeves, 1995	GBAN-AF123673		
Veronicaceae				
Amphianthus pusillus	C. W. dePamphilis 90.33 (PAC) Olmstead et al., 1992;		GBAN-AF14776	GBAN-AF248249
Antirrhinum majus L.	Young, Steiner, and dePamphilis, 1999	GBAN-L11688	GBAN-L36414	GBAN-U48766
Angelonia pubescens	C. W. dePamphilis 94.03 (PAC)	GBAN-AF123672	GBAN-L36418	GBAN-AF248250
Bacopa caroliniana B.L. Rob.	C. W. dePamphilis 90.137 (PAC)	GBAN-AF123670	GBAN-AF123674	GBAN-AF248251
Chelone obliqua L.	Young, Steiner, and dePamphilis, 1999	GBAN-AF026824	GBAN-L36392	GBAN-U48770
Callitriche hermaphroditica L.	Olmstead and Reeves, 1995	GBAN-L36441	GBAN-AF123675	GBAN-O55159
Collinsia grandiflora Lindley	Wolfe and de Pamphilis, 1998	GBAN-AF026825	GBAN-AF123677	GBAN-AF248252
Digitalis grandiflora Mill.	Olmstead and Reeves, 1995	GBAN-L01902	GBAN-AF123680	GBAN-U48767
D. purpurea L.	Olmstead et al., 1993; Young, Steiner, and dePamphilis, 1999		GBAN-L36396	GBAN-AF248253
Globularia cordifolia L.	L. Hufford, voucher unknown	GBAN-AF124558	GBAN-AF188182	GBAN-O55163
Gratiola pilosa Michx.	Young, Steiner, and dePamphilis, 1999	GBAN-AF026827	GBAN-L36399	GBAN-AF055161
Hemiphragma heterophyllum Wall.	Young, Steiner, and dePamphilis, 1999	GBAN-AF123667	GBAN-AF124557	GBAN-AF055164
Hippuris vulgaris L.	Olmstead and Reeves, 1995	GBAN-L36443	GBAN-AF188183	GBAN-AF248254
Plantago lanceolata L.	Olmstead and Reeves, 1995	GBAN-L36454	GBAN-AF123683	GBAN-AF248255
P. major L.	R. A. Smith 101	GBAN-AF236128	GBAN-L36401	GBAN-U48768
Tetranema mexicana Benth.	C. W. Morden 1075 (IND) Young, Steiner, and dePamphilis, 1999	GBAN-L36453	GBAN-L36408	GBAN-AF055154
Veronica arvensis L.	Olmstead and Reeves, 1995	GBAN-AF026835	GBAN-AF123692	na
V. persica Poir.		GBAN-L11680	GBAN-L36419	na
Scrophulariaceae s.l. unassigned				
Mimulus aurantiacus Curtis	Young, Steiner, and dePamphilis, 1999	GBAN-L14398	GBAN-AF188186	GBAN-Z00044
Outgroups				
Borago officinalis L.	Olmstead et al., 1992		GBAN-L36393	
Gentiana procera Holm	Olmstead et al., 1993		GBAN-L36400	
Nicotiana tabacum L.	Olmstead et al., 1993	GBAN-Z00044	GBAN-Z00044	GBAN-Z00044

[a] References are for accessions used in previous studies; vouchers are for accessions first used here (herbarium where deposited in parentheses).

[b] The prefix GBAN- has been added to GenBank accession numbers to link the online version of American Journal of Botany to GenBank, but is not part of the actual accession number.