THE EVOLUTION OF PARASITISM IN SCROPHULARIACEAE/ OROBANCHACEAE: PLASTID GENE SEQUENCES REFUTE AN EVOLUTIONARY TRANSITION SERIES[1]

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ABSTRACT

Parasitic plants in Scrophulariaceae and Orobanchaceae have been traditionally depicted as forming a linear evolutionary series beginning with hemiparasitism and ending with holoparasitism. The genera *Lathraea, Harveya,* and *Hyobanche* have been viewed as transitional links between the parasitic members of Scrophulariaceae and the strictly holoparasitic habit of the traditional Orobanchaceae. Phylogenetic analyses of plastid *rps*2 and *mat*K sequences were performed. The transitional genera are not transitional to the traditional Orobanchaceae, but represent multiple independent origins of holoparasitism. Within Scrophulariaceae, the two traditional subfamilies Rhinanthoideae and Antirrhinoideae are defined by the arrangement of the corolla lobes during aestivation. However, neither of the two subfamilies is monophyletic in our analyses, suggesting that corolla lobe position is a homoplastic character. While the traditional Orobanchaceae are monophyletic, tribes Buchnereae and Rhinantheae are clearly not, and genus *Orobanche* probably is not. Clades of parasitic genera correspond well with biogeographic provinces. One strongly supported clade contains the parasitic Scrophulariaceae, the traditional Orobanchaceae, and *Lindenbergia.* It is proposed that this clade be defined as the Orobanchaceae.

Parasitic angiosperms are found in 16 families and live in diverse habitats, ranging from tropical forests to arctic islands (Musselman & Press, 1995). Recent work on several parasitic groups has explored their anatomy, physiology, ecology, and molecular biology and the control of economically significant parasites (Press & Graves, 1995). Parasites exhibit dramatic adaptations. Some lack leaves, stems, roots, and the ability to photosynthesize. For many lineages of non-photosynthetic parasites, study of their evolution is impeded by the very fact that they are so specialized and so different from their nearest photosynthetic relatives. In many cases the identity of those relatives remains unknown (Nickrent & Duff, 1996; Nickrent et al., 1998). The evolution of parasitism can best be studied in a group where near relatives are known, and different degrees of parasitic specialization are encountered. The group with the greatest range in parasitic specialization is found within the Scrophulariaceae/Orobanchaceae. This family pair includes nonparasites, both facultative and obligate hemiparasites (photosynthetic plants that obtain water, nutrients, or photosynthates from their host), and holoparasites (non-photosynthetic, obtaining all photosynthates from their host). Much has been learned about the anatomy and physiology of parasitism in this group, especially in *Striga* Loureiro and *Orobanche* L., which are important pests of crop plants (Press & Graves, 1995). Reliable information on phylogenetic relationships among the various nonparasites, hemiparasites, and holoparasites would enable us to better understand the anatomical, physiological, and genetic changes that occur during the evolution of parasitism and the loss of photosynthetic ability.

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Boeshore (1920: 171) argued for a close relationship between the Scrophulariaceae (figworts) and the traditional Orobanchaceae (broomrapes), describing in detail an evolutionary transition series:

> "All macroscopic and microscopic details suggest that the parasitic Scrophulariaceae and Orobanchaceae form a continuous and parasitically degrading morphological series that show transitional steps from green nearly autotrophic plants like *Melampyrum, Rhinanthus,* and *Euphrasia* to increasingly condensed and degraded genera like *Bartsia* and *Harveya,* on to *Lathraea,* that has been shown to be placed by some botanists in Scrophulariaceae, by others in Orobanchaceae, thence through species of *Orobanche* to *Epiphegus,* and finally *Aphyllon* and *Conopholis.*"

According to Boeshore, the evolutionary series among parasitic figworts and broomrapes involved progressive enlargement and consolidation of haustoria (the connection to the host), shortening of the vegetative stem, reduction of leaves, simplification of the inflorescence, change from few, large seeds to numerous, small seeds, and a reduction in the ovary from two locules to one. Weber constructed a different evolutionary series based on haustorial anatomy (Weber, 1980), but otherwise, Boeshore's depiction of the group's evolution has endured remarkably well to the present. Later authors have cited Boeshore and reiterated his ideas. The parasitic Scrophulariaceae were viewed as "a pointer to . . . Orobanchaceae" (Hutchinson, 1969: 630); Kuijt (1969: 95) observed, "Many features of Orobanchaceae appear to be already foreshadowed in their figwort relatives," and Cronquist (1981: 940) stated, "The evolutionary journey toward parasitism obviously begins in Scrophulariaceae; the Orobanchaceae merely occupy the house at the end of the road."

Recent results from DNA sequencing show a more complicated history for the group. Phylogenies based on the plastid genes *rps*2 and *rbc*L (dePamphilis et al., 1997; Wolfe & dePamphilis, 1998; Nickrent et al., 1998) show that parasitism evolved a single time in the figwort/broomrape family pair. Thus all the hemi- and holoparasites, taken together, form a monophyletic group. This agrees with Boeshore, whose evolutionary series also contains a single origin of parasitism. Within this parasitic clade, however, evolution has not proceeded as Boeshore envisioned. The *rps*2 phylogeny also shows that the transitional genera (*Lathraea, Harveya, Hyobanche*) are not transitional at all, but occupy their own branches among the parasitic figworts, representing separate losses of photosynthesis, independent of the broomrapes (dePamphilis et al., 1997). It is not surprising that Boeshore had developed a different view of the group's evolution based on morphology. Many of the morphological characters that bear on phylogeny are probably adaptations to the parasitic lifestyle, and most involve reduction or loss of structures. This makes it difficult to interpret relationships within the group. It seems that, as the separate lineages independently specialized as parasites, they converged (sensu Eldredge & Cracraft, 1980) on similar morphologies. This paper includes additional DNA sequence data, enabling us to more rigorously test whether the parasites form a monophyletic group and whether the holoparasites form multiple groups. Because the five basal branches of the parasitic clade were left unresolved in the *rps*2 study (dePamphilis et al., 1997), we have, in this study, included a wider sample of taxa for *rps*2 and added characters from another plastid gene, *mat*K. This gives us the increase in resolution needed to provide a more detailed phylogeny.

A new definition of Orobanchaceae will be presented. For that reason, the common name "broomrapes" will be used for the traditional Orobanchaceae.

Throughout this paper we will use the classification system of Wettstein (1897), which, although dated (Thieret, 1967), is the best worldwide treatment of Scrophulariaceae. There are two minor exceptions to our use of Wettstein's system. We have included his subfamily Pseudosolaneae in the Antirrhinoideae (Thieret, 1967), and we use the name Buchnereae Benth. for his tribe Gerardieae Benth., following Pennell's (1935: 379) "desire that the tribal designation be based on a generic name unquestionably applying to a member of this group." Scrophulariaceae are divided into two subfamilies, Antirrhinoideae and Rhinanthoideae, "which differ fundamentally in the single character of the former having the posterior (corolla) lobes, and the latter the anterior lobes external in aestivation." (Pennell, 1935: 40). This corolla aestivation character has been studied throughout floral development for a number of figwort genera (Armstrong & Douglas, 1989), and the ontogenetic patterns were consistent within each subfamily. However, a study of *rbc*L and *ndh*F sequences (Olmstead & Reeves, 1995) found the Antirrhinoideae to be polyphyletic. In that study, the Rhinanthoideae, which consist of two nonparasitic tribes (Digitaleae and Veroniceae) and two parasitic tribes [Buchnereae and Rhinantheae Benth. (= Euphrasiae Benth.)], were represented only by two nonparasites, *Digitalis* and *Veronica.* Thus, neither the position of the parasites nor the monophyly of the Rhinanthoideae could be

addressed. Phylogenies of *rps*2 (dePamphilis et al., 1997, and unpublished) and *rbc*L (Wolfe & dePamphilis, 1998) indicate that the parasitic and nonparasitic Rhinanthoideae each form a clade, and the two clades are separated by many other taxa including Bignoniaceae and Lamiaceae. Corolla aestivation pattern is also used to separate the two parasitic tribes of subfamily Rhinanthoideae. Of the three abaxial lobes, the center one folds over the two outer ones in the Buchnereae, and the outer two fold over the center one in the Rhinantheae (Armstrong & Douglas, 1989). The membership of these tribes has been stable since their inception.

The broomrapes or traditional Orobanchaceae (Beck von Mannagetta, 1930) consist entirely of holoparasites and have long been hypothesized to have affinities either with Scrophulariaceae or the Gesneriaceae. Gesneriaceae have been considered the closest relatives of the broomrapes by some authors (Baillon, 1891; Fritsch, 1895; Wettstein, 1897; Beck von Mannagetta, 1930), emphasizing the unilocular placentation of both families. Hallier (1903) and Bellini (1907) both proposed formal inclusion of the broomrapes in subfamily Rhinanthoideae of Scrophulariaceae. Boeshore (1920) also found the broomrapes to be similar to the parasitic figworts, not only in the parasitic habit and the presence of haustoria, but in many other features, such as: (1) plants similarly reduced, with very short vegetative axes and linear inflorescence axes; (2) leaves reduced to tooth-like scales; (3) sepals and stamens with tapered, multicellular hairs interspersed with capitate-glandular hairs; (4) parallel anther lobes growing downward into stiff, awn-like horns; and (5) nectary often a median knob in line with the floral axis. Cronquist (1981) referred to the Orobanchaceae as "... derived from Scrophulariaceae," but, due to its parasitic habit and parietal placentation, retained it as a separate family. Finally, a cladistic analysis of DNA sequences from the plastid gene *rps*2 showed the Orobanchaceae to be firmly ensconced within the parasitic figworts, which are not monophyletic (dePamphilis et al., 1997). Despite the lack of monophyly, we use the name Scrophulariaceae and its common name, figworts, as they have been traditionally used, until a reclassification is published.

This study assessed the monophyly of three groups: genus *Orobanche*, the broomrapes, and the broomrapes plus parasitic figworts. If monophyletic, the study also sought the sister group of each clade. The position of *Schwalbea* was also examined. *Schwalbea* was thought to be among the most "primitive" of parasitic figworts, based on its fifth (posterior) sepal, septicidal dehiscence of the capsule, and two bractlets subtending the flower (Pennell, 1935).

These questions were addressed using two plastid genes: *rps*2 and *mat*K. These are two of the small set of plastid genes that are found intact in all of the taxa in this group, even in the holoparasites. Although the ribosomal protein gene *rps*2 is new to plant phylogenetic analysis (dePamphilis et al., 1997), *mat*K has become widely used and has many advantages for use in phylogenetic studies (Hilu & Liang, 1997). It is large (~ 1500 bp) and rapidly evolving (Wolfe et al., 1992; Olmstead & Palmer, 1994), changing 2 or 3 times as fast as *rbc*L (Steele & Vilgalys, 1994; Johnson & Soltis, 1995), thus providing many substitution characters. The changes in *mat*K are distributed more evenly among the three codon positions and throughout its length than in other, more conserved genes (Johnson & Soltis, 1994; Hilu & Liang, 1997). As a coding region, *mat*K is easier to align than non-coding regions, yet most data sets have a few small indels (insertions or deletions) of 3 to 15 bp, providing additional characters. Interestingly, although the *trn*K locus is absent from *Epifagus* (Morden et al., 1991), and possibly several other Orobanchaceae taxa (N. D. Young & C. W. dePamphilis, unpublished), we found an intact *mat*K gene in all Orobanchaceae and Scrophulariaceae tested, including holoparasites such as *Epifagus* (dePamphilis & Palmer, 1990; Wolfe et al., 1992) and *Conopholis* (Colwell, 1994).

Materials and Methods

Sampling

Multiple taxa were chosen from each of the five major lineages of parasitic figworts and broomrapes found previously (dePamphilis et al., 1997). We also included *Schwalbea*, a monotypic genus that may represent a sixth parasitic lineage (Pennell, 1935). Both parasitic figwort tribes, Rhinantheae and Buchnereae, were well sampled. From the broomrapes, we sampled *Epifagus*, *Conopholis*, *Boschniakia*, *Cistanche*, and all four sections of *Orobanche*: *Gymnocaulis* (*O. uniflora* and *O. fasciculata*), *Nothophyllon* (*O. corymbosa*), *Orobanche* (*O. caryophyllaceae*, *O. cernua*, and *O. hederae*), and *Trionychon* (*O. ramosa*).

*rps*2 phylogeny showed that the nearest relatives to the parasites are clearly not the Digitaleae (dePamphilis et al., 1997), as was previously thought. We therefore sampled members of all of Wettstein's (1897) figwort tribes except two small ones, Aptosimeae and Manuleae, for which material was not available. Additional sampling outside the

figworts was based on previous *rps*2 phylogenies (dePamphilis et al., 1997, and unpublished), and emphasized lineages that appeared to be closely related to the parasites. We also included *Lindenbergia,* which, although not parasitic, shares some floral similarities with the parasitic figworts (Brühl, 1920). Trees were rooted with *Ligustrum* (Oleaceae) and *Nicotiana* (Solanaceae). The taxa used are listed, along with collection information and the GenBank accession numbers of the sequences, in Table 1.

DNA ISOLATION, AMPLIFICATION, AND SEQUENCING

The CTAB method (Doyle & Doyle, 1987) was used to isolate total DNA from plant leaf or stem tissue. Amplification of *rps*2 followed dePamphilis et al. (1997), and *mat*K sequences were amplified using 1X Taq Extender buffer (Stratagene), 0.2 mM each dATP, dCTP, dGTP, and dTTP (Pharmacia), 3.6 mM $MgCl_2$, 0.32 μM each primer (see Fig. 1), 0.25 unit of Taq DNA polymerase, 0.25 unit of Taq Extender coenzyme (Stratagene), and ~ 500 ng of total DNA in a 50 μL volume. Figure 1 shows the primary *mat*K primers (Genosys) used. Additional species-specific primers were occasionally needed, especially for *Orobanche.* PCR products were purified in 1% agarose gels using Qiaquick columns according to the manufacturer's instructions (Qiagen). Sequences were generated by two methods: with the ABI 377 autosequencer (P. E. Biosystems) according to the manufacturer's instructions (with the exception that reactions were done in 10 μL, rather than the standard 20 μL volume) and manually, with the Sequenase (U.S. Biochemicals) double-stranded method (dePamphilis et al., 1997). Both strands were sequenced, and all ambiguities clarified by individual examination using Sequencher 3.0 software (GeneCodes, Ann Arbor, MI). Sequences were translated to verify that the protein-coding regions contained no internal stop codons, which would signify a possible sequencing error or pseudogene sequence.

ALIGNMENT

The *rps*2 alignment was simple, requiring only two small indels. The *mat*K alignment was more complicated, requiring 14 locations with indels ranging from 3 to 21 bp, some of which varied among taxa in length and exact position. Initially 22 alignments were produced, using Clustal W 1.4 (Thompson et al., 1994) and the following variations: gap opening penalties (GOP) ranging from 5 to 30, gap extension penalties (GEP) from 1 to 10, with and without transition/transversion weighting, with the NJ guide tree replaced by a "conservative" parsimony tree (derived from a data set with length-variable regions removed), with complex length-variable regions coded as multistate characters, as in Baum et al. (1994), or with length variation divided into characters based on the longest possible independently varying units (Young, 1998), and, finally, protein translations were aligned and the DNA alignments altered to match.

The 22 alignments thus produced were evaluated according to the consistency among characters [the rescaled consistency (RC) index of Farris (1989)] on the resulting maximum parsimony (MP) trees (Wheeler, 1995). This provided an objective optimality criterion: whichever alignment yielded the MP tree(s) with the highest RC was considered the best alignment.

PHYLOGENETIC ANALYSIS

We used two methods of phylogenetic analysis in the program PAUP: maximum parsimony (MP) and maximum likelihood (ML). For *rps*2, positions homologous to positions 48–660 of the *Nicotiana* gene were used [*Nicotiana* plastid genome positions 16275–16887 (Shinozaki et al., 1986)], and for *mat*K, *Nicotiana* gene positions 297–1286 were used (*Nicotiana* plastid genome positions 2425–3414). Because there were more than twice as many taxa sampled for *rps*2 than for *mat*K, we analyzed each gene alone and in two different combined analyses: one with only those taxa sequenced for both genes, and one with all the taxa. In analyses of the *mat*K-only and small-merger matrices, heuristic searches were performed with 100 random addition orders and TBR branch swapping. The *rps*2-only and large-merger matrices were too large for a full heuristic search, so we used a search strategy designed to sample tree space thoroughly in large data sets (Catalan et al., 1997). In the *mat*K-only analysis, the effect of the two regions with overlapping gaps was investigated by repeating the analysis with the gaps removed. Bootstrap support (Felsenstein, 1985) was estimated from the *mat*K-only and small-merger matrices, using the same parameters as the heuristic search (Fig. 3), and at least 500 replicates (Figs. 2, 4). For the *rps*2-only and large-merger matrices, each bootstrap replicate was limited to five random addition orders and five fully swapped trees each (Figs. 2, 4). Bremer support values (Bremer, 1988) were calculated for all four matrices. Topological constraints were used to find the number of extra steps that would be added to the MP trees under specific hypotheses (Table 2). Maximum-likelihood analyses were conducted on

the *mat*K-only and small-merger matrices; the *rps*2-only and large-merger matrices had too many taxa. The substitution model included a transition/transversion ratio of 2.0 and base frequencies estimated from the data (Hasegawa et al., 1985). Analyses were conducted using test version 4.0d54 of PAUP*, with permission of the program's author, David L. Swofford. The aligned data can be obtained from TreeBASE (study accession number S402) at http://www.herbaria.harvard.edu/treebase/.

Results

Although a cpDNA phylogeny cannot be assumed identical to the organismal phylogeny (e.g., Doyle, 1992), introgression or lineage sorting are not likely to be problems above the level of genera, so we expect close correspondence between the cpDNA phylogenies and the organismal phylogeny. The four data matrices used in the phylogenetic analyses are detailed in Table 2. The first, referred to as "*rps*2-only," contains *rps*2 gene sequences from 63 taxa. The second matrix (*mat*K-only) contains *mat*K sequences from 26 of those taxa (plus *Cistanche*, just recently obtained). The third matrix (small-merger) combines sequences for the 26 taxa that have data from both genes (all of the taxa in the *mat*K analysis except *Cistanche*, which had only two-thirds of its *mat*K gene sequenced, due to technical difficulties). This type of analysis (many characters, few taxa) is expected to provide the strongest support for clades on trees (Sanderson, 1989). The fourth matrix (large-merger) also excludes *Cistanche*, but contains all 63 remaining taxa; about 39% of the data are missing. This type of analysis (many taxa, fewer characters per taxon) can be expected to have lower support for clades on trees, but can give at least a preliminary indication of relationships for all the taxa.

The strict consensus tree resulting from the *rps*2-only MP analysis (Fig. 2) supports the monophyly of the parasitic figworts and broomrapes and indicates that *Lindenbergia* is the nearest relative to the parasites, among those plants sampled. Within the parasites, early branching events are unresolved, but the transitional genera (*Lathraea*, *Harveya*, and *Hyobanche*) clearly do not group with the broomrapes. Constraining them to do so adds 14 steps to the tree (Table 2).

The best alignment of the *mat*K sequences, resulting in MP trees with a RC = 0.380, was generated with Clustal W alignment parameters GOP = 15, GEP = 5, transition/transversion weighting on, and the seven resulting indel characters were coded as in Baum et al. (1994). The indels, all in multiples of three bases, were distributed throughout the gene, but were more common near the ends. Because it had the highest RC, this alignment was chosen for the *mat*K-only, small-merger, and large-merger analyses. Similar alignment parameters produced RC values only slightly lower and yielded the same phylogenetic trees. However, parameters that differed substantially (such as GOP < 5 or GOP > 25) produced RC values less than 0.375 and led to less resolved consensus trees.

The *mat*K-only MP analysis resulted in six shortest trees, which differ only in the placement of *Schwalbea* and *Cistanche*. The strict consensus tree (Fig. 3) resolves the basal branches of the parasitic clade better than the *rps*2-only analysis. The *mat*K-only analysis supports the monophyly of traditional Orobanchaceae, whose sister group is the *Striga-Harveya* clade (though support measures are not high). It also indicates that the genus *Orobanche* is diphyletic (polyphyletic, forming two clades), with moderately high support. It also resolves the *Castilleja* clade as sister to the *Melampyrum* clade. The ML (maximum likelihood) tree differs only in that *Antirrhinum* and *Veronica* form a monophyletic group rather than a paraphyletic one. This indicates that the parsimony algorithm may have allowed *Veronica* and *Nicotiana* to be attracted to each other due to their long branches (Felsenstein, 1978). That *Antirrhinum* belongs with *Veronica* is supported by both the *rps*2-only and large-merger analyses, which break up these branches with the addition of more taxa (Figs. 2, 4). When the two regions containing overlapping gaps were removed from the analysis, as well as the gap characters generated by these regions, the strict consensus MP tree differed by a single feature: the sister relationship of two of the outgroups, *Hemimeris* and *Verbascum*, becomes unresolved.

The small-merger analysis (Fig. 3) gives additional support to the findings of the previous two analyses. *Cistanche* has been removed, but otherwise the resulting MP trees have exactly the same topologies. Again, the ML analysis conflicts only in the placement of *Veronica*. The monophyly of the parasites (hemi- plus holo-) is strongly supported, as is the position of *Lindenbergia* as sister group to the parasites. The holoparasites are clearly polyphyletic. Although the broomrapes (as sampled) are monophyletic, *Harveya* and *Lathraea* do not form a clade with them. Instead, each of these holoparasites is revealed as a close relative of green hemiparasites. *Harveya* is related to *Alectra* and *Striga* (and others of tribe Buchnereae; Fig. 4). *Lathraea* is in a clade with *Tozzia* and *Melampyrum* (and oth-

Table 1. Specimens used for DNA sequencing, with family (and subfamily and tribe for Scrophulariaceae) according to Wettstein (1897), DNA number, voucher numbers (in parentheses), localities (in quotes), herbarium of deposition in square brackets, and GenBank accession numbers for *rps*2 and *mat*K sequences.

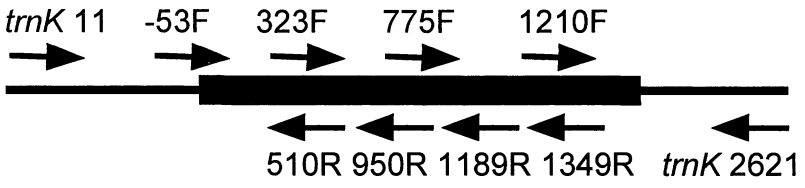
Family/subfamily/tribe	Species	DNA number/voucher	*rps*2	*mat*K
Scrophulariaceae				
Pseudosolaneae				
(we include in Antirrhinoideae)				
Verbasceae	*Verbascum blattaria* L.	CWD 90.117 "Davidson Co., Tennessee, USA" [PSU]	VBU48763	
	Verbascum thapsis L.	CWD 89.201 "Washtenaw Co., Michigan, USA" [PSU]		AFO52002
	Leucophyllum frutescens I. M. Johnston	CWD 95.21 "Austin Co., Texas, USA" [PSU]	AFO55156	
Antirrhinoideae				
Hemimeridae	*Hemimeris sabulosa* L.	(*K. E. Steiner 2387*) "Cape Province, S. Africa" [PSU]	HSU48765	AFO51985
Calceolarieae	*Calceolaria* sp.	CWD 90.203 "ex hort., Indiana U., USA" [PSU]	AFO55162	
Antirrhineae	*Antirrhinum majus* L.	CWD 90.204 "ex hort., U. Michigan, USA" [PSU]	AMU48766	AFO51978
Cheloneae	*Scrophularia californica* Cham. & Schlect.	CWD SS20 "Foster, California, USA" [PSU]	SCU48762	
	Chelone obliqua L.	CWD SS15 (*C. W. Morden 853*) "ex hort., Indiana U., USA" [PSU]	COU48770	
	Paulownia tomentosa Steud.	CWD SS24 "cultivated, Indiana U., USA" [PSU]	AFO55255	AFO51997
Gratioleae	*Mimulus aurantiacus* Renjifo	CWD 90.11 "cultivated, UC Berkeley, USA" [PSU]	AFO55154	
	Lindenbergia phillipinensis Benth.	CWD 98.01 (*J. G. Armstrong 1163*) "cult., Vanderbilt U., USA" [PSU]	AFO55151	AFO51990
	Gratiola pilosa Michx.	CWD 90.34 (no voucher)	AFO55163	
Selagineae	*Selago thunbergii* Choisy	CWD 90.21 "ex hort., UC Berkeley #62.1022, USA" [PSU]	AFO55158	
Rhinanthoideae				
Digitaleae	*Hemiphragma heterophyllum* Wall.	CWD 90.118 "ex hort., UC Berkeley #74.1009, USA" [PSU]	AFO55161	
	Veronica arvensis L.	CWD 92.201 "Davidson Co., Tennessee, USA" [PSU]	VAU48768	AFO52003
	Digitalis purpurea L.	CWD 93.41 "ex hort., Vanderbilt U., USA" [PSU]	DPU48767	
Gerardieae Benth.				
(we use Buchnereae Benth.)				
	Melasma scabrum Berg.	(*K. E. Steiner 2250*) "Farm Huisrivier, Cape Province, S. Africa" [PSU]	MSU48743	
	Alectra orobanchoides Benth.	(*K. E. Steiner 2278*) "Hhluhluwe, Natal, R. S. Africa" [PSU]	AOU48741	
	Alectra sessiliflora var. *sessiliflora* (Vahl.) O. Kunze	(*K. E. Steiner 2446*) "Ysterklip, Cape, R. S. Africa" [PSU]	ASU48742	AFO51977
	Macranthera flammea Pennell	CWD 90.140 (*J. R. Allison & A. K. Gohlson 5053*) "Liberty Co., FL, USA" [UGA]	AFO55139	

Table 1. Continued.

Family/subfamily/tribe	Species	DNA number/voucher	*rps*2	*mat*K
	Seymeria pectinata Pursh	CWD 94.142 (*J. R. Allison 4260b*) "Head Co., Georgia, USA" [UGA]	AFO55141	AFO51999
	Agalinis tenuifolia (M. Vahl.) Rafin.	CWD 90.129 "Monroe Co., Indiana, USA" [PSU]	ASU48738	
	Sopubia cana Harv.	CWD 94.152 (*K. E. Steiner 2473*) "Sani Pass, Natal Province, S. Africa" [PSU]	SCU48748	
	Buchnera floridana Gandoger	CWD 90.13 (*J. R. Allison & A. K. Gohlson 4452*) "Jackson Co., Florida, USA" [UGA]	BFU48744	
	Cycnium racemosum Benth.	(*A. Batten 1121*) "Mt. Kemp, Cape Province, S. Africa" [A. Batten]	CRU48745	
	Striga asiatica (L.) Kuntze	CWD 94.98 (no voucher) "cultivated, Whiteville, North Carolina, USA"	SAU48746	AFO52000
	Striga gesnerioides (Willd.) Vatke ex Engl.	(*G. Sallé 13D*) "Mali, parasitic on cow pea" [PSU]	SGU48747	
	Harveya capensis Hook.	(*K. E. Steiner 2432*) "Onderboskloof, S. Africa" [PSU]	AFO55142	
	Harveya purpurea Harv.	(*K. E. Steiner 2433*) "Cape Province, S. Africa" [PSU]	HPU48749	AFO51984
	Hyobanche sanguinea L.	(*K. E. Steiner 2536*) "20.5 km E of turnoff to Daskop, Cape Province, S. Africa" [NBG]	HSU48750	
Rhinantheae	*Castilleja lineariifolia* Benth.	CWD 90.93 (*Heckard & Chuang 6743*) [UCB]	CLU48739	AFO51981
	Orthocarpus bracteosus Benth.	(*Heckard & Chuang 6757*) "California, USA" [UCB]	AFO55140	
	Triphysaria versicolor Fisch. & Mey.	CWD 90.17 [PSU]	AFO55137	
	Melampyrum sylvaticum L.	(*W. Wetschnig 2 Aug. 1992*) "Styria, E. Alps, Austria" [PSU]	AFO55148	AFO51991
	Tozzia alpina L.	CWD 93.38 (*W. Wetschnig 2 Aug. 1992*) "Styria, E. Alps, Austria" [PSU]	TAU48754	AFO52001
	Lathraea clandestina L.	(*M. W. Chase 2605*) "ex hort., Kew R.B.G." [KEW]	LCU48755	AFO51989
	Euphrasia spectabilis Phil.	CWD 93.39 (*W. Wetschnig 2 Aug 1992*) "Styria, E. Alps, Austria" [PSU]	ESU48752	
	Parentucellia viscosa L.	(*C. W. dePamphilis, L. Heckard & T. I. Chuang 90.89*) "Marin Co., California, USA" [PSU]	PVU48753	
	Bartsia alpina L.	CWD 93.37 (*W. Wetschnig 2 Aug. 1992*) "Styria, E. Alps, Austria" [PSU]	BAU48751	
	Pedicularis attolens A. Gray	(*W. Wetschnig s.n. 2 Aug. 1992*) "Styria, E. Alps, Austria" [PSU]	AFO55149	
	Pedicularis foliosa L.	(*W. Wetschnig s.n. 2 Aug. 1992*) "Styria, E. Alps, Austria" [PSU]	PFU48740	
	Rhinanthus aristatus Čelak.	(*W. Wetschnig s.n. 2 Aug. 1992*) "Styria, E. Alps, Austria" [PSU]	RCU48756	

Table 1. Continued.

Family/subfamily/tribe	Species	DNA number/voucher	*rps*2	*mat*K
	Lamourouxia viscosa	CWD SS16 (*R. Ornduff 9660*) "cultivated, UC Berkeley, USA" [UCB]	AFO55138	
	Schwalbea americana L.	CWD 95.22 (*K. Kirkman s.n.*) "Albany, Georgia, USA" [PSU]	AFO55150	AFO51998
Orobanchaceae	*Cistanche phelypaea* L.	CWD 96.501 (*J. A. Hoder 1996*) "Coutino, Spain" [PSU]	AFO56149	
	Conopholis americana Wallr.	CWD 90.231 "Monroe Co., Indiana, USA" [PSU]	CAU48759	
	Epifagus virginiana Barton	CWD 88.01 "Washtenaw Co., Michigan, USA" [PSU]	EPFCPCG	EPFCPCG, AFO51982
	Boschniakia hookeri Walp.	CWD 94.61 "Marin Co., California, USA" [PSU]	BHU48757	AFO51979
	Boschniakia strobilacea A. Gray	CWD 94.153 (*G. Cohn s.n.*) [PSU]	BSU 48758	AFO51980
	Orobanche caryophyllaceae Sm.	(*M. W. Chase 2769*) "cultivated, KEW R.B.G., London" [KEW]	AFO55145	AFO51992
	Orobanche cernua Loefl.	CWD s.n. (*L. J. Musselman s.n.*) "Valdulapalli, India" [PSU]	AFO55147	AFO56147
	Orobanche corymbosa (Rydb.) Ferris	(*Heckard & Chuang 6751*) "Mono Co., California, USA" [PSU]	OCU48760	AFO51993
	Orobanche fasciculata Nutt.	CWD 94.151 (*Heckard 6754*) "Inyo Co., California, USA" [PSU]	AFO55143	AFO51994
	Orobanche hederae Duby	(KEW 2836) "cultivated, KEW R.B.G., London" [KEW]	AFO55146	AFO51995
	Orobanche ramosa L.	CWD 94.149 (*K. E. Steiner s.n.*) "Kirstenbosch, Capetown, S. Africa" [PSU]	ORU48761	AFO56148
	Orobanche uniflora L.	(*C. W. dePamphilis, L. Heckard & T. I. Chuang 94.15*) "Mt Tamalpais, California, USA" [PSU]	AFO55144	AFO51996
Bignoniaceae	*Kigelia pinnata* DC.	CWD 90.78 "ex hort., Missouri Botanical Garden #897541, USA" [PSU]	KSU48764	AFO51988
	Schlegelia parviflora (Oerst.) Monachino	CWD 90.41 (*A. Gentry #14221*) "cultivated, Missouri Botanical Garden, USA" [PSU]	AFO55152	
Callitrichaceae	*Callitriche hermaphrodita* L.	(*C. T. Philbrick 3022*)	AFO55159	
Gesneriaceae	*Kohleria digitiflora*	CWD 90.57 "ex hort., Missouri Botanical Garden 894763, USA" [PSU]	AFO55164	
Hippuriaceae	*Hippuris vulgaris* L.	(*C. T. Philbrick 305A*) [CA]	AFO55160	
Myoporiaceae	*Myoporum parvifolium* R. Br.	CWD 90.43 "ex hort., Missouri Botanical Garden #896655, USA" [PSU]	AFO55157	
Oleaceae	*Ligustrum japonicum* Buch.-Ham. ex D. Don	CWD SS69 (*J. D. Palmer CPS704*) "ex hort."	LJU48769	
Solanaceae	*Nicotiana tabacum* L.	CWD s.n. "cultivated, Vanderbilt U., USA" [PSU]	CHNTXX	CHNTXX
Verbenaceae	*Verbena bonariensis* L.	*R. G. Olmstead* 464	AFO55153	



trnK 11	CTCAACGGTAGAGTACTCG
trnK 2621	AACTAGTCGGATGGAGTAG
matK -53F	CTTGTTTTGRCTNTATCGCACTATG
matK 323F	TATTCTCAAATCATATCRGAGGG
matK 775F	TCTTGAACGAATCTATTTCTRYGG
matK 1210F	GGGCATCCCATTAGTAAGNC
matK 1349R	CTTTTGTGTTTCCGAGCYAAAGTTC
matK 1189R	CGGTTACTAATGGGATGCCC
matK 950R	CCACARCGAAAAATRMCATTGCC
matK 510R	GAAGAGTTTGAACCAAKAYTTCC

Figure 1. Map of the *mat*K gene (thick line) and flanking regions (thin line) with primer sites indicated. Primer sequences are listed 5′ to 3′; some contain IUPAC ambiguity codes. *trn*K 11 was designed by G. Learn; *trn*K 2621 was designed by K. Steele (Steele & Vilgalys, 1994).

ers of tribe Rhinantheae). *Tozzia* is therefore not an evolutionary transition to *Lathraea,* though they are fairly close relatives. The genus *Orobanche* is diphyletic (Fig. 3). Two New World species group with *Boschniakia,* and four Old World species group with *Epifagus.* The *rbc*L phylogeny also supports *Orobanche* diphyly (Wolfe & dePamphilis, 1998). However, the support values for the New World clade are low, so this result must be regarded as preliminary.

The strict consensus tree resulting from the large-merger analysis (Fig. 4) is more resolved and generally better supported than that from the *rps*2-only analysis, but is otherwise completely congruent with it. The relationships at poorly supported nodes, especially among the outgroups, should be regarded as very preliminary. Neither subfamily of Scrophulariaceae is monophyletic, and this is not only because the broomrapes, mints, and other families derive from within the subfamilies. The tribe Digitaleae sensu Wettstein, which includes *Veronica* and *Hemiphragma,* forms a clade with the tribe Antirrhineae and its relatives rather than with the rest of the subfamily Rhinanthoideae. Likewise neither parasitic tribe is monophyletic. Constraint analyses show that many extra steps would have to be added to make the subfamilies and parasitic tribes monophyletic (Table 2). Surprisingly, there is strong support for a clade that includes members of both the Rhinantheae and the Buchnereae. All of the genera in this clade have their center of distribution in North America (Mabberley, 1997).

Discussion

These analyses of *rps*2 and *mat*K gene sequences allow us to better understand the phylogeny and thus the evolution of parasitism within the figworts and broomrapes. The number of characters and taxa we have utilized exceeds that of previous studies. Out of the 27 taxa contained in the *rps*2 tree and the *mat*K tree, there are only two conflicts. The combination of the data sets is therefore supported (Mason-Gamer & Kellogg, 1996). The monophyly of the parasitic figworts and broomrapes is strongly supported in all four analyses. The monophyly of the parasites also indicates a single origin of parasitism. It is not yet clear how many times haustorial parasitism has evolved in other groups, but an estimate of at least 11 times (Nickrent et al., 1998) suggests that the habit may be relatively uncommon in flowering plants. However, once hemiparasitism has been established, the loss of photosynthesis (and evolution of holoparasitism) may be more common.

Holoparasitism has arisen five independent times in the figwort/broomrape clade. In addition to the *Harveya, Lathraea,* and broomrape lineages, it has also occurred once within the genus *Alectra* and once within the genus *Striga* (dePamphilis et al.,

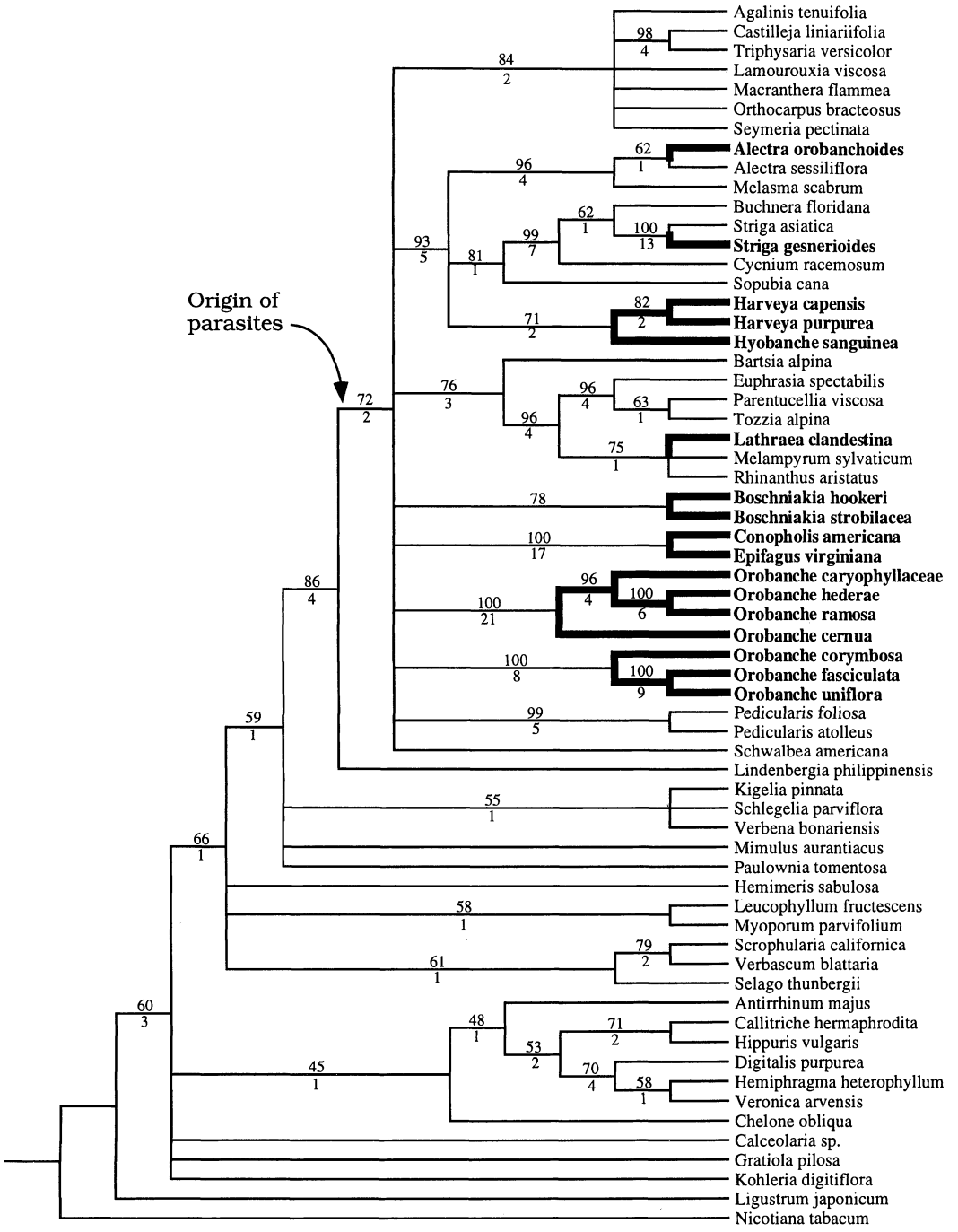


Figure 2. Phylogeny of figworts and broomrapes based on *rps2* gene sequences. The strict consensus tree is shown, with bootstrap values (above branch) based on 500 replicates and Bremer support values (below branch). Bold font typeface and bold font branch segments indicate holoparasitic taxa.

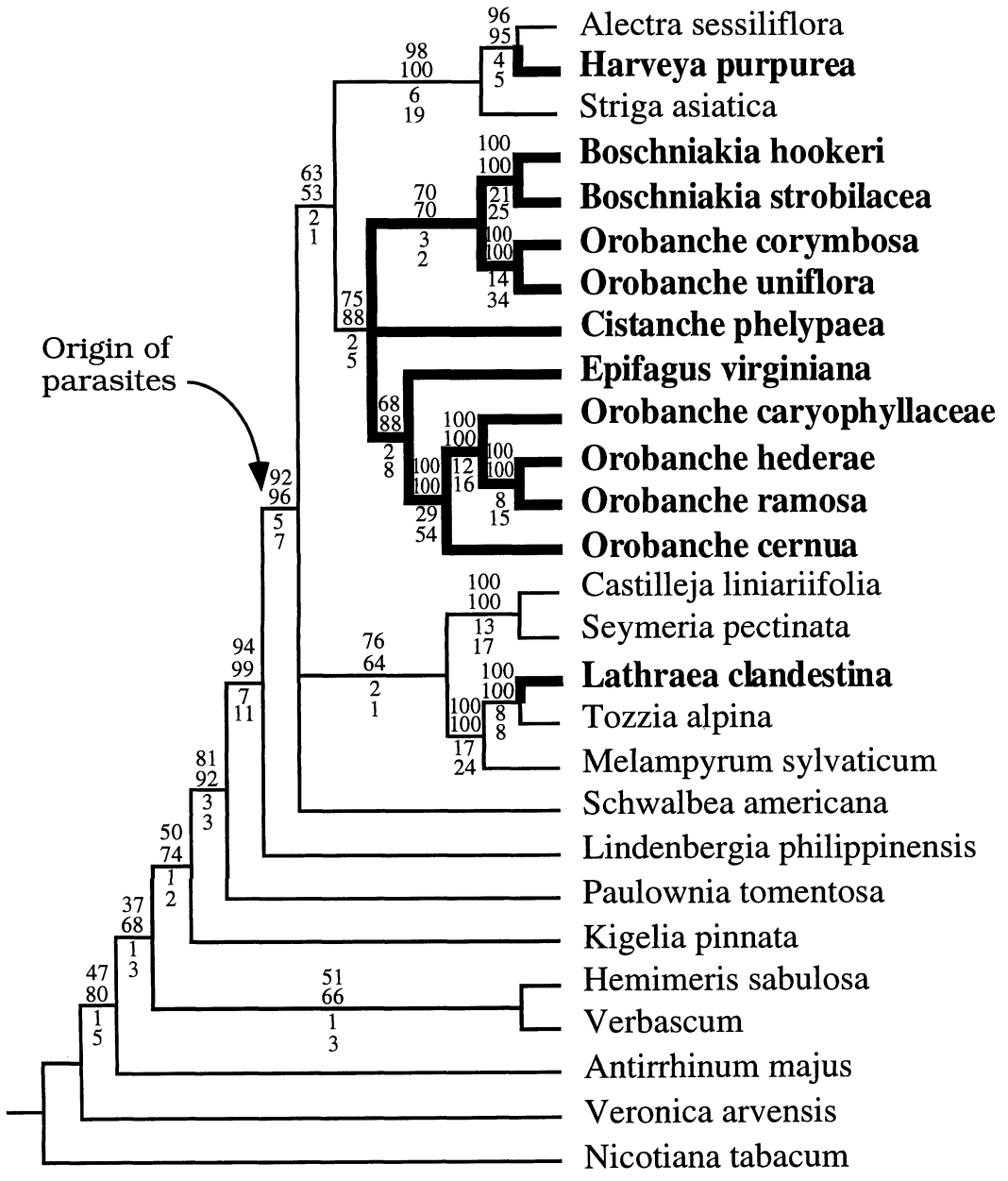


Figure 3. Results of both the *mat*K-only analysis and the small-merger analysis. The topology shown is the strict consensus tree from the *mat*K-only analysis. *Cistanche* and *Schwalbea* have unresolved placements; when these taxa are removed, a single, fully resolved tree is found. The small-merger combined analysis of *rps*2 and *mat*K yielded a strict consensus tree with this same topology, except that *Cistanche* was not included in that analysis. The four numbers displayed at each node represent, top to bottom, the *mat*K bootstrap value (based on 1500 replicates), the small-merger bootstrap value (based on 838 replicates), the *mat*K Bremer support value, and the small-merger Bremer support value. In one case, different species were used to represent a genus: *Verbascum blattaria* was sequenced for *rps*2; *V. thapsis* was sequenced for *mat*K. Bold font typeface and bold font branch segments indicate holoparasitic taxa.

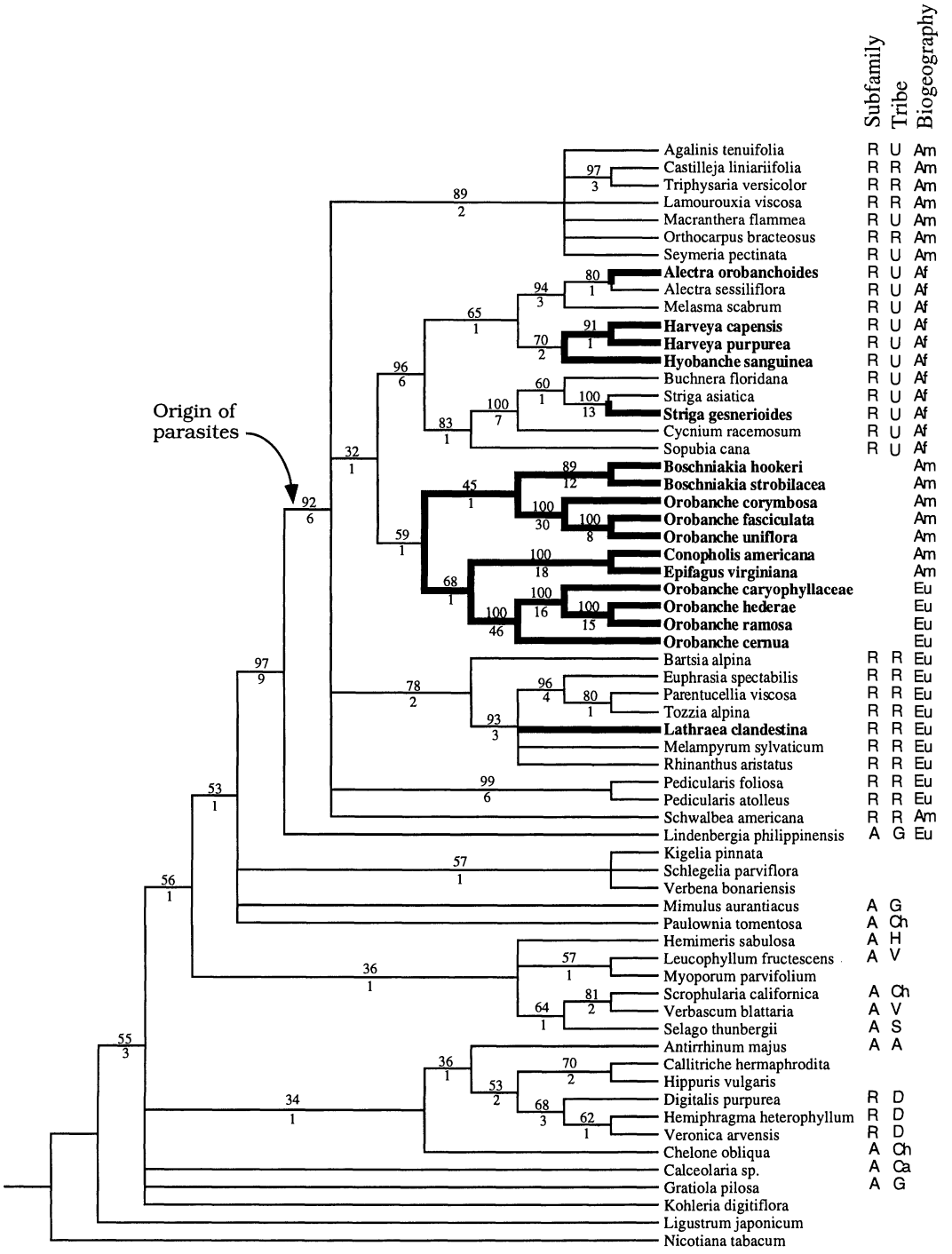


Figure 4. Strict consensus tree from the large-merger analysis. Bootstrap values (above) based on 500 replicates and Bremer support values below. Bold font typeface and bold font branch segments indicate holoparasitic taxa. Subfamily codes: A = Antirrhinoideae, R = Rhinanthoideae. Codes for Wettstein's tribes: U = Buchnereae, R = Rhinantheae, G = Gratioleae, Ch = Cheloneae, H = Hemimeridae, V = Verbasceae, S = Selagineae, A = Antirrhineae, D = Digitaleae, Ca = Calceolarieae. Biogeography codes—taxa have their centers of distribution in the following province: Am = Americas, Af = Africa, Eu = Eurasia.

Table 2. Descriptive measures from parsimony analyses and results of monophyly constraint analyses. Small = small merger, Large = large merger, MP = maximum parsimony.

	*rps*2	*mat*K	Small	Large
No. of taxa	63	27	26	63
No. of characters	617	1205	1822	1822
No. of MP trees	15000	6	3	13941
Tree length	727	1421	1823	2137
CI	0.552	0.669	0.677	0.627
RC	0.370	0.380	0.398	0.381
Constraints[1]:				
F-S	20	21	34	36
SF-A	15	15	22	29
SF-R	19	23	37	38
T-B	6	17	22	25
T-R	6	17	22	22
G-O	0	9	8	7
B&TG	14	54	67	67

[1] The number of extra steps added when the following groups are constrained to be monophyletic (Swofford, 1993): F-S = family Scrophulariaceae (excluding broomrapes), SF-A = subfamily Antirrhinoideae, SF-R = subfamily Rhinanthoideae, T-B = tribe Buchnereae, T-R = tribe Rhinantheae, G-O = genus *Orobanche*, B&TG = broomrapes with the transitional genera *Harveya*, *Lathraea*, and *Hyobanche* (if present on tree).

1997). In all cases, holoparasitic taxa have been derived from hemiparasitic ancestors. This suggests that the loss of photosynthesis may only occur subsequent to the evolutionary origin of the haustorium, the key character defining a parasitic strategy.

THE EVOLUTIONARY SERIES IS NOT SUPPORTED

The transitional genera *Lathraea*, *Harveya*, and *Hyobanche* are each more closely related to green hemiparasitic lineages than they are to the broomrapes. The placement of *Lathraea* with the *Melampyrum* group is found in 100% of bootstrap replicates and all trees up to 23 steps longer (Fig. 3). The grouping of *Harveya* (and presumably *Hyobanche*, based on Fig. 4) with hemiparasites of the *Striga* group is also extremely well supported (bootstrap value = 100, Bremer support value = 19). This reveals that the characters used previously to group these taxa with the broomrapes (e.g., loss of green color, reduction of leaves, the condensation of the vegetative axis, and reduction of cell number in the ovary) are all homoplastic and may be correlated with holoparasitism. The past practice of grouping the transitional genera with the broomrapes may be due to the fact that their specialization involves the loss or reduction of many features, and convergently reduced or lacking features will seem to be homologies due to common ancestry.

OTHER SYSTEMATIC CONCLUSIONS

Olmstead and Reeves (1995; Reeves & Olmstead, 1998) began the disintegration of the Scrophulariaceae by reporting that there are at least two major lineages of figworts. In studies of the chloroplast genes *ndh*F and *rbc*L, they identified the fact that *Antirrhinum*, *Digitalis*, and *Veronica* form a clade distinct from *Scrophularia*, *Verbascum*, and *Selago*. We have identified five additional clades of nonparasitic figworts. *Leucophyllum* represents a clade sister to the Myoporaceae. *Mimulus* and *Paulownia* represent clades that are not in the parasitic figwort clade, but are closer to it, Verbenaceae, and Bignoniaceae than they are to the *Scrophularia* clade. Finally, *Calceolaria* and *Gratiola* represent two clades whose positions are unresolved. Each of these lineages may end up as a family of its own or may be included with one of the other families, depending on its position on future, more resolved, phylogenies. A combined analysis is being conducted, using *rps*2, *ndh*F, and *rbc*L and extensive sampling of nonparasitic Scrophulariaceae and related lineages (R. G. Olmstead, C. W. dePamphilis, A. D. Wolfe, N. D. Young, W. J. Elisens & P. A. Reeves, unpublished).

Based on Figure 4, we can see that neither of the traditional subfamilies (Antirrhinoideae, Rhinanthoideae) are monophyletic. When trees are constrained to contain a monophyletic subfamily, they are at least 15 steps longer (Table 2). In order to circumscribe monophyletic groups, the subfamilies must be broken up, and their members placed into at least five new or re-defined families, separated by other existing families (R. G. Olmstead, C. W. dePamphilis, A. D. Wolfe, N. D. Young, W. J. Eli-

sens & P. A. Reeves, unpublished). One redefined family will combine taxa out of both subfamilies.

Digitalis and *Veronica,* classified by Wettstein (1897) in the Rhinanthoideae, are herein (Fig. 3) more closely related to *Antirrhinum* than to the remainder of Rhinanthoideae. This implies that the floral aestivation character used to distinguish Rhinanthoideae from the other subfamily, Antirrhinoideae (Bentham, 1846; Bentham & Hooker, 1876; Wettstein, 1897; Armstrong & Douglas, 1989), has changed more than once in the evolution of the group: *Digitalis, Veronica,* and their relatives probably represent an independent origin of the rhinanthoid corolla aestivation type: the traditional Rhinanthoideae are clearly polyphyletic. This placement of *Digitalis* and *Veronica* agrees not only with *ndh*F/*rbc*L phylogeny (Olmstead & Reeves, 1995) but also with pollen morphology. Pollen exine structure is tectate with reticulate sculpturing in *Digitalis, Veronica, Antirrhinum,* and *Chelone,* a structure that is otherwise rare in the family (Mink-in & Eshbaugh, 1989).

The nearest relative to the parasitic figworts and broomrapes is the figwort genus *Lindenbergia,* not Gesneriaceae. Gesneriaceae (here represented by *Kohleria*) must therefore have derived unilocular placentation independently from Orobanchaceae. *Lindenbergia,* a southeast Asian genus, has traditionally been placed in the tribe Gratioleae in subfamily Antirrhinoideae (Bentham & Hooker, 1876) despite corolla aestivation similar to parasitic figworts and broomrapes (Cooke, 1903; Duthie, 1903–1920; Brühl, 1920; J. E. Armstrong, unpublished). Brühl (1920) has suggested that *Lindenbergia* shares a closer relationship with the parasitic figworts than with the Gratioleae, which agrees with our analysis (Figs. 2–4). *Lindenbergia* is here shown to be the sister group to the parasites, confirming its floral similarity. The clade containing all parasitic figworts, broomrapes, and *Lindenbergia* is strongly supported. In the small-merger analysis, it has a bootstrap value of 99 and a Bremer support value of 11. In addition, this clade has a defining (though not unique) morphological synapomorphy: anterior lobes external in corolla aestivation. This group warrants family status, and the appropriate name is Orobanchaceae, a conserved name (Greuter et al., 1994). We recommend expanding the Orobanchaceae and suggest the following clade definition:

> Orobanchaceae are the least inclusive clade that contains *Orobanche uniflora, Schwalbea americana,* and *Lindenbergia philippinensis.*

This definition, along with the changes suggested in Olmstead et al. (R. G. Olmstead, C. W. dePamphilis, A. D. Wolfe, N. D. Young, W. J. Elisens & P. A. Reeves, unpublished), forms a new taxonomic system, which is compared to Wettstein's (Wettstein, 1897) in Table 3. Orobanchaceae now include hemiparasites and nonparasites, as well as holoparasites.

Within the parasites, *Schwalbea* is among the basal lineages, as suggested by its morphology (Pennell, 1935), but its exact position remains unresolved. The two parasitic tribes Buchnereae and Rhinantheae are each not monophyletic (Fig. 4). *Agalinis* (= *Gerardia), Macranthera,* and *Seymeria,* the New World representatives of Buchnereae Benth. 1846 (= Gerardieae Benth. & Hook., 1846), are part of a clade that is otherwise made up of genera of Rhinantheae with centers of distribution in North America. This clade is an example of the strong biogeographic pattern seen in this phylogeny (Fig. 4). Clades on the parasitic part of the phylogeny tend to be made up of genera with their centers of distribution (Mabberley, 1997) in the same biogeographic province (Fig. 4). There are large clades of taxa centered in Africa and Eurasia that include both hemiparasites and holoparasites. Even within the broomrapes a pattern emerges, with two clades of taxa centered in the Americas and one in Eurasia.

In Figures 3 and 4, the broomrapes are monophyletic. Their sister group (Fig. 3) is the *Striga-Harveya* group. However, this is not as well supported as our other conclusions and must be regarded as preliminary. The other potential sister groups are the *Tozzia-Lathraea* and *Castilleja-Seymeria* groups. Moderately well supported is the finding that *Orobanche* comprises at least two groups: one allied with *Boschniakia,* the other allied with *Epifagus* and *Conopholis* (Fig. 4). In the small-merger analysis (Fig. 3), these two new groupings are supported by bootstrap values of 70 and 88, respectively. The current classification of *Orobanche* (Beck von Mannagetta, 1930; Collins, 1973; Heckard & Chuang, 1975) contains two Old World sections and two New World sections. Beck von Mannagetta (1890) united the two New World sections into one branch of the genus, and the two Old World sections to form the other. Cytology indicates that one of the Old World sections, section *Orobanche,* has chromosome numbers that are generally $2n = 38$, while the other three sections have chromosome numbers that are nearly always $2n = 24, 48, 72,$ or 96 (Heckard & Chuang, 1975). To be consistent with Beck von Mannagetta's scheme, the cytology suggests that section *Orobanche* might be monophyletic and derived from the other Old World section, *Trionychon* (including *O. ramosa,* $2n$

Table 3. Genera of the Scrophulariaceae/Orobanchaceae complex used in this study, deposited according to the taxonomic systems of Wettstein (1897), and Olmstead et al. (R. G. Olmstead, C. W. dePamphilis, A. D. Wolfe, N. D. Young, W. J. Elisens & P. A. Reeves, unpublished).

Wettstein	Olmstead et al.
Schrophulariaceae	Scrophulariaceae
Pseudosolaneae	*Verbascum*
Verbasceae	*Leucophyllum*
Verbascum	*Scrophularia*
Leucophyllum	*Selago*
Antirrhinoideae	
Hemimeridae	Antirrhinaceae
Hemimeris	*Antirrhinum*
Calceolarieae	*Chelone*
Calceolaria	*Gratiola*
Antirrhineae	*Hemiphragma*
Antirrhinum	*Veronica*
Cheloneae	*Digitalis*
Scrophularia	*Callitriche*
Chelone	*Hippuris*
Paulownia	
Gratioleae	Calceolariaceae
Mimulus	*Calceolaria*
Lindenbergia	
Gratiola	Orobanchaceae
Selagineae	*Lindenbergia*
Selago	*Melasma*
Rhinanthoideae	*Alectra*
Digitaleae	*Macranthera*
Hemiphragma	*Seymeria*
Veronica	*Agalinis*
Digitalis	*Sopubia*
Gerardieae	*Buchnera*
Melasma	*Cycnium*
Alectra	*Striga*
Macranthera	*Harveya*
Seymeria	*Hyobanche*
Agalinis	*Castilleja*
Sopubia	*Orthocarpus*
Buchnera	*Triphysaria*
Cycnium	*Melampyrum*
Striga	*Tozzia*
Harveya	*Lathraea*
Hyobanche	*Euphrasia*
Rhinantheae	*Parentucellia*
Castilleja	*Bartsia*
Orthocarpus	*Pedicularis*
Triphysaria	*Rhinanthus*
Melampyrum	*Lamourouxia*
Tozzia	*Schwalbea*
Lathraea	*Cistanche*
Euphrasia	*Conopholis*
Parentucellia	*Epifagus*
Bartsia	*Boschniakia*

Table 3. Continued.

Wettstein	Olmstead et al.
Pedicularis	*Orobanche*
Rhinanthus	
Lamourouxia	No family designation, near Orobanchaceae
Schwalbea	*Mimulus*
Orobanchacae	*Paulownia*
Cistanche	
Conopholis	Bignoniaceae
Epifagus	*Kigelia*
Boschniakia	
Orobanche	No family designation, near Bignoniaceae
Bignoniaceae	*Schlegelia*
Kigelia	
Schlegelia	Verbenaceae
Callitrichaceae	*Verbena*
Callitriche	
Gesneriaceae	Gesneriaceae
Kohleria	*Kohleria*
Hippuriaceae	
Hippuris	
Myoporiaceae	
Myoporum	
Verbenaceae	
Verbena	

= 24). Our plastid phylogeny (Fig. 4) supports the Old World–New World division, but finds a paraphyletic relationship of section *Orobanche* (*O. caryophyllaceae, O. hederae,* and *O. cernua,* all $2n$ = 38) to section *Trionychon* ($2n$ = 24). This indicates that the Old World branch of the genus may have begun with $2n$ = 38 and later experienced a reduction in section *Trionychon* to $2n$ = 24. No counts have been reported for *Epifagus, Conopholis,* or *Boschniakia. Cistanche* has $2n$ = 40 (Hambler, 1956).

Having a classification that reflects monophyletic relationships will be a great advantage, especially to comparative biologists. These phylogenetically defined groups may even be easier to identify based on morphological characters, compared to the traditional family Scrophulariaceae, which is recognized by symplesiomorphies such as the presence of endosperm, capsular fruit, and strongly zygomorphic flowers. Furthermore, *Antirrhinum majus* serves as a model organism in the field of developmental biology (Coen & Nugent, 1994; Bradley et al., 1996). Such work can be placed in an illuminating context by the study of its relatives and their phylogeny (Reeves & Olmstead, 1998).

Knowing that photosynthesis has been lost multiple times in Orobanchaceae opens up many opportunities for comparative analysis. It allows for the comparison of rates of DNA base substitution (dePamphilis et al., 1997), plastid genome structural evolution (dePamphilis, 1995), as well as rates of loss of particular photosynthetic genes (Wolfe & dePamphilis, 1997). Haustorial anatomy and physiology, host plant use, morphology, as well as the genetic changes that have accompanied parasitism, can all be investigated using the phylogeny for reference. Awareness of the multiple, independent origins of holoparasitism provides us with a powerful comparative framework in which to study the process of parasitic evolution.

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