Pat Reeves collecting water-starwort, *Callitriche verna*. All photographs by R. Olmstead unless otherwise credited.

WHATEVER HAPPENED TO THE SCROPHULARIACEAE?

by Richard G. Olmstead

Science has been hard on some of our best-loved plant families recently. Perhaps none have been hit harder than the figwort family, Scrophulariaceae.

My interest in the Scrophulariaceae started innocently enough when an undergraduate student working in my lab, Pat Reeves, decided to look to see where three genera of aquatic plants, *Callitriche*, *Hippuris*, and *Hydrostachys* belonged phylogenetically, that is, were they part of the same branch of an evolutionary lineage. Cronquist, whose classification scheme dominated our views of plant relationships for the last 30 years of the 20th century, assigned each of these genera to its own family and all three to the order Callitrichales. Some preliminary results from my research indicated that *Callitriche* might be closest to the "scroph" genera *Antirrhinum* (snapdragon) and *Digitalis* (foxglove), rather than near the mint family, Lamiaceae, as others had thought. I suggested to Pat that he should sample a number of other representatives of the Scrophulariaceae, as well as some other members of Cronquist's orders Lamiales and Scrophulariales.

The very first results seemed to indicate that something was amiss. Two groups of genera, normally assigned to Scrophulariaceae, seemed to form distinct branches on the resulting evolutionary tree, but did not come out together. While we could not be confident of the relationships among all the families included in that study, we were confident that these two groups of genera were as distant from each other as any of several major families in this part of the "tree of life" were from each other, including the Lamiaceae, Acanthaceae, Bignoniaceae, Verbenaceae,

Water-starwort, *Callitriche verna* (top left), and Mare's-tail, *Hippuris vulgaris* (above), are now included in the Veronicaceae.

TREE OF LIFE

The metaphor of the "tree of life" has been used ever since Darwin's time to conceptualize the pattern of relationships among species. A classification that reflects the tree of life would be hierarchical in nature, with branch tips representing species and the branches themselves representing groups in the classification. Our present system of classification consists of a series of ever-more-inclusive ranks. Closely related species make up a genus ("twig" on the tree metaphor), one or more genera make up a family (larger branch), and so on down the tree towards the "trunk" with order, class, phylum, and kingdom.

In order for the resulting classification to accurately reflect the evolutionary relationships or *phylogeny*, each named group in the classification must connect to the rest of the tree at a single point (or "saw cut," if we are pruning the "tree"). Each such group is termed *monophyletic* (literally "one branch"), whereas groups that consist of two or more branches pruned separately from the tree are termed *polyphyletic*. Those that require one cut to prune a branch, but then another cut to exclude one of the smaller branches from that branch are termed *paraphyletic*. Traditional classifications are largely monophyletic, but typically have some polyphyletic groups and many paraphyletic groups.

DNA SEQUENCES

Most of the evidence for plant evolutionary relationships in recent years has come from a comparison of the DNA sequences of various genes, usually those found in the chloroplast of the plant cell. A single gene, or even three genes as used in the study on which this article is based, are but a small fraction of the entire genetic material in a plant (its genome). However, the variation in DNA sequences for even a gene of modest length—for example 1,000 nucleotides long—is likely to have more information about evolutionary relationships than the anatomy and morphology on which traditional classifications are based. It is also easier to compare using new computer analyses. In the study of Olmstead et al. (2001), three genes totaling more than 4,200 nucleotides were compared for 65 species of plants in the Scrophulariaceae and related families.

and Gesneriaceae (Olmstead and Reeves 1995). Ever since Darwin said that classification should be "genealogical," taxonomists have tried their best to continually revise classifications to reflect the natural order of evolutionary relationships, the pattern we call *phylogeny* today. A classification that reflects phylogeny would be one in which all of the species assigned to a given group—whether a genus, family, or any other group we may want to name—share a more recent common ancestor with each other than with any species outside the group. This is what is called a monophyletic group (see sidebar on page 14).

For most of the 150 years since Darwin's ideas transformed the way we think about living things, the changes in our classifications have come gradually. However, the pace of change has picked up dramatically in recent years due to the combined effect of new, more rigorous ways of assessing phylogenetic relationships and the tremendous influx of data from DNA sequences (see sidebar above).

That first study was modest in scope: after all, it was an undergraduate thesis project! I knew that I would have to dramatically increase the sampling in order to nail down the extent to which changes would need to be made in our classification of the Scrophulariaceae. At this point I contacted a group of researchers at Vanderbilt University—Claude dePamphilis, Andrea Wolfe, and Ned Young, who were studying the parasitic members of the Scrophulariaceae and Orobanchaceae—to better understand the origin and evolution of parasitism. (Parasitism in this case is the ability to develop the parasitic connections called haustoria to the roots of other plants.)

In Pat's study, we had not sampled any of the parasitic scrophs, not wanting to interfere with their research. However, Claude, Andi, and Ned had some tantalizing evidence that the parasites, including such well known western wildflowers as *Pedicularis* (lousewort), *Castilleja* and *Orthocarpus* (paintbrush and owl's clover), and *Orobanche* (broom-rape), all formed a closely related group of plants that was not close to either of the two groups we had identified.

As fate would have it, our two labs had only a partially overlapping set of DNA sequences, so we undertook the job of backtracking and obtaining all of the DNA sequence data we would need for all of the species we had sampled. We also added some more species to the list to try to sample as many of the tribes (rank more inclusive than genus, but smaller than family) of Scrophulariaceae as possible from around the world. In all, we sampled 39 genera of scrophs, representing 24 tribes, along with representatives of 15 closely related families for a recently published study (Olmstead et al. 2001). Our data consisted of DNA sequences for three genes and totaled more than 4,200 nucleotides of DNA. Since the body of evidence for this study was obtained, numerous other species have been sampled and additional genes have been

The genus *Castilleja* (purple owl's-clover, *C. exserta*, shown here) is now included in the Orobanchaceae family along with broom-rapes and louseworts. Photograph by J. Vale; its use courtesy of Jepson Herbarium, UC.

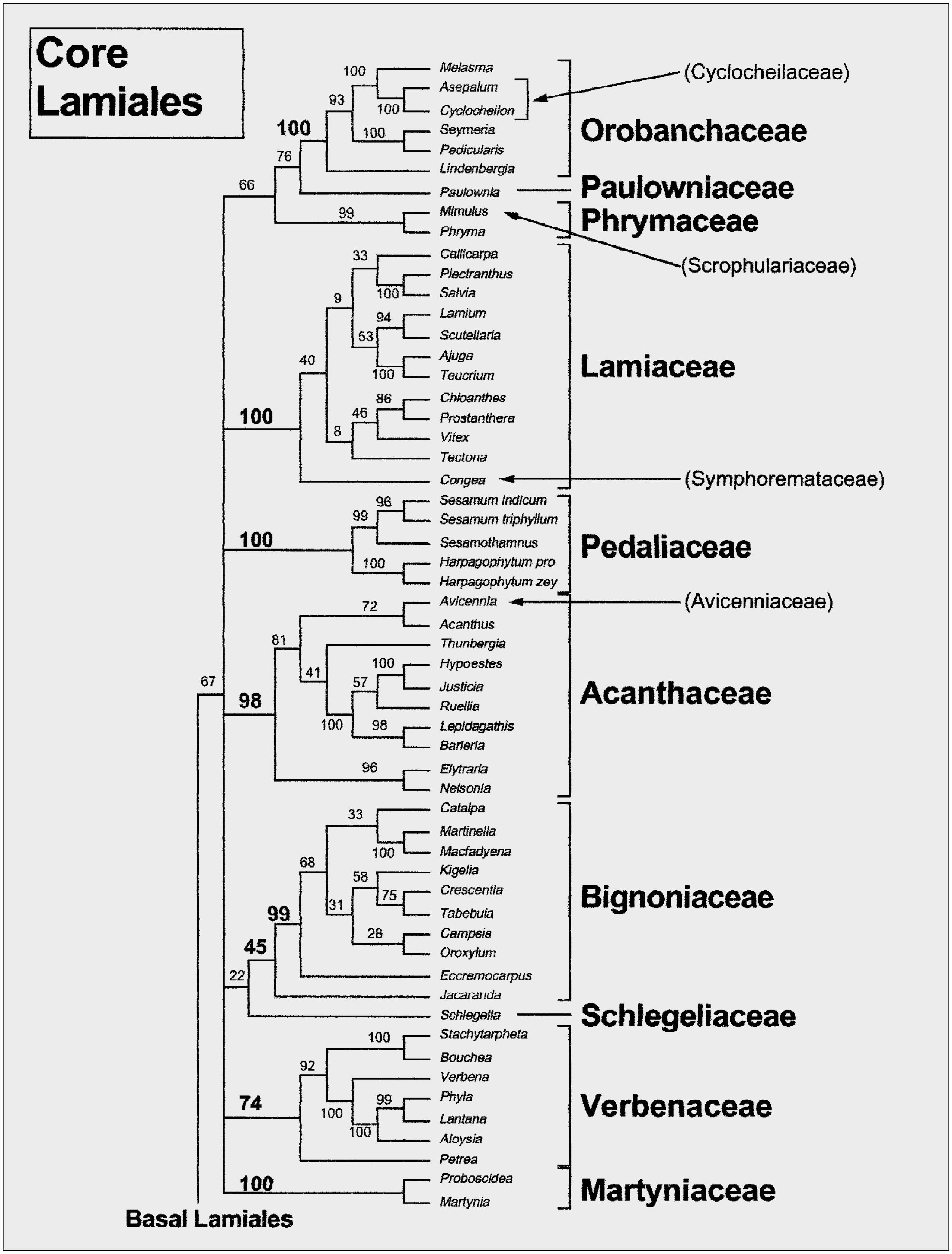
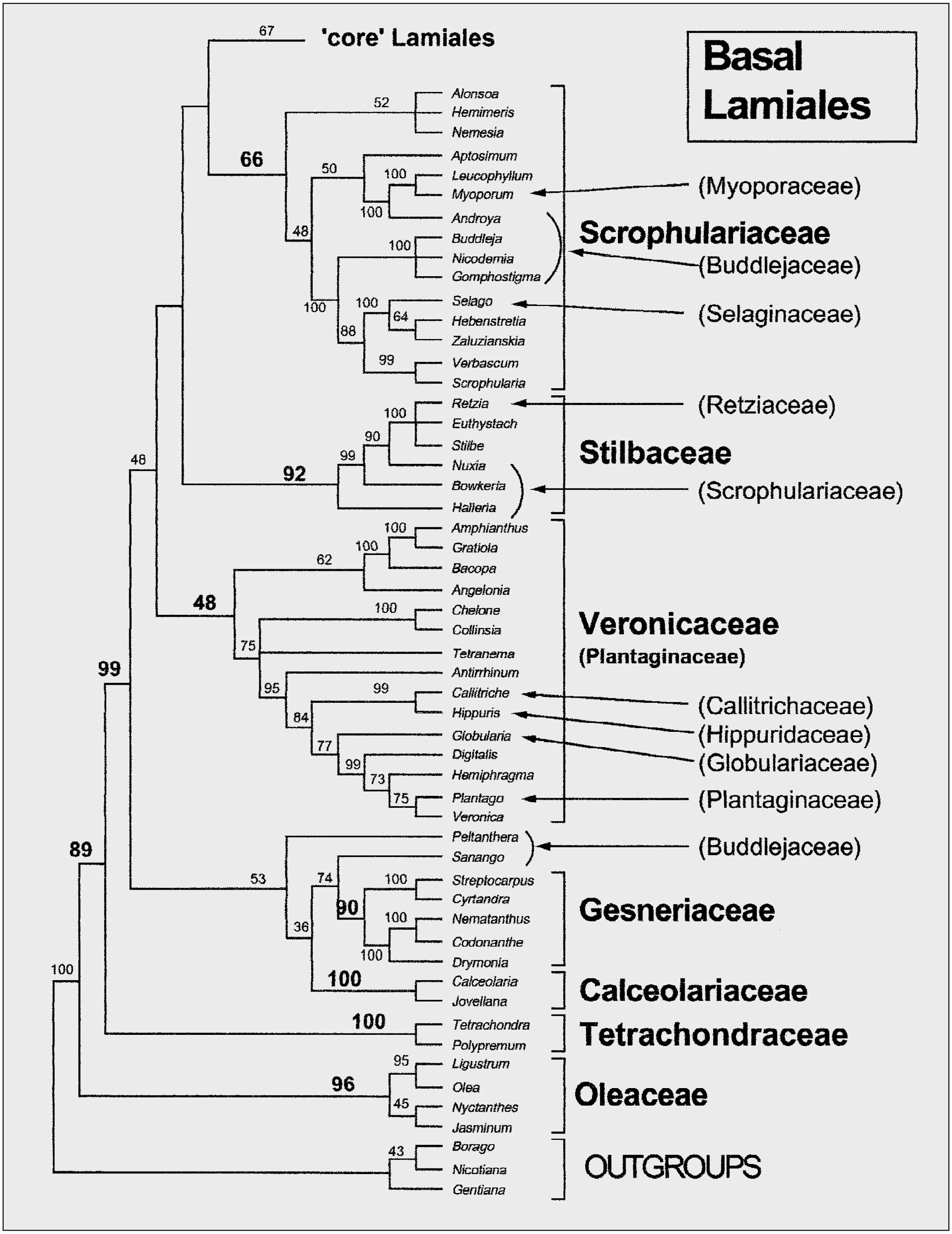


Figure 1. This tree represents the consensus relationships based on the DNA sequences of two genes from the chloroplast genome. Areas of uncertainty regarding relationships are indicated by the unresolved nodes on the tree. Numbers on the branches represent



bootstrap values (scale 0 - 100) which provide a relative measure of support for individual branches. The names on the right in bold are the names of the major groups (indicated by brackets) recognized in our study, while those in parentheses with arrows indicate former family placement of some genera.

TABLE 1. GENERA INCLUDED IN CALLITRICHACEAE, HIPPURIDACEAE, OROBANCHACEAE, PLANTAGINACEAE, AND SCROPHULARIACEAE IN *THE JEPSON MANUAL* (1993) WITH THEIR NEW FAMILY ASSIGNMENTS

Orobanchaceae (Broom-rape Family)

- *Bellardia*
- *Boschniakia* (ground-cone)
- *Castilleja* (Indian paintbrush, owl's clover)
- *Cordylanthus* (bird's-beak)
- *Orobanche* (broom-rape)
- *Orthocarpus* (owl's clover)
- *Parentucellia*
- *Pedicularis* (lousewort)
- *Triphysaria*

Veronicaceae or Plantaginaceae (Veronica or Plantain Family)

- *Antirrhinum* (snapdragon)
- *Bacopa* (water-hyssop)
- *Callitriche* (water-starwort)
- *Collinsia*
- *Cymbalaria*
- *Digitalis* (foxglove)
- *Dopatrium*
- *Galvezia*
- *Gratiola* (hedge-hyssop)
- *Hebe*
- *Hippuris* (mare's-tale)
- *Keckiella*
- *Kickxia* (fluellin)
- *Limosella* (mudwort)
- *Linaria* (toadflax)
- *Maurandya*
- *Mohavea*
- *Nothochelone*
- *Penstemon* (beardtongue)
- *Plantago* (plantain)
- *Stemodia*
- *Synthyris* (kittentails)
- *Tonella*
- *Veronica* (speedwell, brooklime)

Scrophulariaceae (Figwort Family) includes Buddlejaceae (Loganiaceae) and Myoporaceae

- *Buddleja* (butterfly bush)
- *Myoporum*
- *Scrophularia* (figwort)
- *Verbascum* (mullein)

Phrymaceae

- *Mimulus* (monkey flower)

Unassigned

- *Lindernia* (false pimpernel)

As a member of the genus that the family is named for, lance-leaved figwort, *Scrophularia lanceolata* (above), remains in the Scrophulariaceae. Photograph by M. Denton. • Moth mullein, *Verbascum blattaria* (below right), is still included in the Scrophulariaceae.

sequenced, but the picture that I am about to describe of the demise of the Scrophulariaceae has not been altered substantially by any of those results.

Prior molecular systematic studies had determined that Cronquist's Scrophulariales and Lamiales (minus the Boraginaceae), along with the small orders Callitrichales and Plantaginales, formed a monophyletic group, all of which is now referred to as Lamiales (Olmstead et al. 1993). Flowers of plants in the Lamiales tend to be pretty uniform in their appearance, typically having corollas with bilateral symmetry (often two-lipped, or bilabiate) that are five-parted, and superior ovaries consisting of two fused carpels. Perhaps the most notable outlier in the group is the olive family (Oleaceae), which have four-parted flowers with two stamens and radial symmetry. The atypical floral morphology of the Oleaceae is not so difficult to explain, considering that its placement in the phylogenetic tree is at the very base of the Lamiales.

Most of the families related to the Scrophulariaceae can be identified by distinguishing traits that are unique to the family (e.g., winged seeds without endosperm in Bignoniaceae; retinacula for ejecting seeds from the fruit in Acanthaceae), or traits that are clearly uniquely derived, even if shared by some more distantly related family (parietal placentation in Gesneriaceae; ovule number reduced to two per carpel and each carpel divided by a "false septum" in Lamiaceae and Verbenaceae). However, the Scrophulariaceae seem to be characterized by unspecialized floral traits that may represent ancestral traits within the Lamiales.

Even though work to sort out all of the details of evolutionary relationships within the Lamiales is continuing, a sufficiently robust picture of relationships has emerged to enable us to identify major lineages suitable for recognition in a revised classification. The picture that emerges is one that shows some families mixed up with other families (e.g., Lamiaceae and Verbenaceae), some families nested within other families (e.g., Myoporaceae and Buddlejaceae within Scrophulariaceae), and yet other families that are assemblages of unrelated lineages that will need to be dismembered (e.g., Pedaliaceae and Scrophulariaceae).

In some cases, adjusting the classification will mean little more

than changing the rank of some groups (e.g., family to subfamily or vice versa), whereas in other cases, new taxa will need to be named or named taxa will need to be altered dramatically in their circumscription. In any event, the changes that are being made will be a test of how well the traditional Linnaean system of classification, which has served the taxonomic community for 250 years, will hold up in the face of efforts to develop a new system of nomenclature (the method of applying names in a classification, not the classification itself), but this is a subject for another time!

The Scrophulariaceae, as traditionally conceived, is worldwide in distribution and consists of approximately 275 genera and over 5,000 species. The emerging classification for the plants traditionally assigned to Scrophulariaceae will represent at least seven groups that will bear the rank of family. These will vary tremendously in size and several of them will have a distinct geographic focus. Some of these are newly recognized (e.g., Calceolariaceae, Veronicaceae), while others represent expansions of previously recognized families (e.g., Orobanchaceae, Stilbaceae). They are characterized as follows.

Scrophulariaceae. For those of us here in the western US, the Scrophulariaceae will become one of those families that we will encounter on rare occasions in our native flora. Two genera native to North America, *Leucophyllum* in the deserts of the southwest and Mexico, and *Scrophularia* (figwort), are the only native genera that remain in this family; the weed, mullein (*Verbascum*), will be our most frequently encountered scroph. Both *Scrophularia* and *Verbascum* belong to a small group of genera within the Scrophulariaceae that is distributed in north temperate regions, mostly in Eurasia.

Most of the rest of this family is in the southern hemisphere, particularly South Africa, where four tribes are endemic. Australia has a substantial representation of the Scrophulariaceae in the form of the Myoporaceae, a group that is traditionally considered a distinct family, but now is seen to belong nested within the Scrophulariaceae. Buddlejaceae also is included in this group and is distributed in Africa, Asia, and the New World. Excluding Myoporaceae and Buddlejaceae from Scrophulariaceae would make the remaining group paraphyletic.

Most North American botanists think of *Verbascum* as an oddball for the family due to its nearly radially symmetric flowers. However, most of the Scrophulariaceae, as we now define them, exhibit radial corolla symmetry, so it is *Scrophularia*, with its bilabiate corolla, that is the real oddball. Buddlejaceae, Myoporaceae, and most of the South African scrophs all have corollas that are radially symmetric, or nearly so.

Orobanchaceae. For many years the Orobanchaceae have been accepted somewhat reluctantly as the bastard stepchild of the Scrophulariaceae. It was no secret that they were related to the scrophs and probably derived from them, but the distinctive suite of traits associated with being an obligate parasite, or holoparasite, seemed sufficient to merit their own taxonomic designation.

However, recent molecular sys-

Clustered broom-rape, *Orobanche fasciculata* (top), is included in the Orobanchaceae along with photosynthetic genera such as *Pedicularis* (louseworts). • Applegate Indian paintbrush, *Castilleja applegatei* (middle). Paintbrushes are now included in the Orobanchaceae. Photograph by M. Denton. • English plantain, *Plantago lanceolata* (bottom), is moved from the Plantaginaceae in the strict sense to a larger family, Plantaginaceae or Veronicaceae, which includes such genera as *Antirrhinum* (snapdragons), *Penstemon*, and *Veronica*.

Monkey flowers, such as the above *Mimulus mephiticus* from Yosemite National Park, are now placed in the Phyrmaceae. Photograph by C.S. Webber; its use courtesy of Jepson Herbarium, UC.

tematic studies legitimize the Orobanchaceae and reunite them with their rightful siblings, the green hemiparasites (capable of both photosynthesis and parasitism), including such well-known natives as the louseworts (*Pedicularis*), Indian paintbrushes (*Castilleja*), and owl's clovers (*Castilleja* and *Orthocarpus*). Not only are all of the parasites (holo- and hemi-) united into a monophyletic group, thus providing the basis for their acceptance in our classification, they are not particularly close to any of the other major groups of scrophs. Therefore, this is not simply a matter of arbitrarily splitting off a distinctive branch of a larger Scrophulariaceae; these form a distinct and distinctive lineage of plants.

The work of dePamphilis, Wolfe, and Young (dePamphilis et al. 1997; Wolfe & dePamphilis 1997; Young et al. 1999) has shown that parasitism in this group evolved only once. Perhaps more interestingly, they have shown that the loss of chlorophyll and, therefore, the inability to photosynthesize, has occurred multiple times within the descendants of that first parasitic species. Thus the traditional Orobanchaceae, consisting of just the obligate parasites, is not a natural group without also including the hemiparasites with them. Consequently, the Orobanchaceae now assume a much more important role in the flora of western North America.

Veronicaceae. Many of our most familiar scrophs, including snapdragons (*Antirrhinum*), foxglove (*Digitalis*), and such natives as *Collinsia*, *Penstemon*, and *Veronica*, belong to a third major lineage. This group is worldwide in distribution and very diverse. Also in this group are several small groups often recognized as individual families by virtue of having distinctive traits that obscure their true relationships. These include *Plantago* (Plantaginaceae), *Globularia* (Globulariaceae), and two of the aquatic genera mentioned in the beginning of this article that were the initial subject of the undergraduate thesis several years ago, *Callitriche* and *Hippuris* (Olmstead et al. 2000). The third aquatic genus, *Hydrostachys*, is more closely related to the hydrangeas and dogwoods than it is to *Callitriche* and *Hippuris* (Olmstead et al. 2000).

For this group, we have chosen a name calculated to create controversy. The International Code of Botanical Nomenclature requires the use of the earliest validly published name for a family (or genus or species) that is based on the name of a species included in the family. We have chosen Veronicaceae, which is the earliest validly published name (1782) and one that reflects its primary composition as former scrophs, to represent this newly recognized taxonomic group.

However, two rules conspire against this choice. One is a nomenclatural rule that permits exceptions to strict priority, and allows later names to be accepted or "conserved" (in the lingo of taxonomic nomenclature). The second is an obscure rule in an appendix to the

Code of Nomenclature, which further constrains the choice of "conserved" names to a starting date in 1789. The Code requires that Plantaginaceae be used for this group. (Globulariaceae, Callitrichaceae, and Hippuridaceae all are preferred by the Code over any other name, too, but occur later than Plantaginaceae.)

The international committee charged with settling disputes about nomenclature rejected our request to use any name other than Plantaginaceae. However, given that Plantaginaceae already is identified with a well-known and monophyletic group, and recognizing the inadvisability of forcing the many new groups being recognized today to fit into a few Linnaean ranks, we have chosen to ignore the Code and name this group Veronicaceae.

Phrymaceae. If you have studied botany primarily in the western US, chances are you have not heard of this family. As presently conceived, it includes a single genus, *Phryma*, which has either one or two species, depending on whether east Asian and eastern North American populations are considered separate species or not. The plants are small, perennial, understory herbs in deciduous forests with tiny, primarily self-pollinating flowers. The flowers have unusually long curled tips on the calyx and a single-seeded fruit, which accounts for their isolated taxonomic position. However, if you look closely you can see a little monkey face smiling back at you.

The doctoral research of Paul Beardsley at the University of Washington has shown that *Phryma* is, in fact, a monkey flower and is evolutionarily derived from ancestors that we would call *Mimulus* were we to encounter them today. *Phryma*, *Mimulus*, and six to eight other small genera comprise a monophyletic group that is not closely related to any of the other groups of former scrophs. Ironically, neither the name *Mimulus*, nor any of the names of the other small genera, have been published as the root of a family name. Therefore, Phrymaceae has priority at the rank of family for this group. By this point, you are probably cursing out loud. But, bear up—you've just learned a new family!

Are there yet more families? Yes. But these are ones that you won't encounter here in the wilds of the western US. A pretty ornamental plant, with which many of you may be familiar, *Calceolaria* (pocket-book plant), along with two small genera native to South America and New Zealand, are isolated from all other scrophs and comprise a new family, Calceolariaceae (not just new to us provincial botanists here in the western US, but a newly-described family). In South Africa, a few former scroph genera were found to be related to a group called the Stilbaceae and are transferred to that family. Lastly, *Paulownia* (empress tree), a group of six species of large trees from China with showy flowers reminiscent of *Catalpa* and sometimes placed in the Scrophulariaceae, are isolated from any of the other families and are best considered their own family, Paulowniaceae.

As scientists, we plant taxonomists consider ourselves fortunate to be a part of what is one of the eras of greatest advancement in our field since the tremendous age of discovery in the 18th and 19th centuries. At the same time we recognize the fact that many botanists of our generation, amateur and professional alike, may find the changes in plant classification confusing and even counterproductive to the purpose of communicating about biodiversity. However, if our classification is to truly represent the pattern of biodiversity stemming from the evolutionary history of the plants on earth today, changes we make now will result in a classification that will provide a much more stable means of communicating our knowledge of biodiversity many generations into the future.

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