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Phylogeny and Classification of Lamiales with Emphasis on Scrophulariaceae in the Broad Sense

Richard G. Olmstead

Introduction

Sorting relationships in Lamiales is one of the thorniest problems in angiosperm systematics (W. S. Judd and R. G. Olmstead 2004; D. E. Soltis et al. 2011). Relationships among several clades now recognized as families (for example, Angiosperm Phylogeny Group 2016) remain uncertain (Olmstead et al. 2001; B. Oxelman et al. 2005; B. Schäferhoff et al. 2010). Results of phylogenetic studies (for example, L. A. McDade et al. 2008; Olmstead et al. 2009; H. E. Marx et al. 2010; J. R. McNeal et al. 2013; M. Perret et al. 2013; Li Bo et al. 2016) have converged on a classification at the family level that is likely to remain stable. Figure 1 depicts a conservative estimate of relationships at the family level derived from multigene DNA studies (Schäferhoff et al.; N. F. Refulio-Rodriguez and Olmstead 2014).

Traditional circumscriptions of Lamiales often included only Lamiaceae, Verbenaceae, and a few very small segregate or isolated families (G. L. Stebbins 1974; A. L. Takhtajan 1980, 1997) and sometimes included Boraginaceae (for example, A. Cronquist 1981). Some late twentieth-century classifications recognized additional orders that now are included in Lamiales: Callitrichales, Hippuridales, Plantaginales, and Scrophulariales (for example, Cronquist; Takhtajan 1997). R. F. Thorne (1992b) recognized suborder Lamiineae, which was similar to Lamiales of Cronquist, minus Boraginaceae, in a broadly circumscribed Scrophulariales. The Scrophulariales of Thorne, renamed Lamiales in subsequent treatments (Thorne 2000b; Thorne and J. L. Reveal 2007), was similar in circumscription to Lamiales recognized today (Angiosperm Phylogeny Group 2016), with minor differences; Thorne did not include Plocospermataceae Hutchinson and Tetrachondraceae in his Scrophulariales. Recognition of an order Lamiales corresponding to the group with the present circumscription derives from early molecular systematic studies of *rbc*L sequences, in which the clade was first identified and named Lamiales (R. G. Olmstead et al. 1992, 1993) and expanded in many subsequent studies (for example, Olmstead et al. 2001; B. Bremer et al. 2002; B. Oxelman et al. 2005; B. Schäferhoff et al. 2010; N. F. Refulio-Rodriguez and Olmstead 2014).

Adjustments in family circumscriptions in Lamiales include large-scale changes; for example, about one half of Verbenaceae have been transferred to Lamiaceae (P. D. Cantino et al. 1992; S. J. Wagstaff and R. G. Olmstead 1997; R. M. Harley et al. 2004). Small-scale changes have also been made, such as consolidating Avicenniaceae Miquel into Acanthaceae (A. E. Schwarzbach and L. A. McDade 2002), or identifying isolated lineages and recognizing them as distinct families (for example, Schlegeliaceae Reveal from Bignoniaceae, R. E. Spangler and Olmstead 1999; Olmstead et al. 2009; Thomandersiaceae Sreemadhavan from Acanthaceae, A. H. Wortley et al. 2007).

Perhaps the most sweeping changes at the family level have been made to the traditional concept of Scrophulariaceae (for example, A. Cronquist 1981; R. F. Thorne 1992b; A. L. Takhtajan 1997), wherein nine families (Figure 1) have been formed from the traditionally circumscribed family (R. G. Olmstead et al. 2001; B. Oxelman et al. 2005; R. Rahmanzadeh et al. 2005; D. C. Tank et al. 2006;

Orobanchaceae* (100/2000)
Paulowniaceae* (1/7)
Phrymaceae* (13/200)
Mazaceae* (3/36)
Lamiaceae (250/6700)
Schlegeliaceae (4/36)
Verbenaceae (31/840)
Thomandersiaceae (1/6)
Lentibulariaceae (4/310)
Bignoniaceae (82/810)
Acanthaceae (200/3500)
Pedaliaceae (13/79)
Martyniaceae (4/13)
Byblidaceae (1/2)
Linderniaceae* (20/250)
Stilbaceae (11/39)
Scrophulariaceae* (60/1700)
Plantaginaceae* (100/1900)
Gesneriaceae (140/3000)
Calceolariaceae (2/275)
Tetrachondraceae (2/3)
Oleaceae (25/600)
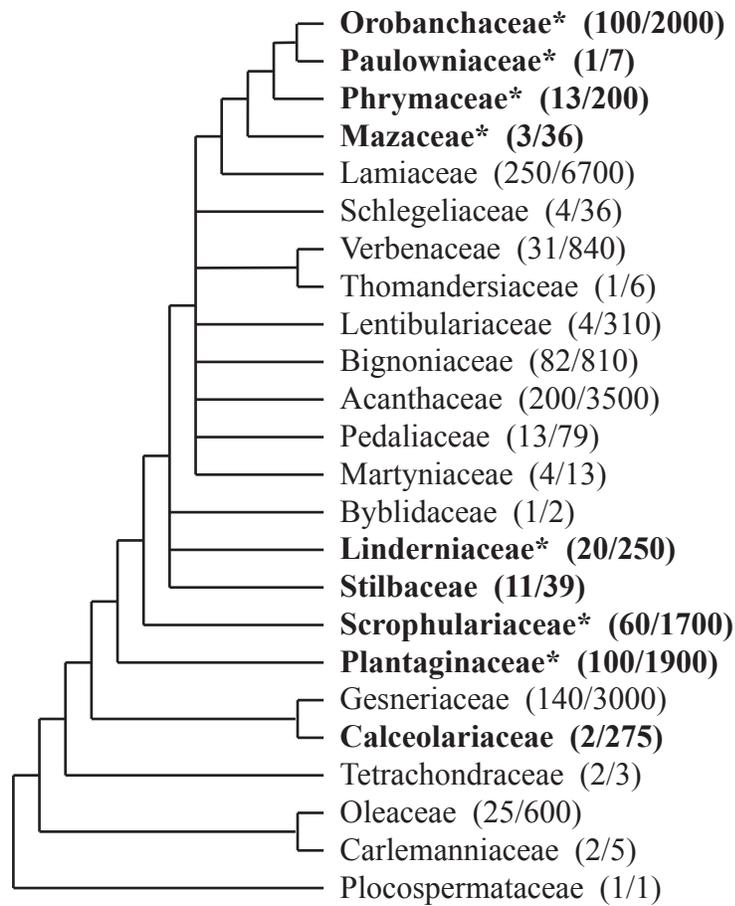Carlemanniaceae (2/5)
Plocospermataceae (1/1)

FIGURE 1. Phylogenetic relationships among clades of Lamiales representing families as recognized by APG III. Families consisting all or in part of Scrophulariaceae in the broad sense are in bold; of those, the ones present in North America are indicated by an asterisk (*). Estimated numbers of genera/species are indicated in parentheses.

J. L. Reveal 2011). Additionally, molecular evidence (Olmstead et al.; Oxelman et al.; B. Schäferhoff et al. 2010; I. C. Gormley et al. 2015) now supports separation of New World Martyniaceae from Old World Pedaliaceae, contrary to some twentieth-century treatments (for example, G. L. Stebbins 1974; Cronquist). The splitting of Scrophulariaceae resulted in enlarging some traditionally small families, including the expansion of Orobanchaceae to accommodate the hemiparasitic members of Scrophulariaceae (J. R. McNeal et al. 2013), expanding Plantaginaceae from two or three genera and about 300 species to more than 100 genera and about 1900 species (D. C. Albach et al. 2005), and transforming the monogeneric Phrymaceae by the addition of a dozen genera and about 200 species (P. M. Beardsley and Olmstead 2002; W. R. Barker et al. 2012).

Splitting Scrophulariaceae

In most twentieth-century classifications (for example, A. L. Takhtajan 1980, 1997; A. Cronquist 1981; R. F. Thorne 1992b), families in Lamiales as circumscribed here were defined by the presence of some distinctive trait or traits (for example, explosive capsules with retinacula in Acanthaceae, winged seeds lacking endosperm in Bignoniaceae, ovules reduced to two per carpel and separated by a false septum in Lamiaceae and Verbenaceae, achlorophyllous parasites in Orobanchaceae, wind pollination in Plantaginaceae, and anthers adherent in pairs in Gesneriaceae Richard & Jussieu). Scrophulariaceae, the largest of the families in those classifications, was defined by a combination of traits (typically bilaterally symmetric flowers with didynamous stamens and two multi-ovulate carpels forming a capsular fruit) that are individually found

in many other families. Despite what may be seen in hindsight as evidence of either polyphyly or paraphyly for a large group without a distinctive defining trait, there is little evidence in writings accompanying the angiosperm classifications from the nineteenth and twentieth centuries that suggests Scrophulariaceae were not considered to be a natural group, apart from inclusion or exclusion of Buddlejaceae, Globulariaceae de Candolle, Lentibulariaceae, Plantaginaceae, Selaginaceae Choisy, and Salpiglossaceae Hutchinson (the last now in Solanaceae).

The earliest molecular evidence to hint that Scrophulariaceae, as traditionally defined, were not monophyletic, came from a study aimed at placing the aquatic genera *Callitriche* and *Hippuris*. That study (R. G. Olmstead and P. A. Reeves 1995) found that the two aquatic genera were sister groups and nested within a group of genera assigned to Scrophulariaceae in the broad sense, including *Antirrhinum*, *Digitalis*, *Plantago*, and *Veronica*. A second group of genera, including *Buddleja*, *Scrophularia*, *Selago* Linnaeus, and *Verbascum*, was distant from the clade containing the aquatic genera. At about the same time, a study of parasitic plants in Orobanchaceae and Scrophulariaceae found that many hemiparasitic plants assigned to Scrophulariaceae in the broad sense were more closely related to the holoparasitic Orobanchaceae than they were to other Scrophulariaceae (C. W. dePamphilis et al. 1997; A. D. Wolfe and dePamphilis 1998). An effort to combine data from these two studies with additional sampling of genera of Scrophulariaceae in the broad sense, and representatives of most families of Lamiales, identified six distinct branches comprising, wholly or in large part, Scrophulariaceae in the broad sense: Calceolariaceae Olmstead, *Mimulus*, Orobanchaceae, Plantaginaceae, Scrophulariaceae in the narrow sense, and Stilbaceae Kunth (Olmstead et al. 2001).

Subsequent studies added additional unsampled genera and further clarified relationships of segregate groups of Scrophulariaceae among Lamiales. *Mimulus* and related genera were assigned to Phrymaceae, along with the Asian genera *Lancea* Hooker f. & Thompson and *Mazus*, as subfam. Mazoideae (named, but not validly published in P. M. Beardsley and R. G. Olmstead 2002), and a group of four Australian genera (Beardsley and W. R. Barker 2005). Beardsley and co-workers (Beardsley and Olmstead; Beardsley and Barker) showed that *Mimulus* was a broadly paraphyletic ancestral group from which the other genera were derived and set the stage for splitting *Mimulus* into monophyletic genera (Barker et al. 2012). Linderniaceae was recognized as another previously unsampled lineage comprising about 15 genera (B. Oxelman et al. 2005; R. Rahmanzadeh et al. 2005). Mazoideae has not been found to occur consistently with Phrymaceae in phylogenies and should not be

included with that family (Oxelman et al.; D. C. Albach et al. 2009; Xia Z. et al. 2009). A clade comprising *Rehmannia* Liboschitz ex Fischer & C. A. Meyer and *Triaenophora* (J. D. Hooker) Solereder occupies an isolated position close to Orobanchaceae and Phrymaceae (Oxelman et al.; B. Schäferhoff et al. 2010). J. L. Reveal (2011) described new families to accommodate each of the last two groups, Mazaceae and Rehmanniaceae Reveal, respectively; the latter was included in Orobanchaceae by Angiosperm Phylogeny Group (2016). Much of this work was contemporaneous with the writing of the treatment of Scrophulariaceae for *The Families and Genera of Vascular Plants* (E. Fischer 2004). In that volume, genera of Scrophulariaceae in the broad sense are included in one family; the genera are organized along lines of the families that were then becoming recognized.

Synopsis of Current Classification

Seven of the nine families derived all, or in large part, from the former Scrophulariales are represented in the flora of North America. Mazaceae and Paulowniaceae are represented by introduced species only; the rest have substantial native representation in North America.

Plantaginaceae contain the largest number of representatives in the flora area and are the largest clade worldwide to emerge from Scrophulariaceae in the broad sense, with about 100 genera and 1900 species. Some formerly recognized families (for example, A. Cronquist 1981) belong here, including Callitrichaceae Link, Globulariaceae, Hippuridaceae Vest, and Plantaginaceae in the narrow sense. Plantaginaceae have been divided into 12 tribes (D. C. Albach et al. 2005). Of these, nine are represented in North America; Angelonieae Pennell, Digitalideae Dumortier, and Russelieae Pennell are represented by introduced species. All genera of Cheloneae D. Don are represented in the flora area, except for *Uroskinnera* Lindley (Central America) and *Pennellianthus* Crosswhite (East Asia) (A. D. Wolfe et al. 2002). Plantaginaceae are primarily Northern Hemisphere in distribution; Angelonieae is South American and is sister to Gratioleae D. Don, which is widely distributed in Northern and Southern hemispheres (Albach et al.). This inclusive clade (Angelonieae + Gratioleae) is sister to the rest of the family, in which the predominantly North American *Chelone* is sister to the other mostly Northern Hemisphere tribes.

Scrophulariaceae in the narrow sense are primarily a Southern Hemisphere group with the greatest generic diversity in southern Africa (P. Kornhall et al. 2001) and another center of diversity in Australia (B. Oxelman et al. 2005). Though much smaller

than the traditional family, this is still a sizable group with about 60 genera and about 1700 species. The former families Myoporaceae R. Brown (distributed primarily in Australia) and Buddlejaceae belong here (R. G. Olmstead and P. A. Reeves 1995; Oxelman et al. 1999, 2005). Despite the Southern Hemisphere origins of Scrophulariaceae, Scrophularieae Dumortier (primarily *Scrophularia* and *Verbascum*) has diversified extensively in the Northern Hemisphere with over 500 species. *Buddleja* also has diversified in the Northern Hemisphere with significant diversity in the New World as far north as the southwest United States, and in the eastern Himalayas. A synopsis of Scrophulariaceae (D. C. Tank et al. 2006) recognized eight tribes; five of these occur in the flora area. Four tribes, Aptosimeae Bentham & Hooker f., Hemimerideae Bentham, Limoselleae Dumortier, and Teedieae Bentham, are entirely or predominantly southern African in distribution. Only the Australian Myoporeae Reichenbach and their sister group, Leucophylleae Miers, of North America and Central America are absent from Africa. Despite the traditional image of bilaterally symmetric corollas, most Scrophulariaceae have radially symmetric corollas; bilateral symmetry is derived in *Scrophularia*, Leucophylleae, and *Eremophila* R. Brown and relatives (Myoporeae).

Linderniaceae are predominantly an African group and one of the last of the Scrophulariaceae segregate families to be recognized (R. Rahmanzadeh et al. 2005); the family comprises 20 genera and about 250 species. Two genera of Linderniaceae are native to North America: *Micranthemum* with three native species and *Lindernia* with six species (D. Q. Lewis 2000). *Torenia* is represented in the flora area by one introduced species.

Mazaceae include *Mazus*, an East Asian and Australian genus of about 33 species, with two adventive species in the flora area, along with *Lancea* (two species in China) and *Dodartia* Linnaeus (one species in Russia). Similarities with *Mimulus* led to classification of *Mazus* and *Lancea* with *Mimulus* in Scrophulariaceae tribes Gratioleae (R. Wettstein 1891–1893) or Mimuleae Dumortier (F. W. Pennell 1920c) and reflect the close relationship inferred by P. M. Beardsley and R. G. Olmstead (2002), who assigned them to Mazoideae (an unpublished name) in Phrymaceae. Subsequent research (B. Oxelman et al. 2005) showed that relationship to be close but not to be monophyletic.

Phrymaceae received the largest proportional increase of any family in Lamiales following recircumscription, going from a single species, *Phryma leptostachya*, recognized in many late twentieth-century classifications (for example, R. F. Thorne 1992b; A. L. Takhtajan

1997), to 13 genera and nearly 200 species (P. M. Beardsley and R. G. Olmstead 2002; W. R. Barker et al. 2012). Most of the increase came with the recognition that the clade that included *Mimulus* in the broad sense also encompassed several smaller genera, including *Phryma* nested within a paraphyletic *Mimulus* (Beardsley and Olmstead; Beardsley and Barker 2005), and that Phrymaceae was the only available name at the rank of family. Distribution of Phrymaceae is predominantly within the flora area with greatest diversity in western North America; Phrymaceae also have a second center of diversity in Australia. The type of *Mimulus* is the eastern North American *M. ringens*, which is not a part of the western North America radiation. Redrawing of generic boundaries in Phrymaceae (Barker et al.) led to the reclassification of all western North American species into *Diplacus* and *Erythranthe*, and unispecific *Mimetanthe*, which shares some synapomorphies with *Diplacus*. The phylogenetic work by Beardsley and co-workers (Beardsley and Olmstead; Beardsley et al. 2004) implicitly suggested that *Diplacus* and *Erythranthe* represent separate radiations in western North America from ancestors outside of the region. Acceptance of *Diplacus* and *Erythranthe* is consistent with the pattern of diversification.

Paulowniaceae comprises a single genus, *Paulownia*, an East Asian genus of seven species of large trees assigned to Scrophulariaceae by G. Bentham (1876); it was placed in Bignoniaceae in some classifications (A. L. Takhtajan 1980; A. Cronquist 1981; R. F. Thorne 1983). J. E. Armstrong (1985) examined morphological and anatomical characters and argued that the preponderance of evidence allied *Paulownia* with Scrophulariaceae, which swayed most late twentieth-century classifications (Thorne 1992b; Takhtajan 1997). Molecular phylogenetic evidence showed that it does not belong with Bignoniaceae, Scrophulariaceae, or any other family; it forms an isolated lineage near Lamiaceae, Orobanchaceae, and Phrymaceae. T. Nakai (1949) had previously established the family Paulowniaceae. One species, *P. tomentosa*, is naturalized in North America.

The remaining families segregated from Scrophulariaceae in the broad sense do not occur naturally in the flora area. Calceolariaceae Olmstead, with two genera and about 275 species, occur primarily in Andean South America and in New Zealand with two species of *Jovellana* Ruiz & Pavón (U. Molau 1988; S. Andersson 2006; A. Cosacov et al. 2009). Stilbaceae are native to South Africa; the family has a core of about five genera with ericoid foliage and small radially symmetric flowers. Molecular phylogenetic studies have expanded Stilbaceae by including *Retzia* Thunberg (B. Bremer et al. 1994) and former members of Scrophulariaceae, *Halleria* Linnaeus

(R. G. Olmstead et al. 2001), and Bowkerieae Barringer (B. Oxelman et al. 2005); the family now includes 11 genera and about 40 species. *Rehmannia* and *Triaenophora* represent a pair of isolated genera comprising about 11 species formerly assigned to Scrophulariaceae in the broad sense, but found to belong close to Orobanchaceae (Oxelman et al.; D. C. Albach et al. 2009; Xia Z. et al. 2009), and described as Rehmanniaceae by J. L. Reveal (2011).

Orobanchaceae are the second largest family of Scrophulariaceae in the broad sense, with about 100 genera and about 1850 to 2100 species (the range is due to widely varying species estimates for *Euphrasia* and *Pedicularis*), and includes the holoparasitic species that have comprised this family in traditional classifications (A. Cronquist 1981; R. F. Thorne 1992b; A. L. Takhtajan 1997) and hemiparasitic species that have been included traditionally in Scrophulariaceae in the broad sense. The non-parasitic *Lindenbergia* Lehmann is included in Orobanchaceae and is sister to the parasitic members; excluding *Lindenbergia* would result in a less well-supported and possibly non-monophyletic group (R. G. Olmstead et al. 2001; A. D. Wolfe et al. 2005; J. R. Bennett and S. Mathews 2006; J. R. McNeal et al. 2013). *Rehmannia* and *Triaenophora*, which share the corolla aestivation character diagnostic of the family, were included in Orobanchaceae by Angiosperm Phylogeny Group (2016). Parasitism is a nearly universal characteristic of Orobanchaceae; it does not provide the defining trait. The so-called rhinanthoid corolla aestivation, wherein the lateral lobes forming the abaxial corolla lip are external in bud, is a shared trait that unites non-parasitic and parasitic members.

J. R. McNeal et al. (2013) identified six major clades of Orobanchaceae (excluding Rehmanniaceae), with *Lindenbergia* composing one of them. The other five major clades are parasitic, and all have representatives in the flora area, even though an origin in Asia is inferred (A. D. Wolfe et al. 2005; McNeal et al.). One clade, centered on *Castilleja* and *Pedicularis*, is predominantly New World in distribution, even though *Pedicularis* is mostly Asian. Conventional circumscriptions of Orobanchaceae restricted the family to the holoparasites and recognized a relationship to the hemiparasitic Scrophulariaceae (for example, A. Cronquist 1981). Phylogenetic studies (C. W. dePamphilis et al. 1997; McNeal et al.) have shown that there are at least three origins of holoparasitism, and each is in a different one of the parasitic clades. There are other lineages in which photosynthetic activity is limited to a brief portion of the life history of the plant; they are often referred to as holoparasites while still meeting the definition of a hemiparasite (McNeal et al.).