

Further disintegration of Scrophulariaceae

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A phylogenetic study of plastid DNA sequences (*ndhF*, *trnL/F*, and *rps16*) in Lamiales is presented. In particular, the inclusiveness of Scrophulariaceae sensu APG II is elaborated. Scrophulariaceae in this sense are mainly a southern hemisphere group, which includes Hemimerideae (including *Alonsoa*, with a few South American species), Myoporeae, the Central American Leucophylleae (including *Capraria*), *Androya*, Aptosimeae, Buddlejaceae, Teediaceae (including *Oftia*, *Dermatobotrys*, and *Freylinia*), Manuleeae, and chiefly Northern temperate Scrophulariaceae (including *Verbascum* and *Oreosolen*). *Camptoloma* and *Phygellius* group with Buddlejaceae and Teediaceae, but without being well resolved to any of these two groups. *Antherothamnus* is strongly supported as sister taxon to Scrophulariaceae. African Stilbaceae are shown to include Bowkeriaceae and *Charadrophila*. There is moderate support for a clade of putative Asian origin and including Phrymaceae, *Paulownia*, *Rehmannia*, *Mazus*, *Lancea*, and chiefly parasitic Orobanchaceae, to which *Brandisia* is shown to belong. A novel, strongly supported, clade of taxa earlier assigned to Scrophulariaceae was found. The clade includes *Stemodiopsis*, *Torenia*, *Micranthemum* and probably *Picria* and has unclear relationships to the rest of Lamiales. This clade possibly represents the tribe Lindernieae, diagnosed by geniculate anterior filaments, usually with a basal swelling.

KEYWORDS: Lamiales, *ndhF*, phylogeny, *rps16*, Scrophulariaceae, *trnL/F*.

INTRODUCTION

Lamiales sensu APG II (The Angiosperm Phylogeny Group, 2003) constitute a major clade of flowering plants, with approximately 17,800 species (Judd & al., 2002) and 23 families, representing 12.3% of eudicot diversity (Stevens, 2001, onwards). They belong to a major asterid clade, the Lamiids (Bremer & al., 2002). The monophyly of Lamiales is comparatively uncontroversial and well supported by molecular (e.g., Backlund & al., 1998; Oxelman & al., 1999; Albach & al., 2001; Bremer & al., 2002) and phytochemical data (Jensen, 1992; Scogin, 1992). Morphologically, they typically are characterized by opposite leaves, sympetalous zygomorphic flowers, oligosaccharides, frequent production of 6-oxygenated flavones, embryos of Onagrad type and capsular fruits. Although the majority of the many well-known families in Lamiales are well supported (e.g., Olmstead & al., 2001), the relationships among them are obscure, despite several recent molecular studies (e.g., Olmstead & Reeves, 1995; Backlund & al., 1998; Oxelman & al., 1999; Olmstead & al., 2001).

The concept “Scrophulariae” occurs in Durande, *Notions Elém. Bot.*: 265. 1782, but the name of the family is conserved as Scrophulariaceae Jussieu. The most

influential classifications for the 19th century concept of Scrophulariaceae were those of Bentham (1846), who in addition recognized Selaginaceae Choisy, Wettstein (1895), who recognized Lentibulariaceae, Plantaginaceae, and Orobanchaceae as separate families, and Hallier (1903), who made the broadest circumscription of the family.

Even if doubts sometimes were expressed regarding the naturalness of Scrophulariaceae (e.g., Thieret, 1967; Barringer, 1984), it was not until the study by Olmstead & Reeves (1995) based on plastid *ndhF* and *rbcL* gene sequences that the concept of Scrophulariaceae needed a revolutionary revision in order to fit within a phylogenetic framework. Olmstead & Reeves (1995) discovered two clearly separated clades consisting of scrophulariaceous taxa. One clade (“scroph I”) included *Buddleja* L., *Selago* L., *Verbascum* L. and the type genus *Scrophularia* L., whereas the other (“scroph II”) included *Antirrhinum* L., *Digitalis* L., *Veronica* L., *Plantago* L., *Hippuris* L., and *Callitriche* L. In their study the enigmatic woody *Paulownia* Siebold & Zucc., which was usually classified in Scrophulariaceae but transferred to Bignoniaceae Juss. by Takhtajan (1980), and *Schlegelia* Miq., which was originally placed in Bignoniaceae but argued by Armstrong (1985) to fit better within

Scrophulariaceae, were both left in uncertain positions within Lamiales.

Several subsequent molecular phylogenetic studies focusing either on other taxa of Lamiales or more inclusive groups (e.g., Soltis & al., 1998; Olmstead & al., 2000, 2001; Albach & al., 2001; Bremer & al., 2002) have confirmed the general pattern revealed by Olmstead & Reeves (1995).

The parasitic plants that have variously been placed in Scrophulariaceae or Orobanchaceae Vent. have been shown to constitute an additional monophyletic group (dePamphilis & al., 1997; Wolfe & dePamphilis, 1998; Young & al., 1999). Olmstead & al. (2001) merged the *rbcL/ndhF* dataset and the data from the plastid *rps2* gene that had been useful for the recognition of Orobanchaceae. They also added some other taxa, and were thereby able to identify an additional distinct scrophulariaceous clade, Calceolariaceae. In addition, *Halleria* L. was shown to group with Stilbaceae Kunth, and *Mimulus* L. did not belong to any of these five clades of ex-Scrophulariaceae taxa. Beardsley & Olmstead (2002) identified *Mimulus* as a member of another clade, Phrymaceae. *Globularia* L. has been demonstrated to belong to the Veronicaceae clade sensu Oxelman & al. (1999), Olmstead & al. (2001), Kornhall & al. (2001), and Bremer & al. (2002).

Olmstead & al. (2001) recognized five distinct phylogenetic lineages composed mainly of taxa previously assigned to Scrophulariaceae. Nevertheless, several of their included genera (e.g., *Schlegelia*, *Paulownia*, *Mimulus*) did not group with any of these five lineages. This fact, together with the restricted sampling (39 of ca. 280 genera in Scrophulariaceae in a traditional sense, (see Watson & Dallwitz, 1992 onwards), calls for more extensive sampling of genera previously assigned to Scrophulariaceae.

Scrophulariaceae sensu APG II (2003) constitutes approximately what Olmstead & Reeves (1995) identified as the “scroph I” clade, that is, Buddlejaceae K. Wilh. (see Oxelman & al., 1999 for phylogenetic circumscription), Manuleeae Benth. (incl. Selagineae, see Kornhall & al., 2001), the two large genera *Scrophularia* and *Verbascum*, plus the tribe Hemimerideae Benth. and Myoporaceae R. Br., including *Androya* Perrier (Oxelman & al., 1999; Bremer & al., 2002). The assignment of Hemimerideae to this clade has received only weak support in previous studies. Several taxa that have been suggested to have affinities with Myoporaceae (e.g., *Capraria* L., *Anticharis* Endl. and *Peliostomum* E. Mey.) have not been studied by molecular methods. The inclusiveness of Manuleeae is not entirely clear, and perhaps most importantly, a more extensive sampling of taxa that have been attributed to Scrophulariaceae is needed before a revised classification of Scrophulariaceae in

accordance with phylogenetic relationships can be presented. Fischer (2004) has presented a tentative classification of all genera belonging to Scrophulariaceae sensu lato (i.e., approximately in the sense of Hallier, 1903) into tribes and informal higher groups (“families”).

The aims of this study are to identify the inclusiveness of the “scroph I” clade, sensu Olmstead & Reeves (1995), using plastid DNA sequence data and to infer relationships of representatives of major groups of taxa previously assigned to Scrophulariaceae, for which plastid DNA sequences have not been obtained before.

MATERIAL AND METHODS

Sampling. — Most previous molecular studies have used sequences of *rbcL* and *ndhF*, but many other plastid loci have been introduced as well, including *rps2* (dePamphilis & al., 1997), *trnL/F* intron/spacer region (Freeman & Scogin, 1999; McDade & Moody, 1999), *rps16* (Wallander & Albert, 2000; Bremer & al., 2002), *matK* (Bremer & al., 2002), *trnT/F* spacer region, and the *trnV* spacer (Bremer & al., 2002). We use the *ndhF*, *trnL/F*, and *rps16* DNA sequence regions, partly because a substantial number of bulk Lamiales taxa have already been sequenced for these, and partly because previous studies have indicated that these regions are particularly informative in Lamiales. Initially, we included all Lamiales taxa with sequence information from either of the *trnL/F*, *rps16*, or *ndhF* regions deposited in EMBL/Genbank as per May 2002. However, because of uneven representation, we used an exemplar approach for groups whose monophyly is not in question. In addition, we tried to obtain material for DNA extraction from representatives of genera that have been suggested to be related to Scrophulariaceae sensu APG II. We also included representatives from other parts of Scrophulariaceae sensu Hallier and from Stilbaceae. We tried to find additional representatives of every taxon, and sequence at least one of the three regions to verify the taxonomic identity of the sequences. Information on sampled taxa, and EMBL/Genbank accession numbers can be found in the Appendix.

Sequencing. — Most of the sequencing was performed either at the Evolutionary Biology Centre, Uppsala University, Sweden, or at the Department of Botany, University of Washington, Seattle, U.S.A. Total DNA was usually extracted from dried plant material using some variant of a standard CTAB/Chloroform extraction protocol, often followed by DNA purification using the Qiaquick PCR purification kit (Qiagen). Polymerase chain reactions (PCR) of the targeting regions were performed using combinations of primers published in Taberlet & al. (1991), Oxelman & al. (1997,

1999), Kornhall & al. (2001), and Popp & Oxelman (2001). For *trnL/F* two new primers were constructed: *trnLBOC*: GGCGRAATYGGTAGACGCTACG, and *trnLBOF-R*: CCAGATTTGAACTGGTGACACGAG. PCR products were purified using the Qiaquick PCR purification kit (Qiagen). In some instances, when the products were present as faint bands only, we used nested primers in a second round of PCR in order to obtain enough products for sequencing. Automated sequencing was performed either on an ABI377 (Applied Biosystems), or on a MegaBACE 1000 capillary machine (Amersham Pharmacia Biotech) using manufacturer's protocols.

Assembly and alignment. — Sequences were assembled using Sequencher v. 3.1.1 (GeneCodes Corporation). The resulting sequences were aligned using the guidelines in Oxelman & al. (1997), and the "simple gap coding" described by Simmons and Ochotorenna (2000).

Phylogenetics. — Separate phylogenetic analyses were performed for each region initially. Taxa for which only one region has been sequenced are not presented in any of the results, unless the relationships found have not previously been reported in the literature. We made three combined analyses: one where all taxa were included, regardless of completeness (A1), one where at least two of the three regions were available for each terminal taxon (A2), and one with taxa where all three regions were available (A3). For all analyses, we used maximum parsimony as the optimality criterion, and the program PAUP* ver. 4.0b10 (Swofford, 1999) to find the most parsimonious trees using the TBR search algorithm and 50 random sequence addition replicates, saving a maximum of ten trees in each. For each analysis, the strict consensus tree was calculated from these trees. Bootstrapping was performed with 500 replicates, TBR search, and three random additions per replicate. No more than 10 trees were saved per random addition replicate.

A Bayesian phylogenetic analysis was performed on dataset A2 using MrBayes v. 3.0b4 (Huelsenbeck & Ronquist, 2001) on computers running Linux. The appropriateness of different models was evaluated using

the program Modeltest v. 3.06 (Posada and Crandall, 1998). We ran the program for 1,000,000 generations, four parallel chains, and with every 100th tree saved.

In order to detect possible incongruence resulting from analyses of the three regions, a partitioned Bremer support analysis (PBS) was conducted using TreeRot v. 2 (Sorensen, 1999). The trees are rooted with sequences from Oleaceae Hoffsgg. & Link, which has been shown to be sister to the rest of Lamiales in previous chloroplast DNA phylogenies (Savolainen & al., 2000; Bremer & al., 2001, 2002; Olmstead & al., 2001).

RESULTS

The number of sequences, previously unreported sequences generated by us, aligned positions, parsimony informative positions and indels, consistency index (CI), and retention index (RI), for each region and the combined analyses are summarized in Table 1. The strict consensus tree from 129 most parsimonious trees found in the A2 parsimony analysis (at least two sequenced regions available) is shown with bootstrap percentages above branches in Figs. 1–6. Details of the summary tree in Fig. 1 are shown in Figs. 2–6, and referred to in the discussion of each group. The strict consensus tree from the A3 analysis is shown in Fig. 7 with bootstrap and PBS values. Table 2 shows the bootstrap support for comparable nodes in the three analyses. In most cases there is higher bootstrap support in the A2 and A3 analyses than in the A1 analysis, and the differences are often considerable. The A2 and A3 analyses had similar support levels for comparable groups.

The Bayesian analysis was performed under a general time reversible model with a proportion of invariant sites and a gamma distribution, as selected by Modeltest. The first 100,000 generations were discarded as burn-in. The frequencies of different nodes are indicated below branches on the A2 trees in Fig. 1–6. Generally, the parsimony and the Bayesian analyses are highly congruent, usually with considerably higher frequencies in the Bayesian analysis. Cases where the parsimony and Bayesian analyses differ include the position of

Table 1. Tree statistics in the form of number of sequences, previously unreported sequences generated by ourselves, aligned positions, parsimony informative sites and indels, consistency index (CI), and retention index (RI), for each region and the combined analyses.

Region	Number of sequences	First time reported	Number of aligned positions	Number of informative substitutions	Number of informative indels	CI	RI
<i>ndhF</i>	235	56	2231	1136	20	0.28	0.62
<i>trnT-F</i>	170	83	1190	501	18	0.46	0.68
<i>rps16</i>	109	106	1076	425	25	0.49	0.60
A3	83		4497	1456	43	0.47	0.52
A2	144		4497	1742	51	0.42	0.62
A1	299		4497	2063	61	0.34	0.63

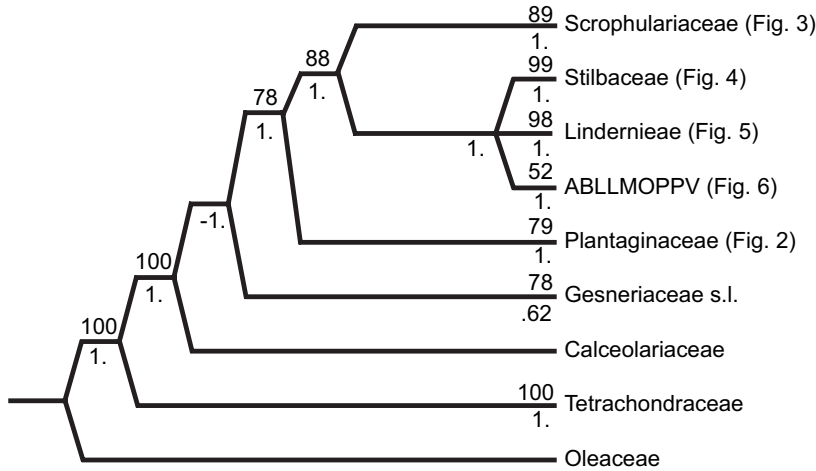


Fig. 1. Simplification of the strict consensus tree from the 129 most parsimonious trees found in the A2 analysis with bootstrap values, when >50%, given above nodes. Bayesian posteriors under a GTR + gamma model are given below branches. Calceolariaceae is represented by a single sequence in this analysis. References to detailed subtrees are given inside parentheses. ABLLMOPPV = Acanthaceae, Bignoniaceae, Lamiaceae, Lentibulariaceae, Martyniaceae, Orobanchaceae, Pedaliaceae, Phrymaceae, and Verbenaceae.

Calceolaria, which in the parsimony analysis is sister group to the core of Lamiales (excluding Tetrachondraceae and Oleaceae, Fig. 1). The bootstrap support for this is less than 50%. In the Bayesian analysis, *Calceolaria* ends up as sister group to Gesneriaceae with a posterior probability of 1. *Colpias* and *Alonsoa* form a monophyletic group with .56 posterior probability, whereas they form a poorly (51% bootstrap frequency) supported grade in the parsimony analysis (Fig. 3). The positions of *Rehmannia* and *Paulownia* are both poorly resolved within the ABLLMOPPV clade by the parsimony bootstrap analysis, but form consecutive sister groups to Orobanchaceae with 100% posterior probabilities in the Bayesian analysis.

DISCUSSION

The general structure of the tree in Figure 1 is in accordance with previously published chloroplast DNA phylogenies of Lamiales (e.g., Oxelman & al., 1999; Olmstead & al., 2001; Bremer & al., 2002). With Oleaceae designated as the outgroup, Tetrachondraceae appears as sister to the rest. In agreement with Olmstead & al. (2001) and Bremer & al. (2002), there is emerging support that the bulk of “core” Lamiales does not include Calceolariaceae, Gesneriaceae Rich. & Juss., *Sanango* Bunting & Duke, and *Peltanthera* Benth. The relationships among these are, however, contradictory. The PBS analysis (Fig. 7) gave conflicting numbers for two nodes (*Calceolaria* L. as sister group to the rest of Lamiales including Gesneriaceae, and monophyly of *Sanango*/

Streptocarpus Lindley), and in both cases the *rps16* data are in conflict with *trnL/F* and *ndhF*. We have rechecked the identities of these sequences as well as the alignment, and we can find no obvious explanation to this pattern. There is support both from *ndhF* (bootstrap) and *trnL/F* (bootstrap) for a sister-group relationship between *Sanango* and Gesneriaceae. The support for *Peltanthera* as sister taxon to *Sanango* and Gesneriaceae is weak

Table 2. Comparison of bootstrap support for nodes in the different analyses. Numbers in parentheses are number of taxa in the node. A1 tree not shown.

Group	A1	A2	A3
<i>Alonsoa</i> ... <i>Verbascum</i>	<50 (99)	95 (93)	99 (28)
<i>Diclis</i> ... <i>Hemimeris</i>	98 (5)	100 (5)	100 (2)
<i>Alonsoa</i> ... <i>Hemimeris</i>	<50 (9)	55 (6)	53 (6)
<i>Diascia</i> ... <i>Nemesia</i>	100 (3)	100 (3)	100 (2)
<i>Alonsoa</i> ... <i>Nemesia</i>	<50 (13)	72 (10)	72 (6)
<i>Androya</i> ... <i>Verbascum</i>	<50 (87)	72 (48)	71 (22)
<i>Androya</i> ... <i>Peliostomum</i>	76 (17)	75 (9)	67 (8)
<i>Aptosimum</i> ... <i>Peliostomum</i>	99 (5)	100 (3)	100 (2)
<i>Androya</i> ... <i>Leucophyllum frutescens</i>	62 (11)	100 (6)	100 (6)
<i>Myoporum</i> ... <i>Leucophyllum frutescens</i>	75 (10)	100 (5)	100 (5)
<i>Leucophyllum</i> ... <i>Capraria</i>	64 (5)	100 (2)	100 (2)
<i>Myoporum</i> ... <i>Eremophila</i>	94 (5)	100 (3)	100 (3)
<i>Bontia</i> ... <i>Myoporum</i>	56 (4)	75 (2)	73 (2)
<i>Camptoloma</i> ... <i>Verbascum</i>	54 (70)	93 (39)	100 (14)
<i>Dermatobotrys</i> ... <i>Teedia</i>	56 (5)	90 (3)	92 (3)
<i>Oftia</i> ... <i>Teedia</i>	78 (2)	88 (2)	92 (2)
<i>Buddleja</i> ... <i>Emorya</i>	81 (7)	100 (6)	100 (3)
<i>Verbascum</i> ... <i>Selago</i>	54 (46)	92 (35)	98 (6)
<i>Manuleopsis</i> ... <i>Selago</i>	92 (36)	84 (26)	93 (3)
<i>Antherothamnus</i> ... <i>Verbascum</i>	55 (8)	100 (5)	100 (3)
<i>Verbascum</i> ... <i>Scrophularia</i>	50 (7)	98 (4)	92 (2)

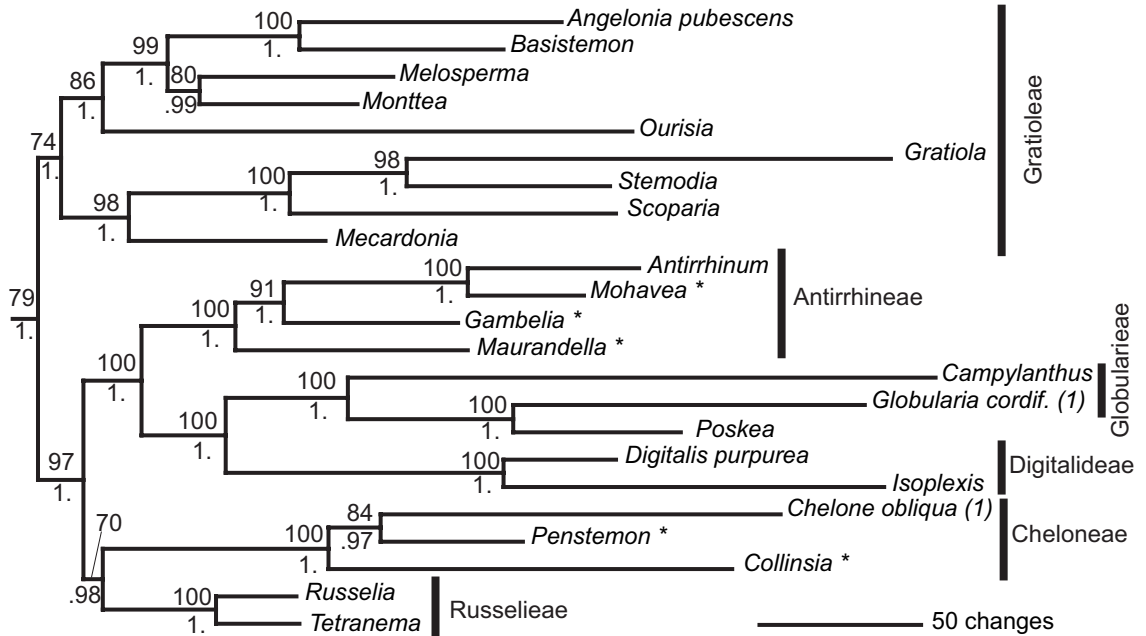


Fig. 2. Phylogram of one of the most parsimonious trees from the A2 analyses with taxa from Plantaginaceae. Bootstrap values, when >50%, are indicated above branches. Bayesian posteriors under a GTR + gamma model are given below branches. * denotes sequences combined from different vouchers. Species epithets and occasionally numbers within parentheses are given to differentiate between DNA samples within genera, when there are more than one (see Appendix).

from our data, only *trnL/F* contributes with positive support in the three-gene dataset (Fig. 7). However, both of these relationships are supported by *rbcL* (Oxelman & al., 1999). Bremer & al. (2002) report strong support for these relationships, and Jensen (2000) report biochemical evidence that may indicate a relationship between *Peltanthera* and Gesneriaceae.

In the following discussion, we will present and discuss more detailed results from particular groups of the “core” Lamiales. We use the family classifications of Olmstead & al. (2001) and APG II and the tribal rank to classify the genera studied taxonomically. We also discuss some still unsequenced scrophulariaceous taxa and their putative relationships.

Plantaginaceae (Veronicaceae sensu Olmstead & al. 2001). This group (Fig. 2) was originally recognized by Olmstead & Reeves (1995) as “Scroph II” and was corroborated with more taxa in Olmstead & al. (2001). The group is supported in the cpDNA tree (parsimony bootstrap: A2 - 79%; A3 - 91%), and there is also support for a sister-group relationship to the rest of the core Lamiales. Within Plantaginaceae, there are several well-supported subgroups. Cheloneae Benth., comprised solely of New World taxa, have been identified by several previous molecular studies, including restriction sites (Wolfe & al., 1997, 2002), the *trnL* intron (Freeman & Scogin, 1999), *ndhF*, *rbcL*, and *rps2* (Olmstead & al.,

2001), and *matK* and nrDNA ITS sequences (Wolfe & al., 2002). In our A2 tree, as well as in Wolfe & al., (2002), and some of the trees obtained by Olmstead & al. (2001), Russelieae (*Russelia* Jacq. and *Tetranema* Benth.) forms the sister group to Cheloneae, although in none of the trees the relationship receives strong support.

Our study and Olmstead & al. (2001) also concur that Cheloneae and Russelieae form a monophyletic group with Antirrhineae Dumort., *Digitalis*, *Globularia*, *Poskea* Vatke, *Campylanthus* Roth., *Hippuris*, *Callitriche*, *Plantago*, *Hemiphragma* Wallich., and *Veronica*. This clade consists of several well-delimited groups, of which several often have been recognized at the family level (Globulariaceae DC., Hippuridaceae Vest., Callitrichaceae Bercht. & J. Presl. and Plantaginaceae sensu stricto). Ghebrehiwet & al. (2000) examined Antirrhineae cpDNA relationships in detail, and the sister-group relationship to the rest of the above-mentioned genera is well established.

Globularia and *Poskea* form a strongly supported group, which is corroborated by morphological data (Barringer, 1993). *Campylanthus* is resolved as sister to these, a result which needs corroboration from other data. The proximity of *Campylanthus* to Digitalideae Dumort. has been suggested (see Hjertsson, 2003) and has also been substantiated in a phytochemical study by Rønsted & Jensen (2002) and a brief molecular study by

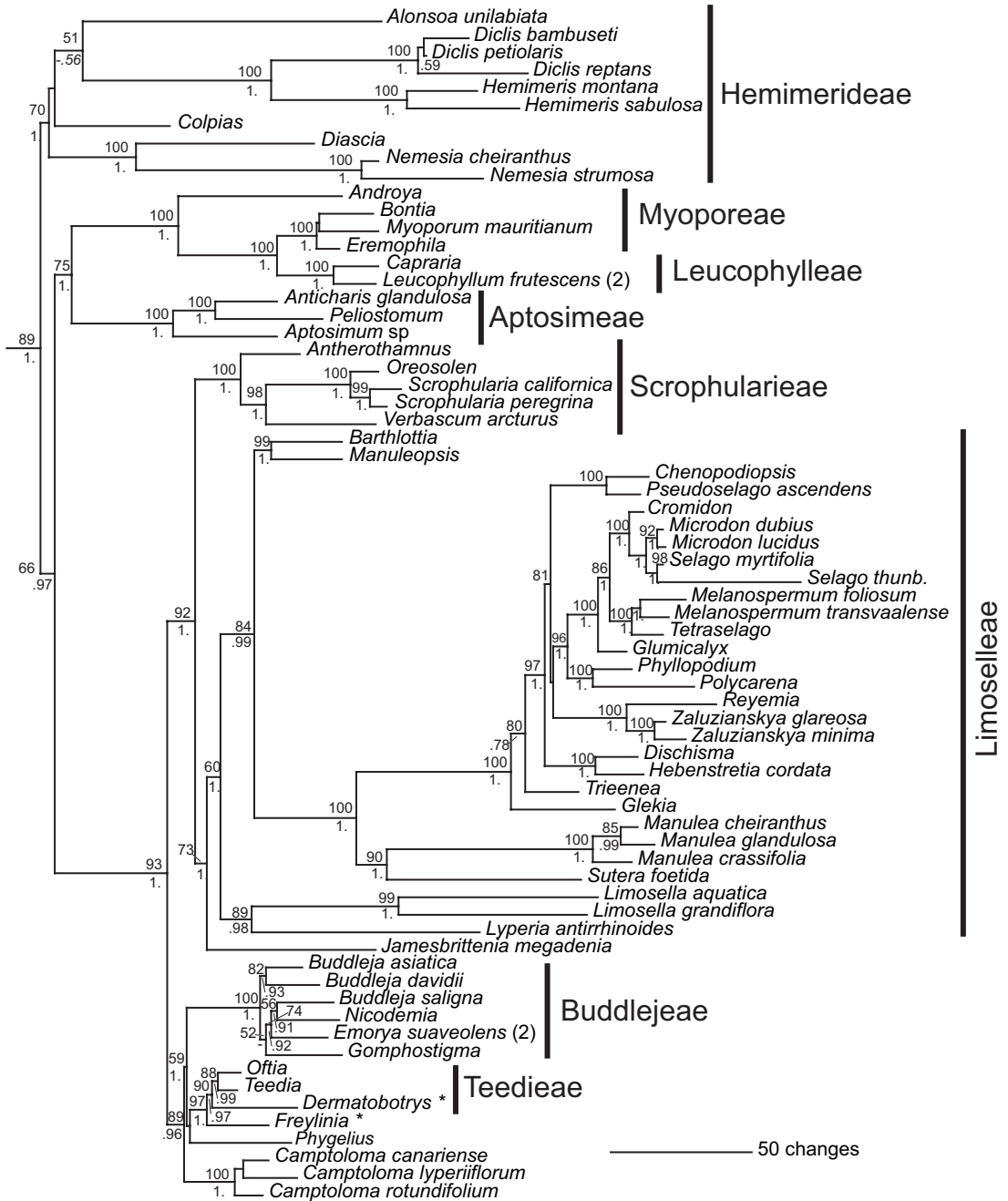


Fig. 3. Phylogram of one of the most parsimonious trees from the A2 analysis with taxa from Scrophulariaceae sensu APG II. Bootstrap values, when >50%, are given above nodes (or on the left when this was not possible). Bayesian posteriors under a GTR + gamma model are given below branches. * indicates sequences combined from different vouchers. Species epithets and occasionally numbers within parentheses are given to differentiate between DNA samples within genera, when there are more than one (see Appendix).

Hjertsson (1997).

Digitalis appears paraphyletic in relation to *Isoplexis* Loudon according to *ndhF* data (results not shown). According to ITS data (Albach, 2001), *Erinus* L. is also part of Digitalideae.

Digitalideae and Globularieae form strongly sup-

ported monophyletic groups irrespective of whether divergent Callitrichaceae and Plantaginaceae s.s. are included. *Hemiphragma* appears to belong to the Plantagineae/Veroniceae clade rather than to Digitalideae (Olmstead & al., 2001).

The least understood group in the former

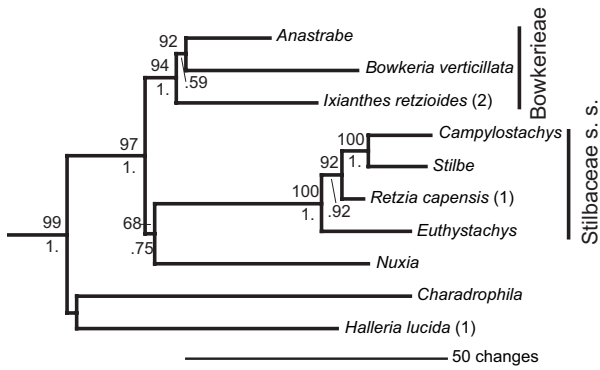


Fig. 4. Phylogram of one of the most parsimonious trees from the A2 analysis with representatives from Stilbaceae. Bootstrap values, when >50%, are indicated above the nodes. Bayesian posteriors under a GTR + gamma model are given below branches. Species epithets and occasionally numbers within parentheses are given to differentiate between DNA samples within genera, when there are more than one (see Appendix).

Scrophulariaceae is probably Gratioleae. The circumscription of Gratioleae by Bentham has been discussed by Wettstein (1895) and Thieret (1967) among others. Olmstead & al. (2001) reported monophyly of *Amphianthus* Torrey, *Bacopa* Aublet, and *Gratiola* L., and a weakly supported relationship with *Angelonia* Bonpl. We present data that assign *Scoparia* L., *Stemodia* L., and *Mecardonia* Ruiz & Pavón to Gratioleae. Many genera remain to be tested for their relationships with this group, with many members occurring in Neotropical areas. This is even more evident for Angelonieae. The close relationship between *Angelonia* and *Basistemon* Turcz. previously suggested by Barringer (1993) is corroborated, and these in turn form a strongly supported group together with the South American genera *Monttea* and *Melosperma*, consecutively followed by a less strongly supported relationship with the Neotropical *Ourisia* Comm. Plantaginaceae deserve more study, in particular with respect to Gratioleae/Angelonieae. Recently, Plantaginaceae have been studied in more detail by Albach & al. (2005).

Scrophulariaceae sensu APG II (Fig. 3). — The concept of Scrophulariaceae has changed considerably with the greater understanding gained via molecular phylogenetics. Olmstead & al. (2001) and APG II (2003) delimit the family to a clade consisting of Scrophularieae Dumort., Manuleeae Benth., Buddlejaceae Bartl. (Buddlejaceae sensu Oxelman & al. 1999), Myoporeae Rchb., Leucophylleae Miers, Aptosimeae Benth. & Hook. f., and Hemimerideae Benth. In this study, we confirm the monophyly of this assemblage (89% bootstrap support in our A2 analysis; 99% in our A3 analysis; Fig. 7). We add one to several taxa to each of the tribes relative to previ-

ous molecular studies. In addition, tribe Teedieae Benth. is identified as a member of this clade. In the following text, each of the tribes is discussed in more detail.

Scrophularieae. — The close relationship between *Scrophularia* and *Verbascum* has been suggested by previous molecular studies, as well as similarities in seed and embryo characteristics (Thieret, 1967), and leaf anatomy (Lersten & Curtis, 1997). The close relation between *Oreosolen* Hook. f. and *Scrophularia* (Fig. 3) is here reported for the first time, but is not surprising from a morphological point of view. Both the Himalayan *Oreosolen* and the closely related *Nathaliella* B. Fedtsch. from Central Asia agrees well with *Scrophularia* in floral morphology and general leaf architecture. The relationship also makes sense biogeographically, since *Scrophularia*, *Oreosolen*, and *Verbascum* all have mainly Northern Hemisphere distributions, as opposed to most other Scrophulariaceae. The strong support for a sister-group relationship between the South African *Antherothamnus* N. E. Br. and *Scrophularia/Oreosolen/Verbascum* is not evident morphologically. The position of *Antherothamnus* is robust to method of analysis, i.e., neighbor-joining, maximum likelihood, and Bayesian inference with various evolutionary models (results not shown), and is supported by all three chloroplast genes (Fig. 7). Similar to *Scrophularia*, *Antherothamnus* has a fully developed staminode, a character that is not found in Manuleeae. *Antherothamnus* now appears to be a link between southern African ancestors and Eurasian Scrophularieae. [N.B. The *Scrophularia macrantha* trnL sequence on EMBL/Genbank is probably a misidentification, because it groups with Collinsieae with strong support.]

Limoselleae. — Manuleeae encompass Selagineae Wettstein (Selaginaceae Choisy) since the latter are nested within Manuleeae (Kornhall & al., 2001). However, the unexpected finding that *Limosella* L. also is nested within this group (Fig. 3, Kornhall & Bremer, 2004) suggests that the correct name for this tribe is Limoselleae Dumort., for priority reasons (Kornhall & Bremer, 2004). *Limosella* includes small plants that, as the vernacular English name, mudworts, indicates, grow in wet areas. Their choice of habitat and the small seeds might have facilitated a global distribution of the taxon by migrating birds (Darwin, 1872). The inclusion of Selagineae in Limoselleae on molecular evidence is also strongly corroborated by morphological characters. The “Selagineae” gynoeceum, with one ovule per locule has apparently emanated several times within Limoselleae. Maintaining a separate Selagineae and Cronquist’s (1981) view that Selagineae belonged in the Globulariaceae results from accepting the uni-ovulate ovary as a cardinal character. In spite of several authors pointing to the close connection between the two tribes based on

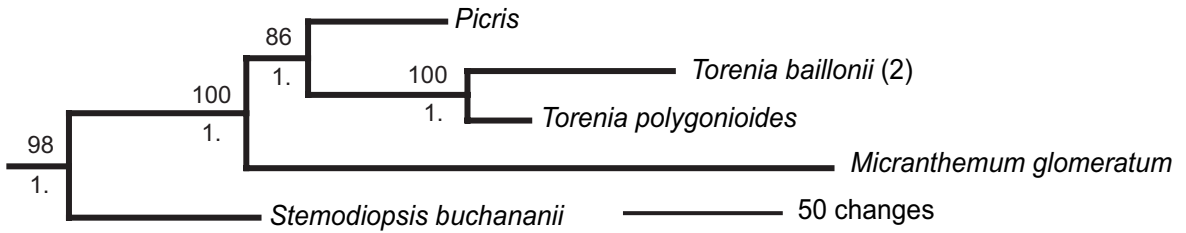


Fig. 5. Phylogram of one of the most parsimonious trees from the A2 analysis with taxa from Lindernieae. Bootstrap values, when >50%, indicated above nodes. Bayesian posteriors under a GTR + gamma model are given below branches. Species epithets and occasionally numbers within parentheses are given to differentiate between DNA samples within genera, when there are more than one (see Appendix).

other characters (Junell, 1961; Argue, 1993; Hilliard, 1994), the two tribes were not united before Kornhall & al. (2001). Long branches characterize the basal parts of Limoselleae, and the relations between *Jamesbrittenia*, *Limosella* and *Lyperia* are not totally clear. Kornhall & Bremer (2004) elaborate in more detail on the interpretation of morphological features of *Limosella* in relation to the chloroplast phylogeny found here.

Buddlejeae. — The results presented here corroborate the circumscription of Buddlejeae by Oxelman & al. (1999). Wallick & al. (2000) have, with more extensive sampling of *Buddleja* and *trnL* intron sequences, further strengthened the suggestion that *Buddleja* might be paraphyletic.

Teedieae. — There is strong support for a monophyletic group, Teedieae, consisting of the genera *Oftia* Adans., *Teedia* Rudolphi, *Dermatobotrys* Bolus, and *Freylinia* Colla. This group in turn forms a strongly supported monophyletic group consisting also of the likewise woody Buddlejeae, *Phygellus* Mey., and *Camptoloma* Benth. Wallick & al. (2000) reported a sister-group relationship between Buddlejeae and Teedieae, but they included only *Teedia* and *Oftia* in their study, so their results are in agreement with ours. *Phygellus* and, in particular, *Camptoloma* are poorly resolved with respect to Teedieae and Buddlejeae. Barringer (1993) segregated the African woody taxa Teedieae, Freylinieae, and Bowkerieae from Cheloneae where Bentham originally placed them. Freylinieae sensu Barringer included also *Phygellus*, *Antherothamnus*, and *Manuleopsis* Thell., a relationship that is not supported by the present study. Barringer did not consider the epiphytic *Dermatobotrys* in this context. Morphologically, Buddlejeae, Teedieae, and *Phygellus* all have anthers with separate thecae, whereas the anther thecae of *Camptoloma* are confluent (as in Manuleeae and Scrophularieae). It is difficult to determine the polarity of this character. Possibly, the leafy inflorescences typical for *Oftia*, *Teedia*, *Dermatobotrys*, and *Freylinia* could be interpreted as a synapomorphy for this group, as the bracts in Buddlejeae

and *Camptoloma* are small, and in *Phygellus* gradually turning leaf-like. We conclude that *Teedia*, *Oftia*, *Dermatobotrys*, and *Freylinia* are best accommodated in Teedieae, whereas at the present it cannot be determined which relationship *Phygellus* and *Camptoloma* have to Teedieae and Buddlejeae. Teedieae, Buddlejeae, *Camptoloma*, *Phygellus*, Manuleeae, and Scrophularieae form a very strongly supported monophyletic group based on chloroplast DNA sequences.

Myoporeae. — With small variations, Myoporeae have generally been circumscribed as a mostly Southern Hemispheric taxon including *Myoporum* Sol., *Eremophila* R. Br., and *Bontia* L. *Oftia*, here considered to belong to Teedieae (see above), has sometimes been considered to belong here (e.g., Wettstein, 1895), but Dahlgren & Rao (1971) rejected this based on morphological evidence. The monophyly of *Myoporum*, *Eremophila*, and *Bontia* is here strongly supported, a result consistent with the palynological data presented by Niezgoda & Tomb (1975) and anatomical data presented by Carlquist & Hoekman (1986).

Leucophylleae have been suggested to be related to Myoporeae (palynological data: Niezgoda & Tomb, 1975; anatomical data: Carlquist & Hoekman, 1986; molecular data: Oxelman & al., 1999; Olmstead & al., 2001). The close relationship between *Leucophyllum* Bonpl. and *Capraria* L., both with Latin American distribution, reported by Niezgoda & Tomb (1975) based on palynological evidence, and by Lersten & Curtis (2001) based on anatomical evidence is here corroborated. [N.B. The *Leucophyllum minus trnL* sequence deposited on EMBL/Genbank (AF034878) does not form a monophyletic group with our *Leucophyllum frutescens* sequence. Instead the *trnL* sequence labeled *Pedicularis procera* (AF034877) groups with *L. frutescens*. The identities of the *L. minus* and *P. procera* sequences need confirmation.]

Karrfalt & Tomb (1983) and Lersten & Beaman (1998) claimed homology between the oil cavities found in several *Leucophyllum* species and those found in the

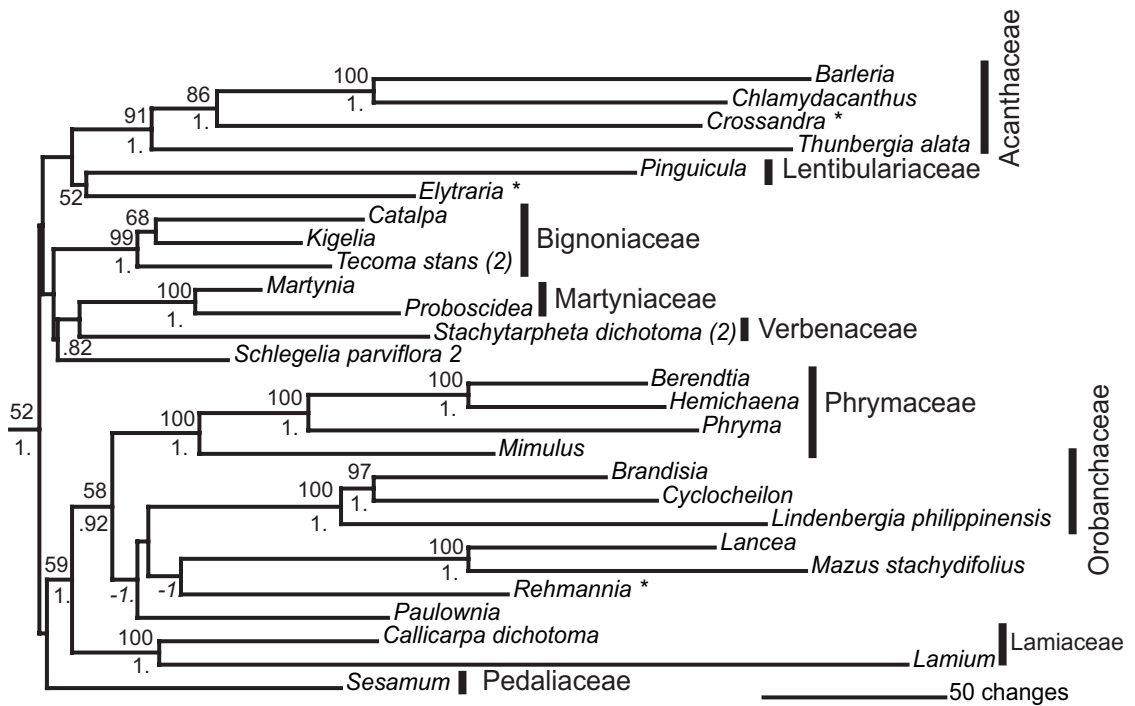


Fig. 6. Phylogram of one of the most parsimonious trees from the A2 analyses with representatives for the families Acanthaceae, Bignoniaceae, Lamiaceae, Lentibulariaceae, Martyniaceae, Orobanchaceae, Pedaliaceae, Phrymaceae and Verbenaceae. Bootstrap values, when >50%, are indicated above nodes. Bayesian posteriors under a GTR + gamma model are given below branches. * denotes sequences combined from different vouchers. Species epithets and occasionally numbers within parentheses are given to differentiate between DNA samples within genera, when there are more than one (see Appendix).

three Myoporeae genera, a hypothesis that is consistent with the results presented here. However, Lersten & Beaman (1998) found no cavities in *L. revolutum* Rzed. or in *Eremogeton grandiflorus* Standl. & L. O. Williams, which is generally considered to be closely related to *Leucophyllum*. More detailed molecular phylogenetic studies on *Leucophyllum*, *Eremogeton*, and the little known *Faxonanthus* Greenm. from Guatemala, are warranted.

There is a strongly supported sister-group relation between Myoporeae and Leucophylleae and between that group and the Madagascar genus *Androya* (100% in both the A2 and the A3 analyses).

Aptosimeae. — The circumscription of Aptosimeae, characterized by alternate leaves and a dilated corolla tube, is one of the very few that seems to have been stable over the history of Scrophulariaceae classification. Bentham included *Aptosimum*, *Peliostomum* (sometimes included in *Aptosimum*), and *Anticharis*, and has been followed by Wettstein among others. Monophyly of Aptosimeae is strongly supported by our chloroplast DNA sequences, and a sister group relation with *Androya*, Leucophylleae, and Myoporeae is moderately supported. All have 3-colpate, diporate

pollen (Erdtman 1952; Niezgodna & Tomb, 1975; Punt, 1980). Thus, these features may be synapomorphies for this group (“Myoporaceae”).

Hemimerideae. — The chiefly South African tribe Hemimerideae (including *Alonsoa*) is supported by our data (70% bootstrap) and conforms well to the circumscription reviewed in Steiner (1996), except that the chloroplast DNA sequence data support an inclusion of *Colpias* E. Mey. Steiner & Whitehead (1996) argued that *Colpias* rather should belong Bowkerieae, based on a basic chromosome number of $x = 20$, a tubular corolla, and a staminode corresponding to a fifth fertile stamen, characters that are shared with Bowkerieae. The amphiatlantic *Alonsoa* groups weakly with *Diclis* and *Hemimeris*. All have a basic chromosome number of $x = 7$ (Steiner, 1996). The South American species of *Alonsoa* form a monophyletic group based on *ndhF* sequences (results not shown). Their shared ancestry is further supported by having $2n = 56$ instead of $2n = 28$ in the South African taxa. Thus, the interpretation of the absence of oil secretion in South American species of *Alonsoa* as a synapomorphy (Steiner, 1996) is reinforced. Whether the origin of *Alonsoa* predates the breakup of Gondwanaland needs to be further investigated. *Nemesia*

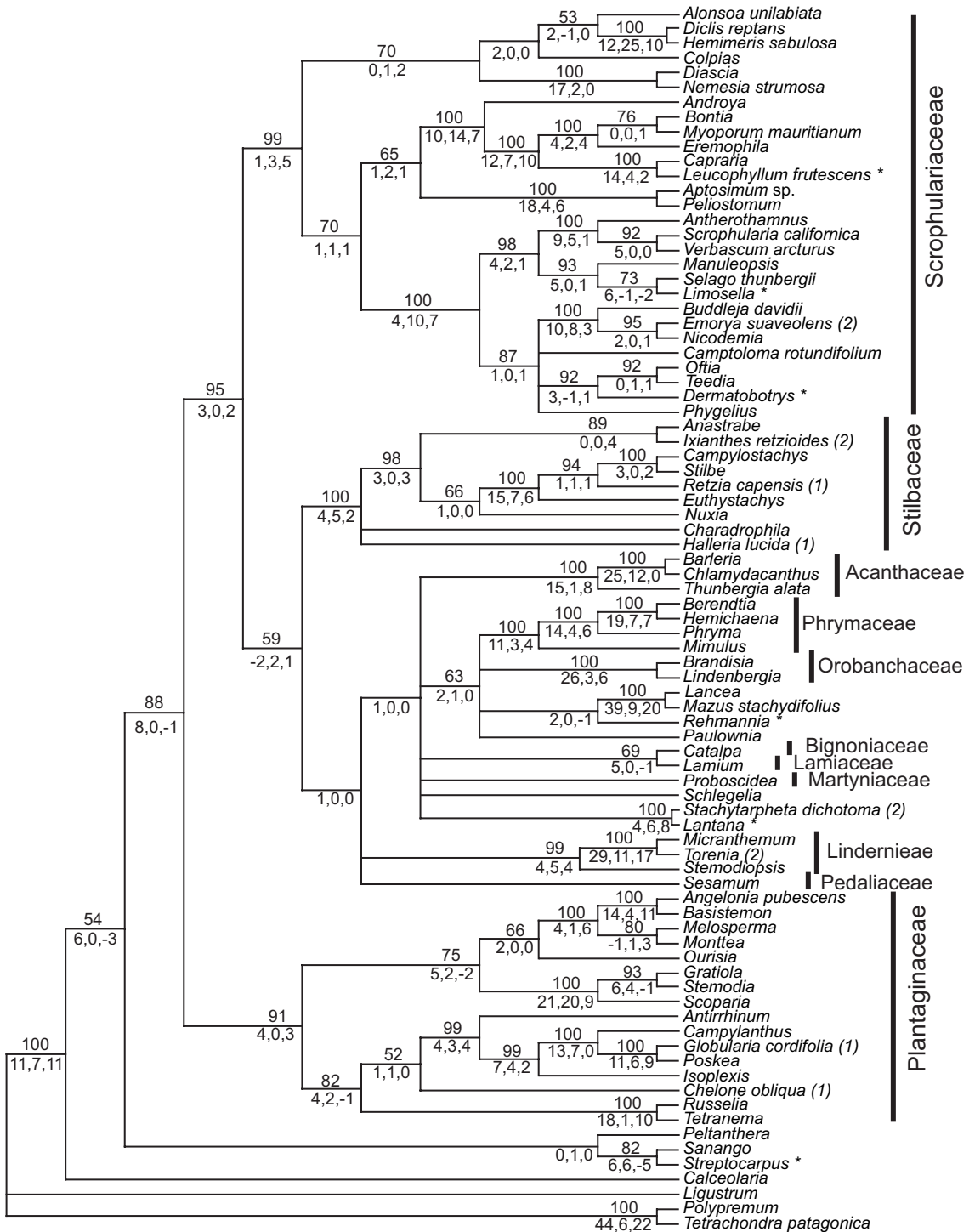


Fig. 7. Strict consensus tree from the A3 analysis. Parsimony bootstrap values, when >50%, are indicated above nodes. Below nodes the values from the PBS analysis are indicated in the following order: *ndhF*, *trnL/F* and *rps16*. *denotes sequences combined from different vouchers. Species epithets and occasionally numbers within parentheses are given to differentiate between DNA samples within genera, when there are more than one (see Appendix).

Vent. is here shown to be closely related with *Diascia*. Steiner (1996) showed that these taxa share the basic chromosome number $x = 9$, but emphasized differences

in the folding of lateral corolla lobes in bud and cited unpublished chloroplast *rps2* gene sequences as evidence for *Nemesia* belonging outside Hemimerideae. Our data

reject this, and instead favor the similarities in the androecium pointed out by Hilliard & Burt (1984) as being homologous for *Nemesia* and *Diascia*.

Stilbaceae. — The circumscription of this family continues to expand (Fig. 4). Bremer & al. (1994) presented molecular data that supported the close relationship between *Retzia* Thunb. and *Stilbe* Berg. proposed by Goldblatt & Keating (1976) on morphological grounds. Oxelman & al. (1999) showed that *Nuxia* Comm., previously classified in Buddlejaceae, also belong here. These findings are supported by phytochemical data (Damtoft & al., 1984; Frederiksen & al., 1999). Olmstead & al. (2001) added *Halleria*, and in this study we show that the chloroplast DNA phylogeny supports putting the tribe Bowkerieae (*Bowkeria*, *Anastrabe*, and *Ixianthes*; Barringer, 1993) and *Charadrophila* Marloth here. *Charadrophila capensis* is a rare plant found in shade on permanent wet cliffs in the Cape Province. Its position has been disputed ever since Marloth's original description (Marloth, 1898). He placed it in Scrophulariaceae, but Engler, in a note to the original description placed it in Gesneriaceae, which it resembles superficially. Weber (1989) followed Marloth and placed it in Scrophulariaceae arguing for a close relationship with *Alonsoa*. Its position in Stilbaceae was unexpected, and detailed studies are needed in order to establish homology hypotheses in this expanded circumscription of Stilbaceae.

Lindernieae. — A novel strongly supported clade of taxa earlier assigned to Scrophulariaceae was found that includes *Stemodiopsis* Engl., *Torenia* L., *Micranthemum* Desf., and probably *Picria* Lour. (= *Conobea*) and has unclear relationships to the rest of Lamiales (Fig. 4). This clade probably represents the tribe Lindernieae, diagnosed by geniculate anterior filaments, usually with a basal swelling. If this character proves to be synapomorphic, then for example also *Lindernia* All., *Craterostigma* Hochst., *Crepidiorhopolon* E. Fischer, *Hartliella* E. Fischer, and *Artanema* D. Don. may belong here. Neither *Stemodiopsis*, *Picria*, or *Micranthemum* possess the characteristic basal swelling of the base of the filament, but like *Stemodiopsis*, they have geniculate or curved anterior filaments/staminodes. Lindernieae have mostly been classified in Gratiolieae (Fischer, 1997), but have a pretty distinct morphology (e.g., alveolate endosperm of a certain type; Fischer, 1992).

Orobanchaceae, Phrymaceae and relatives. — There is moderate support for a clade including Phrymaceae, *Paulownia*, *Rehmannia* Libosch., *Mazus* Lour., *Lancea* Hook. f. & Thomson, and chiefly parasitic Orobanchaceae, to which *Brandisia* Hook. f. & Thomson, previously not known to be parasitic, is shown to belong (Fig. 6). The inclusion of *Lancea* and *Mazus* in Phrymaceae, as advocated by Beardsley & Olmstead (2002), is not supported here.

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Appendix. Taxon table with EMBL/GenBank accession numbers. An asterisk after the accession number indicates sequences here reported for the first time. Voucher collector and number, with herbarium acronym in parentheses, are given for those DNA samples that have previously not been reported in the literature. New sequences with no voucher information are based on the same DNA sample as those previously deposited on GenBank.

Taxon; Voucher; Accession number *ndhF*; Accession number *trnL*; Accession number *rps16*.

Ajuga reptans L.; L36391; -; - *Alonsoa auriculata* Diels; *Molau 1629* (GB); AJ617587*; -; - *Alonsoa meridionalis* Kuntze; *Asthalm 9* (GB); AJ617612*; -; - *Alonsoa peduncularis* (Kuntze) Wettst.; *Wall 15/9 -41* (S); AJ619549*, AJ619550*; - *Alonsoa unilabiata* Ruiz & Pav.ex Steud.; AF188184; AJ608620*; AJ609217*. *Aloysia grattissima* (Gill & Hook) Tronc.; AF130154; -; - *Amphianthus pusillus* Torr.; AF123674; -; - *Amphitecna apiculata* A.H. Gentry; AF102624; -; - *Anarrhinum corsicum* Jord. & Fourn.; AJ245815; -; - *Anastrabe integerrima* E. Mey. ex Benth.; *Wells 3588* (S); AJ619551*, AJ619552*; AJ608633*; AJ609216*. *Kornhall 88* (UPS); -; -; AJ621104*. *Androya decaryi* H. Perrier; AF027276; AJ608619*; AJ609215*. *Angelonia angustifolia* Benth.; *Lundin 1019* (S); AJ617588*; -; - *Angelonia pubescens* Benth.; AF123675; AJ608618*; AJ609214*. *Anisacanthus thurberi* A. Gray; -; AF034864; - *Antherothamnus pearsonii* N.E. Br.; AJ401392; AJ608617*; AJ609213*. *Anticharis glandulosa* Aschers.; *Thulin et al. 8268* (UPS); AJ619553*; -; AJ609212*. *Anticharis linearis* (Benth.) Hochst. ex Asch.; *Wanntorp & Wannorp 963* (S); -; -; AJ609211*. *Antirrhinum majus* L.; L36392; AJ608634*; AJ609218*. *Aptosimum indivisum* Burch.; *Bremer 3508* (UPS); AJ617594*; -; - *Aptosimum* sp.; AF123676; AJ608607*; AJ609210*. *Arrabidaea pubescens* (L.) A.H.Gentry; AF102625; -; - *Asarina procumbens* Mill.; AJ250380; -; - *Avicennia alba* Blume; -; -; AY008820; - *Avicennia germinans* L.; -; -; AY008819; - *Avicennia marina* ssp. *australasica* (Walp.) J.Everett; -; -; AY008821; - *Bacopa caroliniana* Robinson; AF123677; -; - *Barleria prionitis* L.; U12653; AF063118; - *Barthlottia madagascariensis* Fischer; AJ401438; AJ401443; AJ401443; *Bartsia alpina* L.; AF123678; -; - *Basistemon klugii* Barringer; *Plovman & al. 11387* (GB); AJ619554*; AJ619555*; AJ608616*; AJ609209*. *Berendtia laevigata* B.L. Rob. & Greenm.; *Pringle 6294* (S); -; -; AJ608615*; AJ609208*. *Bontia daphnoides* L.; Cult. Pacific Trop. Bot. Gard. #711537004; AJ617613*; AJ608614*; AJ609219*. *Bowkeria cymosa* MacOwan; *Balkwill 1510* (S); -; -; AJ609207*. *Bowkeria verticillata* Druce; *Bremer 3767* (UPS); AJ617589*; -; -; AJ609206*. *Brandisia hancei* Hook. f.; *Kingdon-Ward 318* (UPS); AJ619577*; AJ619578*; AJ608613*; AJ609205*. *Buddleja asiatica* Lour.; AF027277; AF380858; - *Buddleja davidii* Franch.; AF130143; AJ608612*; AJ609204*. *Buddleja indica* Lam.; L36405; AJ608581*; AJ609158*. *Buddleja saligna* Willd.; AJ401396; AJ401397; AJ609203*. *Byblis liniflora* Salisb.; AF482605; AF482533; - *Calceolaria mexicana* Benth.; AF123679; AJ608611*; AJ609202*. *Callicarpa dichotoma* Raesch.; L36395; AF363665; - *Callicarpa japonica* Thunb.; AF130148; -; - *Callitriche hermaphrodita* (1) L.; L47329; -; - *Callitriche hermaphrodita* (2) L.; L36396; -; - *Callitriche verna* L.; L47330; -; - *Campsis radicans* Seem.; AF130144; -; - *Camptoloma canariense* (Webb & Berth) Hilliard; AJ401398; AJ401399; AJ401445; AJ401449. *Camptoloma hypericiflorum* (Vatke) Hilliard; AJ401401; AJ296514; - *Camptoloma rotundifolium* Benth.; AJ401431; AJ401432; AJ401450; AJ296515; AJ609201*. *Campylanthus salsoloides* Roth.; *Bremer 3864* (UPS); AJ619556*; AJ608600*; AJ609200*. *Campylostachys cernua* Kunth.; *Kornhall 135* (UPS); AJ617611*; AJ608621*; AJ609199*. *Capraria biflora* L.; Cul. Utrecht Bot. Gard. 92GRO151g; AJ617610*; AJ608608*; AJ609198*. *Caryopteris bicolor* (Roxb. ex Hardw.) D.J.Mabberley; U78680; -; - *Caryopteris divaricata* Maxim.; U78679; -; - *Castilleja integra* A. Gray; -; -; AF118794; - *Catalpa speciosa* Warder ex Engelm.; L36397; AJ608599*; AJ609197*. *Chaenorhinum minus* L.; AJ250381; -; - *Charadrophila capensis* Marloth; *Kornhall 131* (UPS); AJ617590*; AJ608622*; AJ609196*. *Chelone obliqua* (1) L.; AF123680; AF034875; AJ609220*. *Chelone obliqua* (2) L.; AJ245814; -; - *Chenopodiopsis retrorsa* Hilliard; AJ401421; AJ296502; - *Chilopsis linearis* Sweet; -; -; AF034889; - *Chlamydocanthus lindavianus* H.Winkler; AJ249408; AJ608598*; AJ609195*. *Clerodendrum trichotomum* C.P.Thunberg ex A. Murray; AF130146; -; - *Codonanthe carnosa* (Gardner) Hanst.; -; -; AY047147; - *Codonanthe digna* Wiehler; AF130156; -; - *Collinsia grandiflora* Dougl. ex Lindl.; AF188182; -; - *Collinsia heterophylla* R.Grah.; -; -; AF034873; - *Colpias mollis* E. Mey.; *Jensen IOK-19-99* (C); AJ617591*; AJ608623*; AJ609194*. *Conoeba multifida* (Michx.) Benth.; *Fisher 1929* (UPS); -; -; AJ608597*; - *Cordylanthus kingii* S.Wats.; -; -; AF118795; - *Creoscentia portoricensis* Britton; AF102627; -; - *Cromidion decumbens* (Thunb.) Hilliard; AJ401403; AJ296503; -; - *Crossandra nilotica* Oliver; U12656; -; - *Crossandra pungens* Lindau; -; -; AF061825; - *Picria fer-tellae* Lour.; *Liang 63174* (GB); -; -; AJ608609*; AJ609193*. *Cybistax donnell-smithii* (Rose) Seibert; AF102628; -; - *Cyclocheilon somaliense* Oliver; *Thulin 8364* (UPS); -; -; AJ608624*; AJ609192*. *Cydista aequinoctialis* Miers; AF102629; -; - *Cymbalaria muralis* Gaertn. Mey. & Scherb.; AJ250382; -; - *Cyrtandra hawaiiensis* C.B. Clarke; AF130159; -; - *Dermatobotrys saundersii* Bolus; *Kornhall 136* (UPS); AJ617592*; -; -; AJ609191*. *Dermatobotrys saundersii* Bolus; *Jensen IOK-19-99* (C); -; -; AJ608596*; - *Dermatobotrys saundersii* Bolus; -; -; AF608635*; AJ609221*. *Diascia capsularis* Benth.; AJ617593; AJ608595*; AJ609190*. *Diels bambusetii* R.E. Fries; *Jonsell 2212* (UPS); AJ617614*; AJ608639*; - *Diels pettiolaris* Benth.; *Wanntorp & Wannorp 955* (S); -; -; AJ608594*; AJ609189*. *Diels reptans* Benth.; *Kornhall 104* (UPS); AJ619557*; AJ608593*; AJ609188*. *Digitalis grandiflora* Lam.; L36399; -; - *Digitalis purpurea* L.; AF130150; AF034871; - *Dischisma ciliatum* Choisy; AJ401412; AJ296501; - *Drymonia serrulata* (Jacq.) Mart.; -; -; AY047152; - *Drymonia strigosa* Wiehler; AF130158; -; - *Eccremocarpus scaber* Ruiz & Pav.; AF102630; -; - *Elythria cremata* Vahl.; U12657; -; - *Elythria imbricata* Vahl.; -; -; AF061819; - *Emorya suaveolens* (1) Torr.; -; -; AF327415; - *Emorya suaveolens* (2) Torr.; AF027278; AJ608625*; AJ609187*. *Eremophila bignoniflora* F. Muell.; *Olmstead 794* (WTU); AJ617609*; AJ608592*; AJ609186*. *Euthystachys abbreviata* A. DC.; AF147715; AJ608626*; AJ609185*. *Freylinia lanceolata* (L.) F. G. Don; *Rourke 1991* (S); -; -; AJ609184*. *Freylinia tropica* S. Moore; AJ401402; -; - *Galvezia fruticosa* J.F.Gmel.; AJ250383; -; - *Gambelia speciosa* Nutt. syn. *syn Galvezia* sp.; AJ250384; -; - *Gambelia speciosa* A.Gray; -; -; AF118796; - *Glekia krebsiana* (Benth.) Hilliard; AJ401422; AJ296519; - *Globularia cordifolia* (1) L.; AF027282; AJ430930; AJ431055. *Globularia cordifolia* (2) L.; AF124557; -; - *Globularia nudicaulis* L.; AF123681; -; - *Glumicalyx flanaganii* (Hiern) Hilliard; AJ401413; AJ296497; - *Gmelina hystrix* Schult. ex Kurz; U78692; -; - *Gomphostigma scoparioides* Turcz.; AF027279; -; -; AJ609183*. *Gratiola pilosa* Michx.; AF188183; AJ608591*; AJ609182*. *Halleria lucida* (1) L.; AF188185; AJ608590*; AJ609181*. *Halleria lucida* (2) L.; *Williams 2316* (S); -; -; AJ609180*. *Hebenstretia cordata* L.; AJ401414; AJ296499; - *Hebenstretia dentata* L.; AF027285; -; - *Hemichaena fruticosa* Benth.; *Castillo 1745* (S); AJ617595*; AJ608632*; AJ609179*. *Hemimeris montana* L.f.; *Wall 18/9-1938* (GB); -; -; AJ609230*. *Hemimeris montana* L.f.; *Goldblatt 2787* (S); AJ617596*; -; -; AJ609178*. *Hemimeris sabulosa* L.f.; AF123682; AJ608638*; AJ609227*. *Hemiphragma heterophyllum* Wall.; AF123683; -; - *Hippuris vulgaris* L.; L36401; -; - *Holmskioldia sanguinea* Retz.; U78693; -; - *Howelliiella ovata* (Eastw.) Rothm.; AJ250385; -; - *Isoplexis canariensis* Steud.; *Thulin 9945* (UPS); AJ617597*; AJ608589*; AJ609177*. *Ixianthes retzioides* (1) Benth.; *Adamson 4298* (BOL); -; -; AJ609176*. *Ixianthes retzioides* (2) Benth.; *Wolfe 97.118*; AJ617598*; AJ608636*; AJ609222*. *Jacaranda sparrei* A.H.Gentry; AF102631; -; - *Jamesbrittenia dissecta* Kuntze; AJ401435; AJ401436; -; - *Jamesbrittenia filicaulis* (Benth.) Hilliard; AJ401439; -; - *Jamesbrittenia megadenia* Hilliard; AJ401404; AJ296511; - *Jasminum fluminense* Vell.; -; -; AF102445; - *Jasminum mesnyi* Hance; AF130162; -; - *Jovellana* sp.; AF123684; -; - *Justicia carnea* Lindl.; AF130155; -; - *Justicia longii* Hilsenb.; -; -; AF063135; - *Keckelia antirrhinoides* (Benth.) Straw; -; -; AF034876; - *Kickxia elatine* (L.) Dumort.; AJ245816; -; - *Kickxia gracilis* D.A.Sutton; AJ245817; -; - *Kigelia africana* Benth.; AF102632; AF034880; - *Lamium purpureum* L.; U78694; AJ608588*; AJ609175*. *Lancea tibetica* Hook. f. & Thoms.; *Ho et al. 158* (E); AJ617599*; AJ608610*; AJ609174*. *Lantana camara* L.; -; -; AF225294. *Lantana camara* L.; -; -; AY008824; - *Lantana horrida* H.B. & K.; AF130152; -; - *Leucocarpus alatus* D.Don; -; -; AJ609173*. *Leucocarpus perfoliatus* Benth.; AJ617600*; -; - *Leucophyllum frutescens* (1) I.M.Johnst.; -; -; AF380873; - *Leucophyllum frutescens* (2) I.M.Johnst.; AF123685; -; -; AJ609172*. *Leucophyllum frutescens* (3) I.M.Johnst.; *Schallert 18738* (S); -; -; AJ609171*. *Leucophyllum minus* A.Gray; -; -; AF034878; - *Ligustrum vulgare* L.; AF130164; AF231848; AF225257. *Limosella aquatica* L.; *Dvorak 1556* (UPS); AJ619558*; AJ608587*; - *Limosella grandiflora* Benth.; AJ550552; -; -; AJ609170*. *Linaria amethystea* (Lam.) Hoffm. & Link; AJ250386; -; - *Linaria canadensis* Dum.-Cours.; -; -; AF034867; - *Lindenbergia indica* (L.) Vatke; AF027286; -; - *Lindenbergia philippinensis* Benth.; AF123686; AJ608586*; AJ609169*. *Lyperia antirrhinoides* (L.f.) Hilliard; AJ401405; AJ296521; - *Lyperia tristis* Benth.; AJ401406; -; - *Mabrya acerifolia* (Pennell) Elisens; -; -; AF118797; - *Macfadyena unguis-cati* (L.) A.H.Gentry; AF102633; -; - *Manulea cheiranthus* L.; AJ401418; AJ401419; AJ401446; AJ401452. *Manulea*

Appendix (continued).

Taxon; Voucher; Accession number *ndhF*; Accession number *trnL*; Accession number *rps16*.

crassifolia Benth.; AJ401428 AJ401429; AJ401453 AJ296518. *Manulea glandulosa* Philips; AJ401395; AJ296520; -. *Manulea tomentosa* Hiern; AJ401394; -. *Manuleopsis dinterii* Thell.; AJ401410; AJ401454, AJ296500; AJ609168*. *Martynia annua* L.; AF190906; AF067065; -. *Maurandella antirrhiniflora* (Willd.) Rothm.; AJ250387; -. *Maurandella antirrhiniflora* (Willd.) Rothm.; -. *Maurandella scandens* A. Gray; AJ245818; -. *Mazus reptans* N.E. Br.; -. AF479004; -. *Mazus stachydifolius* Maxim.; *Ulanova* & *Basargin* 6936 (S); AJ619559*; AJ607432*; AJ607433*; AJ609167*. *Mecardonia flagellaris* (C. & S.) Rossow; *Iversen et al.* 87345 (UPS); AJ617601*; AJ608627*; AJ609166*. *Melanospermum foliosum* (Benth.) Hilliard; AJ401415; AJ401456, AJ296507; -. *Melanospermum transvaalense* (Hiern) Hilliard; AJ401426, AJ401427; AJ401455, AJ296508; -. *Melasma scabrum* Berg.; AF123687; -. *Melosperma andicola* (Gill.) Benth.; *Wall* 21/12-1946 (GB); AJ617602*; AJ608585*; AJ609165*. *Micranthemum glomeratum* (Chapm.) Shinners; *Tehler et al.* 22 (S); AJ617603*; AJ608584*; AJ609164*. *Micranthemum umbrosum* (J.F.Gmel.) Blake; -. AF034879; -. *Microdon dubius* (L.) Hilliard; AJ401411; AJ296491; -. *Microdon lucidus* Choisy; AJ401416; AJ296492; -. *Mimulus ringens* L.; AF188186; AJ608606*; AJ609163*. *Misopates orontium* (L.) Raf.; AJ250388; -. *Mohavea breviflora* Coville; -. AJ609223*. *Mohavea confertiflora* (DC.) A. A. Heller; AJ250389; -. *Monttea chilensis* Gay; *Frödin* 246 (UPS); AJ617604*; AJ608583*; AJ609162*. *Myoporium insulare* R.Br.; -. AJ299258. *Myoporium mauritianum* A.DC.; L36403; AJ608582*; AJ609161*. *Myoporium montanum* R.Br.; -. AJ296513; -. *Nematanthus longipes* DC.; AF130157; -. *Nematanthus strigillosus*; -. AY047148; -. *Nemesia cheiranthus* E. Mey. ex Benth.; *Kornhall* 19 (UPS); AJ617605*; -. AJ609160*. *Nemesia strumosa* Benth.; AF123688; AJ608631*; AJ609159*. *Nothochelone nemorosa* (Douglas ex Lindl.) Straw; -. AF034874; -. *Nuxia* sp.; AF027280; AJ608605*; AJ609157*. *Nyctanthes arbor-tristis* L.; AF130161; -. *Oftia africana* Bocq. ex Baill.; *Bremer* 1152 (S); AJ617606*; AJ608585*; AJ609156*. *Olea europaea* L.; AF027288; AF231866; -. *Olea europaea* L.; AF130163; -. *Ophiocolea floribunda* (Boj. ex Lindl.) H.Perrier; AF102634; -. *Oreosolen unguiculatus* Hemsl.; *Paulsen* 1921 (S); AJ617607*; -. AJ609155*. *Oroxylum indicum* (L.) Kurz; AF102635; -. *Ourisia poeppigii* Benth.; *Meudt & Lopes* 13 (TEX); AJ619560*; AJ619561*; AJ619562*; AJ608579*; AJ609154*. *Paliavana prasinata* Benth. & Hook.f.; AF130160; -. *Pandorea jasminoides* Schum; AF102636; -. *Paulownia tomentosa* (Thunb.) Steud.; L36406; AJ608578*; AJ609153*. *Pedicularis foliosa* L.; AF123689; -. *Pedicularis procera* A. Gray; -. AF034877; -. *Peliotostomum leucorrhizum* E. Mey. ex Benth.; *Skarpe* 1976-12-16 (UPS); AJ619563*; AJ619564*; AJ608577*; AJ609152*. *Peltanthera floribunda* Benth.; AF027281; AJ608576*; AJ609151*. *Penstemon centranthifolius* Benth.; -. AF034883; -. *Penstemon* sp.; *Oxelman* 2338 (WTU); -. AJ619565*; -. *Petitia domingensis* Jacq.; U78697; -. *Phryma leptostachya* L.; AJ617586*; AF478988/ AJ430928; AJ609150*. *Phygelius capensis* E.Mey. ex Benth.; *Oxelman* 2339 (UPS); AJ617608*; AJ608575*; AJ609149*. *Phyla incisa* Small; AF130153; -. *Phyllopodium cuneifolium* (L.f.) Benth.; AJ401430; AJ401457, AJ296496; -. *Pinguicula* sp.; *Erixon* 54 (UPS); -. AJ608604*; AJ609148*. *Plantago lanceolata* L.; AF130151; -. *Podranea ricasoliana* Sprague; AF102637; -. *Polycarena formosa* Hilliard; AJ401423; AJ296515; -. *Polypremum procumbens* L.; AJ011986; AJ431063; AJ430938. *Poskea socotrana* (I.B.Balf.) G.Taylor; *Thulin & Gifri* 8670 (UPS); AJ617585*; AJ608574*; AJ609147*. *Prasium majus* L.; U78700; -. *Premna microphylla* Turcz.; U78701; -. *Proboscidea louisianica* (Mill.) Thell.; AF123690; AJ608573*; AJ609146*. *Pseuderanthemum alatum* Radlk.; -. AF063130; -. *Pseudoselago ascendens* (E.Mey.) Hilliard; AJ401433; AJ296495; -. *Pseudoselago serrata* (P.J.Bergius) Hilliard; AJ401440; -. *Pseudoselago subglabra* Hilliard; -. AJ296498; -. *Radermachera frondosa* Chun & How; AF102638; -. *Ramonda myconii* (L.) Rchb.; -. *Rehmannia angulata* Hemsl.; *Hedberg* 29.9.1943 (UPS); AJ619566*; -. AJ609145*. *Rehmannia elata* N.E.Brown; -. AJ608572*; -. *Retzia capensis* (1) Thunb.; AF027289; AJ608628*; AJ609144*. *Retzia capensis* (2) Thunb.; AF147716; -. *Reyemia chasmantiiflora* Hilliard; AJ401425; AJ296505; -. *Rhodochiton atrangoneum* (Zucc.) Rothm.; AJ250390; -. *Ruellia californica* I.M.Johnst.; -. AF063115; -. *Ruellia ciliata* Hornem.; U12664; -. *Ruellia retrorsa* Greene; *Penell* Aug 1995 (S); AJ619567*; AJ608571*; AJ609143*. *Salvia divinorum* Epling & Jativa; U78703; -. *Sanango* sp.; AF027283; AJ608603*; AJ609142*. *Sarmienta scandens* Pers.; -. *Schlegelia parviflora* (1) (Oerst.) Monach.; -. AY008825; -. *Schlegelia parviflora* (2) (Oerst.) Monach.; L36410; AJ608570*; AJ609141*. *Schweinfurthia pterosperma* A.Br.; AJ250391; -. *Scoparia dulcis* L.; *Ryding* 683 (UPS); AJ619568*; AJ619569*; AJ608569*; AJ609140*. *Scrophularia californica* Cham. & Schlecht.; L36411; AF118802*; AJ609224*. *Scrophularia macrantha* Greene ex Stiefelham; -. AF034865; -. *Scrophularia nodosa* L.; -. AF118803; -. *Scrophularia peregrina* L.; *Julin* 23.4.1985 (UPS); -. AJ608568*; AJ609139*. *Selago canescens* L.f.; L36412; AJ608637*; AJ609225*. *Selago corymbosa* L.; -. AJ401458, AJ296494; -. *Selago myrtifolia* Hilliard; AJ401420*; AJ296500; -. *Selago setulosa* Rolfe; -. AJ296493; -. *Sesamum indicum* L.; L36413; AJ608602*; AJ609226*. *Seymeria pectinata* Pursh; AF123691; -. *Sinningia schiffneri* Fritsch; -. *Stachytarpheta dichotoma* (1) Vahl; -. AY008823; -. *Stachytarpheta dichotoma* (2) Vahl; L36414; AJ608567*; AJ299259. *Stemodia glabra* Oerst.; *Nordenstam & Anderberg* 967 (S); AJ617584*; AJ608566*; AJ609138*. *Stemodiopsis buchananii* Skan; *Iversen & Martinsson* 89169 (UPS); AJ619570*; AJ619571*; AJ619572*; AJ608565*; AJ609137*. *Stemodiopsis humilis* Skan; *Ngoni* 177 (P); -. AJ609229*. *Stilbe albiflora* E.Mey.; AF027287; AJ608629*; AJ609136*. *Streptocarpus caulescens* Vathe; *Erixon* 35 (UPS); -. AJ608601*; AJ609135*. *Streptocarpus holstii*; L36415; -. *Strobilopsis wrightii* Hilliard & B.L.Burt; AJ401441; -. *Sutera foetida* Roth; AJ401407, AJ401408; AJ296510; -. *Sutera hispida* (Thunb.) Druce; AJ550566, AJ550567; -. *Sutera patriotica* Hiern; AJ401393; -. *Syringa emodi* Wall.; AF130228; -. *Syringa vulgaris* L.; -. AF231882; -. *Tecoma stans* (1) Juss.; -. AY008826; -. *Tecoma stans* (2) Juss.; AF130145; AF034888; -. *Tecomaria capensis* (Thunb.) Spach; -. AY008827; -. *Tectona grandis* L.f.; AF027284; -. *Teedia lucida* (Ait.) Rud.; *Bremer* 3545 (UPS); AJ617582*; AJ608561*; AJ609127*. *Tetrachondra hamiltonii* Petrie; -. AJ609134*. *Tetrachondra patagonica* Skottsb.; AF027272; AJ430939; AJ609133*. *Tetraclea coulteri* A. Gray; AF130147; -. *Tetranema mexicanum* Benth.; AF123692; AJ608630*; AJ609132*. *Tetraselago longituba* (Rolfe) Hilliard & Burt; AJ401417; AJ296506; -. *Teucrium fruticans* L.; U78686; -. *Thunbergia alata* Bojer ex Sims; U12667; AJ608564*; AJ609131*. *Thunbergia erecta* T.Anders.; -. AJ609228*. *Torenia bailonii* (1) Godefroy ex Andre; *Oxelman* 2367 (UPS); -. AJ608563*; -. *Torenia bailonii* (2) Godefroy ex Andre; *Olmstead* 98-52 (WTU); AJ617583*; AJ608562*; AJ609130*. *Torenia polygonioides* Benth.; *Klackenb. & Lundin* 624 (S); AJ619573*; AJ619574*; -. AJ609129*. *Trieneena glutinosa* (Schltr.) Hilliard; AJ401400; AJ401459; AJ296516. *Uncarina grandidieri* (Baill.) Stapf; -. AF363667; -. *Verbascum arcturus* L.; AJ619575*; AJ619576*; AJ401460, AJ296522; AJ609128*. *Verbascum nigrum* L.; -. AF118804; -. *Verbascum thapsus* L.; L36417; -. *Verbena bonariensis* L.; L36418; -. *Verbena urticifolia* L.; -. AY008822; -. *Veronica catenata* Pennell; L36419; -. *Vitex agnus-castus* L.; AF130149; -. *Zaluzianskya benthamiana* Walp.; AJ401409; -. *Zaluzianskya glareosa* Hilliard & Burt; AJ401424; AJ401448, AJ296504; -. *Zaluzianskya katharinae* Hiern; AF123693; -. *Zaluzianskya minima* (Hiern) Hilliard; AJ401437; AJ401447; -.

Combined sequences:

Codonanthe digna/carnosula; AF130156/AY047147/- *Collinsia grandiflora/heterophylla*/-; AF188182/AF034873/- *Crossandra nilotica/pungens*/-; U12656/AF061825/- *Dermatobotrys saundersii*; AJ617592*; AJ608635*; AJ609191*. *Drymonia strigosa/serrulata/strigosa*; AY047152/AY047152/AF130158. *Elytraria cremata/imbricata*/-; U12657/AF061819/- *Freylinia tropicalis/lanceolata*; AJ401402/-AJ609184*. *Gambelia speciosa/peciosa*/-; AJ250384/AF118796/- *Lantana horrida*; AF130152; AY008824; AF225294. *Leucophyllum frutescens*; AF123685; AF380873; AJ609172*. *Limosella grandiflora/aquatica/grandiflora*; AJ550552/AJ608587*/AJ609170*. *Maurandella antirrhiniflora/antirrhiniflora*/-; AJ250387/AF118798/- *Mohavea confertiflora/-breviflora*; AJ250389/-AJ609223*. *Nematanthus longipes/strigillosus*/-; AF130157/AY047148/- *Penstemon* sp./centranthifolius/-; AJ619565*/AF034883/- *Rehmannia angulata/elata/angulata*; AJ619566*/AJ608572*/AJ609145*. *Streptocarpus holstii/caulescens/caulescens*; L36415/AJ608601*/AJ609135*.