Phylogenetic placement of *Triaenophora* (formerly Scrophulariaceae) with some implications for the phylogeny of Lamiales

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Phylogenetic placement of *Triaenophora* together with *Rehmannia* were explored with DNA sequence data from five regions (*rbcL*, *ndhF*, *rps16*, *trnL-F*, nr ITS) in two combined data matrices. One (*rbcL*, *ndhF*, *rps16*) represented a wide sampling across most families of Lamiales. The other data matrix represented two DNA regions partly unalignable across Lamiales (*trnL-F*, nr ITS) plus *rps16*, which proved to be variable enough to give resolution at a smaller taxonomic level in Lamiales. *Triaenophora rupestris* and *Rehmannia* are sister taxa, composing a strongly supported clade, which is sister to all representative genera of Orobanchaceae. Furthermore, Paulowniaceae and Phrymaceae are next to Orobanchaceae and *Triaenophora-Rehmannia* clade. All of the above taxa are only distantly related to Scrophulariaceae and/or Plantaginaceae (sensu APG II). *Mazus* and *Lancea* would be excluded from Phrymaceae. The resulting phylogeny is in agreement with other data such as phytochemistry and provides a framework for further investigation of character evolution in Lamiales.

KEYWORDS: Lamiales, Orobanchaceae, phylogeny, *Rehmannia*, Scrophulariaceae sensu lato, *Triaenophora*

INTRODUCTION

The phylogeny and circumscription of families in Lamiales remains one of the most problematic topics in angiosperm systematics (Judd & Olmstead, 2004). Especially, the disintegration of Scrophulariaceae (Olmstead & Reeves 1995; Olmstead & al., 2001; Albach & al., 2005; Oxelman & al., 2005) led to the recircumscription and recognition of a number of new families. Still, a number of genera have never been investigated phylogenetically and therefore their affinities remain unknown. The position of these genera is necessary for the interpretation of character evolution in Lamiales.

One such genus is *Triaenophora* Soler., with currently two accepted species, which is distinguished from any other related genus by its five trifid calyx lobes and bilocular ovary. *Triaenophora rupestris* (Hemsl.) Soler. (Fig. 1) was segregated from *Rehmannia* Libosch. ex Fisch. & C.A. Mey. by Solereder (1909) together with *Titanotrichum* but while the former two genera were kept in Digitalideae the latter was removed to Gesneriaceae, a position later supported by DNA-based phylogenetic analyses (Smith & al., 1997; Albach & al., 2001). *Triaenophora rupestris* is now on the red list of endangered species of China, and only occasionally seen at cliff faces of Hubei and Sichuan, China. Only recently, Li & al. (2005) published a second species, *T. shennongjiaensis* X.D. Li, Y.Y. Zan & J.Q. Li, which they said differs from *T. rupestris* in having densely glandular leaves, dentate bract margins, and pale yellow petals that are retuse or rarely obtuse at their apices, although only petal color seems to be a reliable character for differentiation (H.-Q. Li, pers. obs.). Another species of *Triaenophora*, *T. integra* (H.L. Li) Ivanina, that is often recognized (e.g., Hong & al., 1998) has recently been shown to be conspecific with *T. rupestris* based on detailed morphological and cultivation analysis (Li & al., 2008). Li & al. (2005, 2007) studied the morphological characters of leaf epidermis and the allozyme variability of *T. rupestris* and *T. shennongjiaensis* together with species from *Rehmannia*, and suggested a sister relationship between these two genera. Unfortunately, only *T. rupestris* was available for DNA sequencing but given the close similarity of the two species, there is little doubt about the monophyly of the genus. Furthermore, Xia & al. (2009) included T. *shennongjiaensis* in their study and retrieved maximum support for their sister group relationship in all analyses.

Triaenophora has traditionally been placed in Digitalideae next to *Rehmannia* and *Digitalis* L. in Scrophulariaceae s.l. (von Wettstein, 1891; Solereder, 1909; Li, 1948; Hong & al., 1998). This position was also suggested based on a detailed morphological analysis (Wang & Wang, 2005). However, recent molecular systematic studies based on plastid DNA sequence data showed that

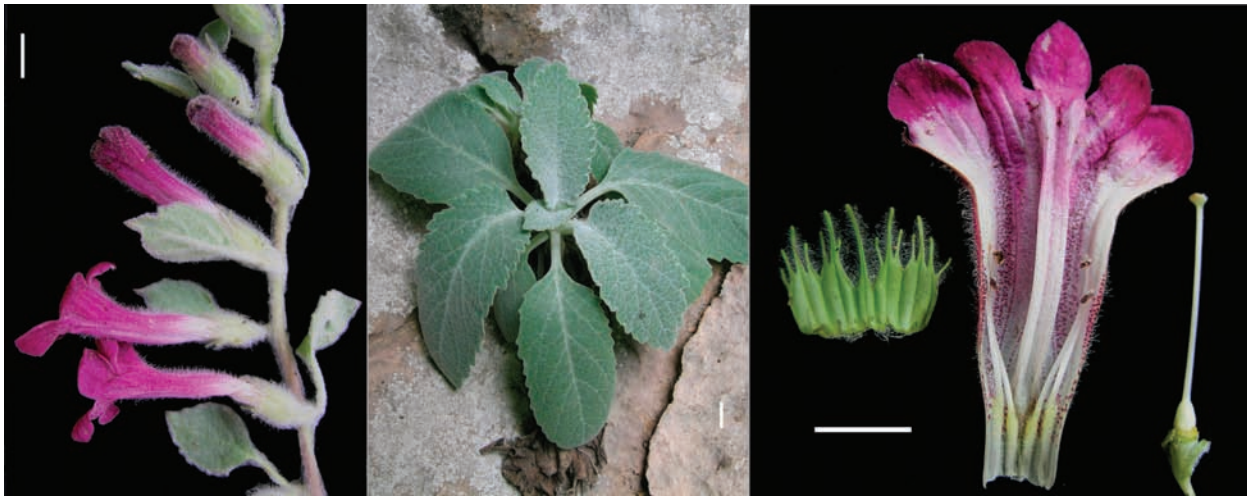

Fig. 1. Raceme, young plant, and dissected flower of *Triaenophora rupestris* (voucher: *Hongqing LI 2006998*, HSNU). Scale bars = 1 cm.

the traditionally circumscribed Scrophulariaceae are polyphyletic and placed *Digitalis* in a new position in Plantaginaceae (Olmstead & Reeves, 1995, Olmstead & al., 2001; Albach & al., 2005). *Rehmannia* was not sampled in these studies but its position in a more derived clade of the Lamiales was shown by Oxelman & al. (2005) and Albach & al. (2007). However, *Triaenophora* has never been sampled in molecular systematic studies before.

The goal of this study is (1) to identify the relationship of *Triaenophora* using *T. rupestris* as a representative of the genus to *Rehmannia* using all six species and (2) to explore the possible phylogenetic position of these two genera in Lamiales. We discuss the implications of these analyses for the phylogeny and evolution of Lamiales. In order to achieve this goal, we analyze five DNA regions, the plastid coding genes *rbcL* and *ndhF*, the noncoding plastid *trnL-F* region and the *rps16* intron, and the nuclear ribosomal ITS region. All regions have been used before in phylogenetic studies of Lamiales and Scrophulariaceae and/or Orobanchaceae (e.g., Olmstead & Reeves, 1995; Manen & al., 2004; Albach & al., 2005; Oxelman & al., 2005) and proved valuable at different taxonomic levels.

MATERIALS AND METHODS

Taxon sampling. — The broader familial phylogenetic placement of *Triaenophora* was conducted on the background of Lamiales (APG II, 2003). The information on all sampled taxa and GenBank accession numbers can be found in the Appendix. Materials of *Triaenophora rupestris* were collected by Hongqing Li from Jianshi, Hubei, China in July 2006 (voucher: *Hongqing LI 2006998*) and from Xingshan, Hubei, China in September 2007 (voucher: *Hongqing LI 2007901*); the material of *Brandisia hancei* Hook. f., *Buchnera cruciata* Buch.-Ham. ex D. Don., *Lantana camara* L., *Mazus stachydifolius* (Turcz.) Maxim., *Pedicularis verticillata* L. and *Rehmannia chingii* H.L. Li. were also obtained for the research, the vouchers of above plants are *Hongqing Li 20071720*, *Hongqing Li 20041001*, *Kun Yan 2007002*, *Kun Yan 2007001*, *Hongqing Li 2007524*, *Xjlj 2007-42*, and *Hongqing Li 20040601*, respectively. All vouchers are deposited in Herbarium of East China Normal University (HSNU).

The *ndhF*, *rbcL*, and *rps16* DNA sequence regions were selected for this phylogenetic analysis, partly because many key taxa of Lamiales had already been sequenced for these regions, and partly because previous studies indicated that these regions are informative in Lamiales (Olmstead & al., 1995; Oxelman & al., 2005). The sampling strategy for the broad phylogenetic analysis of Lamiales using *rbcL*, *ndhF* or *rps16* regions included all families of Lamiales, for which these DNA regions had already been sequenced including *Rehmannia chingii* plus our sequence of *Triaenophora*. For the families (e.g., Gesneriaceae and Scrophulariaceae s.l.) having possible closer relationships with *Triaenophora* on morphology, more representatives had been selected. Outgroups included a representative of the closely related order Solanales. For this purpose, several new sequences of *rbcL* (four), *ndhF* (two) and *rps16* intron (five) were generated. Finally, we included in total 39 genera for the broader phylogenetic analysis.

Based on the results of the first analysis, we compiled a data matrix for *rps16*, *trnL-F*, and ITS DNA sequences regions for a more focused phylogenetic study. In addition to the sequences available in GenBank for those genera in the same clade as *Triaenophora* with high bootstrap value,

we sequenced several new sequences of the *trnL-F* region (six), ITS (two) and *rps16* intron (two in addition to those mentioned above). However, the P8 loop of the *trnL-F* spacer and ITS sequences of *Aeginetia*, and *Boschniakia* (both Orobanchaceae) were too divergent for inclusion in the dataset and were excluded. For *Rehmannia*, all six species were selected. In this part, we include in total 27 species from 22 genera.

DNA sequencing. — Total genomic DNA was extracted from dry leaf samples according to the hexadecyltrimethylammonium bromide (CTAB) procedure of Doyle & Doyle (1987). Polymerase chain reactions (PCR) of *ndhF* was performed in four pieces using primers –47F and 925R, 4F and 1350R, 1200F and 2065R, and 1811F and +606R (Oxelman & al., 1999; Kornhall & al., 2001) except for *Triaenophora rupestris*, for which -47F had to be replaced by 40F (Kornhall & al., 2001). The *rbcL* gene was amplified in two pieces using primers 5′F and Z895R and Z674F and 3′R (Bremer & al., 2002). The *trnL-F* region was amplified using primers c and f of Taberlet & al. (1991), the ITS region using primers 17SE (Sun & al., 1994) and ITS4 (White & al., 1991), and the *rps16* intron using primers rpsF and rpsR2 (Oxelman & al., 1997). PCR products were purified using the TIANgel purification kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocols. Sequencing reactions of both strands were carried out on an ABI 3730 automatic sequencer (Applied Biosystems, U.S.A.). Sequencing primers were the same as amplification primers. Sequences were first aligned by Clustal X (Jeanmougin & al., 1998), followed by manual corrections. All gaps were treated as missing data.

Phylogenetic analyses. — Both matrices were analyzed with PAUP* 4.0b10 (Swofford, 2002) using the maximum likelihood criterion. The appropriateness of different models was evaluated using Modeltest v. 3.8 (Posada & Crandall, 1998; Posada, 2006) based on AIC (Akaike information criterion) for both analyses. For the broad analysis, ten runs of random taxon addition (10 replicates) starting from random trees using tree bisection reconnection (TBR) were conducted with MulTrees (keeping multiple shortest trees) in effect and no tree limit. In addition, three runs of nearest-neighbour interchange (NNI) branch swapping, followed by TBR branch swapping on the optimal tree from the NNI branch swapping analysis were conducted. Bootstrap percentages were assessed using 1,000 replicates and TBR-branch swapping. Following recommendations by Morrison (2007) the focused analysis was conducted using 5 runs of NNI branch swapping, one starting from the BioNJ-tree (Gascuel, 1997), the other four starting from random trees, followed by TBR branch swapping on the optimal tree from the NNI branch swapping, all with MulTrees in effect and no tree limit. Bootstrap percentages were assessed using 1,000 replicates and NNI branch swapping.

RESULTS

The combined dataset of *rbcL*, *ndhF* and *rps16* contains 4,721 aligned characters. The optimal model used for analysis is TVM+I+Γ. The optimal tree is shown in Fig. 2. TBR branch swapping found in all three cases a better tree than NNI branch swapping alone. According to this analysis *Triaenophora* and *Rehmannia* are well supported sister genera (100% bootstrap support, BS). They are sister to Orobanchaceae (90% BS). Orobanchaceae and *Triaenophora*/*Rehmannia* form a moderately supported clade with *Paulownia*, *Mazus* Lour. and Phrymaceae (71% BS) and are well separated from Scrophulariaceae s.str. and Plantaginaceae. This was the focus group used in the subsequent analysis. Notably, we found that *Calceolaria* was inserted in Gesneriaceae.

The more focused analysis using the combined dataset of *rps16*, *trnL-F* and ITS comprises 2,856 aligned characters. The optimal model identified by Modeltest with a weight of 0.95 was TrN+Γ. All runs found the same optimal tree (Fig. 3) and TBR-branch swapping did not find a better tree. Compared to the broad analysis the optimal tree from the focused analysis again supports the monophyly of Orobanchaceae and *Triaenophora*/*Rehmannia* (95% BS). Other relationships in this clade are weakly supported such as *Lancea* Hook. f. & Thomson and *Mazus* being sister to all other genera and Phrymaceae sister to Orobanchaceae, *Rehmannia* and *Triaenophora*. Most importantly, *Triaenophora rupestris* is sister to all species of *Rehmannia* in a clade that receives 100% BS and is not nested in that genus (100% BS), thus supporting its continued recognition at the generic level.

DISCUSSION

Rehmannia and *Triaenophora* were previously suggested to be closely related to Digitalideae in Scrophulariaceae. Speta (1979) was the first to cast some doubt about this relationship based on the shape of the nuclear protein bodies. Our analyses reject the hypothesis of a relationship with Digitalideae strongly for both genera (Fig. 2) as has previously been done for *Rehmannia* alone (Oxelman & al., 2005; Albach & al., 2007). A previous analysis based on allozyme data (Li & al. 2007) obtained a high genetic identity between *Triaenophora* and *Rehmannia* but could not address the issue of monophyly of both genera due to the lack of close relatives that could confirm the monophyly of *Triaenophora*/*Rehmannia* or test the hypothesis that *Triaenophora* is nested in *Rehmannia*. Our analyses including all species of *Rehmannia*, one of two species of *Triaenophora* and a wide assemblage of other possibly related taxa within Lamiales strongly support that *Triaenophora* and *Rehmannia* are monophyletic and sister

genera (Fig. 3). We are convinced that the inclusion of the other species of *Triaenophora* would not change the conclusion based on their morphological similarity and the analysis by Xia & al. (2009), which included also *T. shennongijaensis*. The monophyly of the clade comprising *Rehmannia* and *Triaenophora* is well supported by morphological characters as well as phytochemistry, morphology, and life history. Both share their perennial life history, leaves, stems and perianth covered with cellulous glandular hairs (Hong & al., 1998; Li & Li, 2006), two bracteoles subtending the flower (Wang & Wang 2005), chromosome number $2n = 28$ (or tetraploid $2n = 56$) (Li & al., 2007; Yan & al., 2007), and a very similar assemblage of chemical compounds including shared unique iridoid glucosides (Jensen & al., 2008). They furthermore occur sympatrically in the same region of China (Hong &

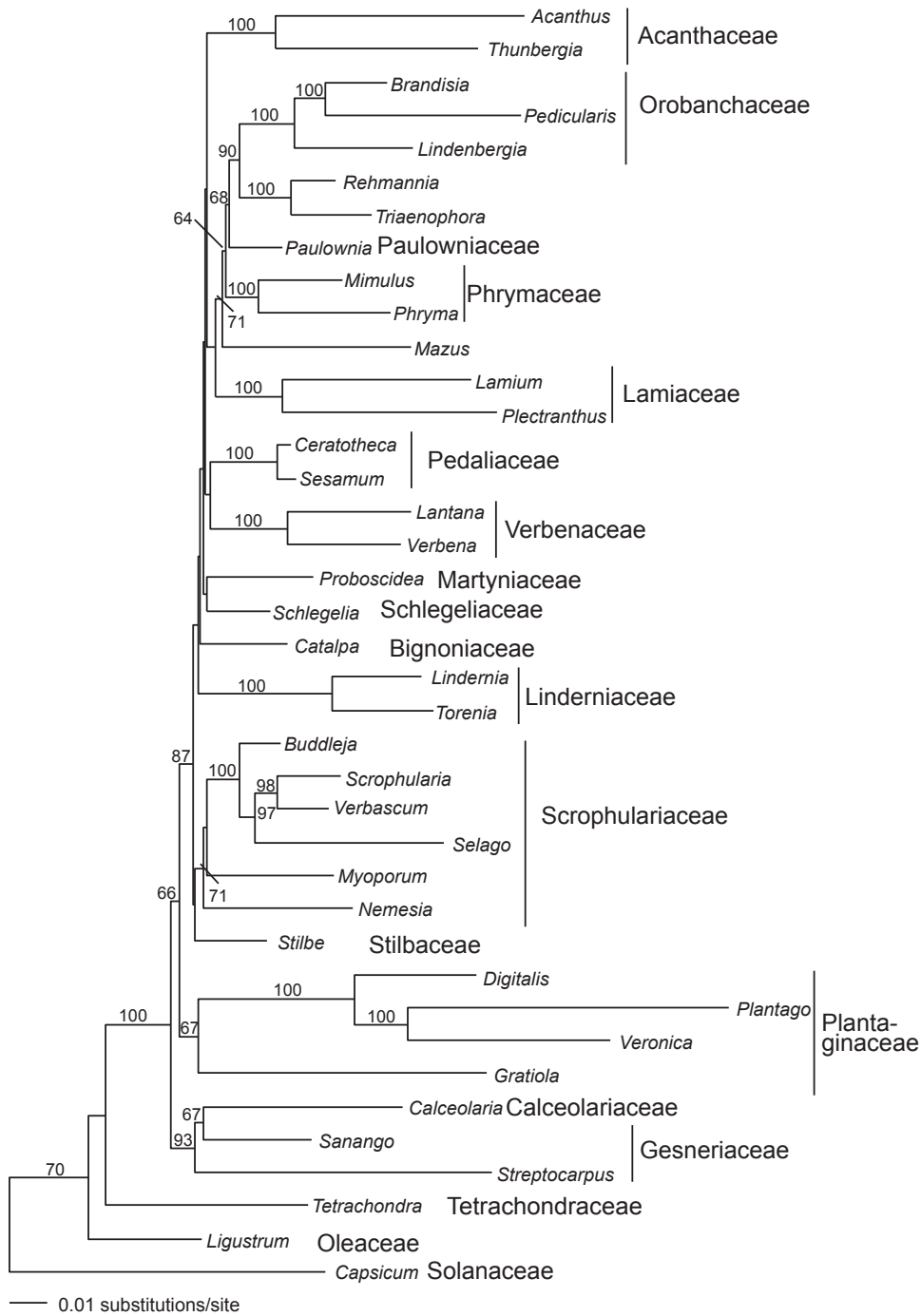

Fig. 2. Optimal tree from the Lamiales-wide analysis using *rbcL*, *ndhF* and the *rps16* intron. Numbers above branches indicate bootstrap support. Branch lengths are scaled according to inferred evolutionary changes.

al., 1998). Despite these similarities the two genera are distinguishable by the trifid (versus entire) calyx lobes and bilocular (versus unilocular) ovary of *Triaenophora*.

The present analyses further establish firmly the phylogenetic position of *Triaenophora* and *Rehmannia* and both in turn as sister to Orobanchaceae. Previous analyses (Oxelman & al., 2005; Albach & al., 2007) have not resolved the placement of the troublesome *Rehmannia*. Oxelman & al. (2005) indicated weak bootstrap support (58 BS) for a clade including Phrymaceae, *Paulownia*, *Rehmannia*, *Mazus*, *Lancea*, and chiefly parasitic Orobanchaceae. Here, we support the removal of *Rehmannia* from Digitaleae and address the question of relationships among its relatives with more focused sampling of taxa. The same clade was again found with strong support in a parallel study by Xia & al. (2009). We refer to this clade

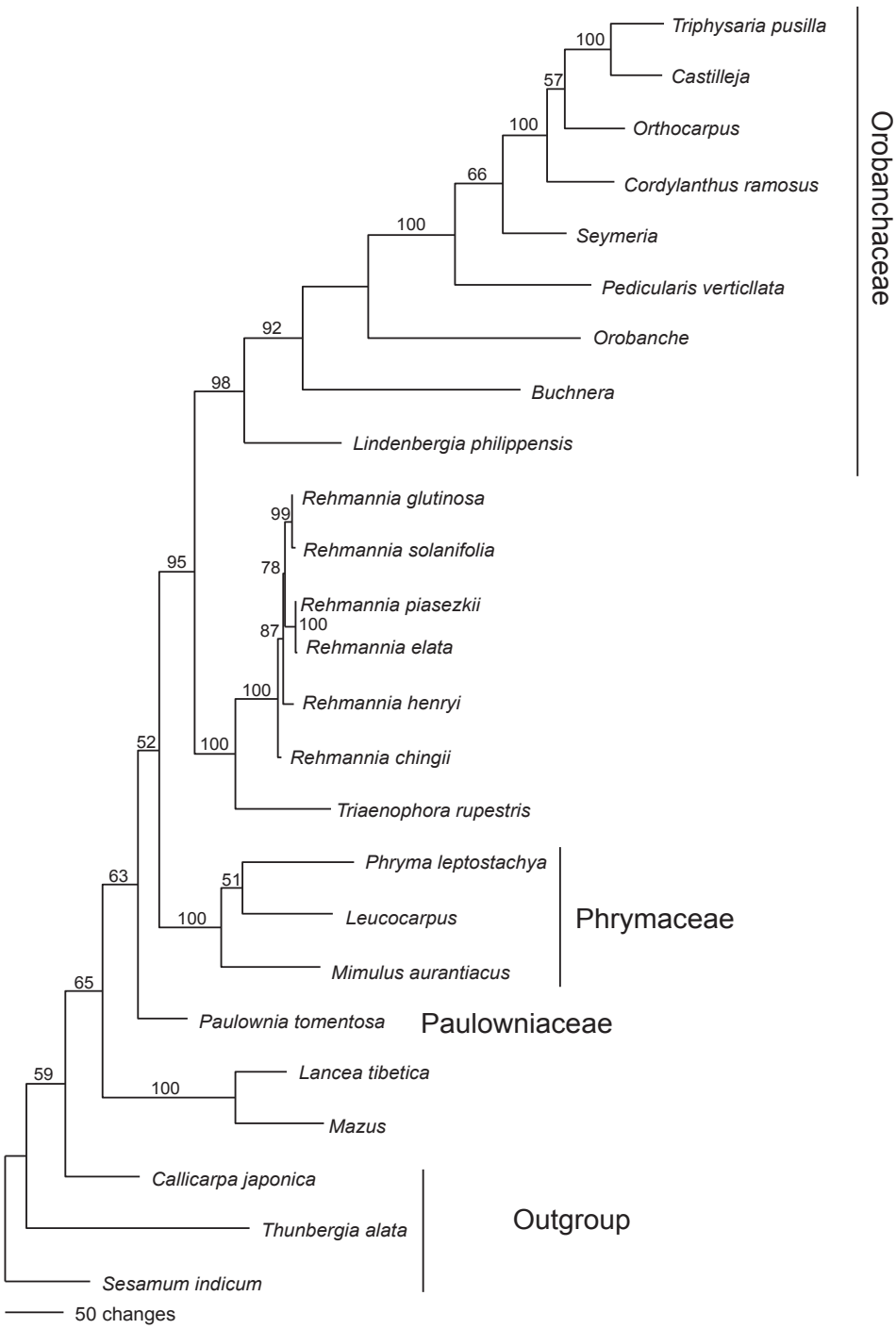

Fig. 3. Optimal tree from the focussed analysis using the *rps16* intron, the *trnL-F* region and the ITS region. Numbers above branches indicate bootstrap support. Branch lengths are scaled according to inferred evolutionary changes.

in the following as the Orobanchaceae-Phrymaceae clade for the ease of discussion.

The close relationship of *Lancea* and *Mazus* to *Rehmannia* as shown in Oxelman & al. (2005) is not supported here. In our focus analysis, we found that these two genera are sister to the remaining Orobanchaceae-Phrymaceae with 95% BS for the clade. This confirmed that *Lancea* and *Mazus* should be excluded from Phrymaceae (Beardsley & Olmstead, 2002; Oxelman & al., 2005) but further analysis is warranted.

Relationships within Orobanchaceae are generally congruent with previous analyses (e.g., Oxelman & al., 1999; Olmstead & al., 2001; Bennett & Matthews, 2006) with *Lindenbergia* sister or nested among the first branching Orobanchaceae. *Lindenbergia* differs from other Orobanchaceae by being non-parasitic but shares certain floral characters with Orobanchaceae (rhinanthoid corolla aestivation, reflexed corolla lobes; Hartl, 1955). An analysis of the distribution of iridoid glucosides in this clade (Fig. 3) shows that both *Rehmannia*/*Triaenophora* and most Orobanchaceae (incl. *Lindenbergia phillipensis* Benth. [Kooiman, 1970]) as well as *Paulownia* (Damtoft & Jensen, 1993) are mainly characterized by aucubin and catalpol and their derivatives while they lack harpagide and 6-rhamnopyranosyl-catalpol and their esters, the latter being characteristic for many Scrophulariaceae s.str. Apparently, neither *Mazus* nor *Phryma* seem to have been investigated for iridoids, but *Lancea tibetica* (Su & al., 1999) and *Mimulus cardinalis* (Jensen, unpub.) do not contain them, while *Dodartia orientalis* (Maksudov & al., 1995) and *Leucocarpus perfoliatus* (Ozaki & al., 1979) only contain biosynthetically primitive iridoid glucosides. Since most genera in Lamiales are characterised by the presence of the biosynthetically advanced iridoid aucubin and/or catalpol, this might indicate that ancient members of Phrymaceae at one point lost the ability to biosynthesize iridoids, and later, some of them regained the ability but only to a limited degree.

The current analysis raises questions regarding the familial status of some former genera of Scrophulariaceae, namely *Lancea*, *Mazus*, *Rehmannia* and *Triaenophora*. While it is clear that they are not closely related to Scrophulariaceae and should not be considered part of that family, their incorporation in some other family does not seem to be justified and none of them has been described in its own family so far. *Lancea* and *Mazus* are the first branching clade in the Orobanchaceae-Phrymaceae clade and cannot be included in any existing family under the rule of monophyly. The situation differs for *Rehmannia* and *Triaenophora*, which could be included in Orobanchaceae, with which it forms a strongly supported clade. The inclusion, however, does not seem to be justified based on morphological grounds, although neither is the inclusion of *Lindenbergia* in Orobanchaceae (Young & al., 1999).

As mentioned above, Speta (1979) raised some doubt about the inclusion of *Rehmannia* in Digitalideae based on the occurrence of protein crystal stacks in *Rehmannia* rather than round crystals as in other Digitalideae. Interestingly, such crystal stacks are also found in *Mimulus*, *Paulownia*, *Lindenbergia* and *Odontites* (Orobanchaceae) but also some taxa now considered members of Scrophulariaceae s.str. (Speta, 1979).

The resulting phylogenetic hypothesis presented here bears serious implications for our understanding of the evolution of the group. Whereas a detailed morphological or phytochemical analysis of the group still awaits more detailed studies of the corresponding characters in key taxa, the present contribution reveals an important biogeographic pattern with an origin of the Orobanchaceae-Phrymaceae clade in the Eastern Himalayan region. This is supported by the restriction of *Paulownia*, *Triaenophora* and *Rehmannia* to this region and *Lancea* and *Mazus* having its centre of diversity and centre of distribution in China. *Lindenbergia* is proposed to have originated in this region as well (Hjertson, 1995). Other early branching Orobanchaceae likewise occur in this area (e.g., *Monochasma*, *Siphonostegia*; Bennett & Matthews, 2006). A biogeographic scenario for Phrymaceae is currently not available but the present analysis is compatible with the idea of an origin in Eastern Asia, where *Phryma* is still found today, and subsequent dispersals and diversification in Australia and North America (Beardsley & Olmstead, 2002).

The phylogenetic hypothesis also has some implications regarding chromosome number evolution. Schneeweiss & al. (2004) hypothesized an origin of *Orobanche* from an ancestor with $x = 5$ and $x = 6$ based on $x = 12$, 19, and 20 being the most commonly found numbers in *Orobanche* and relatives. Such an ancestor, however, seems to be older than the most recent common ancestor of all members of the Orobanchaceae-Phrymaceae clade, because all of them have higher chromosome numbers. *Lindenbergia* has $x = 16$ (Hjertson, 1995), *Rehmannia*/*Triaenophora* have $x = 14$ (see above), *Paulownia* has $n = 20$ (*IPCN*), Phrymaceae have ancestrally $n = 8$ (Beardsley & al., 2004) and *Mazus* has $x = 10$ (*IPCN*). Comparing these numbers to the phylogeny in Fig. 3, one may infer that a polyploidization based on $n = 10$ gave rise to the most recent common ancestor of Orobanchaceae, *Rehmannia*/*Triaenophora* and *Paulownia*. This hypothesis will need to be substantiated in the future.

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LITERATURE CITED

Albach, D.C., Li, H.Q., Zhao, N. & Jensen, S.R. 2007. Molecular systematics and phytochemistry of *Rehmannia* (Scrophulariaceae). *Biochem. Syst. Ecol.* 35: 293–300.

Albach, D.C., Meudt, H.M. & Oxelman, B. 2005. Piecing together the "new" Plantaginaceae. *Amer. J. Bot.* 92: 297–315.

Albach, D.C., Soltis, P.S., Soltis, D.E. & Olmstead, R.G. 2001. Phylogenetic analysis of asterids based on sequences of four genes. *Ann. Missouri Bot. Gard.* 88: 163–210.

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Bot. J. Linn. Soc.* 141: 399–436.

Beardsley, P.M. & Olmstead, R.G. 2002. Redefining Phrymaceae: the placement of *Mimulus*, tribe Mimuleae, and *Phryma*. *Amer. J. Bot.* 89: 1093–1102.

Beardsley, P.M., Schoenig, S.E., Whittall, J.B. & Olmstead, R.G. 2004. Patterns of evolution in western North American *Mimulus* (Phrymaceae). *Amer. J. Bot.* 91: 474–489.

Bennett, J.R. & Mathews, S. 2006. Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. *Amer. J. Bot.* 93: 1039–1051.

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Källersjö, M. & Barkhordarian, E. 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molec. Phylog. Evol.* 24: 274–301.

Damtoft, S. & Jensen, S.R. 1993. Tomentoside and 7-hydroxytomentoside, two iridoid glucosides from *Paulownia tomentosa*. *Phytochemistry* 34: 1636–1638.

Doyle, J.L. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11–15.

Gascuel, O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molec. Biol. Evol.* 14: 685–695.

Hartl, D. 1955. Das Vorkommen rhinanthoider Knospendeckung bei *Lindenbergia* Lehm., einer Gattung der Scrophulariaceae-Antirrhinoideae. *Pl. Syst. Evol.* 102: 80–83.

Hjertson, M.L. 1995. Taxonomy, phylogeny and biogeography of *Lindenbergia* (Scrophulariaceae). *Bot. J. Linn. Soc.* 119: 265–321.

Hong, D.Y., Yang, H., Jin, C.L. & Holmgren, N.H. 1998. Scrophulariaceae. Pp. 1–212 in: Wu, Z.Y. & Raven, P.H. (eds.), *Flora of China*, vol. 18. Science Press, Beijing.

Jeanmougin, F., Thompson, J.D., Gouy, M. & Higgins D.G. & Gibson T.J. 1998. Multiple sequence alignment with Clustal X. *Trends Biochem. Sci* 23: 403–405.

Jensen, S.R., Li, H.-Q., Albach, D.C. & Gotfredsen, C.H. 2008. Phytochemistry and molecular systematics of *Triaenophora rupestris* and *Oreosolen wattii* (Scrophulariaceae). *Phytochemistry* 69: 2162–2166.

Judd, W.S. & Olmstead, R.G. 2004. A survey of tricolpate (eudicot) phylogenetic relationships. *Amer. J. Bot.* 91: 1627–1644.

Kooiman, P. 1970. The occurrence of iridoid glucosides in the Scrophulariaceae. *Acta Bot. Neerl.* 19: 329–340.

Kornhall, P., Heidari, N. & Bremer, B. 2001. Selagineae and Manuleeae, two tribes or one? Phylogenetic studies in the Scrophulariaceae. *Pl. Syst. Evol.* 228: 199–218.

Li, H.L. 1948. A revision of the genus *Rehmannia*. *Taiwania* 1: 71–82, pl. I–V.

Li, X.D. & Li, J.Q. 2006. Morphology characters of leaf epidermis of the genera *Rehmannia* and *Triaenophora*. *J. Wuhan Bot. Res.* 24: 559–564. [In Chinese with English abstract.]

Li, X.D., Ge, J.W. & Zan, Y.Y., Li, J.Q. 2008. A new synonym of *Triaenophora* (Scrophulariaceae) from China. *J. Wuhan Bot. Res.* 26: 362–365.

Li, X.D., Li, J.Q. & Zan, Y.Y. 2005. A new species of *Triaenophora* (Scrophulariaceae) from China. *Novon* 15:559–561.

Li, X.D., Li, J.Q. & Zan, Y.Y. 2007. Allozymic variability in *Rehmannia* and *Triaenophora* (Scrophulariaceae). *Acta Phytotax. Sin.* 45:561–569. [In Chinese with English abstract.]

Maksudov, M.S., Faskhutdinov, M.F., Umarova, R.U. & Saatov, Z. 1995. Iridoids of *Incarvillea olgae* and *Dodartia orientalis*. *Khim. Prir. Soedin.* 5: 751–752.

Manen, J.-F., Habashi, C., Jeanmonod, D., Park, J.-M. & Schneeweiss, G.M. 2004. Phylogeny and intraspecific variability of holoparasitic *Orobanche* (Orobanchaceae) inferred from plastid *rbcL* sequences. *Molec. Phylog. Evol.* 33: 482–500.

Morrison, D.A. 2007. Increasing the efficiency of searches for the maximum likelihood tree in a phylogenetic analysis of up to 150 nucleotide sequences. *Syst. Biol.* 56: 988–1010.

Olmstead, R.G., dePamphilis, C.W., Wolfe, A.D., Young, N.D., Elisens, W.J. & Reeves, P.A. 2001. Disintegration of the Scrophulariaceae. *Amer. J. Bot.* 88: 348–361.

Olmstead, R.G. & Reeves, P.A. 1995. Evidence for the polyphyly of the Scrophulariaceae based on chloroplast *rbcL* and *ndhF* sequences. *Ann. Missouri Bot. Gard.* 82: 176–193.

Oxelman, B., Kornhall, P., Olmstead, R.G. & Bremer, B. 2005. Further disintegration of the Scrophulariaceae. *Taxon* 54: 411–425.

Ozaki, Y., Johne, S. & Hesse, M. 1979. Natural organic substances. 174. Iridoid glucosides from *Leucocarpus perfoliatus* G. Don. *Helv. Chim. Acta* 62: 2708–2711.

Posada D. 2006. ModelTest Server: a web-based tool for the statistical selection of models of nucleotide substitution online. *Nucleic Acids Res.* 34: W700–W703

Posada, D. & Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Schneeweiss, G.M., Palomeque, T., Colwell, A.E. & Weiss-Schneeweiss, H. 2004. Chromosome numbers and karyotype evolution in holoparasitic *Orobanche* (Orobanchaceae) and related genera. *Amer. J. Bot.* 91: 439–448.

Smith, J.F., Brown, K.D., Carroll, C.L. & Denton, D.S. 1997. Familial placement of *Cyrtandromoea*, *Titanotrichum* and *Sanango*, three problematic genera of the Lamiales. *Taxon* 46: 65–74.

Solereder, H. 1909. Über die Gattung *Rehmannia*. *Ber. Deutsch. Bot. Ges.* 27: 390–404.

Speta, F. 1979. Weitere Untersuchungen über Proteinkörper in Zellkernen und ihre taxonomische Bedeutung. *Pl. Syst. Evol.* 132: 1–26.

Swofford, D.L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*, version 4b10. Sinauer, Sunderland.

Su, B.N., Zhu, Q.X., Gao, K., Yuan, C.S. & Jia, Z.J. 1999. Lignan and phenylpropanoid glycosides from *Lancea tibetica* and their antitumor activity. *Pl. Med.* 65: 558–561.

Sun, Y., Skinner, D.Z., Liang, G.H. & Hulbert, S.H. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theor. Appl. Genet.* 89: 26–32.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three noncoding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Wang, L. & Wang, Y.Z. 2005. Floral development of *Triaenophora* (Veronicaceae) and phylogenetic implications. *Pl. Syst. Evol.* 250: 69–79.

Wettstein, R. von. 1891. Scrophulariaceae. Pp. 39–107 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, vol. IV(3b). Engelmann, Leipzig.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1991. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M., Gelfand, D., Sninsky, J. & White, T. (eds.), *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego.

Xia, Z., Wang, Y.-Z. & Smith, J.F. 2009. Familial placement and relationships of *Rehmannia* and *Triaenophora* (Scrophulariaceae sensu lato) inferred from five different gene regions. *Amer. J. Bot.* 96: 519–530.

Yan, K., Zhao, N. & Li, H.Q. 2007. Systematic relationships among *Rehmannia* (Scrophulariaceae) species. *Acta Bot. Boreal.-Occid. Sin.* 27: 1112–1120. [In Chinese with English abstract.]

Young, N.D., Steiner, K.E. & dePamphilis, C.W. 1999. The evolution of parasitism in Scrophulariaceae/Orobanchaceae: plastid gene sequences refute an evolutionary transition series. *Ann. Missouri Bot. Gard.* 86: 876–893.

Appendix. Taxon table with GenBank accession numbers. An asterisk after the accession number indicates sequences here reported for the first time.

Taxon; accession number of *rbcL*; *ndhF*; *rps16*; *trnL-F*; ITS

Acanthus montanus T. Anderson; L12592; AJ429115; –; –; –. *Acanthus sennii* Chiov.; –; –; DQ059148; –; –. *Aeginetia indica* L.; –; –; EU572719*; EU572720*; AY596819. *Boschniakia himalaica* Hook. f. & Thoms.; –; –; EU366156*; EU366157*; –. *Boschniakia strobilacea* A. Gray; –; –; –; –; AY911215. *Brandisia hancei* Hook. f.; EU366160*; AJ619577 & AJ619578; AJ609205; –; –. *Buchnera cruciata* Buch.-Ham. ex D. Don.; –; –; EU366155*; EU366154*; –. *Buchnera glabrata* Benth.; –; –; –; –; AY911216. *Buddleja davidii* Franchet; L14392; L36394; AJ609204; –; –. *Calceolaria mexicana* Benth.; –; –; AJ609202; –; –. *Calceolaria* sp.; AF123669; AF123679; –; –; –. *Callicarpa japonica* Thunb. –; –; AJ505413; AJ505536; FM163230. *Catalpa fargesii* Bur.; –; –; DQ532491; –; –. *Catalpa* sp.; L11679; L36397; –; –; –. *Capsicum annuum* L.; –; –; EU366158*; –; –. *Capsicum baccatum* L.; U08610; U08916; –; –; –. *Castilleja elmeri* Fern.; –; –; EF103780; EF103858; EF103709. *Castilleja sulphurea* Rydb.; –; –; –; AF479008; AF478944. *Ceratotheca triloba* (Bernh.) Hook. f.; AY919277; AY919281; AF482534; –; –. *Cordylanthus ramosus* Nutt. ex Benth; –; –; EF103803; EF103881; EF103725. *Digitalis obscura* L.; –; –; AY218799; –; –. *Digitalis purpurea* L.; L01902; AF130150; –; –; –. *Gratiola pilosa* Michx.; AF026827; AF188183; –; –; –. *Gratiola neglecta* Torr.; –; –; AY727433; –; –. *Lamium purpureum* L.; U75702; U78694; AJ609175; –; –. *Lancea tibetica* Hook. f. & Thomson; –; –; AJ609174; AF479003; AF478939. *Lantana camara* L.; AF156736; –; EU348856*; –; –. *Lantana horrida* H.B.K.; –; AF130152; –; –; –. *Leucocarpus alatus* G. Don; –; –; AJ609173; –; –. *Leucocarpus perfoliatus* (Kunth) Benth.; –; –; –; AY575545; AY575453. *Ligustrum vulgare* L.; DQ673302; DQ673276; AF225257; –; –. *Lindenbergia philippensis* Benth.; AF123664; AF123686; AJ609169; AJ608586; AY911231. *Lindernia antipoda* (Linn.) Alston.; AB259810; –; –; –; –. *Lindernia brevidens* Skan.; –; –; AY492213; –; –. *Lindernia dubia* (L.) Pennell; –; EF527446; –; –; –. *Mazus reptans* N.E. Br.; –; –; –; AF479004; AF478940. *Mazus stachydifolius* (Turcz.) Maxim.; EU348860*; AJ619559; EU348858* –; –. *Mimulus aurantiacus* Curtis; AF026835; AF188186; AJ609163; AF478982; AF478917. *Mimulus ringens* L.; –; –; –; AY575546; AY575454. *Myoporum mauritianum* A. DC.; L36445; L36403; –; –; –. *Myoporum montanum* R. Br.; –; –; AJ431059; –; –. *Nemesia strumosa* Benth.; AF123663; AF123688; AJ609159; –; –. *Odontites serotinus* Dumort.; –; –; –; AY881136; AY881141. *Orobanche coerulescens* Steph.; –; –; –; AY881137; AY209235. *Orobanche hederae* Vaucher ex Duby; –; –; AJ431050; –; –. *Orthocarpus bracteosus* Benth.; –; –; –; –; AY911243. *Orthocarpus tenuifolius* (Pursh) Benth.; –; –; EF103812; EF103890; –. *Paulownia tomentosa* Steud.; L36447; L36406; AJ431051; AF479005; AF478941. *Pedicularis foliosa* L.; AF026836; AF123689; –; –; –. *Pedicularis verticillata* L.; –; –; EU348857*; EU348859*; AY596818. *Phryma leptostachya* L.; U28881; AJ617586; DQ532451; DQ532486; DQ533808. *Plantago argentea* Chaix; –; –; AJ431056; –; –. *Plantago rugelii* Decne.; AJ389597; AY818915; –; –; –. *Plectranthus barbatus* Andr.; U28882; U78698; –; –; –. *Plectranthus cylindraceus* Hochst. ex Benth.; –; –; AJ505415; –; –. *Proboscidea louisiana* (Mill.) Thell.; L01946; AF123690; AJ609146; –; –. *Rehmannia chingii* H.L. Li.; EF544598*; EF522187*; DQ856488; DQ856494; DQ069313. *Rehmannia elata* N.E. Br.; –; –; DQ856490; DQ856496; DQ069315. *Rehmannia glutinosa* (Gaert.) Libosch. ex Fisch. & C.A. Mey.; –; –; DQ856491; DQ856497; DQ069312. *Rehmannia henryi* N.E. Br.; –; –; DQ856491; DQ856497; DQ272447; *Rehmannia piasezkii* Maxim.; –; –; DQ856489; DQ856495; DQ069316. *Rehmannia solanifolia* Tsoong & Chin; –; –; DQ856486; DQ856492; DQ069314. *Sanango* sp.; AJ001763; AF027283; AJ609142; –; –. *Schlegelia parviflora* (Oerst.) Monach.; L36448; L36410; AJ431057; –; –. *Scrophularia arguta* Ait.; –; –; AJ431061; –; –. *Scrophularia* sp.; L36449; L36411; –; –; –. *Selago thomsonii* Rolfe; –; –; AJ431060; –; –. *Selago thunbergii* Choisy; L36450; L36412; –; –; –. *Sesamum indicum* L.; L14408; L36413; AJ609226; AF479010; AF478946. *Seymeria laciniata* (M. Martens & Galeotti) Standl.; –; –; EF103820; EF103898; –. *Seymeria pectinata* (Pursh.) Kuntze; –; –; –; –; AY911253. *Stilbe albiflora* E. Mey.; –; AF027287; –; –; –. *Stilbe ericoides* (L.) L.; –; –; AJ431062; –; –. *Stilbe vestita* P.J. Bergius; Z68827; –; –; –; –. *Streptocarpus caulescens* Vatke; –; –; AJ431043; –; –. *Streptocarpus holstii* Engl.; L14409; L36415; –; –; –. *Tetrachondra patagonica* Skottsberg; AF254787; AF027272; AJ431064; –; –. *Thunbergia alata* Bojer; –; U12667; AJ609131; AJ608564; AF169850. *Thunbergia mysorensis* (Wight) T. Anderson ex Bedd.; AY008828; –; –; –; –. *Torenia baillonii* God.-Leb.; AB259805; AJ617583; AY492227; –; –. *Triaenophora rupestris* (Hemsl.) Solereder.; EF544599*; EF522186*; EF522183*; EF522184*; EF522182*. *Triphysaria pusilla* (Benth.) T.I. Chuang & Heckard; –; –; EF103816; EF103894; EF103738. *Verbascum arcturus* L.; –; –; AJ609128; –; –. *Verbascum thapsus* L.; L36452; L36417; –; –; –. *Verbena bonariensis* L.; L14412; –; –; –; –. *Verbena bracteata* Cav. ex Lag. & Rodr.; –; L36418; –; –; –. *Verbena officinalis* L.; –; –; AF225295; –; –. *Veronica montana* L.; –; –; AY218824; –; –. *Veronica persica* Poir.; L36453; L36419; –; –; –.