# Origin and phylogenetic relationships of the Old World Gesneriaceae with actinomorphic flowers inferred from ITS and *trnL-trnF* sequences

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**Abstract** The phylogenetic placement of the Old World Gesneriaceae genera *Ramonda*, *Conandron*, *Bournea*, *Thamnocharis*, and *Tengia*, all characterized by actinomorphic flowers, has been the subject of much debate. Actinomorphy in Gesneriaceae is rare, with most species exhibiting zygomorphic flowers. The actinomorphic genera have historically been considered "primitive" and lumped in the tribe Ramondeae separate from the remaining Old World Gesneriaceae. In this study, we used nuclear (ITS) and plastid (*trnL-F*) DNA for molecular phylogenetic analysis of these five genera along with representative species across the Cyrtandroideae. Our results show that the actinomorphic genera are scattered over several otherwise zygomorphic clades within Cyrtandroideae, and along with previous data, indicate that Ramondeae is an unnatural group. Floral actinomorphy has evolved convergently in different alliances of Old World Gesneriaceae. *Ramonda* is sister to *Haberlea*, *Bournea* is apparently paraphyletic, *Conandron* seems rather isolated, and *Tengia* is close to *Petrocodon* and sister to a group of *Chirita* sect. *Gibbosaccus* together with *Calcareoboea*. We hypothesize that the evolution from zygomorphy to actinomorphy with novel combinations of characters is possibly due to shifts in pollination strategies, such as a switch from nectar- to pollen-rewards.

**Keywords** flower actinomorphy; Gesneriaceae; nrDNA ITS; phylogeny; polyphyly; *trnL-F*

## ■ INTRODUCTION

Flower actinomorphy in Gesneriaceae was first given taxonomic attention by Fritsch (1893–94). He placed *Ramonda* at the beginning of his treatment of Gesneriaceae in subfamily Cyrtandroideae, and in tribe Ramondeae, believing that it was the most ancestral genus of the family. He argued that flower zygomorphy was derived from actinomorphy, paralleled by stamen reduction from five to four (with one staminode) to two (with three staminodes). However, Fritsch clearly recognized the close relationship of *Ramonda* to zygomorphic genera within the Gesneriaceae, placing *Petrocosmea* and *Saintpaulia* (both with flat-faced, slightly zygomorphic flowers) as well as *Haberlea* and *Corallodiscus* (with strongly zygomorphic corollas) in the tribe Ramondeae. Similarly, he referred *Conandron*, also with actinomorphic flowers, to the beginning of the tribe Didymocarpeae, in the monogeneric subtribe Conandrinae.

Nearly 80 years later, Burtt (1970) suggested that actinomorphic flowers were probably reversions from the zygomorphic state and could not be regarded as ancestral. During the recent decade, the reconstruction of phylogenies from DNA sequences has provided increasing evidence about the evolution of flower symmetry in asterid angiosperms (Donoghue & al., 1998; Ree & Donoghue, 1999; Reeves & Olmstead, 2003; Smith & al., 2004). Molecular phylogenies show that taxa with zygomorphic flowers have evolved several times independently from actinomorphic ancestors within the Asteridae, and zygomorphy also frequently reverted to actinomorphy, especially in the Lamiales (Donoghue & al., 1998; Donoghue & Ree, 2000; Reeves & Olmstead, 2003). Notwithstanding, W.T. Wang (1990) and W.T. Wang & al. (1992) adopted the opinion of Fritsch (1893–94), stating that the actinomorphic corolla preceded the zygomorphic one, that the short corolla tube was primitive and the longer one advanced, and the flowers with stamens all fertile preceded those with one to three staminodes. Moreover, the authors put heavy taxonomic weight on corolla symmetry, redefining tribe Ramondeae to include only the genera with actinomorphic flowers (*Ramonda* Rich., *Conandron* Siebold & Zucc., *Tengia* Chun, *Bournea* Oliv., *Thamnocharis* W.T. Wang) and considering this tribe as most primitive in subfamily Cyrtandroideae.

In clear disagreement, Burtt & Wiehler (1995) did not retain tribe Ramondeae and merged the five genera into tribe Didymocarpeae. Here, however, all genera were arranged in alphabetical order only, without any indication of relationships. Weber (2004) referred the five actinomorphic genera to the informal group of "Didymocarpoid Gesneriaceae" (= Cyrtandroideae excluding Epithemateae), with *Ramonda* attributed to the "European Genera" (containing also *Haberlea* and *Jancaea*) and the other four to the "Advanced Asiatic and Malesian Genera".

Current phylogenetic hypotheses show that flower actinomorphy (only *Bellonia* is truly actinomorphic) has evolved multiple times in parallel in subfamily Gesnerioideae (Smith & al., 2004). This raises the question whether a similar situation occurs in subfamily Cyrtandroideae. Recent molecular phylogenies of this subfamily showed that two actinomorphic genera, *Ramonda* and *Conandron*, do not form a clade (Möller

& al., 1999, 2009; Smith & al., 2004). For clarifying the systematic position and evolution of the cyrtandroid genera with actinomorphic flowers, we have undertaken a molecular phylogenetic analysis including all five actinomorphic genera and a broadly representative sampling of genera in the Cyrtandroideae. The results communicated here may help improving our understanding of the phylogeny reconstruction of the Old World Gesneriaceae and the evolution of zygomorphy and actinomorphy in this family.

## ■ MATERALS AND METHODS

**Taxon sampling. —** We sampled all five genera of the tribe Ramondeae sensu W.T. Wang (1990) including all species of *Bournea*, *Conandron*, *Tengia* and *Thamnocharis*, and two species of *Ramonda*. To examine the putative relatives of the five actinomorphic genera, we selected 44 species of 22 genera in the tribe Didymocarpeae, and representatives of four genera in Trichosporeae and two genera in Epithemateae. Species selection was based on previous morphological and molecular studies (Burtt, 1954, 1963; W.T. Wang, 1990; Burtt & Wiehler, 1995; Smith, 1996; Smith & al., 1997, 2004; Möller & al., 1999, 2009; Mayer & al., 2003; Z.Y. Li & Wang, 2004; C.N. Wang & al., 2004; Weber, 2004; Cronk & al., 2005; J.-M. Li & Wang, 2007). All sampled species of the five actinomorphic genera were newly sampled except for the *trnL-F* DNA sequence of *Ramonda myconi* retrieved from GenBank. Sampled taxa and GenBank accession numbers are listed in the Appendix. Voucher specimens of newly collected materials were deposited in the Herbarium of the Institute of Botany, Chinese Academy of Sciences (PE) except for *R. myconi* and *R. nathaliae* which were deposited in RBGE.

**DNA extraction, amplification and sequencing. —** Total genomic DNA was extracted from silica gel-dried or fresh leaf tissue using the CTAB method of Rogers & Bendich (1988) and used as the template in the polymerase chain reaction (PCR). The entire nuclear ribosomal DNA internal transcribed spacer (ITS) region, including ITS1, 5.8S subunit, and ITS2, and the chloroplast DNA region *trnL-F* were chosen for the phylogenetic analysis. These regions were amplified using the ITS primers ITS1 and ITS4 (Wendel & al., 1995) and the *trnL-F* primers c and f (Taberlet & al., 1991), respectively. Amplification products were purified with a Uniq-10 PCR Purification kit (Sangon Inc., Shanghai, China). All ITS and *trnL-F* sequences were obtained directly using MegaBACE 1000 automatic sequencer (Amersham Biosciences, Sunnyvale, Califonia, U.S.A.) following the manufacturer's protocol. The *trnL-F* was sequenced in both directions using the same primer pairs as for amplification. The ITS1 and ITS4 primers were used to sequence the ITS region in both directions, with additional sequences from internal primers CITS2 (5′ GCATTTCGCTAC GTTCTTCA 3′) and CITS3 (5′ CCATCGAGTCTTTGAAC GCA 3′) when sequences from ITS1 and ITS4 primers did not provide sufficient overlap. Since sequences of ITS and *trnL-F* sampled here were available only for one or another species in *Saintpaulia* and *Whytockia* (see Appendix), we combined sequences from the two species into a single genus in our analyses because there was evidence that the genus was monophyletic and the genus was not a primary focus of our study.

**Sequence alignment and phylogenetic analysis. —** The sequences were aligned using CLUSTAL X (Thompson & al., 1997) and adjusted manually to maximize sequence homology using BioEdit v.5.0.9 (Hall, 1999).

The ITS and *trnL-F* data were further combined into a matrix. The incongruence length difference (ILD) test (Farris & al., 1994) as implemented in PAUP* v.4.0b10 (Swofford, 2003) was performed to assess character congruence between ITS and *trnL-F*, with 1000 replicates, each with 100 random additions with TBR branch swapping. The resulting *P* value was used to determine whether the two datasets contained significant incongruence (0.05).

Parsimony analysis was carried out using maximum parsimony (MP) methods in PAUP* v.4.0b10 (Swofford, 2003). Characters and character-state changes were weighted equally and gaps were treated as missing data. Heuristic searches were performed with 1000 replicates of random addition, one tree held at each step during stepwise addition, tree-bisection-reconnection (TBR) branch swapping, MulTrees in effect, and steepest descent off. To examine the robustness of various clades, we ran a bootstrap analysis (Felsenstein, 1985) with 1000 replicates of bootstrapping using a heuristic search with 1000 replicates of random sequence addition and TBR branch swapping.

Bayesian inference (BI) analyses were conducted using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). Modeltest v.3.06 (Posada & Crandall, 1998) was employed to select an appropriate model of sequence evolution for each DNA dataset from a comparison of 56 models. Four chains of Markov chain Monte Carlo (MCMC) were each run for 10,000,000 generations, and were sampled every 10,000 generations, starting with a random tree. For each run, the first 20% of sampled trees were excluded as burn-in (burn-in = 200). In the majority rule consensus from Bayesian analysis, posterior probability (PP) was used to estimate robustness.

We first conducted cladistic analysis of the combined nrDNA ITS and cpDNA *trnL-F* data from all sampled taxa. The New World Gesnerioideae species *Sinningia incarnata* and *S. lindleyi* were chosen as outgroups for the analysis. Based on these analyses and results of previous molecular phylogenetic studies (Smith, 1996; Smith & al., 1997, 2004; Möller & al., 1999, 2009; Mayer & al., 2003; C.N. Wang & al., 2004; J.-M. Li & Wang, 2007), we conducted a further combined analysis of nrDNA ITS and cpDNA *trnL-F* data with selected taxa, focusing on clades presumably closest to the four Asiatic actinomorphic genera, and with two species of *Corallodiscus* as outgroups.

## ■ RESULTS

**Analysis of combined ITS and *trnL-F* data with all sampled taxa. —** The combined ITS and *trnL-F* datasets consisted of 1808 bp, 685 (37.89%) of which were parsimony informative.

The ILD test gave a value of $P = 0.544$, indicating that the data from the two distinct marker regions did not contain significant incongruence. Modeltest suggested that the TIM + I + G model best fits the combined data. Parsimony analyses resulted in four trees of equal length (L = 3245, consistency index, CI, = 0.507, retention index, RI, = 0.637). The strict consensus of the four MP trees was generally congruent with the majority rule consensus from the Bayesian analysis except that lineage I was sister to lineages II–VI in the Bayesian tree while lineages I–VI were a polytomy in the MP tree in clade G (Fig. 1).

The MP tree comprises seven main clades labeled A–G (Fig. 1). The first clade (A) consists of *Whytockia* and *Rhynchoglossum*, representatives of the tribe Epithemateae (BS = 100%; PP = 100%). Clade B is made up of two species of *Corallodiscus* and sister to other taxa with maximum support (BS = 100%; PP = 100%). Clade C contains the two species of the actinomorphic European *Ramonda* that are well resolved as sister to the zygomorphic European *Haberlea* (BS = 87%; PP = 99%), which is separated with maximum support (BS = 100%; PP = 100%) from the African and remaining Asiatic genera. The two African zygomorphic genera *Streptocarpus* and *Saintpaulia* form clade D that is sister to the remaining Asiatic Cyrtandroideae (BS = 99%; PP = 100%), including the Asian genera with twisted fruits, i.e., *Boea*, *Paraboea*

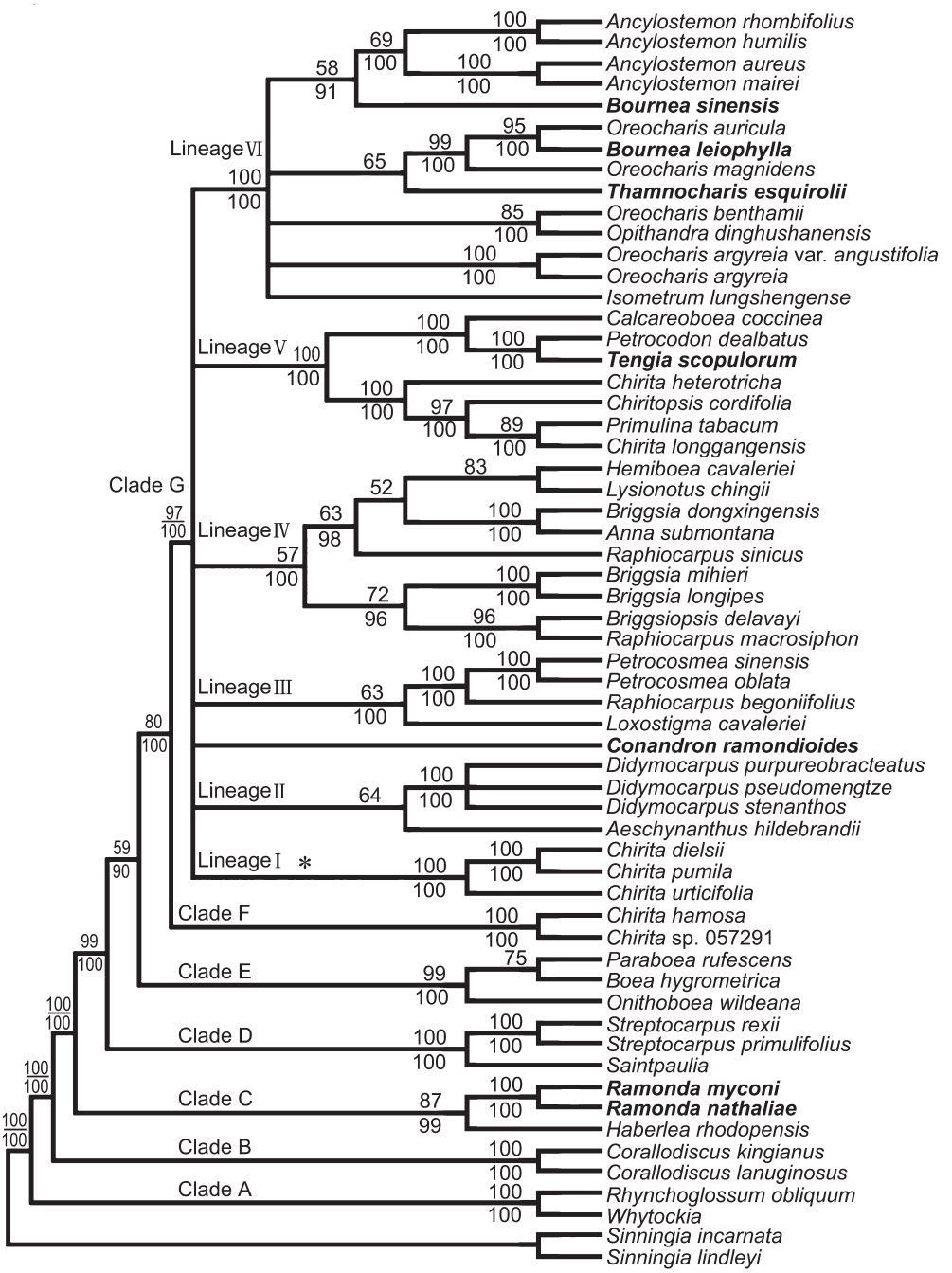


**Fig. 1.** One of four most parsimonious trees generated from analysis of combined ITS and *trnL-F* data for all sampled taxa. Bootstrap (BS) values are above the branches and Bayesian posterior probabilities (PP) below the branches. The asterisk indicates the topological discordance between MP and Bayesian tree. Actinomorphic taxa are in bold letters.

and *Onithoboea* (BS = 99%; PP = 100%). The two species of *Chirita* sect. *Microchirita* (*Chirita hamosa* and *Chirita* sp.) (BS = 100%; PP = 100%) are sister to the remaining groups (clade G) that gets strong support (BS = 97%; PP = 100%) as being monophyletic. Clade G is a polytomy of six lineages along with the monotypic actinomorphic *Conandron* isolated from other lineages. Four members of the tribe Trichosporeae (*Aeschynanthus*, *Lysionotus*, *Loxostigma*, *Anna*) are distributed among different lineages (Fig. 1) and do not constitute a monophyletic group. Lineage I contains three species of *Chirita* sect. *Chirita*, i.e., *Chirita dielsii*, *C. pumila* and *C. urticifolia*, with maximum support (BS = 100%; PP = 100%). The species of *Didymocarpus*, as a branch with maximum support (BS = 100%; PP = 100%), are barely supported (BS = 64%) as sister to *Aeschynanthus*. In lineage III, the diandrous (i.e., flower with two stamens plus three staminodes) zygomorphic *Petrocosmea* and tetrandrous (i.e., flower with four didynamous stamens plus an adaxial staminode) zygomorphic *Raphiocarpus begonifolius* form a strongly supported branch (BS = 100; PP = 100%), which is further grouped as sister to *Loxostigma* (BS = 63%; PP = 100%). Lineage IV, a barely supported clade, comprises nine zygomorphic species, representing six different genera. The two genera with more than a single sampled species are not supported as monophyletic here. In lineage V, the monotypic actinomorphic *Tengia* is sister to *Petrocodon* and together they are sister to *Calcareoboea* (BS = 100%; PP = 100%). These three genera together are sister to the species of *Chirita* sect. *Gibbosaccus* (including *Chiritopsis* and *Primulina*, see J.-M. Li & Wang, 2007 and Möller & al., 2009), i.e., *C. longgangensis*, *Primulina tabacum*, *C. heterotricha* and *Chiritopsis cordifolia*, with maximum support (BS = 100%; PP = 100%). Lineage VI receives maximum support (BS = 100%; PP = 100%) and comprises five branches in a polytomy, i.e., (1) *Isometrum*, (2) *Oreocharis argyreia* vars. *argyreia* and *angustifolia* (BS = 100%; PP = 100%), (3) *O. benthamii* and *Opithandra*, sister to each other (BS = 85%; PP = 100%), (4) two actinomorphic and two zygomorphic species, in which the pentamerous actinomorphic *Bournea leiophylla* is sister to *O. auricula* with strong support (BS = 95%, PP = 100%) and together are sister to *O. magnidens* (BS = 99%; PP = 100%) with *Thamnocharis* weakly supported as sister to all three species (BS = 65%), and (5) the tetramerous actinomorphic *Bournea sinensis* that is weakly supported as sister to the four tetrandrous zygomorphic species of *Ancylostemon* (BS = 58%; PP = 91%).

**Analysis of combined ITS and *trnL-F* data with selected taxa. —** The combined ITS and *trnL-F* matrix consisted of 1706 bp, 439 (25.73%) of which were parsimony informative. The ILD test gave a value of $P = 0.392$, indicating that the data from the two distinct marker regions did not contain significant incongruence. Modeltest suggested that the TIM+I+G model best fits the combined data. The MP analysis yielded one tree (L = 1408, CI = 0.675, RI = 0.721) that was congruent with the majority rule consensus from Bayesian analysis (Fig. 2).

In the MP tree, the monotypic actinomorphic *Conandron* is resolved in a polytomy together with lineages III+IV and V (BS = 61%; PP = 96%). Within lineage V the monotypic actinomorphic *Tengia* is well supported as sister to *Petrocodon* (BS = 99%; PP = 100%), and these two genera are sister to *Calcareoboea* (BS = 100%; PP = 100%). Together, these latter three genera are sister to the species of *Chirita* sect. *Gibbosaccus* (BS = 100%; PP = 100%; Fig. 2). Lineage VI is sister to the remainder of clade G and contains three actinomorphic species that are grouped with the sampled zygomorphic species of *Oreocharis* and *Ancylostemon*. The close relation between the pentamerous actinomorphic *B. leiophylla*, *O. auricula* and *O. magnidens* is also shown herein (BS = 92%–98%; PP = 100%), and together they are sister to *Thamnocharis*, a monotypic actinomorphic genus, with higher support (BS = 72%; PP = 90%) than in the analyses with all sampled taxa (Figs. 1–2). The tetramerous actinomorphic *B. sinensis* is better resolved as sister to *Ancylostemon* (BS = 79%; PP = 99%) while the tetrandrous zygomorphic *O. benthamii* is sister to the diandrous zygomorphic *Opithandra* with stronger support (BS = 97%; PP = 100%). Lineages I–V as a clade are not supported by Bayesian analysis (Fig. 2).

## ■ DISCUSSION

**Phylogenetic analysis. —** The monophyly of the subfamily Cyrtandroideae is well supported by both morphological and molecular data, in which the tribe Epithemateae is sister to the remainder of Cyrtandroideae (Smith & al., 1997; Smith, 2000; Y.Z. Wang & Li, 2002; Y.Z. Wang & al., 2002; Mayer & al., 2003, Möller & al., 2009). The present results based on nrDNA ITS and cpDNA *trnL-F* data are congruent with these previous studies. In addition, the position of *Corallodiscus* as sister to Cyrtandroideae less tribe Epithemateae in these trees is in perfect accordance with its morphological primitiveness. *Corallodiscus* has tetrandrous flowers and septicidally dehiscent capsules, which caused Weber (2004) to place it at the beginning of Didymocarpoid Gesneriaceae (= Cyrtandroideae excluding Epithemateae) in his informal classification. In the recent analysis by Möller & al. (2009), *Corallodiscus* is preceded by *Jerdonia*, a genus of hitherto uncertain familial affiliation. Clades C–F, i.e., the European *Ramonda* and *Haberlea*, African *Streptocarpus* and *Saintpaulia*, three Asian genera with twisted fruits and the representatives of *Chirita* sect. *Microchirita*, are in turn sister to remainder of Cyrtandroideae sampled herein, which are all in agreement with previous studies (Citerne & al., 2000; Mayer & al., 2003; Möller & al., 1999, 2009). The remaining taxa are strongly supported as a monophyletic group (clade G) and include the core groups of the tribe Didymocarpeae and four scattered members of the tribe Trichosporeae, which was defined as the Advanced Asiatic Didymocarpoid Gesneriaceae by Weber (2004) that was also supported by Möller & al. (2009). It has been widely accepted that the presence of four stamens (tetrandrous flower) is ancestral while flowers with two stamens (diandrous flower) is the derived state within Gesneriaceae. Our data here, and those of Möller & al. (2009) support this morphological transition. Apparently, diandrous flowers have evolved independently from tetrandrous flowers in different clades. This morphological shift in stamen number might be

due to the *CYC*-like gene expression that is known to result in stamen repression expanding from the adaxial stamen to lateral or ventral stamens (Gao & al., 2008; Song & al., 2009). The present results further show that the actinomorphic flowers are phylogenetically connected with both types of zygomorphic flowers, tetrandrous and diandrous, respectively.

*Ramonda* has been well resolved with two European zygomorphic genera *Haberlea* and *Jancaea* in previous molecular phylogenies (Möller & al., 1999, 2009; Mayer & al., 2003). Weber (2004) arranged the three closely related genera under "European Genera" because of their isolation from other Cyrtandroideae in both geography and morphology with fruits dehiscing septicidally, which fits well into molecular phylogenies. Möller & al. (1999) suggested that *Haberlea* might be a relic of the stock from which the actinomorphic flowers of *Ramonda* evolved, a hypothesis that is supported by our results.

In contrast, the monotypic genus *Conandron* with actinomorphic flowers is isolated in the molecular phylogeny (see also Möller & al., 2009). Correlatively, *Conandron* is remarkably morphologically distinct from other Didymocarpeae in its actinomorphic corolla with five reflexed lobes, five dorsifixed anthers connate into a tube surrounding the style with each connective having a long apical projection, and lacking a nectary (Fig. 3F; W.T. Wang, 1990; Z.Y. Li & Wang, 2004; Weber, 2004). The relatively isolated position of *Conandron* is indicative of a more ancient derived actinomorphic type that may lack extant sister relatives in the living stock of Cyrtandroideae. However, it deserves further detailed studies with additional sampling in zygomorphic taxa, which may resolve its relationship to other Cyrtandroideae.

*Tengia* has been called a "natural peloria" (Donoghue & al., 1998) because it exhibits an almost perfect actinomorphic

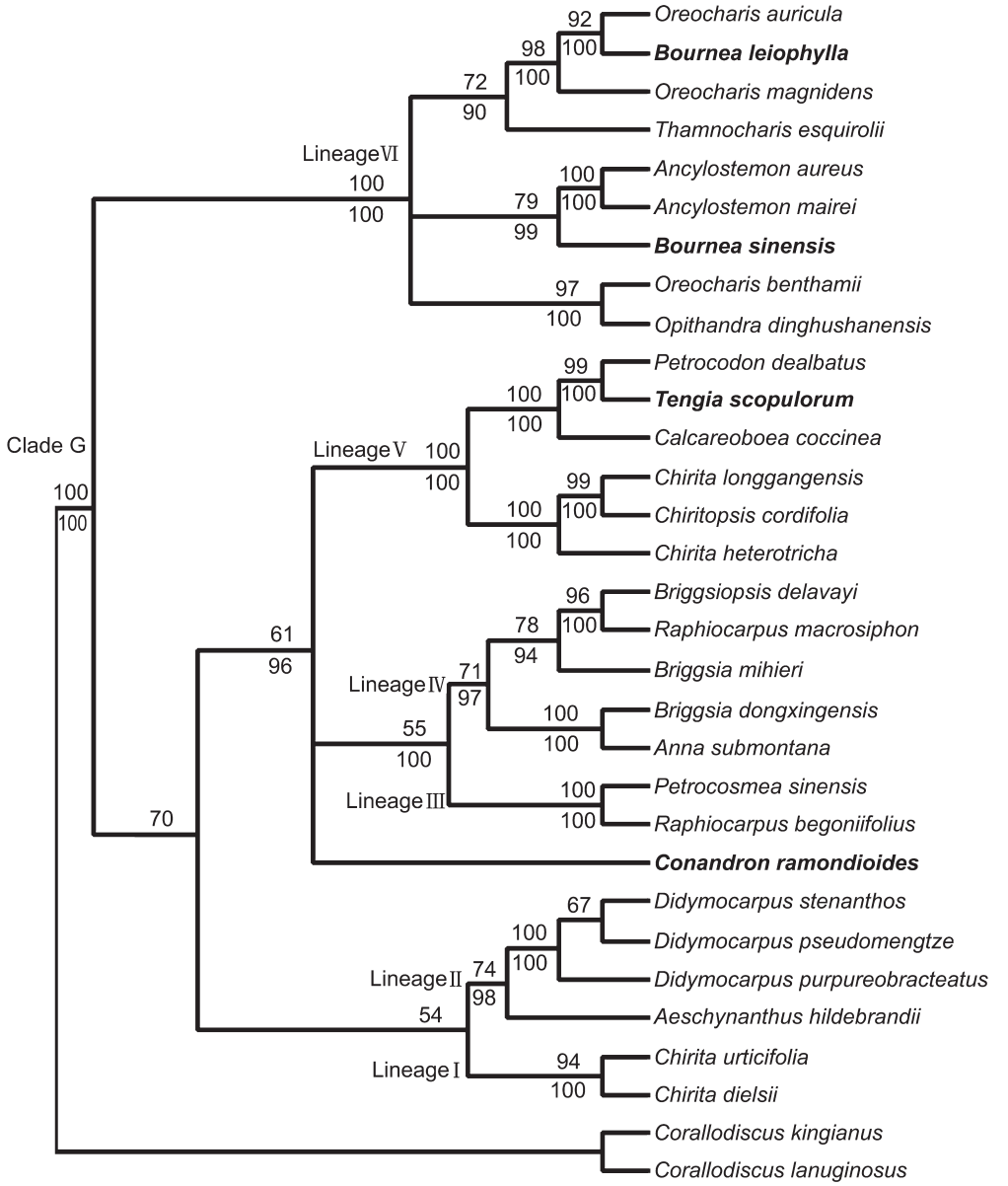


**Fig. 2.** Single most parsimonious tree generated from the combined ITS and *trnL-F* data for selected taxa. Bootstrap (BS) values are shown above the branches and Bayesian posterior probabilities (PP) indicated below the branches. Actinomorphic taxa are in bold letters.

flower from whorl one to whorl three (Z.Y. Li & Wang, 2004). It is deeply nested within the core zygomorphic groups with diandrous flowers in Didymocarpeae, in which it is sister to *Petrocodon*, and further constitutes a monophyletic group with the monotypic genus *Calcareoboea* and *Chirita* sect. *Gibbosaccus*. Plants of *Gibbosaccus* are characterized by strongly zygomorphic flowers with only the two abaxial stamens being fertile plus three staminodes at the lateral and adaxial positions (W.T. Wang & al., 1990, 1998; Z.Y. Li & Wang, 2004). *Calcareoboea* is similar to *Gibbosaccus* in androecium while it has a specialized bilabiate corolla with upper (adaxial) lip of four short teeth and lower (abaxial) lip of a tongue-like single patent lobe (Fig. 3B–C; Z.Y. Li & Wang, 2004; Weber, 2004). The short teeth emerging from the top of the highly fused corolla tube is the synapomorphy shared among the three genera, i.e., *Tengia*, *Petrocodon* and *Calcareoboea* (Fig. 3B–E). *Petrocodon* further exhibits a morphologically transitional form between *Tengia* and *Calcareoboea*, in which its corolla is almost actinomorphic, similar to that of *Tengia* (Fig. 3D–E), while its androecium consists of two fertile stamens at the abaxial position and three staminodes at the lateral and adaxial positions as the androecium of *Calcareoboea* and *Gibbosaccus* (Z.Y. Li & Wang, 2004).

Lineage VI is relatively isolated from other sampled taxa. The tetrandrous flowers in lineage VI, i.e., *Oreocharis*, *Ancylostemon* and *Isometrum*, often have relatively small flowers with narrow cylindric corolla tubes and weakly zygomorphic corolla lobes (W.T. Wang & al., 1990; Z.Y. Li & Wang, 2004). The small flower with narrow corolla base and small petals (or corolla lobes) in the actinomorphic species, i.e., *Thamnocharis*, *Bournea leiophylla* and *B. sinensis*, suggests an evolutionary connection between them and the related zygomorphic taxa in this lineage. In addition, the two zygomorphic genera *Oreocharis* and *Ancylostemon* are widely distributed geographically with a continuous range from southwestern to central and southeastern China (Z.Y. Li, 1996; Z.Y. Li & Wang, 2004). In contrast, the three actinomorphic species are geographically disjunct and remotely scattered over the distribution areas of the two zygomorphic genera. *Thamnocharis* is restricted to a mountain in the southwest of China; *Bournea leiophylla* is restricted to two counties in Fujian, and *B. sinensis* is scattered in western Guangdong, in the southeast of China (Z.Y. Li, 1996). Both nrDNA and cpDNA sequence data strongly support a sister relationship between *Bournea leiophylla*, *Oreocharis auricula* and *O. magnidens*. However, the tetramerous actinomorphic *B. sinensis* forms a branch with *Ancylostemon*, indicating that *Bournea* is paraphyletic with convergent characters of floral actinomorphy. *Opithandra*, a curious diandrous zygomorphic genus with the two lateral

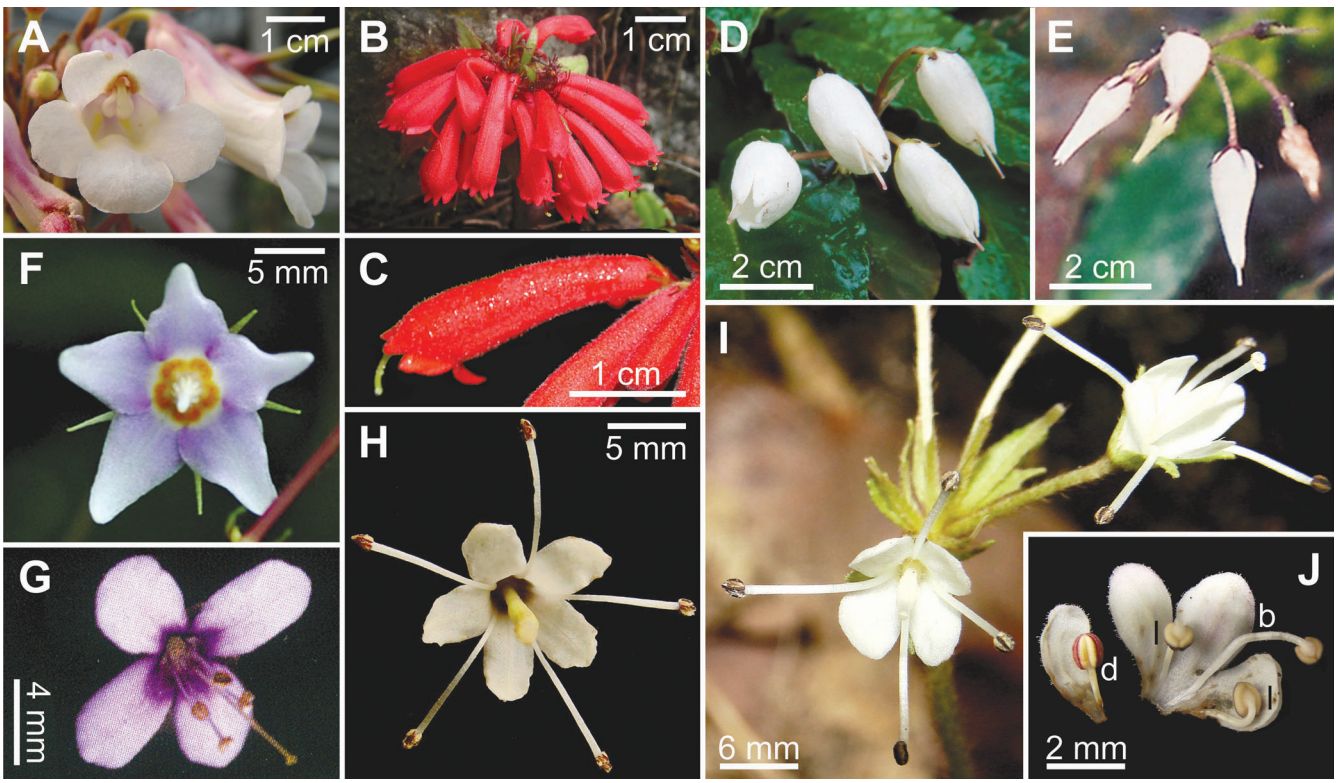


**Fig. 3.** Representative flowers of seven genera in this analysis. **A–E,** morphological shift from zygomorphic flowers of *Chirita* through *Calcareoboea* and *Petrocodon* to the complete peloria of *Tengia*: **A,** *Chirita heterotricha*; **B–C,** *Calcareoboea coccinea*; **D,** *Petrocodon dealbatus* Hance; **E,** *Tengia scopulorum* Chun. **F–J,** flower morphology of actinomorphic genera *Conandron*, *Bournea* and *Thamnocharis*: **F,** *Conandron ramondioides*; **G,** *Thamnocharis esquirolii* (Lévl.) W.T. Wang; **H,** *Bournea leiophylla* W.T. Wang & K.Y. Pan; **I–J,** mature flowers **(I)** and opened corolla of developing flower **(J)** of *Bournea sinensis* Oliv. Abbreviations: d, adaxial petal and stamen; l, lateral petals and stamens; b, abaxial petal and stamen).

(instead of the abaxial) stamens fertile, strongly supported as sister to *O. benthamii*, indicates another evolutionary link of this rare flower type with the tetrandrous flowers of *Oreocharis*, probably through a novel expression domain of *CYC*-like genes in the ventral stamens (Song & al., 2009). *Oreocharis* is polyphyletic.

As outlined above, the scattered distribution of the five actinomorphic genera both in molecular phylogeny and geography, their close sister relationship to species with zygomorphic flowers, and the placement of species with zygomorphic flowers as sister to clade G all suggest that actinomorphy is not ancestral in Gesneriaceae but is instead derived from different zygomorphic lineages (for repeated evolution of actinomorphic flowers in Gesneriaceae, see also discussion in Endress, 1998, 2001).

**The evolution from zygomorphy to actinomorphy. —** Studying morphological characters in light of the molecular phylogeny can enhance our understanding of morphological diversity in relation to the evolutionary history of these clades. Burtt (1970) suggested that *Tengia* might have been derived from some species with diandrous zygomorphic flowers such as *Didymocarpus* through *Petrocodon*. We here further recognize the exact phylogenetic lineage of *Tengia* with its related zygomorphic taxa, i.e., *Petrocodon*, *Calcareoboea* and *Chirita* sect. *Gibbosaccus*. Shifts in floral form are hypothesized to be the result of selection by pollinators (Diggle, 1992). The plants of *Gibbosaccus* usually have an open corolla mouth, with stigma and anthers located almost at the same level below or at the corolla mouth, or with the stigma slightly exserted from the corolla mouth and anthers at the corolla mouth (Fig. 3A). In contrast, in *Tengia* the nearly closed corolla with a keyhole opening from which the stigma is far exserted while all five stamens are completely included within the corolla, makes the stigma and anthers completely separated spatially (Fig. 3E). In this configuration, pollinators may contact only one set of sex organs while visiting the flower, effectively avoiding self-pollination. As Stebbins (1974) suggested, pioneer species usually experience a strong directional selection for any traits that increase the effectiveness of visitors. This combination of characters in *Tengia* might be related to new pollinators, such as small-sized insects, for cross-pollination in the moist and shady habitats that plants of *Tengia* prefer. Meanwhile, the morphological specialization of the *Calcareoboea* flowers might be related to another pollination syndrome corresponding to the long-tongued flies or bees indicated by its long and curved corolla tube (Fig. 3B–C) (Lunau, 2004; Reynolds & al., 2009).

In *Bournea* and *Thamnocharis*, their very short corolla tube with filaments adnate to the corolla, and the adaxial corolla lobes somewhat smaller than the others indicate their evolution from zygomorphic ancestors (Fig. 3G–J) (Zhou & al., 2008). In addition, the flowers of *Thamnocharis* with the two abaxial stamens longer than the others show vestigial traces of zygomorphy in androecial structure (Fig. 3G). The delay of initiation and retardation of early development of the adaxial organs in corolla and androecium in both *B. leiophylla* (Zhou & al., 2008) and *B. sinensis* (Fig. 3H–J) demonstrates a typical early zygomorphic pattern of floral development that is interpreted as a residual zygomorphy due to conserved early expression of *CYC*-like genes (Zhou & al., 2008). Furthermore, the corolla in *B. leiophylla* and *B. sinensis* is campanulate with the lobes widely spreading, while in *Thamnocharis* it is flat-faced (Fig. 3G–I). The five equal-length stamens in *B. leiophylla* are exserted horizontally, while the five or four unequal-length stamens in *Thamnocharis* are ascendant. The three actinomorphic species show some unique features, and other features are shared with zygomorphic *Oreocharis* and *Ancylostemon*. The close relationship between *B. leiophylla* and two species of *Oreocharis* suggests that the actinomorphic flowers of *B. leiophylla* have originated from the zygomorphic flowers of a branch in *Oreocharis*. This suggestion is supported by a recent finding that the altered expression patterns of the floral symmetry genes during floral development is related to the phylogenetic transition from zygomorphy to actinomorphy between *Oreocharis* and *B. leiophylla* (Du & Wang, 2008; Zhou & al., 2008). According to the molecular phylogeny herein, the relationship between *Bournea sinensis* and *Ancylostemon* is supported by our recent observation that the developing stamen filaments in *B. sinensis* frequently have a sharply inflexed apex (Fig. 3J), a feature which is characteristic of *Ancylostemon* (Burtt & Davidson, 1927; W.T. Wang & al., 1990, 1992; Z.Y. Li & Wang, 2004; Weber, 2004). *Bournea sinensis* is apparently different from *B. leiophylla* in its phylogenetic connection among the species in *Oreocharis*/*Ancylostemon* lineage, which still needs further detailed studies with additional sampling among its putative zygomorphic relatives.

As mentioned above, these actinomorphic taxa each indicate that their derived status evolved convergently from different zygomorphic lineages. Their actinomorphic flowers are mostly flat-faced or wide-campanulate without a corolla tube or with a very short corolla tube, such as *Ramonda*, *Conandron*, *Thamnocharis* and *Bournea*. The reduction of corolla tube length might be accompanied by increasing taxonomic diversity of pollinators and reduced specificity of pollen placement on pollinators' bodies (Fenster, 1991; Donoghue & al., 1998; Sargent, 2004). Actinomorphy with flat-faced flowers exhibits the general syndrome of oligandric pollen flowers related to buzz-pollination (Cronk & Möller, 1997; Harrison & al., 1999). It may apply to them with respect to the morphological evolution from zygomorphy to actinomorphy, which represents an evolutionary trend of switching to generalist pollinators. In contrast, the almost closed corolla with the stigma wholly exserted and five stamens completely included, as a novel combination of characters effectively avoiding self-pollination in *Tengia*, represents a distinctive evolutionary pathway from zygomorphy to actinomorphy, which might be related to new pollinators for cross-pollination. Parallelisms, convergent evolution and environmental plasticity all plague the use of morphological characters in systematics. Comparisons of adult structures can often be misleading because unrelated taxa may arrive at an apparently similar adult form through different developmental processes (J.-M. Li & Wang, 2007). Recent evo-devo studies reveal that a late downregulation of *BlCYC1*, as a novel event, could be responsible for the origin of the derived actinomorphy in *B. leiophylla* (Zhou & al., 2008). Detailed investigations of the genes responsible for the derived

actinomorphy with expression pattern and functional analyses will shed more light on mechanisms underlying diverse pathways of the evolution from zygomorphy to actinomorphy.

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**Appendix.** Taxon table with GenBank accession numbers. An asterisk after the accession number indicates sequences here reported for the first time. Voucher collection number, with herbarium acronym in parentheses, are given for those DNA samples that have previously not been reported in the literature.

Taxon; voucher; accession number of ITS; ***trnL-F.***

**OUTGROUP TAXA;** ***Sinningia incarnata*** (Aubl.) Denham; AY047083; AY047142. ***Sinningia lindleyi*** Schauer; AY047084; AY047143. **INGROUP TAXA;** ***Aeschynanthus hildebrandii*** Hemsl.; AY047040; AY047099. ***Ancylostemon aureus*** (Franch.) Burtt; *Liang R.H. 006*, Yunnan, China (PE); GU350657*; GU350688*. ***Ancylostemon humilis*** W.T. Wang; *Liang R.H. SC-YB*, Sichuan, China (PE); GU350633*; GU350665*. ***Ancylostemon mairei*** (Lévl.) Graib; *Liang R.H. YN-QJ*, Yunnan, China (PE); GU350658*; GU350689*. ***Ancylostemon rhombifolius*** K.Y. Pan; *Liang R.H. LRH-07-01*, Sichuan, China (PE); GU350632*; GU350664*. ***Anna submontana*** Pellegr.; FJ501362; FJ501542. ***Boea hygrometrica*** (Bunge) R. Br; FJ501319; FJ501476. ***Bournea leiophylla*** W.T. Wang & K.Y. Pan; *Zhou X.R. ZXR-05-01*, Fujian, China (PE); GU350644*; GU350676*. ***Bournea sinenis*** Oliver; *Tao J.H. TJH-06-01*, Guangdong, China (PE); GU350634*; GU350666*. ***Briggsia dongxingensis*** Chun ex K.Y. Pan; *Wen F. GX-GP*, Guangxi, China (PE); GU350655*; GU350686*. ***Briggsia longipes*** (Hemsl. ex Oliv.) Graib; *Lu Y.X. 00403*, Yunnan, China (PE); GU350653*; GU350684*. ***Briggsia mihieri*** (Franchet) Craib; *Liang R.H. CQ-JFS-03*, Yunnan, China (PE); GU350646*; GU350678*. ***Briggiopsis delavayi*** (Franchet) K.Y. Pan; *Li J.M. SC-HY-01*, Sichuan, China (PE); GU350647*; GU350679*. ***Calcareoboea coccinea*** C.Y. Wu ex H.W. Li; FJ501365; FJ501516. ***Chirita dielsii*** (Borza) Burtt; DQ872838; DQ872818. ***Chirita hamosa*** R. Br.; DQ872839; DQ872822. ***Chirita heterotricha*** Merrill; DQ872826; DQ872816. ***Chirita longgangensis*** W.T. Wang; DQ872833; DQ872809. ***Chirita pumila*** D. Don; DQ872836; DQ872819. ***Chirita urticifolia*** Buch.-Ham. ex D. Don; DQ872835; DQ872821. ***Chirita*** sp. 057291; DQ872840; DQ872823. ***Chiritopsis cordifolia*** D. Fang & W.T. Wang; DQ872845; DQ872803. ***Conandron ramondioides*** Siebold & Zucc.; *Xiao N. XN-03-39*, Zhejiang, China (PE); GU350649*; GU350681*. ***Corallodiscus kingianus*** (Craib) B.L. Burtt; *Liang R.H. SC-ML*, Sichuan, China (PE); GU350630*; GU350663*. ***Corallodiscus lanuginosus*** B.L. Burtt; *Liang R.H. YN-KM*, Yunnan, China (PE); GU350631*; GU350662*. ***Didymocarpus pseudomengtze*** W.T. Wang; *Li J.M. LJM-2005-011*, Yunnan, China (PE); GU444003*; GU444002*. ***Didymocarpus purpureobracteatus*** W.W. Smith; DQ912676; FJ501510. ***Didymocarpus stenanthos*** Clarke; DQ912687; FJ501512. ***Haberlea rhodopensis*** Frivaldszky; AF316898; AJ492296. ***Hemiboea cavaleriei*** Lévl.; FJ501355; FJ501533. ***Isometrum lungshengense*** (W.T. Wang) W.T. Wang & K.Y. Pan; *Liang R.H. GX-LG-01*, Guangxi, China (PE); GU350659*; GU350690*. ***Lysionotus chingii*** Chun ex W.T. Wang; FJ501332; FJ501498. ***Loxostigma cavaleriei*** (Lévl. Et Van.) Burtt; FJ501339; FJ501509. ***Onithoboea wildeana*** Graib; DQ865197; DQ872824. ***Opithandra dinghushanensis*** W.T. Wang; *Lin Q.B. LQB-06-01*, Guangdong, China (PE); GU350643*; GU350675*. ***Oreocharis argyreia*** Chun ex K.Y. Pan; *Wen F. GX-JX-01*, Guangxi, China (PE); GU350638*; GU350670*. ***Oreocharis argyreia*** Chun ex K.Y. Pan var. ***angustifolia*** K.Y. Pan; *Liang R.H. GX-SS-01*, Guangxi, China (PE); GU350639*; GU350671*. ***Oreocharis auricula*** (S. Moore) C.B. Clarke; *Liang R.H. GX-LG-02*, Guangxi, China (PE); GU350640*; GU350672*. ***Oreocharis benthamii*** C.B. Clarke; *Song C.F. GD-DHS*, Guangdong, China (PE); GU350642*; GU350674*. ***Oreocharis magnidens*** Chun ex K.Y. Pan; *Wen F. GX-JX-02*, Guangxi, China (PE); GU350641*; GU350673*. ***Paraboea rufescens*** (Franch.) Burtt; DQ865196; DQ872825. ***Petrocodon dealbatus*** Hance; *Li J.M. LJM-2003-104*, Guizhou, China (PE); GU350636*; GU350668*. ***Petrocosmea sinensis*** Oliv.; *Qiu Z.J. QZJ-2008-41*, Sichuan, China (PE); GU350660*; GU350691*. ***Petrocosmea oblate*** Graib; *Qiu Z.J. Q060923-1*, Sichuan, China (PE); GU350661*; GU350692*. ***Primulina tabacum*** Hance; FJ501352; AJ492300. ***Ramonda myconi*** (L.) Reichenb.; Möller-01, cult. (RBGE); GU350650*; AJ492301. ***Ramonda nathaliae*** Pancic & Petrovic; Möller-02, cult. (RBGE); GU350651*; GU350682*. ***Raphiocarpus begoniifolius*** (Léveillé) B.L. Burtt; *Liang R.H. LY-01001*, Yunnan, China (PE); GU350648*; GU350680*. ***Raphiocarpus macrosiphon*** (Hance) Burtt; *Liang R.H. GX-LC*, Guangxi, China (PE); GU350654*; GU350685*. ***Raphiocarpus sinicus*** Chun; *Liang R.H. GX-SS-02*, Guangxi, China (PE); GU350656*; GU350687*. ***Rhynchoglossum obliquum*** Blume; *Liang R.H. GX-NP-02*, Guangxi, China (PE); GU350652*; GU350683*. ***Saintpaulia brevipilosa*** B.L. Burtt; AF316924; –. ***Saintpaulia velutina*** B.L. Burtt; –; AJ492303. ***Streptocarpus primulifolius*** Gandoger; AY047039; AY047098. ***Streptocarpus rexii*** Lindl.; AF316979; AJ492305. ***Tengia scopulorum*** W.Y. Chun; *Li J.M. LJM-2004-001*, Guizhou, China (PE); GU350637*; GU350669*. ***Thamnocharis esquirolii*** (Léveillé) W.T. Wang; *Li J.M. LJM-2003-015*, Guizhou, China (PE); GU350645*; GU350677*. ***Whytockia bijieensis*** Y.Z. Wang & Z.Y. Li; AH006053; –. ***Whytockia tsiangiana*** (Hand.-Mazz.) A. Weber; –; AJ492289.