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# Floral evolutionary trend of *Petrocodon* (Gesneriaceae) and its significance in classification

LU Yong-Bin<sup>1</sup>, HUANG Yu-Song<sup>2</sup>, XU Wei-Bin<sup>1</sup>, HUANG Jie<sup>1</sup>, LIU Yan<sup>2</sup>,  
XIANG Chun-Lei<sup>3</sup>, ZHANG Qiang<sup>1\*</sup>

( 1. Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, Guangxi, China; 2. Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, Guangxi, China; 3. Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China )

**Abstract:** *Petrocodon* Hance is a genus (ca. 30 species) in the angiosperm family Gesneriaceae, which are mainly distributed in the limestone region of South China. The genus is special for its remarkable floral diversity, and is also one of the most taxonomy-difficult groups in the family. Although the genus was redefined to accommodate species with distinctive floral morphologies from eight other genera based on molecular phylogenetic studies, the evolutionary trends of the floral characters have not been studied comprehensively yet; the major causes of the incongruence between traditional taxonomy and molecular phylogenetic studies remain largely unexplored; and the morphological characters that can support the redefined *Petrocodon* keep unknown, either. In the present study, we coded 35 morphological characters (including 26 floral characters) of 19 *Petrocodon* species and 9 outgroup taxa of *Primulina*, traced the evolutionary trends and addressed the taxonomic implications in *Petrocodon* based on phylogenetic tree. We found that most of the floral characters, especially those used for delimiting traditional genera, evolved frequently and highly homoplasiously within and across the genus, which were the key factors that misled the traditional classifications. Further, we proposed that among all the characters we examined, the shape of filaments and stigmas may be used to distinguish most taxa of *Petrocodon* from its closest relative *Primulina*. The present study indicates that caution must be taken when using these floral traits in future classification of Gesneriaceae. Moreover, extensive examinations of the morphological characters and their evolutionary trends need to be carried out and traced upon denser sampling and more robust phylogenetic tree. More importantly, efforts should also be taken to investigate the underlying molecular regulatory networks and driving forces underpinning the complicated floral evolution of *Petrocodon*. All these will finally improve the understandings of the evolutionary processes and mechanism of this group of typical karst flora and its allies.

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作者简介: 卢永彬 (1987-), 女, 广西灵山人, 硕士, 研究实习员, 主要从事分子系统学研究, (E-mail) luyongbin4@foxmail.com。

\*通信作者: 张强, 博士, 副研究员, 主要从事分子系统学研究, (E-mail) qiangzhang04@126.com。

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# 石山苣苔属(苦苣苔科)花形态演化及分类学意义

卢永彬<sup>1</sup>, 黄俞淞<sup>2</sup>, 许为斌<sup>1</sup>, 黄洁<sup>1</sup>, 刘演<sup>2</sup>, 向春雷<sup>3</sup>, 张强<sup>1\*</sup>

(1. 广西喀斯特植物保育与恢复生态学重点实验室, 广西壮族自治区广西植物研究所, 广西 桂林 541006; 2. 广西植物  
中国科学院

功能物质研究与利用重点实验室, 广西壮族自治区广西植物研究所, 广西 桂林 541006; 3. 东亚植物多样性与  
中国科学院

生物地理学重点实验室, 中国科学院昆明植物研究所, 昆明 650201)

**摘要:** 石山苣苔属(苦苣苔科)约 30 种, 主要分布于我国南部的石灰岩地区。目前该属已知物种数虽少但花形态极其多样, 是该科中分类最为困难的类群之一。基于分子证据, 其它 8 个属中花形态迥异的一些物种被并入石山苣苔属。然而, 该属花形态的演化趋势缺乏系统性的研究, 传统分类对属的界定与分子系统学研究结果相矛盾的原因, 以及是否有形态特征支持新界定的石山苣苔属还不清楚。该研究中, 总共编码了 19 种石山苣苔属植物和 9 种报春苣苔属植物的 35 个形态特征, 其中包括 26 个花部形态特征, 在分子系统树上追踪了它们的演化路径。结果表明: 无论属内还是属间, 多数花部形态特征, 尤其以往属的分类界定特征, 在演化过程中变化频繁且发生了高度同塑性演化, 这是导致传统形态分类不自然的关键因素。此外, 在观察研究的所有特征中, 花丝和柱头的差异可能在石山苣苔属植物共同祖先中经历了演变, 或可用于区分石山苣苔属与其姐妹报春苣苔属的大多数种类。因此, 在苦苣苔科植物的分类学研究中应当慎用这些花部性状作为分类依据, 而且应对形态特征进行广泛地观察研究, 在密集的取样和分辨率更高、更可靠的系统树上追踪它们的演化规律。更为重要的是, 需要进一步研究导致复杂形态性状演化的内在分子调控机理和外在的自然选择动力, 最终更加深入地理解石山苣苔属等典型喀斯特植物的演化过程和机理。

**关键词:** 分类, 花部特征, 苦苣苔科, 同塑性演化, 喀斯特植物, 石山苣苔属

*Petrocodon* Hance (Gesneriaceae), firstly established in 1883, remained as a monotypic genus (only containing *Petrocodon dealbatus*) over a century. It is perennial herb and has campanulate corolla with indistinctly two-lipped limb, being different from most other Gesneriaceae, which usually possess distinctly two-lipped limb with lobes different in size and/or shape. The species is distributed in South and Central China, mostly growing in the crevices of limestone rocks. No more species of the genus were reported in the following century. Till the last decade, several new species of *Petrocodon* were finally discovered and described from South China (Wei, 2007; Wei et al, 2010).

The phylogenetic position and the boundary between *Petrocodon* s.s. and other closely-related genera were revealed by the recent molecular phylogenetic

studies (Weber et al, 2011b; Xu et al, 2014). The monotypic genera (*Calcareaoboea* C. Y. Wu ex H. W. Li, *Paralagarosolen* Wei, *Dolicholoma* D. Fang & W. T. Wang and *Tengia* Chun) and one to a few species from *Wentsaiboea* Fang & Qin, *Primulina* Hance, *Lagarosolen* Wang and *Didymocarpus* Wallich were included into *Petrocodon* and the expanded genus was indicated to be most close to *Primulina* according to the molecular phylogenetic studies based on the chloroplast *trnL-trnF* and nuclear ribosome ITS DNA sequences (Weber et al, 2011b; Xu et al, 2014). The redefinition as well as more new species recently published enlarged *Petrocodon* to contain ca. 30 species (Weber et al, 2011b; Wen et al, 2012; Chen et al, 2014; Hong et al, 2014; Xu et al, 2014; Yu et al, 2015; Guo et al, 2016; Lu et al, 2017). It is more noteworthy is that the expanded

genus represent one of the most varied genera in the Old World, comparable to those in the New World (Weber et al, 2011b).

The morphological traits of the expanded *Petrocodon*, particularly those of floral parts, are remarkably diverse among the lineages of Old World Gesneriaceae. The floral variation of *Petrocodon* covers almost all aspects of the floral characters. For example, the symmetry of corolla includes zygomorphy and almost actinomorphy; most species have corolla limb with two adaxial (dorsal) lobes and three abaxial (ventral) lobes but two species of the genus have corolla limb with a very unusual pattern of four adaxial lobes and one abaxial lobe [corresponding to the terminology of four-toothed upper lip and one-toothed lower lip according to Weber et al (2011b)]; corolla shape is variable from campanulate, tubular, funnelform, urceolate to salverform; corolla coloration is diverse, including pure white, purple, yellow and bright red; stamen numbers per flower are usually two but *Pet. scopulorum* (previous *Tengia scopulorum*) and one recently published species *Pet. huanensis* (Yu et al, 2015) belonging to the genus possess five and four fertile stamens, respectively. The general appearance of the floral diversity of *Petrocodon* is as shown in Fig. 1.

The redefinition of *Petrocodon* and the inclusion of species from other eight genera, could imply that morphological characters, particularly those diagnostic traits the traditional taxonomy was based on, might experience complicated evolution such as homoplasy (i.e. convergence, parallel or reversal evolution), dramatic morphological divergence of closely related species, and/or plesiomorphy remained in different lineages etc. However, little is known about the evolutionary trends of the diverse morphological characters in the expanded genus and their taxonomic significance, though the previous molecular phylogenetic study sampled the majority of the included species (Weber et al, 2011b; Chen et al, 2014). It remains unknown whether there is any floral or vegetative characters that can differentiate *Petrocodon* from its sister genus *Primulina* and other close relatives,

and characterize each of the lineages in the molecular phylogenetic tree within the genus. To explore these issues needs carefully examination of the diverse morphological characters of *Petrocodon*, comparing with other close relatives, and tracing their evolution based on a robust phylogenetic framework suggested by molecular phylogenetic studies.

In this study, we examined and coded 35 morphological characters (including 26 floral traits) of *Petrocodon* and some outgroup taxa of *Primulina*, traced the evolutionary trends of these characters on the phylogenetic tree reconstructed based on DNA sequences downloaded from the public accessible database of National Centre of Biological Information (NCBI). The aims we targeted to explore included: (1) the evolutionary trends how the diverse morphologies evolved; (2) the potential synapomorphy that can characterize and differentiate *Petrocodon* from its close relatives. We also discuss the implications of the inferred morphological evolution for future classification of Gesneriaceae.

## 1 Materials and Methods

### 1.1 Materials examined

A total of 35 morphological characters (including 26 floral characters) of 19 *Petrocodon* species and 9 outgroup species of *Primulina* were examined and coded numerically. The morphological characters were coded and validated according to the original description of the species, the related monographs such as *Flora of China* (Wang et al, 1998), *Plants of Gesneriaceae in China* (Li & Wang, 2005) and *Gesneriaceae of South China* (Wei et al, 2010), and the observation of transplanted individuals and/or the field investigations.

We coded different states of the same character with different numerics. For those possessing mosaic character states, we coded them with all contained states. For example, for the corolla coloration, *Pet. tiandengensis* has corolla with pale blue lobes and white tube, we coded it with these two character states (0

and 2, representing bluish violet and white, respectively.) For the characters lacking of observation and record, we treated them as missing. All the coded traits are listed in Table 1.

## 1.2 Selection of DNA markers and sampling strategy

As the plastid *trnL-trnF* intergenic spacer and nuclear ribosome internal transcribed spacer (ITS) sequences have been most frequently used for phylogenetic analysis in Gesneriaceae, particularly in the subfamily Didymocarpoideae including *Petrocodon* from the Old World (Denduangboripant et al, 2007; Wang et al, 2010; Möller et al, 2011a, b; Weber et al, 2011b; Chen et al, 2014), these two DNA fragments were therefore selected and downloaded from GenBank for the present study.

As shown by some previous studies (Weber et al, 2011b; Chen et al, 2014), the newly redefined genus *Primulina* is the closest relative to *Petrocodon*, therefore, all the *trnL-trnF* and ITS sequences of *Petrocodon* taxa and a few of *Primulina* representatives available from GenBank were included. The sequences of some other Chinese Gesneriaceae, namely *Allocheilos* W. T. Wang, and *Oreocharis mileensis* which also possess the rare corolla character of four adaxial lobes and one abaxial lobe (Fig. 2) were also included. Some other Old World Gesneriaceae, e. g. *Deinocheilos* W. T. Wang and *Gyrocheilos* W. T. Wang with another pattern of special corolla structure (unity of the adaxial lip with normal tri-partition of the abaxial lip) as well as several other *Oreocharis* species were also downloaded and included in the present phylogenetic analyses. Finally, sequences of some representative species of *Lysionotus*, *Cyrtandra* and *Streptocarpus* were included and used as the more distant outgroups according to the previous larger-scale phylogenetic analyses (Roalson & Roberts, 2016). The *trnL-trnF* data included 60 accessions representing 45 species and the ITS data encompassed 54 accessions of 44 species. The GenBank accessions of all the downloaded sequences are as listed in Table S1 in supporting information.

Table 1 Coding and scoring for the characters in this study

No.	Character	Character state
1	Habit	0 herb perennial; 1 herb annual; 2 shrub
2	Stem	0 absent; 1 decumbent; 2 erect
3	Leaf blade form	0 ovate-elliptic; 1 rounded; 2 lanceolate-linear
4	Leaf base form	0 cordate-obliquely or cordate-truncated; 1 cuneiform; 2 rounded
5	Leaf margin	0 entire; 1 non-entire
6	Leaf apex	0 rounded; 1 acute obtuse; 2 acuminate
7	Leaf indumentum	0 non-glabrous; 1 glabrous; 2 adaxial glabrous; 3 abaxial glabrous
8	Inflorescence	0 comprising solitary flower; 1 comprising two or more flowers
9	Floral symmetry	0 zygomorphy; 1 actinomorphy
10	Bract form	0 sublinear; 1 subovate
11	Calyx form	0 non-subovate; 1 subovate
12	Corolla shape	0 campanulate; 1 funnellform-infundibuliform; 2 cylindric-tubular; 3 urceolate-gyalectoid; 4 salver
13	Corolla coloration	0 bluish violet; 1 yellow; 2 white; 3 red; 4 green
14	Color marks inside corolla	0 none; 1 on upper lip; 2 on lower lip; 3 on both upper and lower lips
15	Corolla lobes	0 upper two and lower three; 1 upper four and lower one; 2 five equal lobes
16	Lobe shape	0 rounded; 1 oblong; 2 triangular
17	Lobe apex	0 subrounded; 1 acute; 2 acuminate
18	Lobe reflexed	0 yes; 1 no
19	Corolla tube	0 straight; 1 curved
20	Fertile stamen numbers	0 two; 1 four; 2 five
21	Relative stamen length	0 included; 1 extended out of the corolla tube
22	Filaments	0 straight; 1 curved; 2 geniculate
23	Filament indumentum	0 glabrous; 1 puberulent
24	Filament adnated position	0 above or near the middle; 1 near the base
25	Anther attachment position	0 dorsifixed; 1 basifixed
26	Anther's relation	0 cohesion; 1 divergence
27	Anther form	0 reniform; 1 triangular; 2 subovate
28	Staminode numbers	0 none; 1 one; 2 two; 3 three
29	Pistils	0 included; 1 protruding or equal length with corolla tube
30	Pistil indumentum	0 glabrous; 1 pubescent-pilose
31	Ovary shape	0 linear; 1 narrow elliptic-ovate-round
32	Stigma split	0 solitary; 1 bifid
33	Stigma shape	0 capitate; 1 obtapeziform (chiritooid)
34	Fruit form	0 ovate-elliptic; 1 linear-lanceolate
35	Fruit split numbers	0 none; 1 one; 2 two; 3 three; 4 four

## 1.3 Sequence assembling and phylogenetic analyses

All the sequences were aligned using the software

MUSCLE 3.8.31 (Edgar, 2004), and then manually adjusted using Bioedit 5.0.9 (Hall, 1999). The flank regions which were ambiguously aligned or possessed numerous missing nucleotides for the majority of the accessions were deleted.

The maximum likelihood (ML) in RaxML-VI-HPC (Stamatakis, 2006), maximum parsimony (MP) in PAUP 4.10 (Swofford, 2002) and the Bayesian inference (BI) in MrBayes 3.2.6 (Ronquist et al, 2012) were employed to reconstruct the phylogenies. First, we conducted the ML analyses for each of the two regions under the settings of 1 000 rapid bootstrap searches and thereafter a thorough ML search with the substitution model GTR+G. The congruence of the phylogenetic signals of the two loci were assessed by comparing the two ML majority-rule consensus trees using a bootstrap support value of 70% as an arbitrary threshold, meaning that the nodes showing incongruent relationships between *trnL-trnF* and ITS ML majority-rule consensus trees with bootstrap support value above 70% were assumed to be strong phylogenetic conflict and against combined analysis of the two loci. For the ML analysis of the combined data, the same parameters were settled. For the MP analysis of the combined data, the MP settings were heuristic searches of 1 000 replicates of random sequence addition, tree bisection and reconnection (TBR) swapping and all MP trees saved at each replicate (Multree on); for the bootstrap analysis, 1 000 bootstrap pseudo-replicates were conducted, each with 10 replicates of random sequence addition and a maximum of 5 000 trees saved for each bootstrap pseudo-replicate. For the BI analysis, 100 000 000 generations were run with four chains in two parallel runs and one tree every 5 000 generations were sampled with a burn-in of the first 5 000 trees discarded. The convergence of the two parallel runs was guaranteed by the splitting frequency less than 0.005. All other parameters were set as default.

We reconstructed the ancestral floral morphologies and traced the evolutionary trends based on the molecular phylogenetic tree generated from the combined da-

ta with only *Petrocodon* and *Primulina* taxa preserved using Fitch maximum parsimony implemented in Mesquite 2.01 (Maddison & Maddison, 2007). The phylogenetic tree to which the morphological characters were mapped was modified to comprise only one representative accession per species, with additional repetitive accessions of the same species pruned. All character states were treated as unordered, with all other settings left as default.

## 2 Results and Analysis

### 2.1 Phylogenetic analysis of *Petrocodon* and its allies

The aligned *trnL-trnF* and ITS datasets were 896 and 744 base pairs including 74 and 286 informative sites, and 72 and 123 variable but parsimony uninformative sites, respectively. The combined matrix thus consisted of 1 640 characters including 555 variable sites with 360 informative sites. The parameters of consistency index (CI), retention index (RI) and homoplasy index (HI) were 0.836, 0.904, and 0.164, and 0.553, 0.736 and 0.447 for the *trnL-trnF* and ITS data, respectively.

For the combined data, the ML, BI and MP analyses yielded 50% majority-rule consensus trees with congruent topologies (Fig. 3). The monophyly of *Petrocodon* was recovered with moderate to high support values ( $BS_{ML} = 65\%$ ;  $PP = 0.98$ ;  $BS_{MP} = 100\%$ ), and the genus was fully supported to be the sister of *Primulina* ( $BS_{ML} = 100\%$ ;  $PP = 1.00$ ;  $BS_{MP} = 100\%$ ), which are in line with previous phylogenetic analyses (Weber et al, 2011b; Xu et al, 2014; Guo et al, 2016; Lu et al, 2017). The relationships within *Petrocodon* are as shown in Fig. 3.

### 2.2 Floral evolution in *Petrocodon*

Mapping morphological characters to the molecular phylogeny clearly indicated most of the floral characters evolved frequently and highly homoplasiously in *Petrocodon*. The floral symmetry evolved twice in parallel from zygomorphy into almost actinomorphy (Fig. S1 in supporting information); the ancestral corolla shape

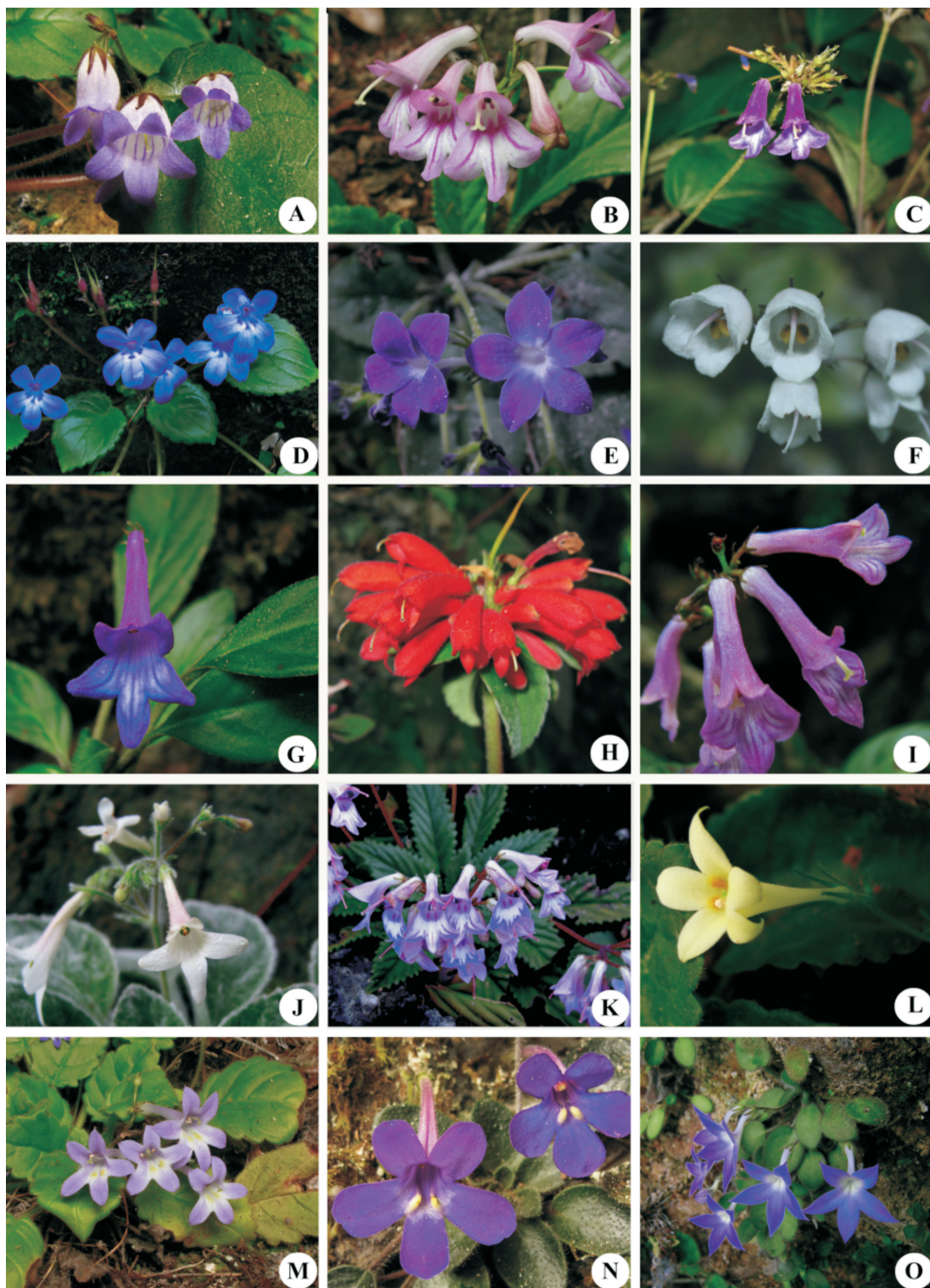


Fig. 1 Floral diversity of *Petrocodon* A. *Petrocodon tiandengensis*; B. *Pet. pseudocoriaceifolius*; C. *Pet. longgangensis*; D. *Pet. lui*; E. *Pet. guangxiensis*; F. *Pet. dealbatus*; G. *Pet. laxicymosus*; H. *Pet. coccineus*; I. *Pet. coriaceifolius*; J. *Pet. niveolanosus*; K. *Pet. hancei*; L. *Pet. hechiensis*; M. *Pet. jingxiensis*; N. *Pet. fangianus*; O. *Pet. jasminiflorus*.

was inferred to be tubular and evolved frequently (a total of 13 times of changes) among variable states, in-

cluding twice convergently into campanulate and thrice into funnelform; the floral coloration was inferred to be

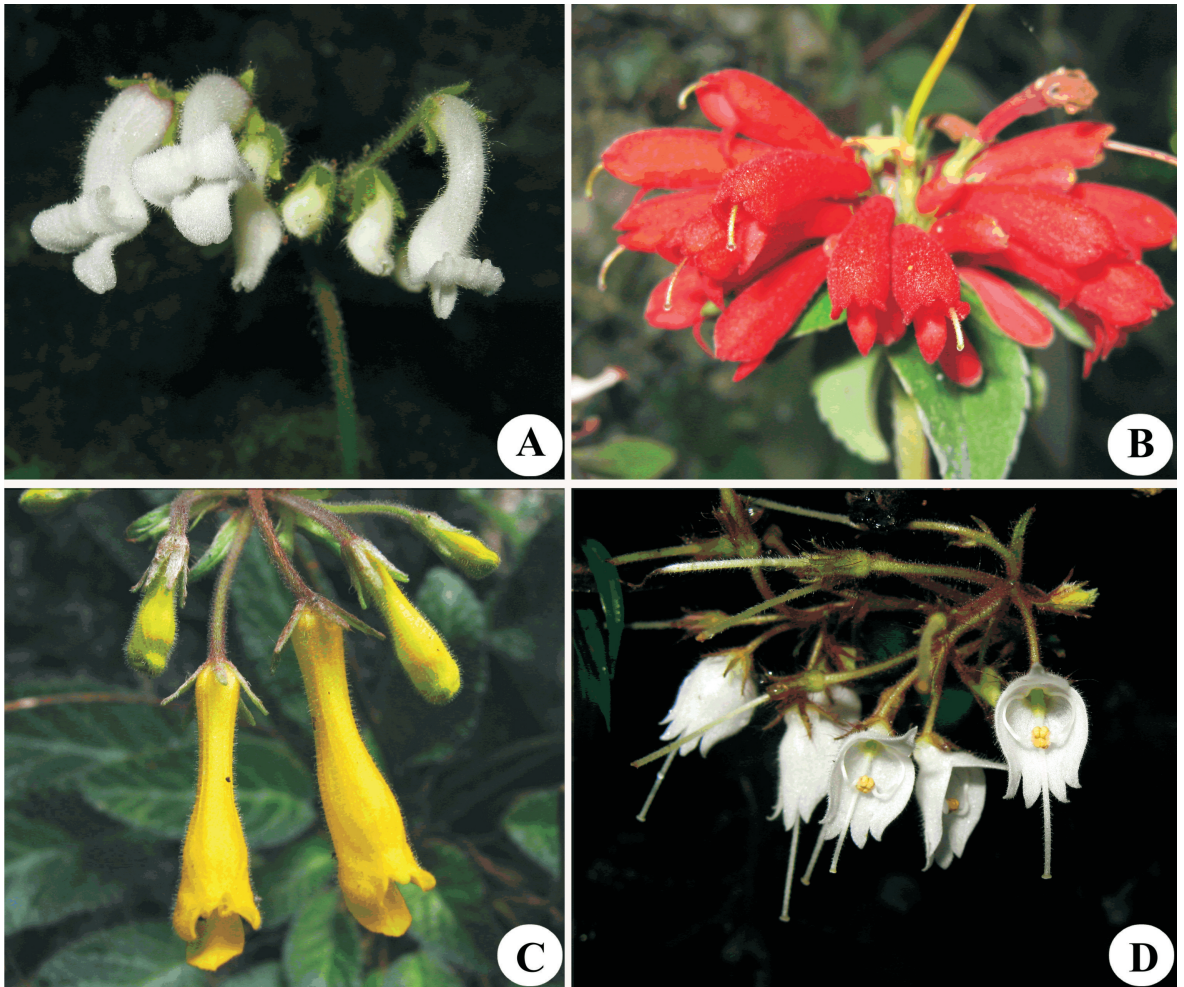


Fig. 2 Parallel evolution of four adaxial corolla lobes and one abaxial lobe in separate Gesneriaceae lineages  
**A.** *Petrolodon retroflexus*; **B.** *Pet. coccineus*; **C.** *Oreocharis mileensis*; **D.** *Allocheilos cortusiflorus*.

purple ancestrally and experienced 13 times of changes, including six independent transformations in parallel into white; even the very unusual corolla structure of four adaxial lobes and one abaxial lobe evolved twice in parallel from the ancestral and common state of two adaxial lobes and three abaxial lobes in *Petrocodon*, with additional same two shifts in other Old World Gesneriaceae (*Allocheilos* and *Oreocharis mileensis*) also from southern China; the stigma also experienced eight changes, seven from bifid into unity (being solitary) in parallel and one reversal back from unity into bifid (Fig. 4). Most other floral characters were also suggested to experience multiple homoplasious changes (data not shown).

### 3 Discussion

#### 3.1 Frequent changes, convergent and parallel evolution of the previously assumed taxonomy-significant floral characters

Mapping the morphological characters to the phylogenetic tree clearly showed that most of the floral characters of *Petrocodon* experienced complicated evolution, i.e. frequent changes and multiple convergences and parallel changes, within and beyond the genus (Fig. 4). This seems especially true for those diagnostic characters that were used to delimit the taxonomic units, which could have misled the traditional taxo-

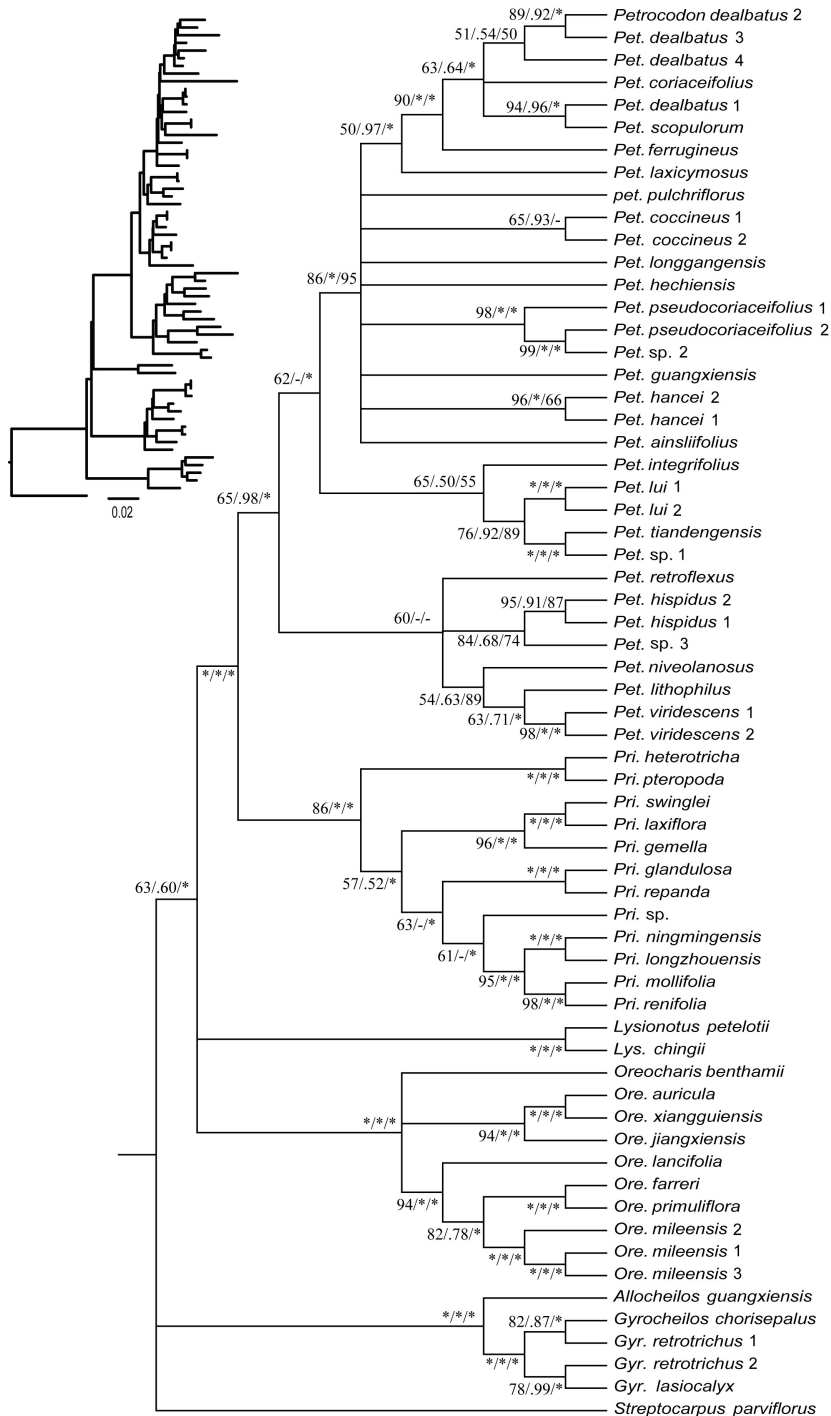


Fig. 3 Maximum likelihood phylogenetic tree based on the combined *trnL-trnF* and ITS data, showing evolutionary relationships of *Petrocodon* and its allies. ML bootstrap support, BI posterior probability and MP bootstrap support values are listed to the corresponding nodes. \* stands for the bootstrap support or posterior probability equal to 100% or 1.00; - stands for the bootstrap support or posterior probability below 50% or 0.50.

onomic treatments of the genera.

Floral (corolla) symmetry varied in Gesneriaceae

and was assumed to be one of taxonomy-significant

characters. Actinomorphic floral corolla symmetry, a



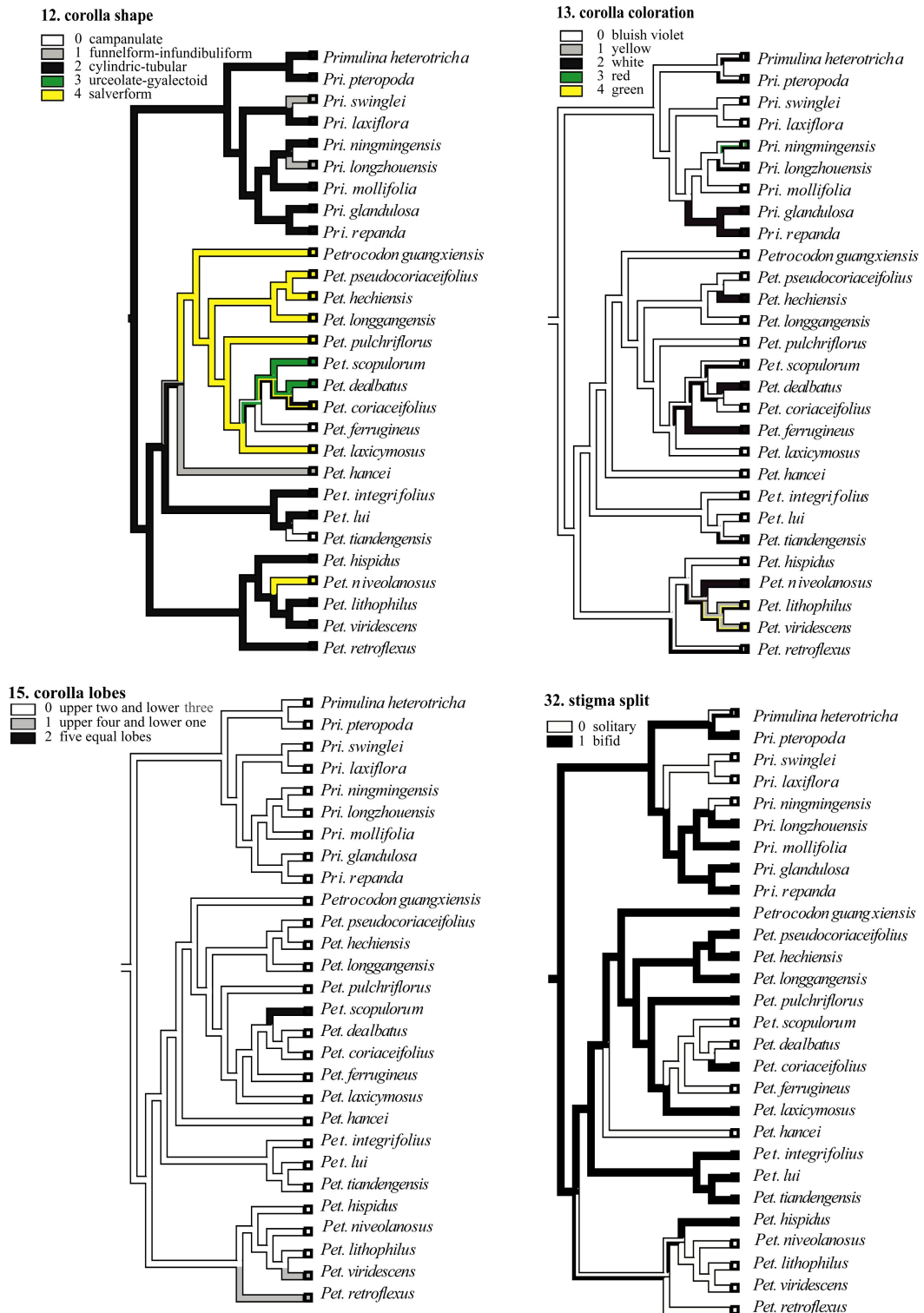


Fig. 4 Evolutionary trends of four key representative floral characters, showing highly homoplasious evolution of these characters

relative rare state possessed only by a few Old World Gesneriaceae, was assumed to be a diagnostic charac-

ter and mainly used to differentiate *Primulina tabacum* from other close relatives, e. g. the previous genus

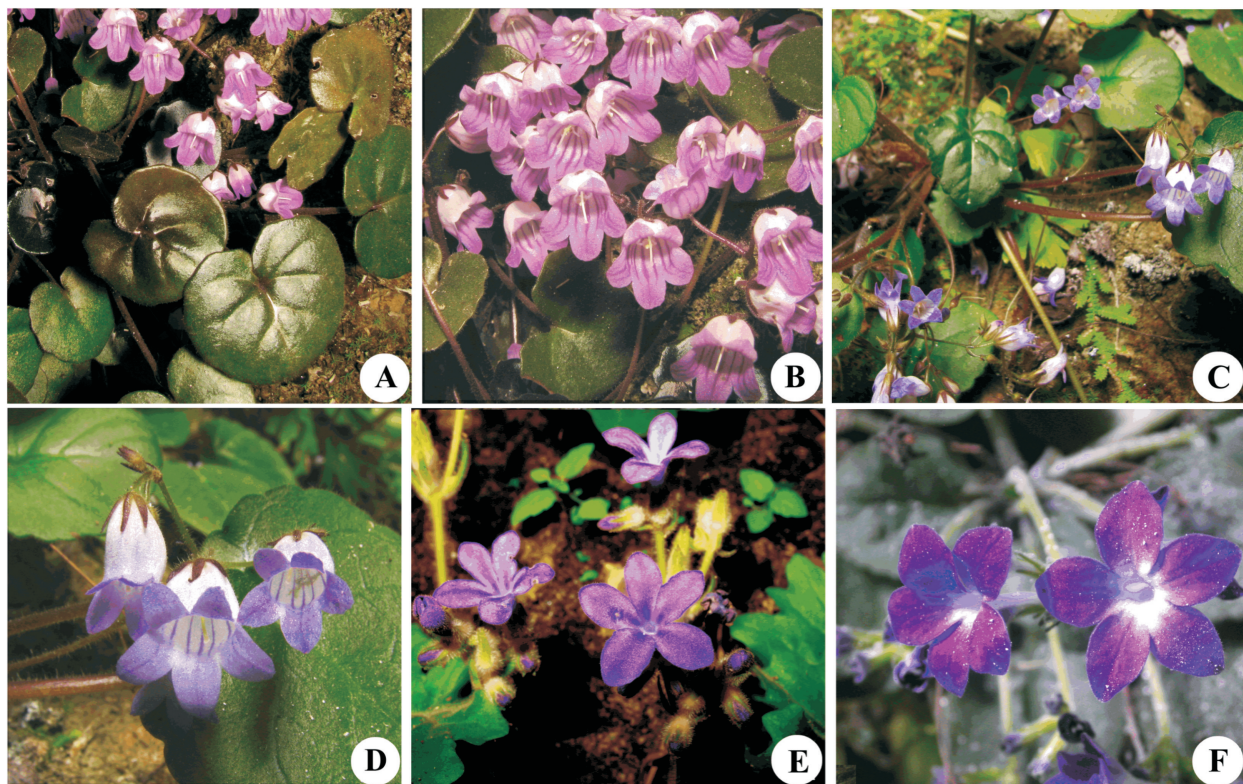


Fig. 5 Two species pairs which are morphologically similar with each other (especially for the floral characters) but are genetically distant A–B. *Primulina renifolia*; C–D. *Petrocodon tiandengensis*; E. *Primulina tabacum*; F. *Petrocodon guangxiensis*.

*Chirita*. Later, another morphologically similar new species *Pri. guangxiensis* which has general floral appearance and also almost actinomorphic corolla like *Primulina tabacum* was described and added to the monotypic genus (Liu et al, 2011). However, the molecular phylogenetic analyses clearly indicated that *Pri. tabacum* was deeply embedded in the redefined genus *Primulina* while *Pri. guangxiensis* was nested within the redefined genus *Petrocodon*, strongly suggesting parallel evolution of floral symmetry from zygomorphy to actinomorphy. Some other Gesneriaceae in China (e.g. the previous *Thamnocharis* Wang, *Bournea* Oliv. and *Conandron* Sieb. & Zucc.) were also characterized by actinomorphic corolla, whereas, these similarities were suggested to be resulted from morphological convergence (factually parallel evolution) rather than common ancestry according to the molecular phylogenetic analyses (Wang et al, 2010). Even in *Petrocodon*, the

floral symmetry transformation from zygomorphy into almost actinomorphy was also suggested to have happened twice independently, leading to *Petrocodon guangxiensis* and *Pet. scopulorum*, respectively. All these suggest that the floral symmetry can evolve rapidly and in parallel and it is not suitable to be used as the diagnostic character to delimit taxonomic units (i.e. genus) in Gesneriaceae.

The corolla shape, a previously assumed taxonomy-significant character, also evolved in parallel and convergence frequently. The previous *Lagarosolen* and *Paralagarosolen* were characterized by narrow and long (i.e. salverform) corolla tube. However, the present ancestral morphological reconstruction indicated that this salverform corolla evolved at least twice in parallel in *Petrocodon* from ancestral tubular, and it also evolved into urceolate and tubular quickly in different lineages. The campanulate corolla that characterized

*Petrocodon* s.s. and *Wentsaiboea* was also suggested to evolve twice convergently within the expanded genus *Petrocodon*, and this convergence also happened between *Petrocodon* and *Primulina*. These suggested that the character of corolla shape (salverform and campanulate) that characterized *Lagarosolen*, *Paralagarosolen*, *Wentsaiboea* and *Petrocodon* s.s. also evolved homoplasiously in *Petrocodon* and its allies.

Other characters that were assumed to be characteristic of the previous genera *Calcareboea*, *Dolicholoma* and *Didymocarpus*, whose taxa have been wholly or partially included into *Petrocodon*, were also suggested to be homoplasious according to the present study. *Dolicholoma*, a previous monotypic genus, possesses narrow corolla tube like the taxa of the previous *Lagarosolen*, but differs from the latter by its deep split corolla lobes. However, this character of deep split lobes was also observed in another recently described new species *Petrocodon ainsliifolius*. According to the previous molecular phylogenetic analysis (Chen et al, 2014), these two species are distantly related to each other, embedding within lineages with quite different characters, respectively, and thus suggesting convergent evolution of the character (discussed also in Weber et al, 2011). *Calcareboea*, a previous monotypic genus, is characteristic of bright red flower and very special corolla structure of four shallowly divided adaxial lobes and one abaxial lobe. Although the bright red flower is unique to this species in *Petrocodon* and was indicated to be derived from ancestral bluish violet, the red flower which was presumed to be associated with ornithophily (Weber et al, 2011b) is also possessed by some taxa of *Aeschynanthus*, another distantly-related genus of Gesneriaceae. The other characteristic of four adaxial lobes was also clearly indicated to have evolved in parallel within *Petrocodon* (at least twice independently) and beyond the genus (additionally twice observed leading to *Allocheilos* and *Oreocharis mileensis*). The frequent changes and highly homoplasious evolution of these previously assumed taxonomy-significant floral characters seemed to be the key factors that accounted for the unnaturalness

of the previous generic classifications, resulted in taxonomic oversplit of genera.

### 3.2 Potential synapomorphy that may characterize and differentiate the redefined *Petrocodon* from its closest relative *Primulina*

Although the redefined genus forms as monophyletic, separated from its closest relative *Primulina*, little is known about the synapomorphies that can support the new classification. Particularly, these newly redefined sister genera not only encompass remarkable diversity, but also include morphologically similar species reclassified into *Petrocodon* and *Primulina*, respectively. For instance, the two morphologically similar taxa, *Pri. tabacum* and the previous *Pri. guangxiensis* (namely *Petrocodon guangxiensis*) were indicated to be embedded in *Primulina* and *Petrocodon*, respectively, according to molecular phylogenetic analysis (Xu et al, 2014). Also, *Wentsaiboea renifolia*, *Wen. luochenensis* and *Wen. tiandenensis* were once placed in the same genus by very similar gross vegetative and floral appearance, however, the molecular phylogenetic studies clearly suggested the former two species should be placed in *Primulina*, while the latter was nested within *Petrocodon* (Wang et al, 2011; Weber et al, 2011b). These morphologically similar taxa divided into two genera confounded the boundary and brought difficulties to uncover potential diagnostic morphological characters that can distinguish and characterize each of the genera.

After extensive comparisons of *Petrocodon* with *Primulina* taxa, we noticed two possible synapomorphic characters that may be used to differentiate these two genera. Firstly, most *Primulina* taxa have geniculate filaments of the two fertile stamens, with an exception of *Pri. tabacum*, which is embedded deeply within the genus and possesses almost straight filaments. This character of geniculate filaments is also shared by some other Gesneriaceae lineages like *Hemiboea* and *Lysionotus* taxa, therefore it at least can be inferred to be ancestral of the genus *Primulina* as a whole, though it remains unknown whether this character shared by different Gesne-

riaceae lineages (genera) is indicative of plesiomorphy or convergent or parallel evolution. In contrast, most *Petrocodon* taxa we observed have straight or slightly curved filaments of the fertile stamens, being different from the commonly geniculate filaments of *Primulina*. However, *Petrocodon retroflexus* we recently published has special filaments, which are geniculate near the base like most *Primulina* taxa but with an additional loop near the tip where the anthers attached (Guo et al, 2016). Therefore, this character seemed also to be homoplasious between these two genera though it may differentiate most *Petrocodon* from *Primulina* taxa.

Secondly, *Primulina* taxa usually have obtrapeziform-like stigma (Chiritoid-type stigma), which is commonly bifid. This type of stigma was assumed to be developed from the lower half of an ancestral bi-parted stigma, with the upper half reduced (Weber et al, 2011a). This characteristic of stigma is also shared by some other distantly-related lineages, particularly those (e.g. *Microchirita*, *Henckelia*, *Liebigia*, etc.) that were unnaturally placed in the previous genus *Chirita* (Weber et al, 2011a). *Petrocodon* taxa, however, have different stigma types which usually have undivided or bi-partitioned capitate stigma(s) [corresponding to the concept of two stigmas in Wang et al (1998)] and may be a derived state in the ancestor of the lineage leading to *Petrocodon*. The capitate-like versus obtrapeziform-like stigma may be used to distinguish *Petrocodon* from *Primulina*.

Anyway, these tentatively proposed characteristics of *Petrocodon* versus *Primulina* need to be tested by more future extensive examinations of these characters in these two genera and the outgroups and inferences of their evolutionary trends based on more robust phylogenetic tree with denser sampling of the taxa.

## 4 Conclusions and Prospects

The present study illustrated complicated morphological evolution in the genus *Petrocodon*, especially for those floral characters that were assumed to be tax-

onomy-significant. Frequent changes and highly convergent and parallel evolution of these traits in the genus and its close relatives seemed to be key factors that have misled the traditional taxonomy, and brought difficulties to identify possible synapomorphic characters that can be used to support the new classifications based on molecular phylogenetic studies. More extensive investigations should be carried out on both morphologies and molecular phylogenetic studies. On one side, the available phylogenetic studies in Gesneriaceae were mainly based on limited plastid and the nuclear ribosome ITS DNA sequences (Wang et al, 2011; Weber et al, 2011a, b), which sometimes were likely to yield low resolution or partial or even misleading relationships. So that, denser samplings of DNA sequences from different genomes as well as taxa are required to reconstruct the highly resolved species tree. On the other side, more morphologies including micro-morphological and anatomical characters, etc. should be examined and added to the present data set, and their evolutionary trends should be traced on robust phylogenetic framework. More importantly, efforts should also be taken to investigate the underlying causes (e.g. molecular regulatory network from the discipline of evolutionary developmental genetics) and its biological senses (e.g. the fitness adapted to natural selection) of the complicated morphological evolution. All these together would help bridge up the gaps between traditional taxonomy and molecular phylogenetic studies and between phenotypes and genotypes, and advance the understandings of the evolution of this group of typical karst flora and its allies.

## 5 Supporting Information

Table S1 GenBank accessions of the sequences used in the present study.

Fig. S1 Evolutionary trends of four selected flower traits, showing highly homoplasious evolution. The ancestral states of the traits were traced using the maximum parsimony method implemented in Mesquite 2.7.

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Table S1 GenBank accessions of the sequences used in the present study

No.	Taxa	<i>trnL-trnF</i>	ITS
1	<i>Allocheilos guangxiensis</i>	HQ632897.1	HQ632994.1
2	<i>Gyrocheilos chorisepalus</i>	HQ632900.1	HQ632997.1
3	<i>Gyrocheilos lasiocalyx</i>	HQ632901.1	HQ632998.1
4	<i>Gyrocheilos retrotrichus</i> 1	HQ632902.1	HQ632999.1
5	<i>Gyrocheilos retrotrichus</i> 2	HQ632903.1	HQ633000.1
6	<i>Lysionotus chingii</i>	FJ501498.1	FJ501332.1
7	<i>Lysionotus petelotii</i>	FJ501496.1	HQ632974.1
8	<i>Oreocharis auricula</i>	GU350672.1	DQ912664.1
9	<i>Oreocharis benthamii</i>	JF697584.1	NA
10	<i>Oreocharis farreri</i>	JF697585.1	JF697573.1
11	<i>Oreocharis jiangxiensis</i>	HQ632933.1	HQ633029.1
12	<i>Oreocharis lancifolia</i>	HQ632924.1	HQ633020.1
13	<i>Oreocharis mileensis</i> 1	HQ327444.1	HQ327460.1
14	<i>Oreocharis mileensis</i> 2	HQ632928.1	HQ633024.1
15	<i>Oreocharis mileensis</i> 3	JF697587.1	JF697575.1
16	<i>Oreocharis primuliflora</i>	HQ632923.1	HQ633019.1
17	<i>Oreocharis xiangguiensis</i>	HQ632932.1	HQ633028.1
18	<i>Petrocodon ainsliifolius</i>	NA	HQ633038.1
19	<i>Petrocodon coccineus</i> 1	KF202299.1	NA
20	<i>Petrocodon coccineus</i> 2	FJ501516.1	FJ501341.1
21	<i>Petrocodon coriaceifolius</i>	HQ632943.1	HQ633039.1
22	<i>Petrocodon dealbatus</i> 1	JX506767.1	JX506959.1
23	<i>Petrocodon dealbatus</i> 2	JF697590.1	JF697578.1
24	<i>Petrocodon dealbatus</i> 3	FJ501537.1	FJ501358.1
25	<i>Petrocodon dealbatus</i> 4	NA	GU350636.1
26	<i>Petrocodon ferrugineus</i>	HQ632946.1	HQ633043.1
27	<i>Petrocodon guangxiensis</i>	JX506791.1	JX506899.1
28	<i>Petrocodon hancei</i> 1	GU350667.1	NA
29	<i>Petrocodon hancei</i> 2	HQ632944.1	HQ633041.1
30	<i>Petrocodon hechiensis</i>	HQ632942.1	HQ633036.1
31	<i>Petrocodon hispidus</i> 1	KF202300.1	NA
32	<i>Petrocodon hispidus</i> 2	KF202301.1	NA
33	<i>Petrocodon integrifolius</i>	HQ632940.1	HQ633037.1
34	<i>Petrocodon laxicymosus</i>	KC765117.1	KC765115.1
35	<i>Petrocodon lithophilus</i>	KF202303.1	NA
36	<i>Petrocodon longgangensis</i>	KC765116.1	KC765114.1
37	<i>Petrocodon lui</i> 1	In prepare	In prepare

No.	Taxa	<i>trnL-trnF</i>	ITS
38	<i>Petrocodon lui</i> 2	HQ632938.1	HQ633035.1
39	<i>Petrocodon niveolanosus</i>	JF697588.1	JF697576.1
40	<i>Petrocodon pseudocoriaceifolius</i> 1	JX506741.1	JX506852.1
41	<i>Petrocodon pseudocoriaceifolius</i> 2	In prepare	In prepare
42	<i>Petrocodon pulchriflorus</i>	KX579059.1	KX579058.1
43	<i>Petrocodon retroflexus</i>	KX579061.1	KX579060.1
44	<i>Petrocodon scopulorum</i>	HQ632947.1	HQ633044.1
45	<i>Petrocodon</i> sp. 1	HQ632945.1	HQ633042.1
46	<i>Petrocodon</i> sp. 2	JF697589.1	JF697577.1
47	<i>Petrocodon</i> sp. 3	HQ632941.1	NA
48	<i>Petrocodon tiandengensis</i>	JX506850.1	JX506960.1
49	<i>Petrocodon viridescens</i> 1	KF202304.1	NA
50	<i>Petrocodon viridescens</i> 2	HQ632939.1	HQ633036.1
51	<i>Primulina gemella</i>	FJ501523.1	FJ501345.1
52	<i>Primulina glandulosa</i>	HQ632948.1	HQ633045.1
53	<i>Primulina heterotricha</i>	DQ872816.1	DQ872826.1
54	<i>Primulina laxiflora</i>	JX506801.1	JX506909.1
55	<i>Primulina longzhouensis</i>	JX506810.1	JX506918.1
56	<i>Primulina mollifolia</i>	JX506755.1	JX506866.1
57	<i>Primulina ningmingensis</i>	JX506822.1	JX506931.1
58	<i>Primulina pteropoda</i>	DQ872817.1	DQ872827.1
59	<i>Primulina renifolia</i>	JX506851.1	JX506737.1
60	<i>Primulina repanda</i>	AJ492292.1	FJ501351.1
61	<i>Primulina</i> sp.	HQ632949.1	NA
62	<i>Primulina swinglei</i>	JX506841.1	JX506950.1
63	<i>Streptocarpus parviflorus</i>	HQ718941.1	HQ719021.1

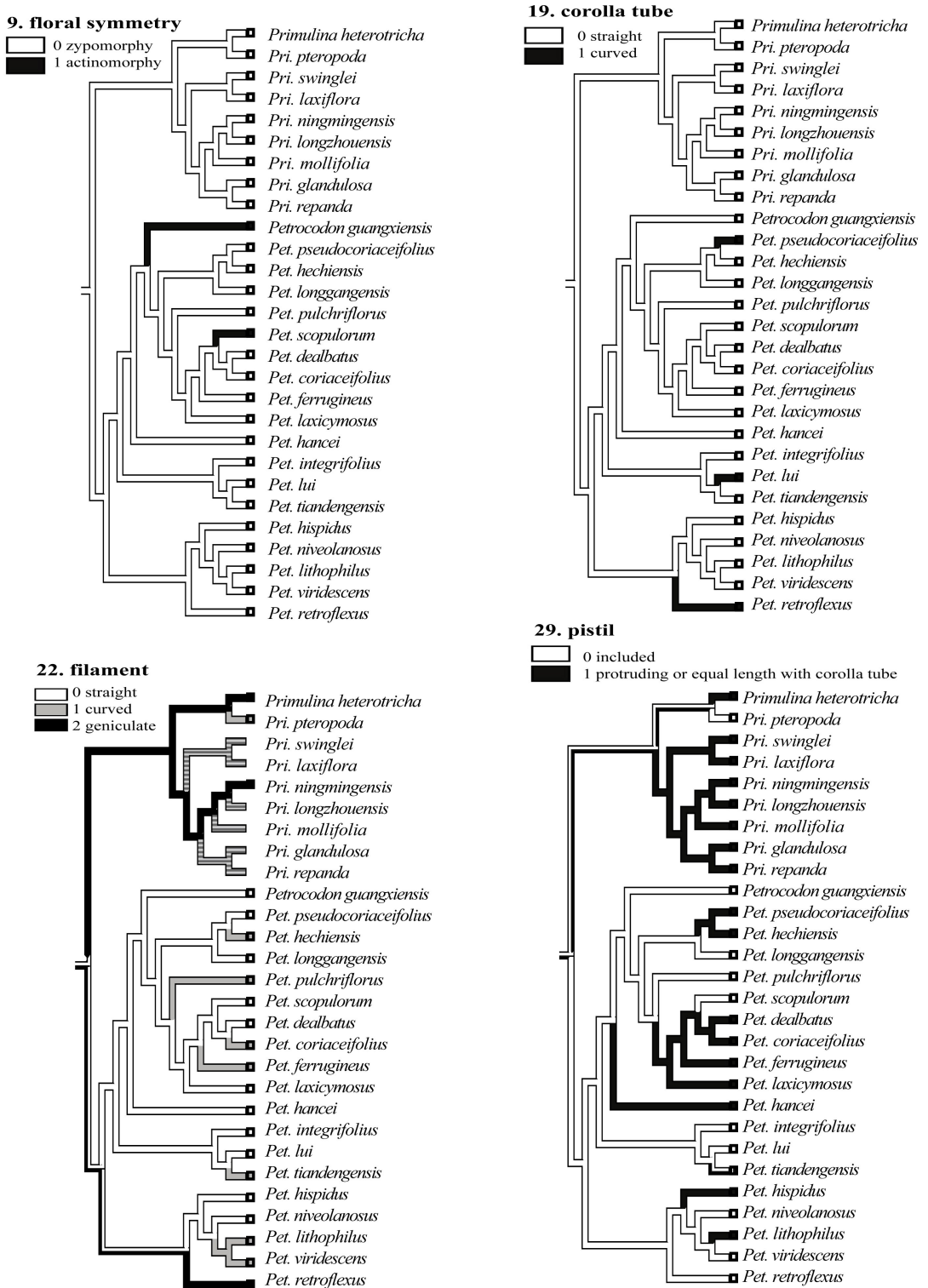


Fig. S1 Evolutionary trends of four selected floral traits, showing highly homoplasious evolution