

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/335627872>

# Reversal versus specialization in floral morphological evolution in *Petrocosmea* (Gesneriaceae)

Article in *Journal of Systematics and Evolution* · September 2019

DOI: 10.1111/jse.12541

CITATIONS

0

READS

196

7 authors, including:



Li Chaoqun

Institute of Botany, Chinese Academy of Science

8 PUBLICATIONS 42 CITATIONS

SEE PROFILE



Mengqi Han

Chinese Academy of Sciences

14 PUBLICATIONS 44 CITATIONS

SEE PROFILE



Yang Dong

John Innes Centre

27 PUBLICATIONS 450 CITATIONS

SEE PROFILE



Peng-Wei Li

Chinese Academy of Sciences

8 PUBLICATIONS 23 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



molecular regulation mechanism of floral symmetry [View project](#)



Cell biology of reproduction in *Capsella rubella* [View project](#)



## Research Article

Reversal versus specialization in floral morphological evolution in *Petrocosmea* (Gesneriaceae)Chao-Qun Li<sup>1,2†</sup>, Tian-Feng Lü<sup>1,2†</sup>, Meng-Qi Han<sup>1,2</sup>, Yang Dong<sup>1</sup>, Peng-Wei Li<sup>1,2</sup>, Yan Liu<sup>3</sup>, and Yin-Zheng Wang<sup>1,2\*</sup> <sup>1</sup>State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China<sup>2</sup>College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China<sup>3</sup>Guangxi Institute of Botany, Guangxi Zhuangzu Autonomous Region and the Chinese Academy of Sciences, Guilin 541006, Guangxi, China<sup>†</sup>These authors contributed equally to this work.

\*Author for correspondence. E-mail: wangyz@ibcas.ac.cn

Received 24 August 2018; Accepted 24 August 2019; Article first published online 4 September 2019

**Abstract** A widely held hypothesis in evolution is that adaptive specialization constrains the potential direction of future evolutionary change and thus may be irreversible, also known as Dollo's law. However, this hypothesis has long been subject to debate in evolutionary biology. Floral specialization is intriguing as it is usually linked to reproductive isolation and could affect speciation. Here, following the discovery of four new taxa, we observed some interesting phenomena of reversal versus specialization in morphology in a clade with the most specialized flowers in the genus *Petrocosmea*. In the phylogenetic tree based on sequences of multiple DNA regions, the morphological reversals, especially the regain of a long corolla tube, are nested within the branches characteristic of normally specialized flowers with a short corolla tube and highly specialized zygomorphy. Our results indicate that the highly specialized floral organ of this clade is still actively evolving in multiple branches toward specialization while reversals to different ancestral states occur in some branches. Great disturbance of ecological environment is likely a crucial factor affecting trait reversibility, such as the rapid uplift of the Himalayan–Tibetan plateau. The four new taxa are treated herein taxonomically. The flowers of this clade represent an interesting model to explore the genetic basis underlying the evolutionary reversal versus specialization and the interplay between genetic factors and environmental variables.

**Key words:** corolla tube, floral symmetry, Gesneriaceae, *Petrocosmea*, reverse evolution.

## 1 Introduction

*Petrocosmea* Oliv. (Gesneriaceae) contains approximately 50 species that are mostly distributed in southwestern China with several species in northern Myanmar and Thailand, and northeastern India (Wang, 1985, 1990, 1998; Li & Wang, 2004; Weber, 2004; Weber et al., 2013; Qiu & Liu, 2015; Qiu et al., 2015; Han et al., 2017, 2018a, 2018b). They usually grow in shady environments under forest on damp limestone hills. Recent molecular phylogenetic analyses clearly defined five major clades (clades A–E) in *Petrocosmea* (Qiu et al., 2015). However, just after the phylogenetic reconstruction, several new taxa were found in some previously unexplored ecological habitats in this small genus. These new taxa all belong to clade C in *Petrocosmea*. However, some of them have flowers distinctively different from those of known species in clade C, implying an interesting evolutionary phenomenon in *Petrocosmea*.

Reverse evolution is a widespread phenomenon in biology. Rudimentary, lost, and aborted organs are extremely common throughout nature and reacquisition of such characters is frequent across all of life. However, it has long been a hotly debated issue in evolutionary biology. The

paleontologist Dollo (1893) proposed the idea that evolution is unidirectional and irreversible, that is, Dollo's law. The modern version of Dollo's law formulated by Simpson (1953) and Gould (1970) means that complex traits, once lost, will not re-evolve in the same lineage. However, the evolutionary biologists opposing Dollo's law claim that evolutionary reversals have been observed both in the wild and in the laboratory, and they doubt if this idea is really true (Collin & Miglietta, 2008). The advent and development of molecular phylogenetics and evolutionary developmental biology has made it possible to test Dollo's law rigorously. This test can be at various biological levels of organization, including phenotypes in morphology, anatomy, structure, function, or behavior and genotypes in gene knockdown, deletion, gain of function, or back mutation (Porter & Crandall, 2003).

In this study, we undertook molecular phylogenetic analyses based on multiple DNA sequences in *Petrocosmea*, focusing on clade C to determine the exact systematic position of the four new taxa. On the basis of the phylogenetic frame, we have discussed the floral morphological evolution in *Petrocosmea*. We suggest that, even though highly specialized, the floral organ of clade C is still actively adaptive and dynamically evolvable toward either

specialization or reversal in response to environmental variables under the background of the rapid uplift of the Himalayan–Tibetan plateau. Our results would shed new light on floral morphological evolution, especially on the issue of evolutionary reversal versus specialization in angiosperms.

## 2 Material and Methods

### 2.1 Plant materials

In the phylogenetic analysis, the four new taxa (*Petrocosmea qionglaiensis* C. Q. Li & Y. Z. Wang, *P. longituba* M. Q. Han & Yan Liu, *P. shilinensis* Y. M. Shui & H. T. Zhao var. *changhuensis* T. F. Lü & Y. Z. Wang, and *P. purpureoglandulosa* Y. Dong & Y. Z. Wang) with an additional 20 species were sampled, which include two species of clade A, two species of clade B, 10 species of clade C, 2 species of clade D, and 2 species of clade E. According to Qiu et al. (2015), *Raphiocarpus* Chun is not only sister to *Petrocosmea* but also has many primitive morphological characters in comparison to *Petrocosmea*. We, therefore, chose *Raphiocarpus begoniifolius* (H. Lév.) B. L. Burtt and *R. petelotii* (Pellegr.) B. L. Burtt as outgroups in the phylogenetic analysis (Table 1).

### 2.2 DNA extraction, polymerase chain reaction amplification, and sequencing

Total DNA was extracted from fresh leaf materials using the CTAB method (Rogers & Bendich, 1988) and used as templates in the polymerase chain reaction. Six chloroplast

regions (*atpI-H*, *matK*, *trnH-psbA*, *rps16*, *trnL-trnF*, *trnT-trnL*) and two nuclear regions (*Petrocosmea* *CYCLOIDEA1D* (Pe-CYC1D) and ITS) were amplified using the primers of Qiu et al. (2015). Polymerase chain reaction was carried out under the following conditions: 94°C for 5 min; 30 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 1 min; and a final extension at 72°C for 10 min. The polymerase chain reaction products were purified using the TIANquick Midi Purification Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol and were directly sequenced. The sequencing primers are the same as amplification primers.

### 2.3 Sequence alignment and phylogenetic analysis

All sequences were aligned and adjusted manually in Geneious Pro 4.8.4 (<http://www.geneious.com>) (Kearse et al., 2012). The data matrices were analyzed with maximum parsimony (MP), implemented in PAUP\* 4.0b10 (Swofford, 2003), and Bayesian inference, implemented in MrBayes 3.2.6 (Ronquist & Huelsenbeck, 2003). In the MP analysis, character states were unordered and all characters were given equal weight. Heuristic searches were carried out with 1000 replicates of random addition, with the default setting of one tree held at each step during stepwise addition, tree bisection–reconnection branch swapping, MulTrees in effect, and steepest descent off. Bootstrap value support for each clade was estimated with 1000 replicates using a heuristic search. In the Bayesian analysis, the optimal model and parameters were determined by MrModeltest 2.3 (Nylander, 2004). Four chains of Markov chain Monte Carlo

**Table 1** Taxa sampled for phylogenetic analysis in this study

Taxa	Voucher specimen	Collection locality (all China)
<i>Petrocosmea grandifolia</i> W. T. Wang	QZJ-2007-037, PE	Yunnan
<i>P. menglianensis</i> H. W. Li	QZJ-2007-026, PE	Yunnan
<i>P. sichuanensis</i> Chun ex W. T. Wang	Q060912-1, PE	Sichuan
<i>P. intraglabra</i> (W. T. Wang) Zhi J. Qiu	QZJ-2007-068, PE	Yunnan
<i>P. huanjiangensis</i> Yan Liu & W. B. Xu	9549, IBK	Guangxi
<i>P. xingyiensis</i> Y. G. Wei & F. Wen	6101, IBK	Guizhou
<i>P. shilinensis</i> Y. M. Shui & H. T. Zhao var. <i>shilinensis</i> Y. M. Shui & H. T. Zhao	66771A, KUN	Yunnan
<i>P. minor</i> Hemsl.	QZJ-2008-54, PE	Yunnan
<i>P. iodoides</i> Hemsl.	QZJ-2007-074, PE	Yunnan
<i>P. leiandra</i> (W. T. Wang) Z. J. Qiu	QZJ-2008-33, PE	Guizhou
<i>P. sericea</i> C. Y. Wu ex H. W. Li	991104, KUN	Yunnan
<i>P. grandiflora</i> Hemsl.	Hancock 115, KUN	Yunnan
<i>P. martinii</i> (H. Lév.) H. Lév.	QZJ-2007-074, PE	Yunnan
<i>P. yanshanensis</i> Z. J. Qiu & Y. Z. Wang	QZJ-2007-077, PE	Yunnan
<i>P. barbata</i> Craib	QZJ-2007-009, PE	Yunnan
<i>P. cavaleriei</i> H. Lév.	QZJ-2007-082, PE	Guizhou
<i>P. oblata</i> Craib	Q060923-1, PE	Sichuan
<i>P. sinensis</i> Oliv.	QZJ-2008-41, PE	Sichuan
<i>Raphiocarpus begoniifolius</i> (H. Lév.) B. L. Burtt	QZJ-2008-026, PE	Guizhou
<i>R. petelotii</i> (Pellegr.) B. L. Burtt	GX_NP_1, PE	Guangxi
<i>P. purpureoglandulosa</i> Y. Dong & Y. Z. Wang	20140613C, PE	Yunnan
<i>P. shilinensis</i> Y. M. Shui & H. T. Zhao var. <i>changhuensis</i> T. F. Lü & Y. Z. Wang	20140619F, PE	Yunnan
<i>P. qionglaiensis</i> C. Q. Li & Y. Z. Wang	2015051611, PE	Sichuan
<i>P. longituba</i> M. Q. Han & Yan Liu	HMQ988, IBK	Guizhou

were run each for 20 million generations in two independent analyses and were sampled every 2000 generations. For each run, the first 25% samples were discarded as burn-in to ensure that the chains reached stationarity. In the majority rule consensus tree from the Bayesian analysis, posterior probability (PP) was used to estimate robustness. For combined sequence data, the incongruence length difference test (Farris et al., 1994) as implemented in PAUP\* 4.0b10 (Swofford, 2003) was undertaken to assess character congruence between chloroplast DNA data and nuclear ribosomal DNA data, with 1000 replicates, each with 100 random additions with tree bisection–reconnection branch swapping. The *P*-value was used to determine whether the two datasets contained significant incongruence ( $\leq 0.05$ ).

#### 2.4 Morphological data collection

Representative living plants of almost all species of *Petrocosmea* have been transplanted from the wild to our greenhouse at the Institute of Botany, CAS. Five to 10 flowers of each population in the four new taxa were measured and compared to their close relatives. For the length of flowers and corolla tubes, the flowers were pinned to a blackboard and photographed using a digital camera (D7200, Nikon, Tokyo, Japan) to obtain images of  $6000 \times 4000$  pixels in dimension. All images were acquired during full bloom.  $R_{(tube)}$  (relative corolla tube length) was used to describe corolla tube length divergence in *Petrocosmea*, that is,  $R_{(tube)} = \text{length of tube} / \text{length of flower} \times 100\%$ . The measurement and calibration was implemented in ImageJ 1.50b. The data shown were the average of three technical replicates with each including nine to 15 flowers from three to five plants in each species. The average relative corolla tube length ( $R_{(tube)}$ ) was calculated and set as the demarcation line of species with long corolla tube ( $R_{(tube)} > \text{mean relative corolla tube length}$ ) and short corolla tube ( $R_{(tube)} < \text{mean relative corolla tube length}$ ). Significance of differences between species with long tube and short tube was tested using the *t*-test ( $P < 0.05$ ) by SPSS 13.0 software (SPSS Inc., Chicago, IL).

### 3 Results

The combined DNA matrix with six chloroplast regions (*atpH*, *matK*, *trnH-psbA*, *rps16*, *trnL-trnF*, *trnT-trnL*) and two nuclear regions (*Petrocosmea* CYCLOIDEA1D (PeCYC1D) and ITS) had aligned sequences of 7227 bp, of which 349 (4.83%) were parsimony informative. The topologies derived from the chloroplast DNA matrix and nuclear ribosomal DNA matrix are congruent with each other. The incongruence length difference test gave a value of  $P = 0.168$ , indicating that the data from the two distinct genome regions did not contain significant incongruence. Modeltest suggested that the GTR model best fit the combined data. One tree from the MP analysis (Length = 1047, Consistency Index = 0.840, Retention Index = 0.776) was congruent with the Bayesian tree (Fig. 1).

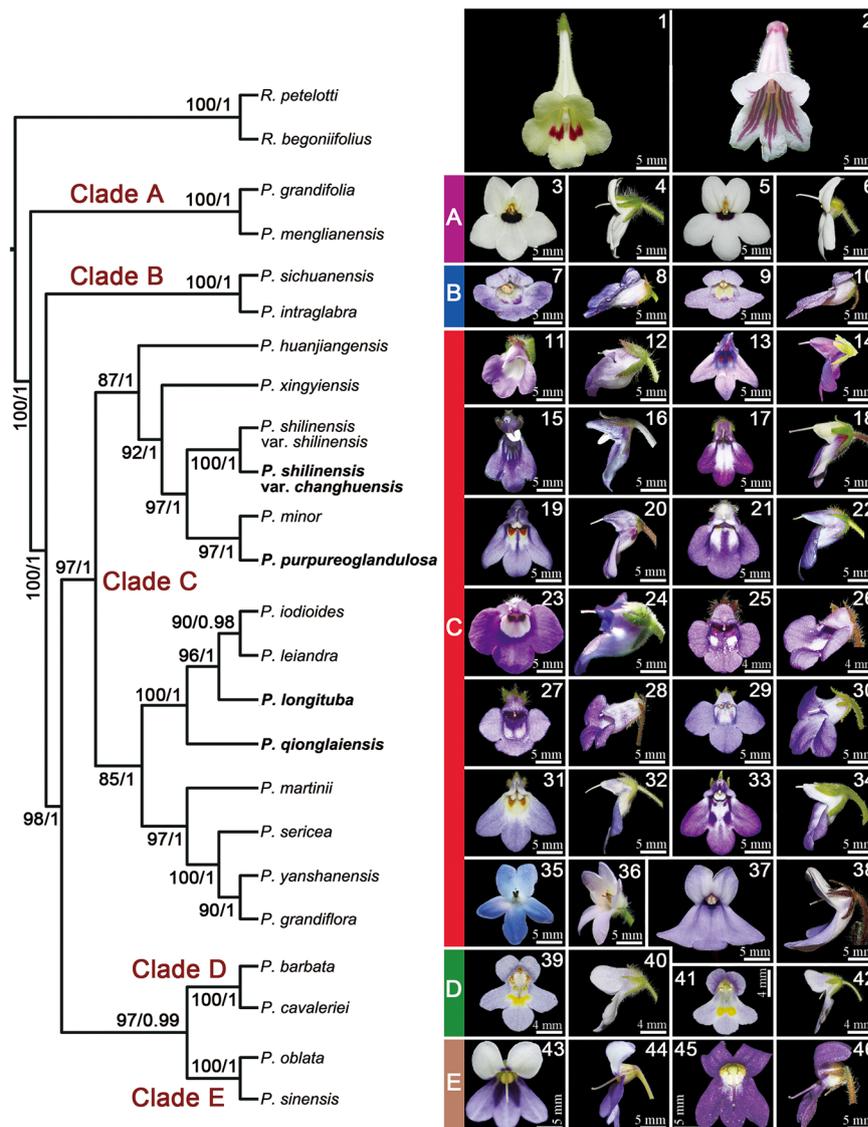
The MP tree consists of five clades, which is consistent with previous results (Qiu et al., 2015). Each clade receives strong or maximum support, and relationships among clades are maximally or strongly supported.

In the MP tree, all four new taxa are unambiguously located in clade C. Clade C (14 taxa) includes two major

lineages with high support (MP–bootstrap support [BS] = 97%; PP = 100%). The first major lineage consists of five species and two varieties, including *Petrocosmea huanjiangensis* Yan Liu & W. B. Xu, *P. xingyiensis* Y. G. Wei & F. Wen, *P. shilinensis* var. *shilinensis*, *P. minor* Hemsl., and two new taxa *P. purpureoglandulosa* and *P. shilinensis* var. *changhuensis*. The second major lineage includes eight species, *P. idodioides* Hemsl., *P. leiandra* (W. T. Wang) Z. J. Qiu, *P. martinii* (H. Lév.) H. Lév., *P. sericea* C. Y. Wu ex H. W. Li, *P. yanshanensis* Z. J. Qiu & Y. Z. Wang, *P. grandiflora* Hemsl., and two new species *P. longituba* and *P. qionglaiensis*. In the first lineage, the new species *P. purpureoglandulosa* is sister to *P. minor* with strong support (MP-BS = 97%; PP = 100%). The new variety *P. shilinensis* var. *changhuensis* is sister to *P. shilinensis* var. *shilinensis* with maximum support. Furthermore, these two branches are clustered together with strong support (MP-BS = 97%; PP = 100%) and come together successively with *P. xingyiensis* (MP-BS = 92%; PP = 100%) and *P. huanjiangensis* (MP-BS = 87%; PP = 100%).

In the first lineage, the two new taxa (*P. purpureoglandulosa* and *P. shilinensis* var. *changhuensis*) are generally similar to other species of this lineage in floral morphology with a series of shared morphological synapomorphies. These combined synapomorphies are: (i) the carinate-plicate structure of the adaxial lip apparently extended forward, longer than or at least equal to the corolla tube, and enclosing the style and two anthers; (ii) each adaxial corolla lobe rolled laterally and reflexed upward; (iii) an angle more than  $60^\circ$  formed between the abaxial lip and carinate-plicate structure; (iv) corolla tube short, approximately 3–4 mm long (approximately  $1/3$  of abaxial lip length); and (v) the lower part of the corolla tube slightly or not inflated at throat. The new variety *P. shilinensis* var. *changhuensis* has a close affinity to *P. shilinensis* var. *shilinensis*, but can be easily recognized by its cuneate leaf base, white filaments, and the inner surface of the abaxial lip with two white stripes at the throat (Fig. 2; Table 2). The new species *P. purpureoglandulosa* has a close affinity to *P. minor*, but can be easily recognized by the broad, ovate lateral corolla lobes, the carinate-plicate structure that is straight on the adaxial side, and purple glandular filaments that are more or less straight. These floral characters of *P. purpureoglandulosa* are distinguished from the triangular or triangular-ovate lateral corolla lobes, the carinate-plicate structure that is inflated on the adaxial side, and white glandular filaments that are strongly geniculate in *P. minor* (Fig. 3; Table 2). In floral morphology, *P. purpureoglandulosa* is also similar to *P. sericea* located in the second major lineage. However, it is distinctively different from the latter in its two white stripes on the inner abaxial surface of the corolla tube extended to the throat and densely purple glandular filaments (Figs. 1, 3; Table 2).

The second major lineage includes two branches. In the first branch, the new species *P. longituba* is sister to *P. iododioides* and *P. leiandra* with strong support (MP-BS = 96%; PP = 100%). Furthermore, they are grouped with the new species *P. qionglaiensis* with maximum support. The remaining four species (*P. martinii*, *P. sericea*, *P. yanshanensis*, and *P. grandiflora*) form another branch. *Petrocosmea yanshanensis* and *P. grandiflora* as a pair of sister species receive strong support (MP-BS = 90%; PP = 100%), strongly supported to come together successively with

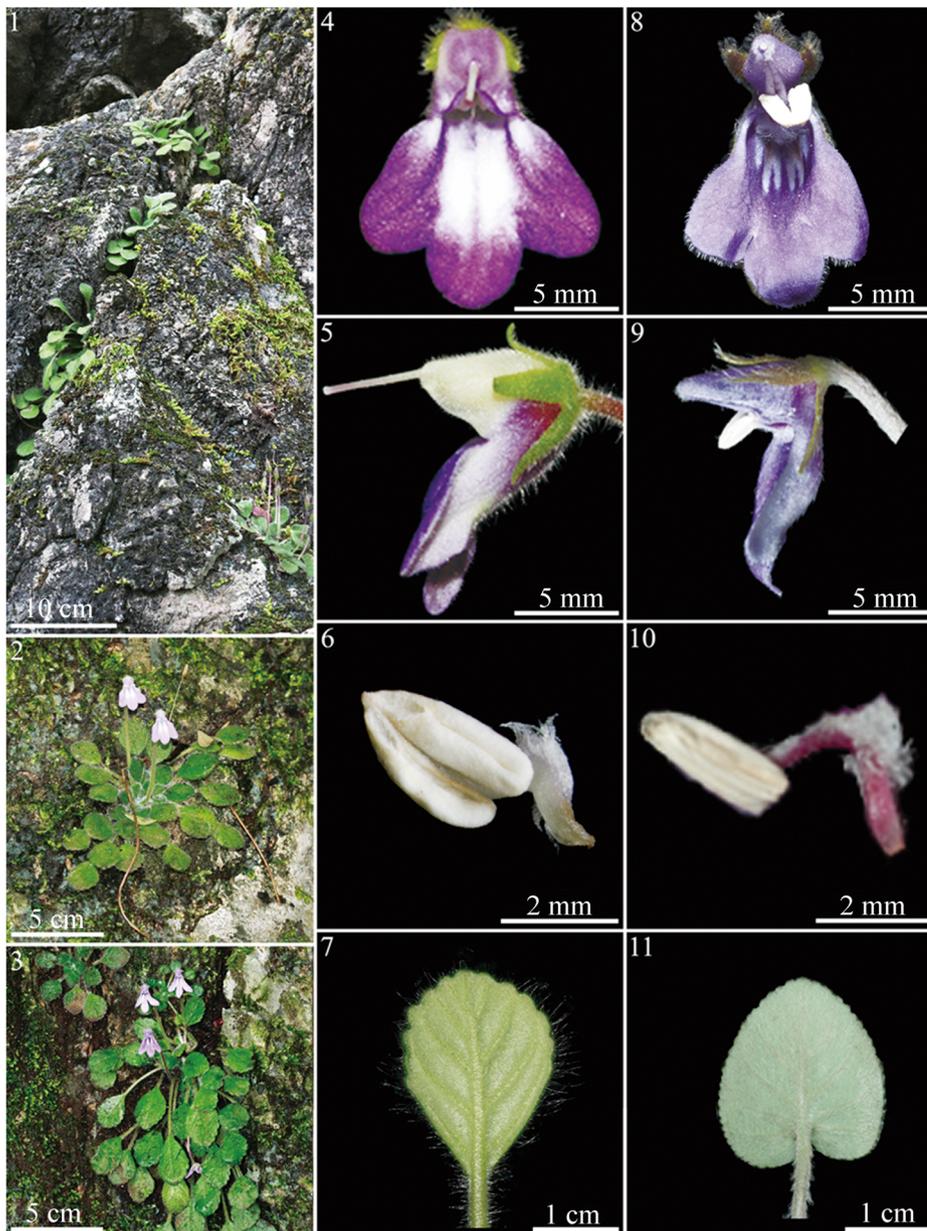


**Fig. 1.** Most parsimonious tree of *Petrocosmea* and photographs of flowers of corresponding taxa. Left, the most parsimonious tree (left) is generated from combined chloroplast DNA and nuclear DNA data. Right, photographs of flowers are the corresponding taxa (bold indicates new taxa) sampled in the phylogenetic analysis. Values next to branches indicate bootstrap support / Bayesian posterior probability. **1, 2.** Outgroups. **1.** *Raphiocarpus petelotii*. **2.** *R. begoniifolius*. **3–6.** Species of clade A. **3, 4.** *Petrocosmea grandifolia*, face (3) and lateral (4) view. **5, 6.** *P. menglianensis*, face (5) and lateral (6) view. **7–10.** Species of clade B. **7, 8.** *P. sichuanensis*, face (7) and lateral (8) view. **9, 10.** *P. intraglabra*, face (9) and lateral (10) view. **11–38.** Species of clade C, including four new taxa. **11, 12.** *P. huanjiangensis*, face (11) and lateral (12) view. **13, 14.** *P. xingyiensis*, face (13) and lateral (14) view. **15, 16.** *P. shilinensis*, face (15) and lateral (16) view. **17, 18.** New variety *P. shilinensis* var. *changhuensis*, face (17) and lateral (18) view. **19, 20.** *P. minor*, face (19) and lateral (20) view. **21, 22.** New species *P. purpureoglandulosa*, face (21) and lateral (22) view. **23, 24.** *P. iodoides*, face (23) and lateral (24) view. **25, 26.** *P. leiandra*, face (25) and lateral (26) view. **27, 28.** New species *P. longituba*, face (27) and lateral (28) view. **29, 30.** New species *P. qionglaiensis*, face (29) and lateral (30) view. **31, 32.** *P. martinii*, face (31) and lateral (32) view. **33, 34.** *P. sericea*, face (33) and lateral (34) view. **35, 36.** *P. yanshanensis*, face (35) and lateral (36) view. **37, 38.** *P. grandiflora*, face (37) and lateral (38) view. **39–42.** Species of clade D. **39, 40.** *P. barbata*, face (39) and lateral (40) view. **41, 42.** *P. cavaleriei*, face (41) and lateral (42) view. **43–46.** Species of clade E. **43, 44.** *P. oblata*, face (43) and lateral (44) view. **45, 46.** *P. sinensis*, face (45) and lateral (46) view. Photographs 15 and 16 by Y. M. Shui.

*P. sericea* (MP-BS = 100%; PP = 100%) and *P. martinii* (MP-BS = 97%; PP = 100%).

The two new species *P. longituba* and *P. qionglaiensis* located in the first branch share a series of morphological synapomor-

phies with *P. iodoides* and *P. leiandra* that can easily be distinguished from other species in clade C. These combined synapomorphies include: (i) the carinate-plicate structure of the adaxial lip slightly extended forward, only enclosing the style



**Fig. 2.** Photographs of *Petrocosmea shilinesis* var. *changhuensis* with comparison of *P. shilinesis*. 1–7. *P. shilinesis* var. *changhuensis*. 1. Habitat. 2, 3. Plants with basal leaves. 4. Flower, face view, showing two white oblong stripes extended to the throat on abaxial corolla lip. 5. Flower, side view. 6. Stamen, showing filaments white. 7. Leaf, showing leaf rhombic, margin crenate, base cuneate, lateral veins abaxially conspicuous. 8–11. *P. shilinesis*. 8. Flower, face view, showing several white stripes on the inner surface of abaxial side of corolla restricted in the tube. 9. Flower, side view. 10. Stamen, showing filaments purple. 11. Leaf, showing leaf cordate, base cordate, margin serrulate, lateral veins abaxially inconspicuous. Photographs 8–11 by Y. M. Shui.

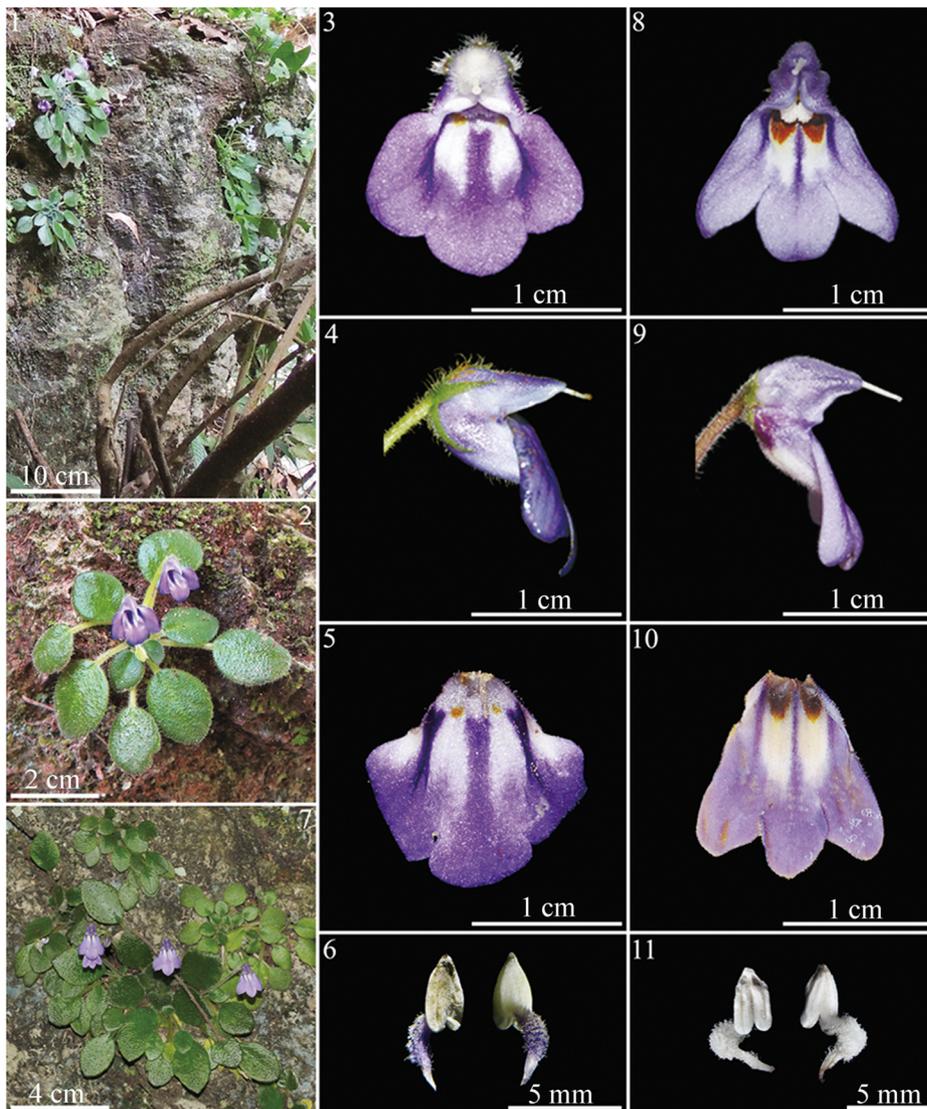
with two anthers exerted from the tube; (ii) the adaxial corolla lobes rolled laterally slightly with lobe base straight; (iii) the abaxial lip extended forward with an angle between it and carinate-plicate structure less than  $30^\circ$ ; and (iv) the corolla tube apparently extended (4–6 mm long,  $1/2$  of the abaxial lip length) with the lower part inflated at throat (Fig. 1). However, *P. longituba* has a longer corolla tube (greater than 6 mm) than any other species in *Petrocosmea*, and *P. qionglaiensis* has petioles of dark red-brown, leaf margin entire, two yellow-green

spots on the corolla tube just under filaments, two long purple stripes on the tube extended to the throat of abaxial lip, and filaments densely milky-yellow glandular, which can be easily recognized and distinguished from other species in this branch (Fig. 4; Table 3).

We used the relative corolla tube length ( $R_{(tube)}$ ) to assess the variation of the corolla tube length in representative species of *Petrocosmea*. The average relative corolla tube length of *Petrocosmea* species is approximately 30% (Fig. 5). Seven species

**Table 2** Morphological comparison of *Petrocosmea shilimensis* var. *changhuensis* and *P. purpureoglandulosa* with *P. sericea*, *P. shilimensis* var. *shilimensis*, and *P. minor*

Character	<i>P. shilimensis</i> var.			
	<i>P. sericea</i>	<i>P. shilimensis</i> var. <i>changhuensis</i>	<i>P. shilimensis</i> var. <i>shilimensis</i>	<i>P. purpureoglandulosa</i>
Leaf blade	Elliptic to ovate	Elliptic, ovate, widely ovate, or orbicular	Round or broadly ovate	Widely elliptic
Leaf base	Broadly cuneate to cuneate	Cuneate to rounded	Cordate	Cuneate to rounded
Leaf adaxial side	Densely appressed puberulent	Densely villous	Sparsely white sericeous	Densely pubescent
Leaf abaxial side	Densely covered with retrorse sericeous indumenta	Densely villous	Densely covered with retrorse sericeous indumenta	Densely pubescent
Leaf margin	Entire	Crenate	Crenate or nearly entire	Entire
Petiole	Densely covered with white retrorse sericeous indumenta	Densely pilose	Covered with white retrorse sericeous indumenta	Densely pilose
Peduncle	Densely appressed puberulent	Pilose	Pubescent	Pilose
Corolla tube	Two little round brown dots on the inner surface of tube below filaments	Two brown triangular spots on the inner surface of tube below filaments and two white oblong stripes extended to the throat of abaxial lip	Six white stripes, two brown spots on the inner surface of tube below filaments	Two little round brown spots on the inner surface of tube below filaments and two white oblong stripes extended to the throat of abaxial lip
Corolla lobes	Carinate-plicate adaxial lip inflated adaxially, abaxial lip white at throat, lateral lobes ovate	Carinate-plicate adaxial lip inflated adaxially, abaxial lip purple at throat, lateral lobes oblong	Carinate-plicate adaxial lip inflated adaxially, abaxial lip purple at throat, lateral lobes ovate-oblong	Carinate-plicate adaxial lip inflated adaxially, abaxial lip white at throat, lateral lobes triangular
Filament	White, slightly inflated and curved at middle, densely transparent glandular	White, slightly inflated and geniculate at middle, long white beard-like villi in the middle on the abaxial side	Purple, slightly inflated and geniculate, long white beard-like indumenta in the middle	White, strong inflated and geniculate at middle, densely transparent glandular-pubescent around the middle



**Fig. 3.** Photographs of *Petrocosmea purpureoglandulosa* with comparison of *P. minor*. 1–6. *P. purpureoglandulosa*. 1. Habitat. 2. Plants with basal leaves. 3. Flower, face view. 4. Flower, side view, showing the carinate-plicate structure straight on the adaxial side. 5. Abaxial corolla lip, showing lateral lobes broad ovate and 2 little round brown spots on the inner surface of corolla tube below filaments. 6. Stamen, showing filaments slightly curved, densely purple glandular. 7–11. *P. minor*. 7. Plants with basal leaves. 8. Flower, face view. 9. Flower, side view, showing the carinate-plicate structure protuberance on the adaxial side. 10. Abaxial corolla lip, showing lateral lobes triangular and 2 big triangular brown spots on the inner surface of corolla tube below filaments. 11. Stamen, showing filaments strongly geniculate, densely white glandular.

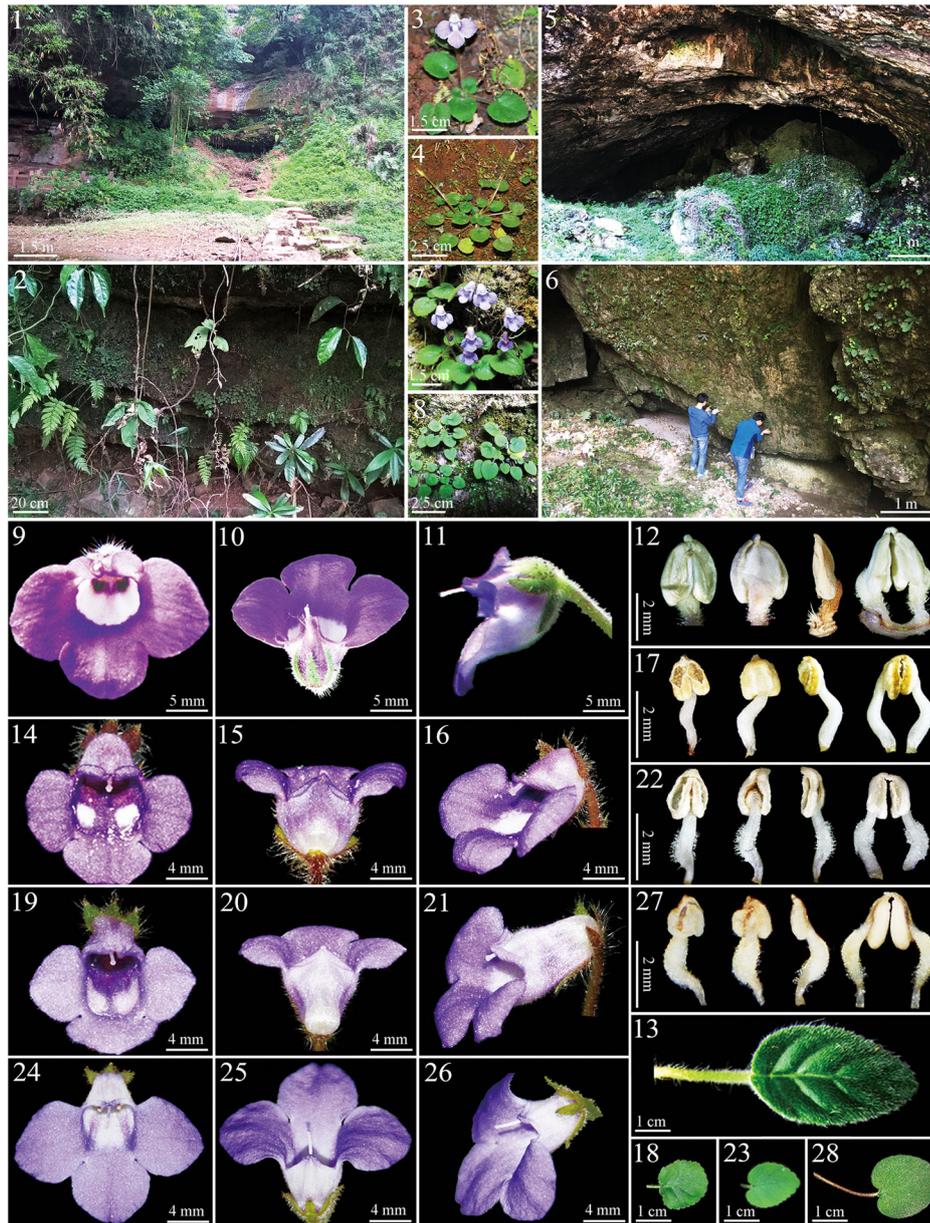
(*P. sichuanensis* Chun ex W. T. Wang, *P. intraglabra* (W. T. Wang) Zhi J. Qiu, *P. huanjiangensis*, *P. iodoides*, *P. leiandra*, *P. longituba*, and *P. qionglaiensis*) have a relative corolla tube length greater than 30%. All seven species belong to clade B or clade C. The other 15 species have a relative corolla tube length less than 30%. The t-test shows that the seven species with long corolla tube are significantly different from species with short corolla tube ( $P < 0.05$ ). *Petrocosmea longituba* has the largest relative corolla tube length (42.1%), more than twice *P. oblata* Craib, which has the smallest relative corolla tube length (19.1%). The species in clades A, D, and E all have relative corolla tube length less than 30% (Figs. 1, 5). Even in clade C, the relative corolla tube length of

*P. longituba* (42.1%) is approximately twice as long as that of *P. grandiflora* (22.2%). The relative corolla tube length ( $R_{(tube)}$ ) is consistent with the absolute corolla tube length (6–7 mm long), twice as long as the average corolla tube length (3–4 mm long) of *Petrocosmea* species (Figs. 1, 5).

## 4 Discussion

### 4.1 Floral morphological reversal versus specialization

Recent phylogenetic studies indicate that the diandrous flower with a short corolla tube (only 3–6 mm in length) characteristic of *Petrocosmea* might have proceeded from a



**Fig. 4.** Photographs of *Petrocosmea qionglaiensis*, *P. longituba* with comparison of *P. iodoides* and *P. leiandra*. 1–4. *P. qionglaiensis*. 1, 2. Habitat, growing on moist shady cliff of limestone hill. 3, 4. Plants with basal leaves. 5–8. *P. longituba*. 5, 6. Habitat, growing on moist shady cliff of limestone hill. 7, 8. Plants with basal leaves. 9–13. *P. iodoides*. 9. Face view. 10. Top view. 11. Side view. 12. Stamen. 13. Leaf. 14–18. *P. leiandra*. 14. Face view. 15. Top view. 16. Side view. 17. Stamen. 18. Leaf. 19–23. *P. longituba*. 19. Face view. 20. Top view. 21. Side view. 22. Stamen. 23. Leaf. 24–28. *P. qionglaiensis*. 24. Face view. 25. Top view. 26. Side view. 27. Stamen. 28. Leaf, showing petiole dark red-brown.

tetrandrous flower with a long corolla tube (usually 3–5 cm in length) of a *Raphiocarpus*-like ancestor (Qiu et al., 2015, see also Fig. 1). Importantly, the phylogenetic reconstruction combined with morphological analyses shows that the flowers of clade C with a short corolla tube are most specialized in *Petrocosmea*. The two adaxial corolla lobes of the adaxial lip extend forward and are fused in nearly their full length with each lobe folded down and rolled up again laterally and then reflected backwards to form a specialized carinate-plicate structure. This carinate-plicate structure

encloses the style, which is tightly pressed against the inner surface to establish a synorganized complex structure that has never been mentioned in the traditional taxonomic literature in Gesneriaceae (Qiu et al., 2015, see also Fig. S1). In the phylogeny of *Petrocosmea*, the two adaxial corolla lobes of the adaxial lip tend to be shortened and specialized from clade A through clade B to clade C in size and shape, that is, successive specialization. However, the morphological reversals take place from clade C to clade D and further to clade E as well as within clade C. These are evident that the

**Table 3** Morphological comparison of *Petrocosmea longituba* and *P. qionglaiensis* with *P. huanjiangensis*, *P. iodoides*, *P. leiandra*, and *P. leiandra*

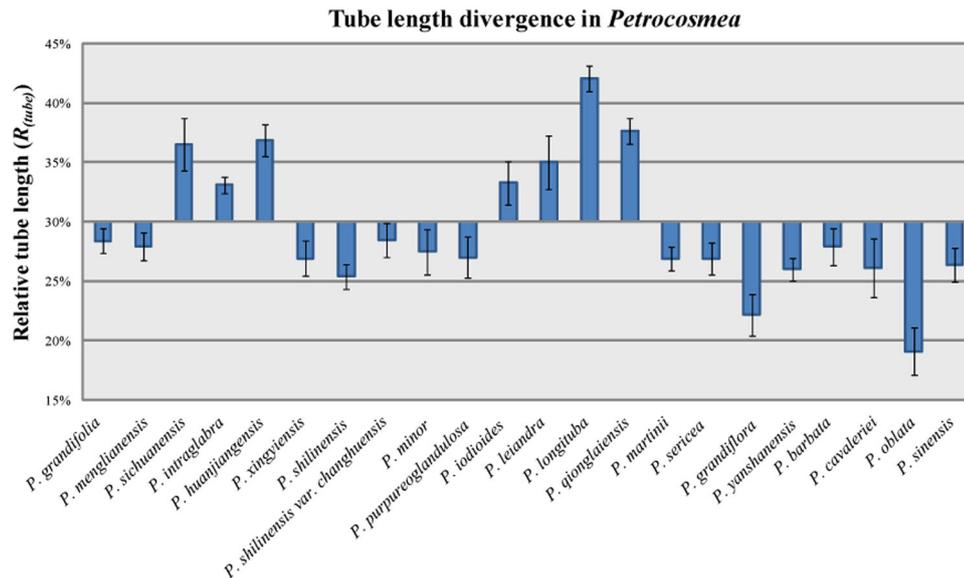
Character	<i>P. huanjiangensis</i>	<i>P. iodoides</i>	<i>P. leiandra</i>	<i>P. longituba</i>	<i>P. qionglaiensis</i>
Leaf blade	Ovate or broadly ovate to subrotund	Ovate or lance-ovate to orbicular	Ovate to elliptic	Cordate	Cordate
Leaf base	Peltate	Cordate	Cordate	Cordate	Cordate
Leaf margin	Crenate or repand	Crenate	Serrate	Entire to crenate	Entire
Corolla tube	Oblique campanulate, two yellow spots on the inner surface of tube just below filaments	Oblique campanulate, two brown spots connected to two wine red spots on the inner surface of tube below filaments	Oblique campanulate, two red spots merged into one on inner surface of tube below filaments and two white spots at the throat of abaxial lip	Short tubular, two white elliptic spots on the inner surface of tube near throat of abaxial lip	Short tubular, two yellow-green spots on tube under filaments and two purple stripes on tube extended to the throat of abaxial lip
Corolla lobes	Carnate-plicate adaxial lip inflated adaxially, abaxial lip white at throat	Carnate-plicate adaxial lip straight, abaxial lip white at throat	Carnate-plicate adaxial lip straight, abaxial lip violet at throat	Carnate-plicate adaxial lip straight, abaxial lip violet at throat	Carnate-plicate adaxial lip straight, abaxial lip white at throat
Filament	Densely lanate	Densely white glandular	Glabrous	Densely white glandular	Densely milky-yellow glandular

flowers of clade D are similar to clade B, and clade E similar to clade A as well as some species of clade C, such as *P. yanshanensis* and *P. grandiflora*, similar to clade A and E (Qiu et al., 2015, see also Figs. 1, S1).

Here, we report that, in the four new taxa recently found in *Petrocosmea*, one (*P. longituba*) has a long corolla tube that is two to three times as long as the average corolla tube length of other *Petrocosmea* (Figs. 1, 4, 5). Another species (*P. qionglaiensis*) also has a corolla tube distinctively longer than the average length of the corolla tube of other *Petrocosmea* (Figs. 4, 5). Surprisingly, the two new species are grouped in a branch with high support, in which *P. qionglaiensis* is located at the basal position and *P. longituba* is closely related to two sister species with a normal corolla tube (slightly longer than the average length of the corolla tube of other *Petrocosmea*) (Figs. 1, 4, 5). This branch pattern implies that the evolutionary event of the corolla tube lengthening might have successively happened in this branch. Meanwhile, the corolla tube shortening proceeded to further specialization and diversification in other branches. Apparently, the successive corolla tube lengthening represents an evolutionary trend of reversal to the *Raphiocarpus*-like ancestor. In addition, the two new species have anthers with longitudinal-poricidal dehiscence that is different from the poricidal dehiscence of anthers in other members of clade C (except for *P. leiandra* and *P. grandiflora*). It would be interesting to know whether or not the two kinds of morphological characters are functionally correlative for adaptation.

According to Porter & Crandall (2003), reverse evolution is the change of a character state to a state similar in appearance to an ancestral state. Since Dollo (1893) proposed the concept of irreversibility of evolution, that is, Dollo's law, further enumerated by Simpson (1953) and Gould (1970), evolution in reverse has been an interesting subject that attracts many biologists' attention. They have attempted to find insights to resolve the issue of whether ancestral characters can be regained or not. Nevertheless, although evidence has continuously strengthened Dollo's law (Zufall & Rausher, 2004; Goldberg & Igić, 2008; Bridgman et al., 2009; Smith & Rausher, 2011), frequent exceptions have also challenged Dollo's law. Especially, these challenges have recently been met in the fields of molecular phylogeny and evolutionary developmental biology, such as: (i) coiled shells re-evolved in gastropods with uncoiled cap-shaped shells (Collin & Cipriani, 2003); (ii) regain of a free living life form of the ancestral lifestyle in house dust mites that re-evolved from a parasitic lifestyle (Klimov & OConnor, 2013); and (iii) an evolutionary reversal from C<sub>4</sub> to C<sub>3</sub> photosynthesis in *Alloteropsis* J. Presl (Poaceae) (Ibrahim et al., 2009).

Approximately one-third of angiosperm species produce flowers with petals fused into a corolla tube with adnate stamens (Erbar & Leins, 1996; APG IV, 2016). The length of the corolla tube is coevolved with the behavior or the shape of the mouth-parts of animal pollinators (Darwin, 1862; Nilsson, 1988). The discovery of the reversal from short corolla tube to the ancestral state of long corolla tube in clade C highlights empirical evidence violating Dollo's law. Even though highly specialized, the floral organs of clade C are still actively evolving in response to environmental



**Fig. 5.** Tube length divergence in *Petrocosmea*. X-axis, species of *Petrocosmea*; Y-axis, relative tube length ( $R_{(tube)}$ ). Line of 30% on Y-axis was set to better show the difference between species with long tube and short tube (30% is the average value of  $R_{(tube)}$ ).

variables. The dynamic evolvability is reflected in multiple branches, in which some branches evolve toward specialization while others reverse into different ancestral states, including regain of the long corolla tube and loss of the highly specialized zygomorphy. The ancestral developmental pathway generating the long corolla tube might have been more or less maintained during a long period of such trait absence preceding the reacquisition of the trait in the face of perturbed environment.

#### 4.2 Adaptive implication of the reverse character long corolla tube

The giant collision of the Indian subcontinent with the Asian plate approximately 50 million year ago could be the most important geological event in the Cenozoic, which has thrown up the highest mountains (Himalayas) and the largest and highest plateau (Tibet) on Earth (Royden et al., 2008; Kind & Yuan, 2010). The rapid uplift of the Himalayan–Tibetan plateau was accompanied with increasing climatic and environmental changes, especially the continued intensification of the East Asian monsoon through the Oligocene to late Miocene (An et al., 2001; Sun & Wang, 2005; Royden et al., 2008). The ancestral area reconstruction in Qiu et al. (2015) indicates that *Petrocosmea* might have originated in the boundary area of India, Myanmar, Thailand, and China, and the most specialized clade C is greatly diversified in the Hengduan Mountain–Yungui Plateau region. This region is located at the eastern shoulder of the Himalayan–Tibetan plateau, the most geologically active region after the collision of the India with Asia, mainly covered with pure carbonate substrate (Zhang & Ding, 2003; Royden et al., 2008). These well-known limestone areas are characteristic of fluctuating ecological environments with an alternation between severe erosion in the rainy season and extreme drought in the long dry season (Yuan et al., 1991; Xu, 1995). The dramatic climate change upon the Himalayan uplift with spatiotemporally heterogeneous ecogeographical environments might have

served as a key driving force for speciation and diversification, which is evident in radical changes of the biota that occurred in these areas since the Eocene or Miocene epoch (Li & Li, 1993; Axelrod et al., 1998; Chapman & Wang, 2002).

Evolutionary change commonly involves an adaptive shift between different ecological niches, including both the physical environment that directly affects survival and the pollinator environment that influences lifetime fecundity in plants (Stebbins, 1970; Johnson, 2010). Pollinator-mediated selection on floral traits is widely regarded as a key mechanism of adaptation and speciation in angiosperms (Johnson, 2010; Van der Niet & Johnson, 2012). Pollination type and behavior usually have a great effect on the floral structure and reproductive success that help plants to establish an adaptive advantage over competitors (Schemske & Bradshaw, 1999; Caruso, 2000). It has been increasingly documented that pollinator or pollinator-behavior shifts are usually associated with reproductive isolation and further speciation (Fenster et al., 2004; Kay & Sargent, 2009; Johnson, 2010; Van der Niet & Johnson, 2012). Considering the above-mentioned facts, we here suggest that the regain of the long corolla tube, correlated with longitudinal-poricidal dehiscence, in clade C of *Petrocosmea* might have been related to adaptation to new pollinators evolved under the background of the uplift of the Himalayan–Tibetan plateau.

Increasing evidence indicates that the strong environmental and climatic impact of the Himalayan uplift led to a fundamental change of the insect fauna in the eastern Himalayan–Tibetan plateau, especially in Hengduan Mountain–Yungui plateau regions where clade C of *Petrocosmea* is presently diversified (Wang, 1990; Yang & Zhao, 2000). A new variant with a specific change in floral morphology would succeed in reproduction only when it fits well into a particular pollinator environment or “pollination climate,” and then goes further to speciation (Johnson, 2010). Therefore, this type of

morphological reversal is likely a product of concerted evolution associated with adaptation to the new insect fauna, such as insect pollinators with elongated tongues. The long corolla tube correlated with longitudinal-poricidal dehiscence as a deviant floral morphology in clade C of *Petrocosmea* could have taken advantage of the new ecological opportunity with interactions in the new insect fauna in response to drastic environmental changes. The Himalayan uplift acts as both the provider of new ecological opportunities and the activator of key innovations, and the adaptive speciation starts at the coincident coupling of the two events that have happened in plants and animals. In a laboratory experiment in *Drosophila*, after reimposing the ancestral environment on 25 diverged populations over 50 generations, an overwhelming tendency to evolutionary reversal took place during 50 generations of selection. However, reverse evolution failed to occur, or was stalled in some populations (Teotónío & Rose, 2000). This experiment indicates that evolution in reverse is achievable, but is a contingent process dependent on genetic backgrounds interacting with environments, that is, genotype-by-environment interaction (Teotónío & Rose, 2000; Porter & Crandall, 2003). This could be particularly true for the ancestral state of the long corolla tube in clade C of *Petrocosmea*. The great disturbance of ecological environment is likely a crucial factor affecting trait reversibility, as revealed herein. Reverse evolution, in fact, is achieved in a special adaptive way in which the variation in a particular phenotype that tends towards the ancestral state successively responds to ancestral environments occasionally emerging in disturbed backgrounds. It would be very interesting to explore the genetic basis underlying such evolutionary phenomena and the interplay between genetic factors and environmental variables in floral morphological evolution.

## 5 Taxonomy treatments

### 5.1 *Petrocosmea shilinesis* Y. M. Shui & H. T. Zhao var. *changhuensis* T. F. Lü & Y. Z. Wang var. nov.

**Type:** China. Yunnan Province: Shilin County, Changhu Town, Changhu Scenic Area, 24°42'44"N, 103°25'3"E, elevation ca. 1920 m a.s.l., on moist shady cliffs under the forest of limestone hills, 16 August 2015, Y. Dong 20140619F (holotype, PE!).

**Diagnosis:** *Petrocosmea shilinesis* var. *changhuensis* is morphologically similar to *P. shilinesis*, but it differs from *P. shilinesis* var. *shilinesis* mainly in leaf rhombic, leaf base cuneate, leaf margin crenate, abaxial lip white at throat, filaments white. A detailed morphological comparison of the two species is shown in Fig. 2.

Perennial herbs, rosettes, with short rhizome and crowded fibrous roots. Leaves 10–40, all basal, crowded; leaf blades rhombic, obovate, or sometimes ovate and elliptic, 1–5 × 0.5–5 cm, apex rounded, base cuneate to rounded, margin crenate, both surfaces densely villous, lateral veins 3–4, abaxially conspicuous; petioles 0.5–7 cm long, densely pilose. Cymes 3–10, one flower per cyme; peduncle 5–8 cm long, densely pilose; bracts 2 at mid-peduncle, lanceolate, 0.4–0.7 cm long, pubescent, pedicel 1.5–5 cm long, densely pilose. Calyx 5-lobed to base, calyx lobes narrowly lanceolate, 4–5.5 mm long, pubescent externally. Corolla zygomorphic, blue to purple, pubescent outside, glabrous inside, with two

brown triangular spots on the inner surface of the corolla tube just below filaments and two white oblong stripes extended to the throat of the abaxial lip; corolla tube campanulate, 3.5–4.5 mm long; adaxial lip 3.5–4.5 mm long, indistinctly bi-lobed, carinate-plicate, inflated adaxially, enclosing the style and two anthers; adaxial lobes 0.5–0.8 mm long, rolled laterally and reflexed backward; abaxial lip ca. 0.8–1.1 cm long, tri-lobed to the middle, lateral lobes oblong, 5–6 × 2.5–3 mm, apex rounded; ventral lobe ovate, 4–5 × 3–4.5 mm, apex rounded. Stamens 2, ca. 4–5 mm long, filaments adnate to corolla tube near base, ca. 2.5 mm long, geniculate, white, covered with ca. 1.5 mm long white beardlike villi in the middle on the abaxial side; staminodes 3, linear, adnate to corolla tube near base, 0.2–0.5 mm long, glabrous; anthers narrowly ovoid, brevirostrate, coherent at apex, white, ca. 3.2 mm long, poricidal-dehiscent apically. Pistil ca. 12 mm long, ovary pubescent, oblique abaxially, ca. 4 mm long; style glabrous or sparsely pubescent at base, ca. 8 mm long. Capsules ovoid, style persistent.

**Phenology:** The new species was observed to flower from August to October, fruit from September to November.

**Distribution and habitat:** *Petrocosmea shilinesis* var. *changhuensis* grows on moist shady cliffs under the forest of limestone hills in Changhu Scenic Area, at ca. 1920 m a.s.l., in northeastern Yunnan (Changhu Town), China.

**Etymology:** The name of this new species is derived from its locality, that is, Changhu Town, Yunnan Province, China.

**Additional specimens examined (paratypes):** China. Yunnan Province: Kunming City, Shilin County, Changhu Town, Changhu Scenic Area, 24°42'44"N, 103°25'3"E, elevation ca. 1920 m a.s.l., on moist shady cliffs under the forest of limestone hills, 20 September 2016, M. Q. Han, Y. Dong & T. F. Lü HMQ1150 (IBK, PE).

### 5.2 *Petrocosmea purpureoglandulosa* Y. Dong & Y. Z. Wang sp. nov.

**Type:** China. Yunnan Province: Kunming City, Shilin County, Banqiao Town, Dadieshui Scenic Area, 24°40'28"N, 103°11'48"E, elevation ca. 1448 m a.s.l., on moist shady cliffs under the forest of limestone hills, 13 June 2014, Y. Dong 20140613C (holotype PE!).

**Diagnosis:** *Petrocosmea purpureoglandulosa* is morphologically similar to *P. minor*, but differs mainly by its wider leaf blades, lateral corolla lobes broad ovate, carinate-plicate structure straight on the adaxial side, filaments slightly curved, purple glandular. A detailed morphological comparison of the two species is shown in Fig. 3.

Perennial herbs, rosettes, with short rhizome, and crowded fibrous roots. Leaves 15–30, all basal, crowded; leaf blades widely elliptic, 0.5–3.5 × 0.4–3 cm, apex rounded, base rounded, margin entire, both surfaces densely pubescent, lateral veins 3–4, distinct on abaxial side; petioles 0.5–6 cm long, densely pubescent. Cymes 3–10, 1–2 flowers per cyme; peduncle 5–8 cm long, densely pilose; bracts 2, lanceolate, 0.5–1 cm long, pubescent, pedicel 2–5 cm long, with dense pubescence. Calyx 5-lobed to base, lobes narrowly lanceolate, 5–7 mm long, pubescent externally. Corolla zygomorphic, blue to purple, pubescent outside, glabrous inside, with 2 little round brown dots on abaxial corolla lip under filaments; corolla tube campanulate,

4–4.5 mm long; adaxial lip ca. 5 mm long, indistinctly bi-lobed, carinate-plicate, straight, enclosing the style; the adaxial lobes 0.6–1.2 mm long, rolled laterally and reflexed backward; abaxial lip 1.2–1.4 cm long, tri-lobed to the middle, lateral lobes broad ovate, 6.5–8.5 × 5.5–7 mm, apex blunt or rounded; ventral lobe ovate 5.5–7.5 × 5.5–7 mm, apex rounded. Stamens 2, ca. 6 mm long, filaments adnate to corolla tube near base, ca. 4 mm long, slightly curved, densely purple glandular; staminodes 3, linear, adnate to corolla tube near base, 0.2–0.5 mm long, glabrous; anthers narrowly ovoid, brevirostrate, coherent at apex, pale-yellowish green, ca. 3 mm long, poricidal-dehiscent apically. Pistil ca. 12 mm long, ovary pubescent, oblique abaxially, ca. 4.5 mm long; style glabrous or sparsely pubescent at base, ca. 4 mm long. Capsules narrowly ovoid, style persistent.

**Phenology:** The new species was observed to flower from August to October, fruit from September to November.

**Distribution and habitat:** *Petrocosmea purpureoglandulosa* is endemic to Yunnan, restricted to growing on moist shady cliffs under the forest of limestone hills, 0.5 km west of Dadieshui Waterfalls in Dadieshui Scenic Area, at 1448 m a.s.l., in northeastern Yunnan (Banqiao Town), China. This species usually grows with *P. barbata* Craib.

**Etymology:** The name of this new species is derived from its character of filaments densely purple glandular, never described in other known species.

**Additional specimens examined (paratypes):** China. Yunnan Province: Kunming City, Shilin County, Banqiao Town, Dadieshui Scenic Area, 24°40'28"N, 103°11'48"E, elevation ca. 1448 m a.s.l., on moist shady cliffs under the forest of limestone hills, 20 September 2016, M. Q. Han, Y. Dong & T. F. Lü HMQ1153 (IBK, PE).

### 5.3 *Petrocosmea longituba* M. Q. Han & Yan Liu sp. nov.

**Type:** China. Guizhou Province: Duyun City, Heilongzhai village, elevation ca. 840 m a.s.l., 26°19'7"N, 107°29'32"E, 15 April 2016, M. Q. Han, Y. Dong & T. F. Lü HMQ988 (holotype IBK!, isotype PE!).

**Diagnosis:** *Petrocosmea longituba* is morphologically similar to *P. leiandra*, but it differs mainly in the corolla tube much lengthened, filaments densely white glandular. A detailed morphological comparison of the two species as well as *P. leiandra* and *P. iodoides* is shown in Fig. 4.

Perennial herbs, rosettes, with short rhizome and crowded fibrous roots. Leaves 6–10, all basal, crowded; leaf blades cordate, 0.3–2 × 0.2–2 cm, apex obtuse, base cordate, margin entire to crenate, both surfaces densely pubescent, lateral veins 4–5; petioles 0.5–4 cm long, densely pubescent. Cymes 3–10, 1–2 flowers per cyme; peduncle 3–8 cm long, densely pilose; bracts 2, lanceolate, 0.3–0.6 cm long, pubescent. Calyx 5-lobed to base, calyx lobes narrowly lanceolate, 4–5 mm long, pubescent externally. Corolla zygomorphic, blue to purple, pubescent outside, glabrous inside; two white elliptic spots near throat on the inner surface of abaxial corolla tube; corolla tube 6–7 mm long, short tubular; adaxial lip ca. 2–2.5 mm long, bi-lobed subapically, carinate-plicate, enclosing the style; the adaxial lobes ca. 0.7 mm long, rolled laterally and reflexed backward; abaxial lip 7–8 mm long, ventricose at throat, tri-lobed to the middle, lateral lobes widely rotund, 5–5.5 × 5–5.5 mm, apex blunt to rounded; ventral lobe ovate, 5–6 × 5–6 mm, apex

rounded. Stamens 2, 3–3.5 mm long, filaments adnate to corolla tube near base, ca. 2–3 mm long, slightly curved, densely white glandular; staminodes 3, linear, adnate to corolla tube near base, 0.2–0.5 mm long, glabrous; anthers ovoid, brevirostrate, coherent at apex, white, ca. 1.5 mm long, longitudinal-poricidal dehiscent nearly to base, introrse. Pistil ca. 7 mm long, ovary pubescent, oblique abaxially, ca. 2 mm long; style glabrous or sparsely pubescent at base, ca. 5 mm long, bending downward at base.

**Distribution, habitat, and ecology:** *Petrocosmea longituba* grows on moist shady cliffs at the entrance to limestone cave, at 840 m a.s.l., Heilongzhai village, Duyun City, Guizhou Province, China.

**Phenology:** The new species was observed to flower from May to July; fruits not seen.

**Etymology:** The name of this new species is derived from its character of long corolla tube.

**Additional specimens examined (paratypes):** China. Guizhou Province: Duyun city, Heilongzhai village, elevation ca. 840 m a.s.l., 26°19'7"N, 107°29'32"E, 10 June 2016, M. Q. Han, L. B. Zhang & X. Y. Miao HMQ1062 (IBK).

### 5.4 *Petrocosmea qionglaiensis* C. Q. Li & Y. Z. Wang sp. nov.

**Type:** China. Sichuan Province: Qionglai City, Pingle Town, 30°20'24"N, 103°18'29"E, elevation ca. 1920 m a.s.l., on moist shady cliffs under the forest of limestone hills, 16 May 2015. *Chaoqun Li 2015051611* (holotype PE!).

**Diagnosis:** Morphologically, *P. qionglaiensis* is similar to *P. leiandra*, but it differs from *P. leiandra* by leaf margin entire, 2 yellow-green spots on the corolla tube just under filaments and 2 long purple stripes on the tube extended to the throat of abaxial lip, filament densely pubescent, anther ovoid, longitudinal-poricidal dehiscent to base. A detailed morphological comparison of the two species as well as *P. longituba* and *P. iodoides* is shown in Fig. 4.

Perennial herbs, rosettes, with short rhizome, and crowded fibrous roots. Leaves 10–25, all basal, crowded; leaf blades cordate, 0.5–2 × 0.3–2 cm, apex obtuse, base cordate, margin entire, both surfaces densely pubescent, lateral veins 4–5; petioles dark red-brown, 0.5–4 cm long, densely pubescent. Cymes 3–10, 1–2 flowers per cyme; peduncle 3–8 cm long, densely pilose; bracts 2, lanceolate, 0.3–0.6 cm long, pubescent. Calyx 5-lobed to base, calyx lobes narrowly lanceolate, ca. 4–5 mm long, pubescent externally. Corolla zygomorphic, blue to purple, pubescent outside, glabrous inside; 2 yellow-green spots on corolla tube just under filaments and 2 purple stripes on corolla tube extended to the throat of abaxial lip; corolla tube 5–6 mm, short tubular; adaxial lip ca. 2–3 mm long, indistinctly bi-lobed, carinate-plicate, enclosing the style; adaxial lobes ca. 0.5–1 mm, rolled; abaxial lip ca. 1–1.2 cm long, ventricose at throat, tri-lobed to the middle, lateral lobes broad ovate, 5.5–6.5 × 5–6 mm, apex rounded; ventral lobe ovate 6–7 × 5.5–7 mm, apex rounded. Stamens 2, ca. 3.5 mm long, filaments adnate to corolla tube near base, ca. 2.3 mm long, slightly curved, densely white glandular; staminodes 3, linear, adnate to corolla tube near base, 0.2–0.5 mm long, glabrous; anthers ovoid, brevirostrate, coherent at apex, white, ca. 1.6 mm long, longitudinal-poricidal dehiscent from apex extend to base, introrse. Pistil ca. 7.2 mm long, ovary pubescent, oblique

abaxially, ca. 3.4 mm long; style glabrous or sparsely pubescent at base, ca. 3.8 mm long, straight.

**Phenology:** The new species was observed to flower from May to July; fruits not seen.

**Distribution and habitat:** *Petrocosmea qionglaiensis* grows on moist shady cliffs of limestone hills beside streams, at 1900–2050 m a.s.l., in Sichuan (Pingle Town), China.

**Etymology:** The name of this new species is derived from its locality, that is, Qionglai City, Sichuan Province, China.

## Acknowledgements

We thank Dr. James F. Smith for constructive comments and language improvements on this article. We also thank Dr. Yu-Min Shui, Dr. Bo Pan, Dr. Li-Bing Zhang and Xin-Yuan Miao for providing photographs and location of related species and help with fieldwork. This study was funded by the National Natural Science Foundation of China (Grant Nos. 31530003 and 31170198).

## References

- An ZS, Kutzbach JE, Prell WL, Porter SC. 2001. Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. *Nature* 411: 62–66.
- APG IV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181: 1–20.
- Axelrod AI, Al-Shehbaz I, Raven PH. 1998. History of modern flora of China. In: Zhang AL, Wu SG eds. *Floristic characteristics and diversity of East Asian plants*. Beijing: Higher Education Press; Verlag: Springer. 43–55.
- Bridgham JT, Ortlund EA, Thornton JW. 2009. An epistatic ratchet constrains the direction of glucocorticoid receptor evolution. *Nature* 461: 515–519.
- Caruso CM. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. *Evolution* 54: 1546–1557.
- Chapman GP, Wang YZ. 2002. *The plant life of China*. Berlin: Springer.
- Collin R, Cipriani R. 2003. Dollo's law and the re-evolution of shell coiling. *Proceedings of the Royal Society B: Biological Sciences* 270: 2551–2555.
- Collin R, Miglietta MP. 2008. Reversing opinions on Dollo's Law. *Trends in Ecology and Evolution* 23: 602–609.
- Darwin C. 1862. *On the various contrivances by which British and foreign orchids are fertilized*. London: Murray. 365.
- Dollo L. 1893. Les Lois de l'évolution. *Bulletin de la Société belge de Géologie, de paléontologie, et de hydrologie* 7: 164–166.
- Erbar C, Leins P. 1996. Distribution of the character states "Early Sympetaly" and "Late Sympetaly" within the "Sympetaleae Tetracyclaeae" and presumably allied groups. *Botanica Acta* 109: 427–440.
- Farris JS, Källersjö M, Kluge AG, Bult C. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35: 375–403.
- Goldberg EE, Igić B. 2008. On phylogenetic tests of irreversible evolution. *Evolution* 62: 2727–2741.
- Gould SJ. 1970. Dollo on Dollo's Law: Irreversibility and the status of evolutionary laws. *Journal of the History of Biology* 3: 189–212.
- Han MQ, Lü TF, Liu Y. 2017. *Petrocosmea magnifica* (Gesneriaceae): A new species from limestone caves in Yunnan, China. *Phytotaxa* 319: 283–288.
- Han MQ, Lü TF, Liu Y. 2018a. *Petrocosmea viridis* sp. nov. of *Petrocosmea* (Gesneriaceae) from Guizhou, China and a supplementary and revised description of *P. minor*. *Nordic Journal of Botany* 36: njb-01566.
- Han MQ, Yuan Q, Lü TF, Jiang H, Liu Y. 2018b. *Petrocosmea chrysotricha* sp. nov. (*Petrocosmea*, Gesneriaceae), a species previously mistaken for *P. begoniifolia* on marlstone cliffs in Yunnan, China. *Nordic Journal of Botany* 36: njb-01664.
- Ibrahim DG, Burke T, Ripley BS, Osborne CP. 2009. A molecular phylogeny of the genus *Alloteropsis* (Panicoidae, Poaceae) suggests an evolutionary reversion from C4 to C3 photosynthesis. *Annals of Botany* 103: 127–136.
- Johnson SD. 2010. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365: 499–516.
- Kay KM, Sargent RD. 2009. The role of animal pollination in plant speciation: Integrating ecology, geography, and genetics. *Annual Review of Ecology, Evolution, and Systematics* 40: 637–656.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.
- Kind R, Yuan X. 2010. Seismic images of the biggest crash on earth. *Science* 329: 1479–1480.
- Klimov PB, O'Connor B. 2013. Is permanent parasitism reversible? Critical evidence from early evolution of house dust mites. *Systematic Biology* 62: 411–423.
- Li XW, Li J. 1993. A preliminary floristic study on the seed plants from the region of Hengduan Mountain. *Acta Botanica Yunnanica* 15: 217–231.
- Li ZY, Wang YZ. 2004. *Plants of Gesneriaceae in China*. Zhengzhou: Henan Science and Technology Publishing House.
- Nilsson LA. 1988. The evolution of flowers with deep corolla tubes. *Nature* 334: 147–149.
- Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.
- Porter ML, Crandall KA. 2003. Lost along the way: The significance of evolution in reverse. *Trends in Ecology and Evolution* 18: 541–547.
- Qiu ZJ, Liu ZY. 2015. *Plants of Petrocosmea in China*. Beijing: Science Press.
- Qiu ZJ, Lu YX, Li CQ, Dong Y, Smith JF, Wang YZ. 2015. Origin and evolution of *Petrocosmea* (Gesneriaceae) inferred from both DNA sequence and novel findings in morphology with a test of morphology-based hypotheses. *BMC Plant Biology* 15: 1–19.
- Rogers SO, Bendich AJ. 1988. Extraction of DNA from plant tissues. *Plant Molecular Biology Manual* A6: 1–10.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Royden LH, Burchfiel BC, van der Hilst RD. 2008. The geological evolution of the Tibetan Plateau. *Science* 321: 1054–1058.
- Schemske DW, Bradshaw HD. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences USA* 96: 11910–11915.
- Simpson GG. 1953. *Major features of evolution*. New York: Columbia University Press.

- Smith SD, Rausher MD. 2011. Gene loss and parallel evolution contribute to species difference in flower color. *Molecular Biology and Evolution* 28: 2799–2810.
- Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: Pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307–326.
- Sun X, Wang P. 2005. How old is the Asian monsoon system? Palaeobotanical records from China. *Palaeogeography Palaeoclimatology Palaeoecology* 222: 181–222.
- Swofford DL. 2003. PAUP\*: Phylogenetic analysis using parsimony (\*and other methods). Version 4. Beta 10. Sunderland: Sinauer Associates.
- Teotónio H, Rose MR. 2000. Variation in the reversibility of evolution. *Nature* 408: 463–466.
- Van der Niet T, Johnson SD. 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology and Evolution* 27: 353–361.
- Wang SY. 1990. Primary discussion on the fauna of Hengduan Mountains, China. *Acta Entomologica Sinica* 33: 94–101.
- Wang WT. 1985. The second revision of the genus *Petrocosmea* (Gesneriaceae). *Acta Botanica Yunnanica* 7: 49–68.
- Wang WT. 1990. *Petrocosmea* Oliver (Gesneriaceae). In: *Flora Reipublicae Popularis Sinicae*. Beijing: Science Press. 69: 305–323.
- Wang WT. 1998. *Petrocosmea* Oliver. In: Wu ZY, Raven PH, Hong DY eds. *Flora of China*. Beijing: Science Press; St. Louis: Missouri Botanical Garden. 18: 302–308.
- Weber A. 2004. Gesneriaceae. In: Kubitzki K, Kadereit JW eds. *The families and genera of vascular plants, Dicotyledons. Lamiales (except Acanthaceae including Avicenniaceae)*. Berlin: Springer. 7: 63–158.
- Weber A, Clark JL, Möller M. 2013. A new formal classification of Gesneriaceae. *Selbyana* 31: 68–94.
- Xu ZR. 1995. A study of the vegetation and floristic affinity of the limestone forests in southern and southwestern China. *Annals of the Missouri Botanical Garden* 82: 570–580.
- Yang XK, Zhao JM. 2000. Fifty years of insect classification in China. *Entomological Knowledge* 37: 1–11.
- Yuan DX, Zhu DH, Weng JT, Zhu XW, Han XR, Wang XY. 1991. *Karst of China*. Beijing: Geological Publishing House.
- Zhang J, Ding L. 2003. East-west extension in Tibetan plateau and its significance to tectonic evolution. *Chinese Journal of Geology* 38: 179–189.
- Zufall RA, Rausher MD. 2004. Genetic changes associated with floral adaptation restrict future evolutionary potential. *Nature* 428: 847–850.

## Supplementary Material

The following supplementary material is available online for this article at <http://onlinelibrary.wiley.com/doi/10.1111/jse.12541/supinfo>:

**Fig. S1.** Photos of representative flower morphologies of the five clades in *Petrocosmea*.