广 西 植 物 Guihaia Apr. 2015, **35**(2):143－150 http://journal.gxzw.gxib.cn

DOI: 10.11931/guihaia.gxzw201402007

黎乾坤,张强,邓涛,等. 广西报春苣苔属一新种及其系统位置[J]. 广西植物,2015,35(2):143－150

Li QK, Zhang Q, Deng T, *et al*. *Primulina bobaiensis*, a new species of Gesnericaeae from Guangxi, China and its phylogenetic placement revealed by the chloroplast *matK* gene[J]. Guihaia, 2015, 35(2):143－150

Primulina bobaiensis, a new species of Gesnericaeae from Guangxi, China and its phylogenetic placement revealed by the chloroplast *matK* gene

LI Qian-Kun[1], ZHANG Qiang[2], DENG Tao[2], PAN Bo[2],
HUANG Yu-Song[2], LI Wen-Lan[1,3*]

(1. *College of Chemistry and Bioengineering*, *Guilin University of Technology*, Guilin 541004, China; 2. *Guangxi Institute of Botany*, *Guangxi Zhuang Autonomous Region and the Chinese Academy of Sciences*, Guilin 541006, China; 3. *College of Life Sciences & Technology of Guangxi University*, Nanning 530005, China)

Abstract: *Primulina* (Gesneriaceae) is an enigmatic group of lithophytic plants, which encompass over 160 species mainly distributed in the limestone region of southern China and nothern Vietnam. A majority of species of the genus have very narrow distribution, which have been found in only one or a few proximate localities. It seems that this genus could even possess much more diversity than presently known as lots of new species have recently been and are being discribed, particularly from its distributional center. In the presnt study, a new species of Gesneriaceae, *P. bobaiensis* from Guangxi, China is described and illustrated. We intuitively judged the higher taxonomic unit, i. e. genus, that the assumed new species should belong to; compared the morphologies of this assumed new species with all other recognized species previously discribed in the genus and deduced possible close relatives of the new species based on morphological similarity. Then we tested its affinities to other sampled species from the same large region and the divergence of DNA sequences between the assumed new species and its closest relatives. Morphologically, this new species is assumed to belong to the genus *Primulina* and can be easily distinguished from all other Gesneriaceae species by its special floral appearance, e. g. lower lip is much longer than upper lip and it has spotted marks on the inside of the upper lip (the majority of other species have striped marks on the inside(s) of the lower and/or upper lips). Subsequently, the taxonomic treatment of being attibuted to *Primulina* and the monophyly of the recircumscribed genus *Primulina* s. l. are also corroborated by the molecular evidence based on the plastid *matK* gene. Within the genus the new species is suggested to be the sister of a lineage comprising *P. swinglei* and *P. laxiflora*. Further examination of the *matK* sequences indicated 5 and 11 base differences of *P. bobaiensis* separating from *P. swinglei* and *P. laxiflora*, respectively, implying potential long period of isolation from these species or high substitution rate of the *matK* gene in these taxa. Moreover, the new species is restricted on Danxia rocks while its closest relatives as well as most of its congeners are confined on limestone rocks, suggesting possible specific edaphic adaptation and isolation of the new species. Further, the high endemism with low abundance and usually morphological and molecular distinctiveness of this and many other species of *Primulina* would also superimpose the importance of conservation of these rare species.

Key words: Gesneriaceae; *Primulina*; new species; phylogeny; *matK*

CLC number: Q949 **Document code:** A **Article ID:** 1000-3142(2015)02-0143-08

收稿日期: 2014-04-23 **修回日期:** 2014-06-27

基金项目: 广西自然科学基金(2012GXNSFBA053067)**;广西林业科技项目(桂林科学**[2014]**第** 27 **号);广西植物功能物质研究与利用重点实验室主任基金**(ZRJJ2013-9)**;广西植物研究所基本业务费项目**(Guizhiye 12011)**;广西植物研究所博士启动基金**(Guizhiye 11006)。

作者简介: 黎乾坤(1986-)**,男,广西梧州人,硕士研究生,从事药用植物资源学研究,**(E-mail)459672720@qq.com。

[*] **通讯作者: 李文兰,博士,从事植物分子生物学研究,**(E-mail)liwenlan@whu.edu.cn。

广西报春苣苔属一新种及其系统位置

黎乾坤[1]，张　强[2]，邓　涛[2]，盘　波[2]，黄俞淞[2]，李文兰[1,3*]

（1. 桂林理工大学 化学与生物工程学院，广西 桂林 541006；2. 广西壮族自治区 中国科学院 广西植物研究所，
广西 桂林 541006；3. 广西大学 生命科学与技术学院，南宁 530005）

摘　要：报春苣苔是一类生长在石灰岩基质上的多年生草本植物，目前已知的种类160余种，主要分布在我国南部和越南北部的石灰岩地区。该属植物许多种类分布极其狭域，往往仅零星分布在某个或少数几个地点。近年来，该属植物许多新种类不断地被发现和报道，预示着该属植物可能有更加丰富的多样性。该研究报道了在广西博白县发现的该属植物一新种。从形态上判断，该新种属于广义报春苣苔属，其具有的一些特别的形态特征，如花冠下唇比上唇长得多，上唇内部具有两个近圆形斑点（其它多数种类具有条形斑点），而易于与本属其它物种区分。而且，基于叶绿体*matK*基因序列重建的系统发育关系表明，该新种的确属于报春苣苔属且广义报春苣苔属是单系类群。该新种与钟冠报春（*P. swinglei*）和疏花报春（*P. laxiflora*）最为近缘，其*matK*基因与后两者分别有5个和11个碱基差异，暗示这些物种间经历了较长时间的独立演化或*matK*基因在这些物种中具有较高的碱基替换速率。另外，该新种只生长在丹霞岩石上，而其近缘种以及同属大多数其它种类都生长在石灰岩基质上，暗示该新种获得了特殊的适应能力以及与其它近缘种间存在生态隔离。该新种以及近年来本属许多其它新种类的发现，进一步增加了对该属植物多样性的认识，以及该属植物的演化与多样性分化之谜。这些物种所具有的高度区域或局域特有性和稀有性，以及形态上的特殊性和遗传上的独立性，进一步强化了对该属植物开展保护的重要性。

关键词：苦苣苔科；报春苣苔属；新物种；系统发育；*matK*基因

The recently recircumscribed genus *Primulina* Hance (Gesneriaceae) contains more than 130 species with large morphological diversities (Weber *et al.*, 2011). It has grown to be one of the species-richest genus of Gensneriaceae in South China and its surrounding regions, one of the distributional centres of the family across the world, with 59 genera and over 460 discribed species found in China alone (Li *et al.*, 2004; Wei *et al.*, 2010). *Primulina* is still expanding rapidly with lots of new species and varieties that have been and are being discribed, mainly from the distrbutional centre of the limestone regions of South China, especially from Guangxi Zhuang Autonomous Region, where dozens of new species have been discribed in the last several years (Xu *et al.*, 2008; Li *et al.*, 2009; Li *et al.*, 2010; Xu *et al.*, 2010; Huang *et al.*, 2011; Wu *et al.*, 2011; Xu *et al.*, 2011a, 2011b; Hong *et al.*, 2012; Huang *et al.*, 2012; Wu *et al.*, 2012; Xu *et al.*, 2012; Chung *et al.*, 2013; Wen *et al.*, 2013; Zhao *et al.*, 2013).

In the present study, we found and discribed a Gesneriaceae population with special floral appearance from the generic distributional centre of Guangxi, China. The new population was found growing on Danxia rocks, but not on limestones, habitat occupied by most others of its congeners. According to its floral structure and chararaters, this new population can be easily attributed to the recently redefined genus *Primulina* and is assumed to represent a new species in the genus after carefully checking the morphologies against all the recognized congeners, including specimens and those discribed in literatures (Wang *et al.*, 1990; 1999, Li *et al.*, 2004; Wei *et al.*, 2010). Whereas, depending on morphological comparisons alone, it is difficult to assess which known species the new species is closely related to due to large morphological diversities and complicated morphological evolution in *Primulina* (Weber *et al.*, 2011).

Traditional taxonomy has been proved to largely contradict with the molecular systematics in Gesneriaceae and the majority of the traditional genera have been revealed as non-monophyly (e.g. Wang *et al.*, 2011; Weber *et al.*, 2011). Further, numerous monotypic genera with distinct morphology have been widely proved to be embedded deeply within other genera and their generic status thus should be abandoned, that again imply large incongruence between phylogenetic relationship and morphological similarity (Weber *et al.*, 2011). The incongruence between phylogenetic relationship and morphological similarity had often led errors in generic level taxonomic treatments for some species and a large proportion of taxa should be trasferred from their genera originally assigned by traditional taxonomy to another according to the molecular phylogenetics (Liu *et al.*, 2010; Möller *et al.*, 2011; Weber *et al.*, 2011). All these would suggest complicated morphological evolution in Gesneriaceae and necessity to combine molecular evidence with taxonomic treatment. Hence we also tested the generic placement and particular intrageneric relationships of the new species to other Gensericeae species from the surrounding areas where the new species was found based on a molecular framework, a matrix of *matK* gene modified from our previous study (Wu *et al.*, 2012).

1 Materials and Methods

We newly sampled the new species and four other *Primulina* taxa within the same region in the present study, each with one individual as the representative of the corresponding species. Further, we also newly sampled 14 taxa representing other 14 genera from the same large region. These newly sampled taxa would be added to our previously obtained *matK* sequences from other 41 *Primulina* taxa mainly from the same large region.

Total genomic DNA was extracted from silica dried leaves using the CTAB protocol (Doyle *et al.*, 1987). DNA sequences were amplified using a PCR procedure of initial denaturation at 94 ℃ for 3 min, followed by 34 cycles of 30 sec at 94 ℃, 40 sec at 52 ℃, and 90 sec at 72 ℃, with a final extension at 72 ℃ for 10 min. PCR products were purified using TIANgel Midi Purification Kit (Tiangen Biotech) according to the manufacturer's instructions and then sequenced on an ABI 3730 DNA Sequencer (Applied Biosystems International, Foster City, CA, USA), using Big Dye Terminator (Applied Biosystems, Shanghai, China). The primers used for amplification and sequencing are the same as in Wu *et al.* (2011).

DNA sequences were manually edited and spliced using the software Bioedit 5.0.9 (Hall 1999) according to the bidirectional chromatograms for each of the sequences. All sequences after edition were aligned in Clustal X 1.81 (Thompson *et al.*, 1997) and adjusted manually in Bioedit 5.0.9. The flank regions at both ends with many missing sites were excluded and the indels within sequences were filled by gaps. Then, the matrix of these newly obtained sequences was added to our previous larger matrix and aligned visually in Bioedit 5.0.9. The genbank accession numbers and sources of the plant materials are as listed in the supplementary material (Table 1) and the final matrix is available upon request from the corresponding author.

Phylogenetic analyses were conducted using neighbor joining (NJ) in Mega 4.0.2 (Tamura *et al.*, 2007), Maximum Parsimony (MP) in PAUP version 4.0b10 (Swofford 2002) and Bayesian Inference (BI) in MrBayes v3.1.2 (Huelsenbeck *et al.*, 2001), respectively. For the NJ method, we chose and calculated Kimura 2-parameter distance with pairwise deletion of the gaps and reconstruct the phylogeny based on the distance matrix. Branch Support (BS_{nj}) was calculated with 100 bootstraps, also using NJ method and the same parameter settings. The MP analysis was conducted using the following settings: 100 replicates of random stepwise addition with tree bisection-reconnection (TBR) branch swapping performed using heuristic searches, with all most-parsimonious trees saved at each replicate (MulTree on). Branch Support (BS_{mp}) was assessed using bootstrap analysis with 100 bootstrap replicates, each with 10 stepwise additions. As the inadequacy of the parsimony informative sites of the *matK* sequences would have yielded excessive equally most parsimonious trees in the tentative MP heuristic search, therefore, 500 000 trees were set as the maximum limit for the first round of heuristic searches based on the original matrix and 50 000 maxi

Table 1 Genbank accession numbers and the plant material sources used in the present study

Taxa	*MatK*	Collector (Voucher)/Location
Aeschynanthus austroyunnanensi	KJ137885	R. C. Hu & Q. Zhang/Huanjiang County,Guangxi,China
Beccarinda tonkinensis	KJ137886	W. B. Xu/Shangsi County,Guangxi,China
Chiritopsis glandulosa	KJ137887	W. B. Xu/Pingle County,Guangxi,China
Chiritopsis sp.	KJ137888	W. B. Xu/Zhongshan County,Guangxi,China
C. lingchuanensis	KJ137889	L. Wu & B. Pan/Lingcuan County,Guangxi,China
C. glandulosa var. *yangshuoensis*	KJ137890	W. B. Xu/Yangshuo County,Guangxi,China
Dayaoshania cotinifolia	KJ137891	N. Z. Huang (Cultivated in Botanical Garden of Guangxi Institute of Botany (GXIB))
Didymocarpus glandulosus	KJ137892	S. S Mo & Q. Zhang/Huanjiang County,Guangxi,China
Gyrocheilos retrotrichum	KJ137893	S. S Mo & Q. Zhang/Huanjiang County,Guangxi,China
Hemiboea cavaleriei	KJ137894	Y. Liu/Lingcuan County,Guangxi,China
Lagarosolen coriaceiforlium	KJ137895	L. Wu & B. Pan/Yangshuo County,Guangxi,China
Lysionotus fengshanensis	KJ137896	S. S Mo & Q. Zhang/Huanjiang County,Guangxi,China
Ophithandra sinohenryi	KJ137897	W. B. Xu/Fangcheng City,Guangxi,China
Oreocharis xiangguiensis	KJ137898	W. B. Xu /Lingcuan County,Guangxi,China
Paraboea swinhoii	KJ137899	S. S Mo & Q. Zhang/Huanjiang County,Guangxi,China
Paraisometrum mileense	KJ137900	Q. Gao/Xingyi County,Guizhou,China
Petrocodon sp.	KJ137901	B. Pan/Jiangyong County,Hunan,China
Priminlina swinglei	KJ137902	B. Pan/cult. In GXIB
Henckelia anachoreta	KJ137903	W. B. Xu/Ningming County,Guangxi,China
Primulina baishouensis	KJ137904	B. Pan/Yongfu County,Guangxi,China
P. bobaiensis	KJ137905	B. Pan/Bobai County,Guangxi,China
P. cordifolia	KJ137906	Q. Gao & B. Pan/Yangshuo County,Guangxi,China
P. dryas	JX195976	A. Chautems & M. Perret 01－019 (G)
P. eburnea	KJ137908	Q. Gao & B. Pan/Guilin City,Guangxi,China
P. fimbrisepala	KJ137909	Q. Zhang/Shaoguan City,Guangdong,China
P. gongchengensis	KJ137910	Y. S. Huang & W. B. Xu (10496)/Gongcheng County,Guangxi,China
P. gueilinensis	KJ137911	Q. Gao & B. Pan/Guilin City,Guangxi,China
P. guihaiensis	KJ137912	Q. Gao & B. Pan/Guilin City,Guangxi,China
P. hedyotidea	KJ137913	W. B. Xu/cult. In GXIB
P. hochiensis	KJ137914	W. B. Xu/cult. in GXIB
P. langshanica	KJ137915	W. B. Xu & B. Pan (10021)/ Quanzhou County,Guangxi,China
P. laxiflora	KJ137916	Y. S. Huang /Longzhou County,Guangxi,China
P. liboensis	KJ137917	W. B. Xu/Guangxi
P. liguliformis	KJ137918	B. Pan/Hechi City,Guangxi,China
P. lijiangensis	KJ137919	W. B. Xu/Yangshuo County,Guangxi,China
P. linearifolia	KJ137920	Y. Liu/Guangxi
P. longangensis	KJ137921	Y. S. Huang /Longzhou County,Guangxi,China
P. longicalyx	KJ137922	L. Wu & B. Pan/Guilin City,Guangxi,China
P. longii	KJ137923	Y. S. Huang& B. Pan/Yongfu County,Guangxi,China
P. lunglinensis var. *amblyosepala*	KJ137924	W. B. Xu/Huanjiang County,Guangxi,China
P. lutea	KJ137925	W. B. Xu/cangwu County,Guangxi,China
P. lutea	KJ137926	S. S. Mo/Hezhou City,Guangxi,China
P. macrodonta	KJ137927	L. Wu & B. Pan/Lingcuan,Guangxi,China
P. medica	KJ137928	B. Pan/Pingle County,Guangxi,China
P. minutimaculata	KJ137929	W. B. Xu/Longzhou County,Guangxi,China
P. debaoensis	KJ137930	W. B. Xu/cult. in GXIB,Guangxi,China
P. ningmingensis	KJ137931	W. B. Xu/ningming County,Guangxi,China
P. pseudoheterotricha	KJ137932	W. B. Xu/Zhongshan County,Guangxi,China
P. pungentisepala	KJ137933	W. B. Xu/Guangxi,China
P. ronganensis	KJ137934	Y. S. Huang & B. Pan/Rongan County,Guangxi,China
P. rongshuiensis	KJ137935	Y. S. Huang & B. Pan/Rongshui County,Guangxi,China
P. shouchengensis	KJ137936	W. B. Xu/Yongfu County,Guangxi,China
P. subrhomboidea	KJ137937	B. Pan/Yangshuo County,Guangxi,China
P. tabacum	KJ137938	W. B. Xu/Hezhou City,Guangxi,China
P. tribracteata var. *zhuana*	KJ137939	W. B. Xu/cult. in GXIB,Guangxi,China
P. wangiana	KJ137940	Y. S. Huang & B. Pan/Rongan County,Guangxi,China
P. wentsaii	KJ137941	W. B. Xu/Ningming County,Guangxi,China
P. yangshuoensis	KJ137942	Q. Gao & B. Pan/Yangshuo County,Guangxi,China
P. yungfuensis	KJ137943	J. Liu et al. /Yongfu County,Guangxi,China
P. sinovietnamica	KJ137944	W. B. Xu/Longzhou County,Guangxi,China

Table 2　Difference of the nucleotides of the new species to its close congeners

Species	32	63	113	149	173	174	298	511	525	567	702	752	834	1005
P. bobaiensis	C	T	C	G	T	C	T	C	A	C	A	C	G	A
P. swinglei	**?**	**?**	C	**A**	T	**A**	**C**	C	A	C	**G**	**T**	G	A
P. laxiflora	**T**	**C**	**T**	G	**G**	C	**C**	**T**	**T**	**T**	**G**	C	**C**	**T**

Note: The first row represents the site numbers in the matrix. The mark (?) represents ambiguous state for the site. The different bases of *P. swinglei* and *P. laxiflora* to the new species are highlighted in bold.

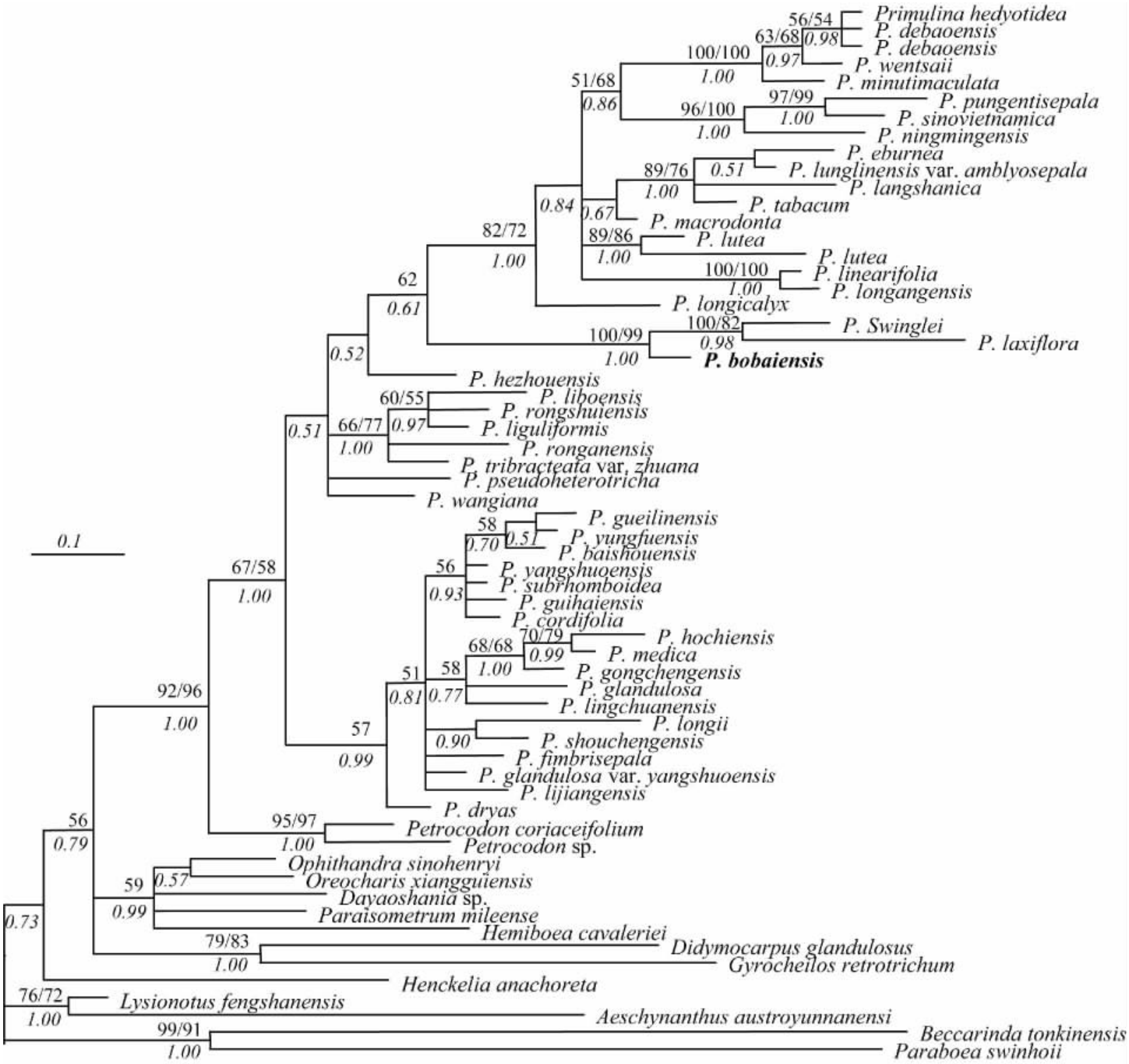

Fig. 1　Phylogenetic placement of the new species *P. bobaiensis* (highlighted in bold) to its close relatives. Nodal supports bigger than 50% from NJ and MP are sequentially (before or behind the left slash) listed above the branches, while PP values bigger than 0.50 from BI method are listed below the branches in italic.

mum trees were set for each of the bootstrap pseudoreplications. Prior the Bayesian analysis, we used Modeltest (Posada *et al.*, 1998; Posada *et al.*, 2004) to select the fittest DNA substitution model based on the Akaike Information Criterion (AIC). Two runs of Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) analyses were performed simultaneously, with a random starting tree and four chains for each run (one cold and three heated). The MCMCMC length was 10 000 000 generations and the chain was sampled every 1000th generation from the cold chain. Bayesian clade posterior probabilities and average branch lengths were calculated based on the sampled trees combined from the two runs after the first 15% of the total trees were discarded as burn-in. The burn-in for each run was validated by plotting the likelihood values against the generations.

2 Results and Analysis

2.1 Phylogenetic placement of the new species

After pruning the flank regions with many missing characters, the *matK* region obtained varies from 950 bp to 1 156 bp for all the sampled 61 accessions with an average GC content of 32% and the final aligned matrix has 1 156 bp in length with 115 informative sites as well as 165 additional variable but uninformative sites. TVM + G model was selected as the fittest substitution model for the *matK* gene by the Modeltest.

Through three methods, we yielded phylogenetic trees with generally consistent topology and the Bayesian 50% majority consensus tree was more resolved than those from the other two methods. Maximum constraint of 500 000 trees was hit in the first replication of MP heuristic searches, with the shortest tree length of 401 steps, consistency index (CI) of 0.79 and Retention index (RI) of 0.84. The results from all three methods indicated that all the *Primulina* taxa sampled in present study formed as a monophyletic group with low NJ and MP bootstrap support, but high Bayesian posterior probability ($BS_{nj}=56\%$; $BS_{mp}=58\%$; $PP=1.00$). The new species was embedded within *Primulina*, being sister ($BS_{nj}=100\%$; $BS_{mp}=99\%$; $PP=1.00$) to a lineage ($BS_{nj}=84\%$; $BS_{mp}=82\%$; $PP=0.98$) composed of *P. swinglei* and *P. laxiflora* (Fig. 1). Further examination of the *matK* genes indicated the new species differed from *P. swinglei* and *P. laxiflora* by 5 and 11 nucleotide bases, respectively, implying potential long-term of isolation between the species or higher substitution rate of the *matK* gene in these taxa (Table 2).

2.2 Taxonomic treatment

P. bobaiensis Q. K. Li, Q. Zhang & W. L. Li, *sp. nov.* Fig. 2 & Fig. 3.

Herbs perennial. Rhizome subterete, 2 – 7 cm long, 1 – 3 cm across. Leaves 5 – 9, crowded at the apex of rhizome, petiolate; petiole applanate, 3 – 12 cm long, 5 – 15 mm across; leaf blade herbaceous, elliptic or oval, 4 – 12 × 2 – 6 cm, apex acute to obtuse, base cuneate to broadly cuneate, margin shallowly serrate, pubescent on both surfaces, lateral veins 3 – 6 on each side, impressed adaxially and prominent abaxially. Cymes 2 – 4, 1 – 3-branched, 5 – 12-flowered; peduncle 5 – 18 cm long, 1 – 3 mm across, pubescent; pedicel 1.2 – 3.1 cm long, pubescent; bracts opposite, oblanceolate, 8 – 22 × 4 – 9 mm, margin repand, apex obtuse, pubescent on both sides. Calyx 5-parted nearly to the base, lobes narrowly lanceolate, 3 – 5 × 0.8 – 1.1 mm, apex acuminate, outside pubescent, inside puberulent, margins entire. Corolla deep purple, 16 – 18 mm long, outside pubescent, inside sparsely puberulent; tube ca. 7 – 9 mm long, ca. 4 mm in diam. at the base, ca. 6 – 8 mm in diam. at the mouth; limb distinctly 2-lipped, adaxial lip 2-parted to over the middle, lobes broadly ovate, 3 – 4 × 3 – 3.5 mm; abaxial lip 3-parted to over the middle, lobes oblong, 6 – 8 × 3 – 4 mm. Stamens 2, adnate to ca. 5 mm above the corolla tube base, filaments linear, 5 – 8 mm long, purple, twisted at over middle, glabrous; anthers reniform, dorsifixed, ca. 2 mm long, glabrous; staminodes 3, adnate to ca. 6 mm above the corolla tube base, lateral ones 2 mm long, apex capitate, middle one 0.5 mm long. Disk annular, ca. 1.1 mm in height, margin repand. Pistil 12 – 15 mm long, ovary 4 – 6 mm long, ca. 1.3 mm across, pubescent; style pubescent, 6 – 8 cm long; stigma obovate, 1 – 1.5 mm long. Capsule not seen. The species is only known growing on Danxia rock from one locality in Wangmao Town, Bobai County, Guangxi, China. It is in flowering from September to October. The specific epithet is derived from the type locality, Bobai County, Guangxi. *P. bobaiensis* is special in floral morphology which can be easily distinguished from all other Gesneriaceae. It is even very different from its sampled rela tives in floral appearance. The details of diagnostic

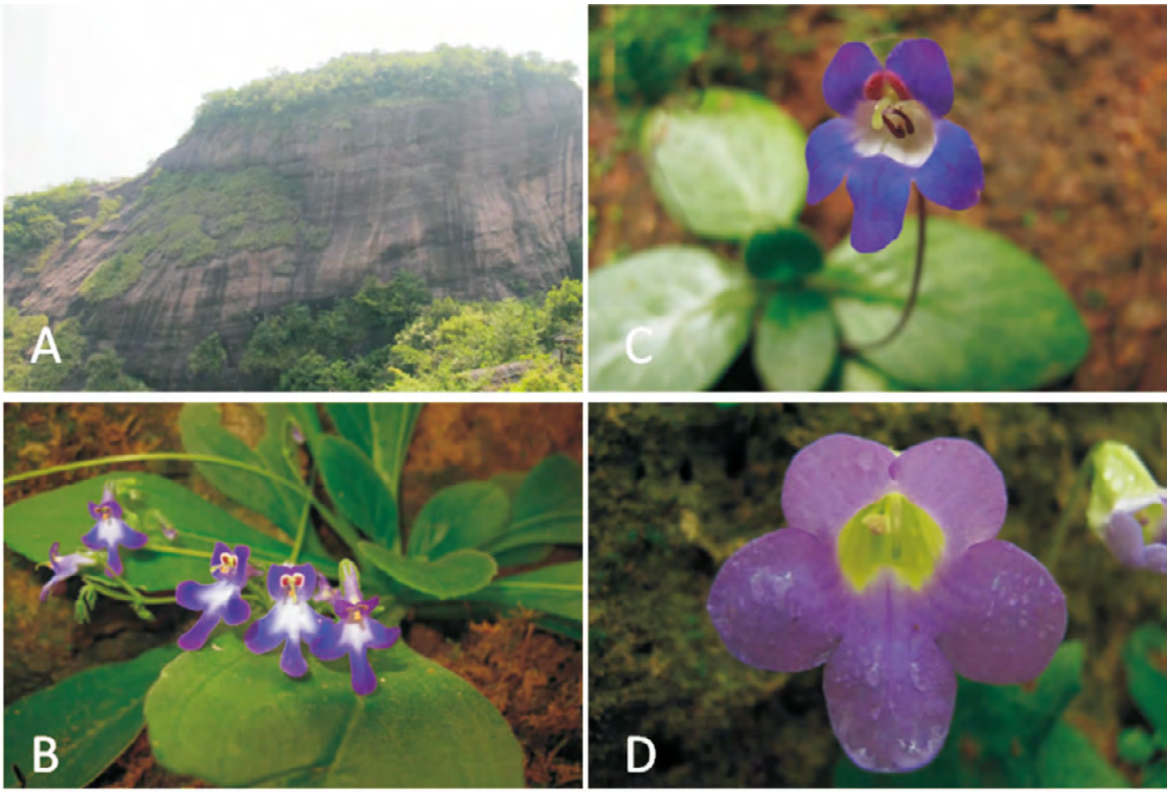

Fig. 2 **A.** Habitat of the new species (*Primulina bobaiensis*) in Danxia landscape; **B.** *P. bobaiensis*; **C.** Flower of *P. swinglei*; **D.** Flower of *P. laxiflora*.

Table 3 Distinguishing morphological characters among *P. bobaiensis*, *P. swinglei* and *P. laxiflora*

	P. bobaiensis	*P. swinglei*	*P. laxiflora*
Corolla	White at mouth, upper lobes pointed at terminal; round spotted upper lobes oblong, upper labia ca. 1/2 in length to the lower.	White at mouth, upper lobes pointed at terminal; striped upper lobes ovate, upper labia slightly shorter than the lower.	Yellow at mouth, upper lobes round at terminal, lower lobes near round, upper labia slightly shorter than the lower.

morphological characters of *P. bobaiensis* to its phylogenetic close relatives of *P. swinglei* and *P. laxiflora* are listed in Table 3.

China, Guangxi, Yulin City, Bobai County, Wangmao Town, Yanshi temple, on rock face of Danxia landscape, rare, elevation 123 m, 9 October 2010, Yu-Song HUANG and Bo Pan100839 (holotype IBK; isotype PE). Guilin Botanical Garden, introduced from Bobai County, Wangmao Town, 31 August 2009, Wei-Bin XU et Bo Pan 091775 (IBK).

3 Conclusion and Discussion

The new species embedded within *Primulina* seems robust, strongly supported by both morphological observation and molecular characters. The status as an independent species is also easy to be confirmed by its very special appearance. According to our knowledge the extremely unsymmetrical labia with the lower one extended to two folds or more in length than the upper one is rare in other recognized Gesneriaceae taxa in this area; the two spotted honey guides on the upper two lobes but not any marks on the lower three lobes are also rare. All the floral characters seem stable as several cultivated individuals on yellow soils in greenhouse show no discernable morphological variations compared with those observed for the wild population on Danxia rocks, unlike many other taxa which show observable or striking morphological variations between and/or within populations (Wei *et al.*, 2010). Although the species status is easy to be confirmed, whereas, the exact relationships and whether there is more closely-related known species for

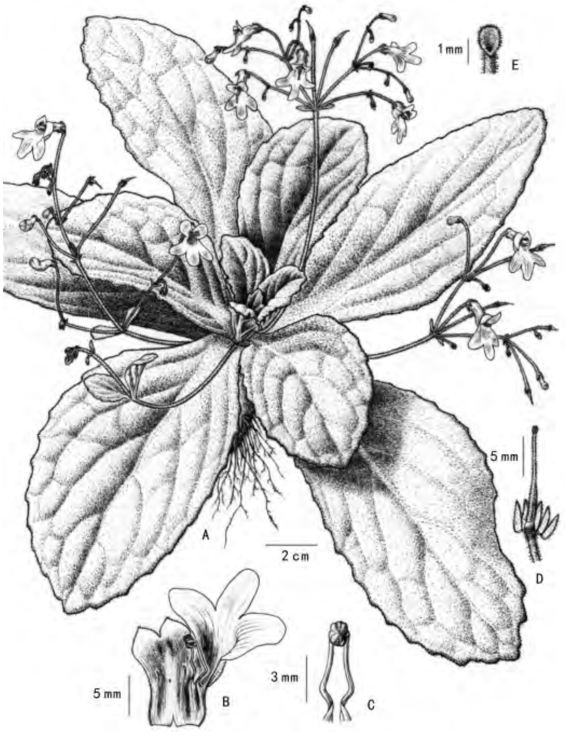

Fig. 3 *P. bobaiensis* Q. K. Li,& W. L. Li **A.** Habit; **B.** Corolla opened showing stamens and staminodes; **C.** Stamens; **D.** Pistil and calyx; **E.** Stigma. (Drawn by ZHU Yun-Xi from the holotype).

the new species still needs further molecular analysis based on denser sampling.

Acknowledgements We are grateful to Mr. Zhu Yun-Xi (IBK) for the handsome drawing. We thank Xu Wei-Bin, Gao Qi, Hu Ren-Chuan, Huang Nin-Zhen and Liu Jing from IBK for collecting the plant materials.

References:

Chung KF, Huang HY, Peng CI, *et al.* 2013. *Primulina mabaensis* (Gesneriaceae), a new species from a limestone cave of northern Guangdong, China [J]. *Phytotaxa*, **92**:40 - 48

DoyleJJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue [J]. *Phytochem Bull*, **19**:11 - 15

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT [J]. *Nucleic Acids Symp Ser*, **41**:95 - 98

Hance HF. 1883. Primulina tabacum Hance [J]. *J Bot*. **21**:169

Hong X, Zhou SB, Wen F. 2012. *Primulina chizhouensis* sp. nov. (Gesneriaceae), a new species from a limestone cave in Anhui, China [J]. *Phytotaxa*, **50**:13 - 18

Huang YS, Xu WB, Liu Y. 2011. *Chirita rongshuiensis*, a new species of Gesneriaceae from northern Guangxi, China [J]. *Taiwania*, **56**:54 - 57

Huang YS, Xu WB, Wu L, *et al.* 2012. *Primulina gongchengensis* (Gesneriaceae), a new species from Guangxi, China [J]. *Ann Bot Fenn*, **40**:107 - 110

Huelsenbeck JP. Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees [J]. *Bioinformatics*, **17**:754 - 755

Li JM,. Möller M. 2009. *Chirita grandibracteata* (Gesneriaceae), a new species from Yunnan, China [J]. *Ann Bot Fenn*, **46**:125 - 129

Li JM, Zhu SX. 2010. *Chirita auriculata* (Gesneriaceae), a new species from China, placed in sect. *Chirita* based on molecular data [J]. *Ann Bot Fenn*, **47**:221 - 225

Li ZY, Wang YZ. 2004. Plants of Gesneriaceae in China [M]. Zhengzhou: Henan Science and Technology Publishing House: 721

Liu Y, Xu WB, Pan B. 2010. *Wentsaiboea tiandengensis* sp. nov. and *W. luochengensis* sp. nov. (Gesneriaceae) from Karst caves in Guangxi, southern China [J]. *Nord J Bot*, **28**:739 - 745

Möller M, Forrest A, Wei YG, *et al.* 2011. A molecular phylogenetic assessment of the advanced Asiatic and Malesian didymocarpoid Gesneriaceae with focus on non-monophyletic and monotypic genera [J]. *Plant Syst Evol*, **292**:223 - 248

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests [J]. *Syst Biol*, **53**:793 - 808

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution [J]. *Bioinformatics*, **14**:817 - 818

Swofford DL. 2002. PAUP* . Phylogenetic analysis using parsimony (* and other methods). Version 4 [M]. Sinauer Associates, Sunderland, Massachusetts

Tamura K, Dudley J, Nei M, *et al.* 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0 [J]. *Mol Biol Evol*, **24**:1 596 - 1 599

Thompson JD, Gibson TJ, Plewniak F, *et al.* 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools [J]. *Nucleic Acids Res*, **25**:4 876 - 4 882

Wang WT, Pan KY, Li ZY. 1990. Gesneriaceae [M] //Wang WT (eds). Flora Reipublicae Popularis Sinicae. Beijing: Science Press: 125 - 581

Wang WT, Pan KY, Li ZY, *et al.* 1998. Gesneriaceae [M] // Wu ZY, Raven PH (eds). Flora of China. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press: 244 - 401

Wang YZ, Mao RB, Liu Y, *et al.* 2011. Phylogenetic reconstruction of *Chirita* and allies (Gesneriaceae) with taxonomic treatments [J]. *J Syst Evol*, **49**:50 - 64

Weber A, Middleton DJ, Forrest A, *et al.* 2011. Molecular systematics and remodelling of *Chirita* and associated genera (Gesneriaceae) [J]. *Taxon*, **60**:767 - 790

Wei YG, Wen F, Möller M, *et al.* 2010. Gesneriaceae of South China [M]. Nanning: Guangxi Science and Technology Publishing House: 777

Wen F, Zhao B, Liang GY, *et al.* 2013. *Primulina lutvittata* (Gesneriaceae), a new species from a limestone cave in Guangdong, China [J]. *Ann Bot Fenn*, **50**:87 - 90

Wu L, Zhang Q, Xu WB, *et al.* 2012. *Primulina guigangensis* (Gesneriaceae): a new species from limestone area in Guangxi, China [J]. *Phytotaxa*, **38**:19 - 23

Wu WH, Xu WB, Nong DX, *et al.* 2011. *Chirita ninminensis*

(下转第 177 页 Continue on page 177)

不同物种在胚乳细胞的长、宽、长宽比以及形状上都呈现差异，表明胚乳细胞特征在一定程度上具有共属分种的意义，但不能很好地反映属以及基因组间的差异。按照染色体组分类方法，含相同染色体组的物种亲缘关系较近。*E. Canadensis*（2）和 *E. sibiricus*（6）都具有 StH 染色体组，亲缘关系很近，但是 *E. Canadensis* 和 *E. sibiricus* 在细胞形状和长宽比上的差异都很大。然而具有同样 P 染色体组的 *A. cristatum*（2）和 *A. mongolicum*（2）和同样具有 St 染色体组的 *P. libanotica*（10）和 *P. spicata*（11）在细胞形状和长宽比上的差异又很小，甚至 *P. libanotica*（10）和 *P. spicata*（11）的长宽比为相同的 1.57。就考察的胚乳细胞的各项指标来看，在一些种间胚乳细胞的的大小和宽厚差异不及形状差异明显。例如 *E. nutans*（5）和 *E. sibiricus*（6）的大小和长宽比差异不大，但是前者主要是椭球形，后者以圆形和长体形为主，而且前者较密后者稀疏。还有些物种各项指标仅有少许差异，如 *P. libanotica*（10）和 *P. spicata*（11），利用胚乳细胞特征分析它们关系时应该慎重。比较本实验 4 个属 11 个物种的胚乳细胞，发现不同属、同一属内不同物种的胚乳细胞在形状和长宽比上都差异较大，且无明显规律，表明胚乳细胞特征不能能较好地反映属以及基因组间的亲缘关系。所以对披碱草属植物的分类地位、起源以及系统进化需要综合分析形态学、细胞学、细胞遗传学以及分子生物学的资料才能作出正确的分析。

参考文献：

Cai LB（蔡联炳）. 2000. Characters of endosperm cells in the seeds of some species of *Roegneria* and their taxonomics ignificance（鹅观草属一些种种子胚乳细胞的特征及其分类学意义的探讨）[J]. *Acta Bot Boreal-Occident Sin*（西北植物学报），**20**（6）：1 070 – 1 075

Dong YC（董玉琛）. 1992. Study and Utilization of Wild Relatives of Wheat in China（小麦野生近缘植物的研究和利用）[M]. Beijing（北京）：China Science and Technology Press（中国科学技术出版社）：39 – 44

Guo BZ（郭本兆），Lu LS（卢莲生），Sun YH（孙永华），*et al.* 1987. Flora of China. 9（3）（中国植物志第 9 卷 3 分册）[M]. Beijing（北京）：Science Press（科学出版社）：52 – 61

Jensen KB. 1989. Cytology and origin of *Elymus abolinii*, and its F1 hybrids with *Pseudoroegneria spicata*, *Elymus lanceotalus*, *E. dentatus* ssp. *ugamicus*, and *E. drobovii*（Poaceae：Triticeae）[J]. *Genome*，**32**（5）：468 – 474

Liu J（刘静），Yang RW（杨瑞武），Zhang HQ（张海琴），*et al.* 2007. Study on diversity of endosperm cells in seeds of *Hystrix* and related genera in Triticaae（猬草属及其近缘属种子胚乳细胞多样性研究）[J]. *J Sichuan Agric Univ*（四川农业大学学报），**25**（1）：14 – 18

Lu BR. 1994. Meiotic analysis of the intergeneric hybrids between *Pseudoroegneria* and tetraploid *Elymus* [J]. *Cathya*，**6**（2）：1 – 14

Lu BR，Bothmer RV. 1990. Intergeneric hybridization between *Hordeum* and asiatic *Elymus* [J]. *Hereditas*，**112**：109 – 116

Lu BR. 1993. Genomic relationships within the *Elymus parviglumis* group（Triticeae：Poaceae）[J]. *Plant Syst Evol.* **187**（1-4）：191 – 211

Wang XL（王晓丽），Tang JY（汤加勇），Liu J（刘静），*et al.* 2008. Diversity of endosperm cells in Triticeae species with StH genomes（小麦族 StH 基因组物种的胚乳细胞多样性）[J]. *J Sichuan Agric Univ*（四川农业大学学报），**26**（1）：11 – 15

Yang RW（杨瑞武），Zhou YH（周永红），Zheng YL（郑有良），*et al.* 2004. Study on diversity of endosperm cells in seeds of *Leymus*（赖草属种子胚乳细胞多样性研究）[J]. *Acta Bot Boreal-Occident Sin*（西北植物学报），**24**（1）：38 – 42

（上接第 150 页 Continue from page 150）

（Gesneriaceae），a new species from Guangxi，China [J]. *Ann Bot Fenn*，**48**：422 – 424

Xu H，Li ZY，Jiang H. 2008. A new species of *Chirita*（Gesneriaceae）from Yunnan，China [J]. *Bot J Linn Soc*，**158**：269 – 273

Xu WB，Huang YS，Wu L，*et al.* 2011a. *Chirita luochengensis*（Gesneriaceae），a new species from limestone areas in northern Guangxi，China [J]. *Brittonia*，**63**：314 – 317

Xu WB，Pan B，Huang YS，*et al.* 2010. *Chirita leprosa* sp. nov.（Gesneriaceae）from limestone areas in Guangxi，China [J]. *Nord J Bot*，**28**：705 – 708

Xu WB，Pan B，Huang YS，*et al.* 2011b. *Chirita lijiangensis*（Gesneriaceae），a new species from limestone area in Guangxi，China [J]. *Ann Bot Fenn*，**48**：188 – 190

Xu WB，Pan B，Liu Y，*et al.* 2012. Two new species，*Primulina multifida* and *P. pseudomollifolia*（Gesneriaceae），from karst caves in Guangxi，China [J]. *Bot Study*，**53**：165 – 175

Zhao B，Pan B，Zhang Y，*et al.* 2013. *Primulina guizhongensis*（Gesneriaceae），a new species from Guangxi，China [J]. *Phytotaxa*，**109**：27 – 35