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PRIMULINA WENII (GESNERIACEAE), A NEW SPECIES FROM CHINA

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Abstract

Primulina wenii (Gesneriaceae) is described and illustrated here as a new species. Morphologically, this new species resembles *P. juliae* (Hance) Mich. Möller & A. Weber, but it is distinct from the latter by some obvious features in leaf blade, bract, indumentum, corolla, filament, stigma and pistil. Nuclear ribosomal internal transcribed spacer (ITS) region and plastid *trn*L-F intronspacer (*trn*L-F) DNA sequence data from the new species and its 22 relatives are used to determine the systematic position of the new species within *Primulina*. Molecular evidence suggests that *P. wenii* is strongly supported as a sister to a clade in which *P. juliae* is included. The conservation status of *P. wenii* is assessed as "Critically Endangered" (CR) according to IUCN Red List categories and criteria.

Introduction

A great number of new species of *Primulina* have been described since 2011 at present the number has rapidly reached more than 150 species in China (Möller *et al*., 2016). Undoubtedly, the limestone areas of South-west and South China and North Vietnam are considered to be of the highest biodiversity and differentiation for *Primulina* (Gesneriaceae) (Wei *et al*., 2010; Wen *et al*., 2015). It is easily understood that very few species of *Primulina* in Fujian province have been recorded. Before 1992, there were two species and one variety, namely *P. pinnatifida* (Hand.-Mazz.) Y.Z. Wang [former *Chirita pinnatifida* (Hand.-Mazz.) Burtt], *Primulina fimbrisepala* (Hand.-Mazz.) Y.Z. Wang (former *Chirita fimbrisepala* Hand.-Mazz.) and *Chirita gueilinensis* W.T. Wang var. *brachycarpa* W.T. Wang (Zhang, 1993). However, *Chirita gueilinensis* var. *brachycarpa* was brought into *Chirita juliae* Hance (Wang *et al*., 1998; Li and Wang, 2004) based on the similar morphological characters of the two congeners. But soon afterwards, *Chirita* along with several other associated genera, namely almost all the species and *Chirita* sect. *Gibbosaccus* C.B. Clarke, two species of *Wentsaiboea* D. Fang & D.H. Qin (*W. renifolia* D. Fang & D.H. Qin (2004) and *W. luochengensis* Yan Liu & W.B. Xu (2010)) and all the species of *Chiritopsis* W.T. Wang (1981) were shifted to *Primulina* Hance (Li and Wang, 2007; Wang *et al*., 2011; Weber *et al*., 2011). It was originally the monotypic genus (Hance, 1883). Subsequently, a new species of *Primulina*, *viz*., *P. xiuningensis* (X.L. Liu & X.H. Guo) Mich. Möller & A. Weber (former *Chiritopsis xiuningensis* X.L. Liu & X.H. Guo) (Liu and Guo, 1989; Weber *et al*., 2011), from Fujian, China was discovered and reported formally in recent years (Geng *et al*., 2014). To sum it up, Fujian province in East China cannot still be considered to have high biodiversity of *Primulina* because only three species were found in this area.

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In 2012, one of the authors found a population of the peculiar Gesneriaceae plants growing on a rocky slope near a stream in a limestone gorge close to Fuzhou city, Fujian province, China. Firstly, we considered it to be *Prmulina juliae* because it is recorded in Ninghua County and Songxi County of Fujian. The next year we re-visited the same location to collect specimens with flowers, and we found that the two similar congeners are actually different. We sent some specimens with flowers and fruits to IBK (Dr. Fang Wen, FW) for identification. He thought they should be an unknown species and have never been seen before. After consulting relevant literature (Wang, 1985; Wang *et al*., 1990, 1998; Li and Wang, 2004; Wei *et al*., 2010; Wang *et al*., 2011; Weber *et al*., 2011; Möller *et al*., 2011, 2016;Xu *et al*., 2012; Möller and Clark, 2013) and checking herbarium specimens of *Primulina* (Some former *Chirita*), 'World checklist of Gesneriaceae' (Skog and Boggan, 2007) and 'The genera of Gesneriaceae' (Weber and Skog, 2007), and consulting Dr. Fang Wen based on his rich experience and knowledge about the identification and taxonomy of *Primulina*, we found the morphology of these newly discovered plants to be similar to *P. juliae* (Hance) Mich. Mich. & A. Weber (Wei *et al*., 2010). However, they differ significantly in some characters. We have determined that they belong to a new species of *Primulina viz. P. wenii*, sp. nov.The new species *Primulina wenii* is described and illustrated below and compared with the morphologically similar *P. juliae* and their conservation status is evaluated.

Material and Methods

An overview of the genus *Primulina* from South, South-west and East China was made. All the available specimens of *Primulina* stored in the following herbaria in China (ANU, BJFC, CDBI, CTC, HGAS, HIB, IBK, IBSC, KUN, PE, SZ) were used as material of *Primulina* from recent fieldwork by the re-searching team of authors in South, South-west and East China (Thiers, 2015). All the morphological characters were studied under dissecting microscopes, and described using the terminology presented by Wang *et al.* (1990, 1998).

Leaf material of the new species was collected in the field and dried by silica gel for DNA extraction (Chase and Hills, 1991). The nuclear ribosomal internal transcribed spacer (ITS) region and the plastid *trn*L-F intron spacer (*trn*L-F) were used as molecular markers. The molecular methods and protocols followed Möller *et al.* (2009, 2011). GenBank accession numbers for ITS and *trn*L-F of the new species are KX985576 and KX985577, respectively (Table 1). To elucidate the phylogenetic affinities of the new species, ingroups (22 species of *Primulina*) and outgroups [*Ornithoboea wildeana* Craib, *Paraboea rufescens* (Franch.) B.L.Burtt] selection were chosen based on recent phylogenetic analyses (Li *et al*., 2007; Möller*et al*., 2011; Weber *et al*., 2011; Kang *et al*., 2014), and from which sequences were available from GenBank (Table 1).

Sequence data were edited and assembled using Lasergene Navigator 7.1 (DNAStar, Madison, Wisconsin, USA) and then aligned with the MEGA 5.1 (Tamura *et al*., 2011) with additional manual refinements where necessary. Phylogenetic analyses were performed using maximum parsimony (MP) method implemented in PAUP* 4.0b10. Heuristic searches were performed using a starting tree built from stepwise in addition with TBR branch swapping and 1,000 random addition replicates. To assess confidence in clades, bootstrap analyses based on 1,000 replicates with 10 random additions per replicate were used.

Table 1. List of species along with accession number of *trn*L-F and ITS sequences used in this study.

Species name	Voucher number	*trn*L-F	ITS
Ornithoboea wildeana Craib	Jia-Mei Li Ljm-04-44	DQ872824	DQ865197
Paraboea rufescens(Franch.) Burtt.	Jia-Mei Li 0185	DQ872825	DQ865196
Primulina bipinnatifida (W.T. Wang) Y.Z. Wang	Guangxi NA/Li J.M.NA	DQ872806	DQ872842
Primulina danxiaensis	DXS04	KF498157	KF498050
Primulina dryas (Dunn) Mich. Möller & A. Weber	T.C. Godfrey 369	FJ501524	FJ501348
Primulina glandulosa (D. Fang, L. Zeng & D.H. Qin) Y.Z. Wang	Jia-Mei Li 054291	DQ872804	DQ872841
Primulina glandulosa var. *yangshuoensis* (Fang Wen, Yue Wang & Q.X. Zhang) Mich. Möller & A. Weber	M. Möller MMO 06-912	HQ632948	HQ633045
Primulina heterotricha (Merr.) Y.Z. Wang	Yin-Zheng Wang 06731	DQ872816	DQ872826
Primulina juliae	JXGF01	KF498228	KF498107
Primulina linearifolia (W.T. Wang) Y.Z. Wang	Jia-Mei Li 11121	DQ872810	DQ872834
Primulina longgangensis (W.T. Wang) Y.Z. Wang	A. Takhtajan & N. Aruzytov 1975	AJ492290	FJ501347
Primulina minutimaculata (D. Fang & W.T. Wang) Y.Z. Wang	Jia-Mei Li 067134	DQ872815	DQ872828
Primulina mollifolia (D. Fang & W.T. Wang) Y.Z. Wang	Jia-Mei Li 054281	DQ872802	DQ872847
Primulina ophiopogoides (D. Fang & W.T. Wang) Y.Z. Wang	Yin-Zheng Wang 06713	DQ872814	DQ872829
Primulina pinnata (W.T. Wang) Y.Z. Wang	Expedition Beijing 896526	FJ501526	FJ501349
Primulinapinnatifida (Hand.-Mazz) Y.Z. Wang	Q.J. Xie J-037	FJ501527	FJ501350
Primulina pteropoda (W.T. Wang) Y.Z. Wang	Yin-Zheng Wang 06731	DQ872817	DQ872827
Primulina repanda var. *guilinensis*	ex Smithsonian Institute 94-083	AJ492292	FJ501351
Primulina spadiciformis (W.T. Wang) Mich. Möller & A. Weber	ex Smithsonian Institute 94-087	AJ492291	FJ501346
Primulina spinulosa (D. Fang & W.T. Wang) Y.Z. Wang	Yin-Zheng Wang 06713	DQ872813	DQ872830
Primulina tabacum Hance	Q.J. Xie & C.X. Ye	AJ492300	FJ501352
Primulina weii Mich. Möller & A. Weber	Jia-Mei Li, Ljm-04-42	DQ872811	DQ872832
Primulina wenii Jian Li & L.J. Yan sp. nov.	Jian Li & F. Wen 20130412-01	KX985577	KX985576
Primulina wentsaii (D. Fang& L. Zeng) Y.Z. Wang	Jia-Mei Li 11630	DQ872812	DQ872831
Primulina xiuningensis (X.L. Liu & X.H. Guo) Mich. Möller & A. Weber	ZJJS01	KF498252	KF498124

Results and Discussion

Taxonomical treatment

Primulina wenii Jian Li & L. J. Yan, **sp. nov.** **(Figs 1 & 2).**

Diagnosis: *Primulina wenii* Jian Li & L. J. Yan differs from its congener, *P. juliae* (Hance) Mich. Möller & A. Weber, by the combination of the following characters: the lateral veins of leaf blade 3 or 4; peduncle densely covered with erectly spreading white eglandular pubescent and villous hairs; bracts 3, outside pubescent and villous, inside pubescent; corolla outside densely white glandular-pubescent, inside sparsely pubescent; filaments glabrous; stigma trapeziform, 2-lobed to irregularly lobed (Table 2)

Type: CHINA. Fujian province, Fuzhou city, Rixi town, 26.35139°N, 119.27056°E, 130 m, on moist tufa and the rocky surface of limestone cliff, 12 April 2013, *Jian Li & F. Wen* 20130412-01 (*Holotype*: IBK!; *Isotype*: IBK!).

Table 2. Morphological comparison of *Primulina wenii* sp. nov. and *P. juliae*.

Characters	*P. wenii*	*P. juliae*
Leaf blade indumentum	Lower surface densely appressed pubescent and veins villous, upper surface densely pubescent and villous	Both surfaces appressedpuberulent
Leaf blade margin	Irregularly serrated in number and size	Dentate to pinnately lobed basally or crenate, apex acute to rounded
Lateral veins of leaf blade	3 or 4, abaxially obviously prominent, adaxially conspicuously sunken	4 or 5, abaxially inconspicuously prominent, adaxially inconspicuous
Peduncle indumentum	Densely covered with erectly spreading white eglandular pubescent and villous hairs	Densely covered with spreading puberulent hairs
Bract number and indumentum	3; outside pubescent and villous, inside pubescent	2; outside puberulent, inside glabrous
Corolla indumentum	Outside densely white glandular-pubescent, inside sparsely pubescent	Outside sparsely puberulent, inside nearly glabrous
Filaments	Glabrous	Glabrous to glandular puberulent near apex
Pistil	Densely covered with white glandular puberulent hairs	Puberulent
Stigma	Trapeziform, 2-lobed to irregularly lobed	Narrowly oblong, 2-lobed

Herbs, perennial. Rhizome cylindric, 2–4 cm long, 0.6–1.0 cm in diam. Leaves 4–6, basal; petiole compressed, 1.9–2.5 × c. 0.8 cm, densely villous; leaf blade pale green to green, oblong to broadly elliptic, 10–20 × 7–14 cm, herbaceous but chartaceous when dried, lower surface densely appressed pubescent and along veins villous, upper surface densely pubescent and villous, base attenuate to cuneate, margin irregularly serrate, ciliate, apex obtuse; lateral veins 3 or 4 on each side of midrib, abaxially obviously prominent, adaxially conspicuously sunken. Cymes 3–4, axillary, 3(5)–7-flowered or more; peduncle green, 8–10 cm long, 2.5–3.0 mm in diam., densely covered with erectly spreading white eglandular pubescent and villous hairs; bracts 3, lateral ones

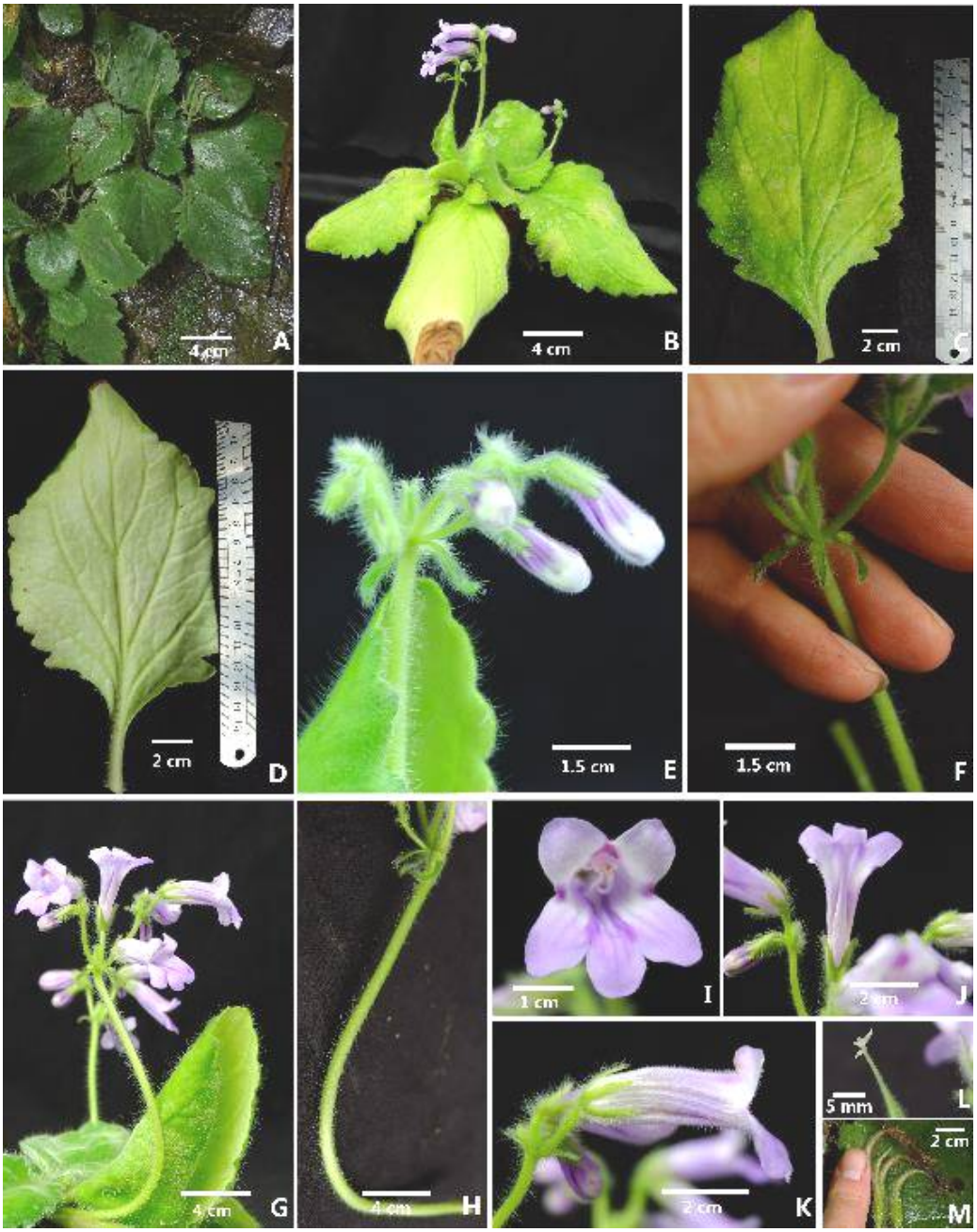

Fig. 1. *Primulina wenii* **sp. nov**. A. Habitat; B. Habit in flowering; C. Leaf blade – adaxial surface; D. Leaf blade – abaxial surface; E. Cyme, buds and bracts; F. bracts; G. Cymes and flowers; H. Peduncle and its indumentum; I. Front view of corolla; J. Upward view of corolla; K. Lateral view of corolla; L. Stigma with dissected lobes; M. Curved immature capsules. A and M: collected from type locality, 22 Jul 2013; B–L: photographed in nursery of Gesneriad Conservation Center of China, Guilin, 12 Apr 2014, by F. Wen.

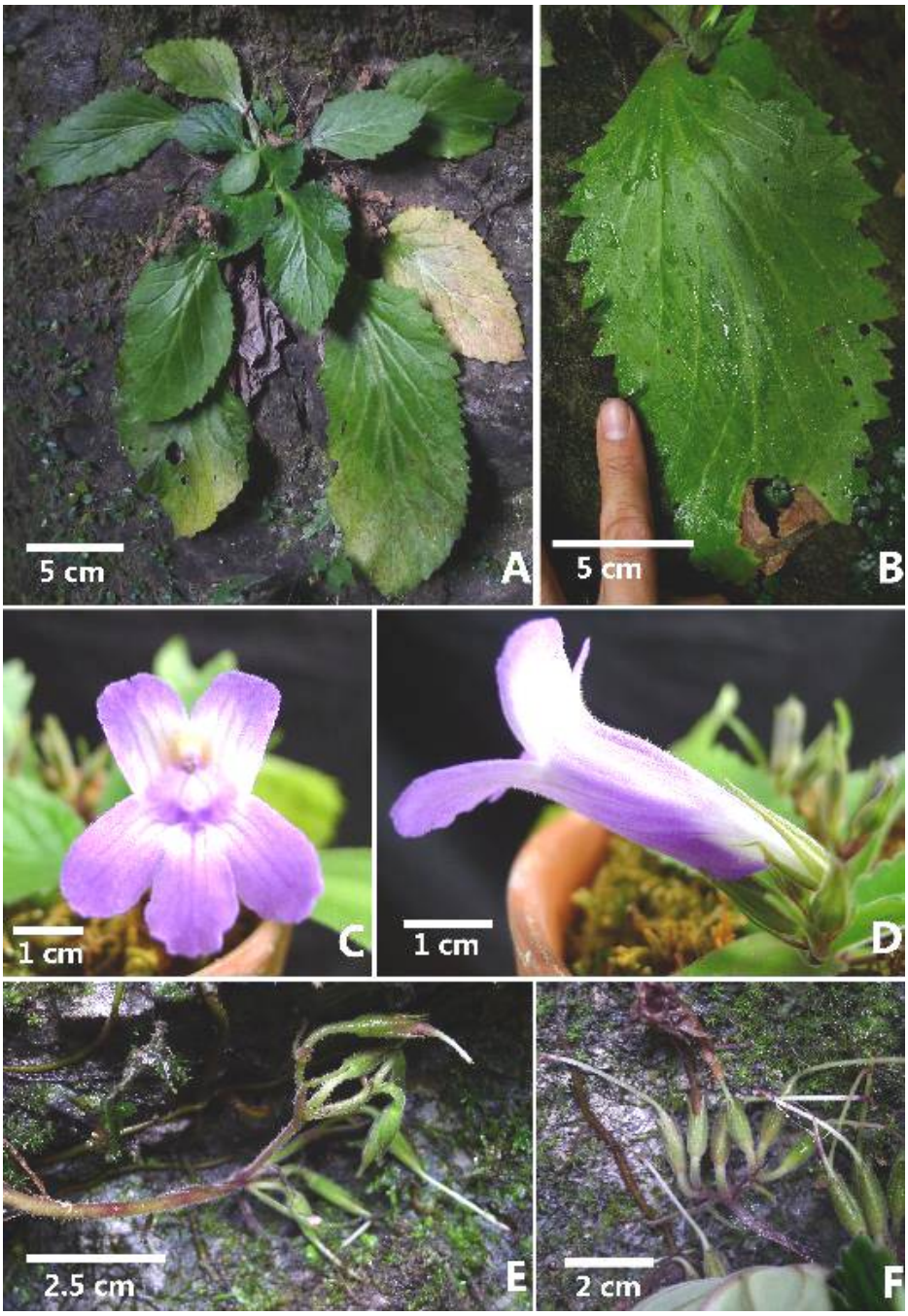

Fig. 2. *Primulina juliae.* A. Habit; B. Leaf blade – adaxial surface; C. Front view of corolla; D. Lateral view of corolla; E. Cyme, calyx lobes and indumentum; F. Young straight capsules. A– B and E– F: photographed in type location by J. Li; C– D: photographed in nursery of Gesneriad Conservation Center of China by F. Wen.

conspicuously opposite, green, linear to lanceolate, 14–16 × 2.5–3.0 mm, the central one smaller, 10–12 × 1.3–1.6 mm, persistent at flowering; outside white pubescent and villous, inside white pubescent, margin entire and ciliate, apex acute. Calyx 5-partite to base; lobes lanceolate, 14–15 × c. 2.5 mm, nearly equal, outside densely erectly white villous and pubescent, inside sparsely shortly pubescent to nearly glabrous, margin entire, apex acute. Corolla outside pale bluish purple, throat with 2 purple stripes, upper portion of inside corolla surface with 2 dark purplish brown swollen spots, 2 swollen spots sparsely covered with short glandular hairs, inside of lobes pale purplish blue with slightly darker purple longitudinal stripes, corolla c. 3.2 cm long, outside densely white glandular-pubescent, inside sparsely pubescent; tube nearly tubular, c. 2.8 cm long,

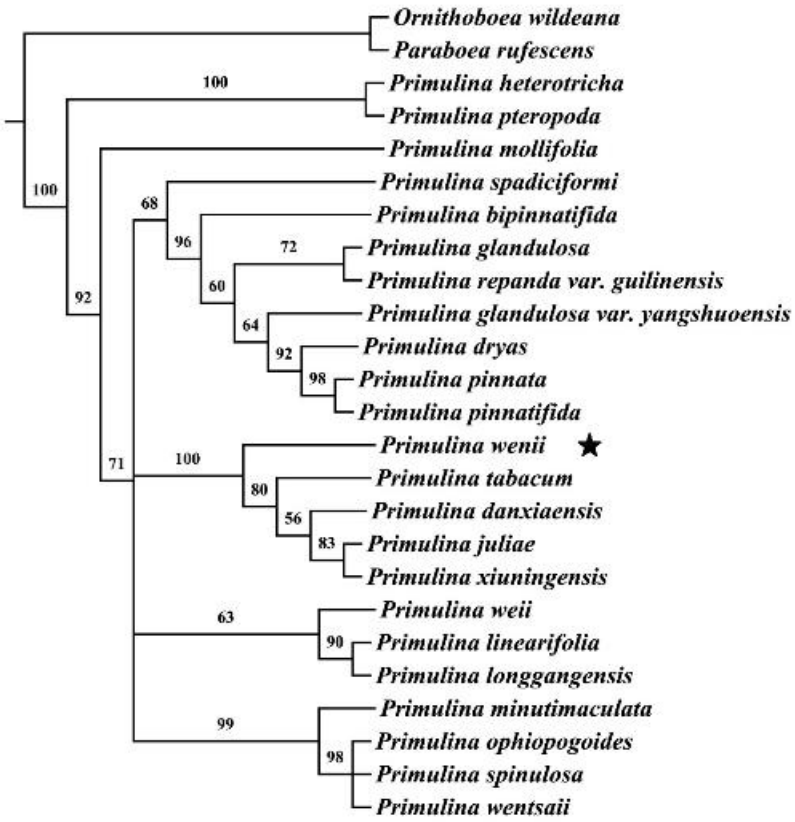

Fig. 3. The strict consensus tree of *Primulina* taxa based on a maximum parsimony (MP) analysis of combined ITS and *trn*L-F. Numbers of the branches indicate bootstrap values > 50% by MP analysis. * indicates the new species.

orifice c. 1.7 cm in diam.; limb distinctly 2-lipped; upper lip 2-lobed, lobes triangular to semicircle, 1.0–1.2 cm long; lower lip 3-lobed, lobes oblong to triangle, 1.0–1.3 cm long. Stamens 2, adnate to c. 1.2 cm above corolla base; filaments white, c. 1 cm long, geniculate c. 3 mm above insertion, glabrous; anthers reniform, slightly constricted at middle, c. 3.5 mm long; staminodes 3, lateral ones adnate to c. 1 cm above corolla base, white, linear, c. 7.5 mm long, glabrous, apex small capitate, central staminode adnate to c. 0.5 cm above corolla base, punctiform, inconspicuous, c. 0.5 mm long. Disk annular, c. 10 mm high; pistil c. 2.3 cm long, densely

covered with white eglandular hairs; style linear, c. 1.3 cm, pubescent; stigma trapeziform, 2-lobed to irregularly lobed, c. 2.5 mm long. Fruit c. 5 cm long, straight to slightly curved, hairy.

Flowering and fruiting period: April – June.

Etymology: The species epithet '*wenii*' is used to commemorate a Chinese botanist, Fang Wen, who studied Gesneriaceae of China for many years, and is devoted to the conservation and taxonomy of Chinese Gesneriaceae plants.

Vernacular name: Chinese: Wēn Shì Bào Chūn JùTái.

Habitat: *Primulina wenii* grows on shaded moist turf of limestone cliff in subtropical limestone evergreen broad-leaf forest on a North-facing slope of a limestone hill at an altitude of about 130 meters. The climate of Fuzhou, Fujian province is the monsoon of subtropical moist marine climate zone. The average annual temperature of Fuzhou is 20.1℃, the average annual precipitation is c. 1718.1 mm.

Notes: East China is not the area with high biodiversity of Gesneriaceae in China. For example, only six new species of Gesneriaceae, namely *Primulina xiziae* Fang Wen, Yue Wang & G. J. Hua (Li *et al.*, 2012), *P. chizhouensis* Xin Hong, S.B. Zhou & F.Wen (Hong *et al*., 2012), *P. suichuanensis* X.L. Yu & J.J. Zhou (Zhou *et al*., 2016), *Didymocarpus dissectus* Fang Wen, Y. L. Qiu, Jie Huang & Y.G. Wei (Wen *et al*., 2013), *Oreocharis striata* Fang Wen & C.Z. Yang (Yang *et al*., 2015) and *Beccarinda baolianis* Q.W. Lin (Lin, 2016) were discovered and described in the past decade. Further, Fujian province, belongs to E China, only possesses three species of *Primulina* before this new one was discovered. This also implies that more field investigations will help more discoveries.

Molecular analysis

The combined matrix had a length of 1,670 characters, 672 for ITS and 998 for *trn*L-F. Of the 254 (15.2%) varied, 257 (15.4%) were parsimoniously informative, including the indels. The maximum parsimony analysis on the combined matrix resulted in two trees of 937 steps in length, a consistency index (CI) of 0.7311, retention index (RI) of 0.6644 and homoplasy index (HI) of 0.2689. The strict consensus tree (Fig. 3) was highly resolved and tree topology was consistent with the previous phylogenetic study by Möller *et al*. (2011) and Kang *et al*. (2014).

DNA analyses of sequence data show that the new species is sister to a clade (BS = 100) comprising of *Primulina tabacum*, *P. danxiaensis*, *P. juliae* and *P. xiuningensis*. Of these species the undescribed species is morphologically most similar to *P. juliae* from which it can be distinguished by certain qualitative and quantitative characters in leaf blade, bract, indumentum, corolla, filament, stigma and pistil. For instance, it is obviously different that the peduncles of *P. wenii* are densely covered with erectly spreading white eglandular pubescent and villous hairs, the number of bracts is three, filaments are glabrous, and pistil is densely covered with white glandular puberulent hairs. The morphological differences between the two species (*P. wenii* and *P. juliae*) are depicted in Table 2.

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