# Two new species of *Primulina* (Gesneriaceae) from limestone karsts of China (#23971)

First revision

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I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.



## Two new species of *Primulina* (Gesneriaceae) from limestone karsts of China

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The limestone karst area of South China is a major biodiversity hotspot of global terrestrial biomes. During extensive field work on the Guangxi limestone formations, two unknown species of Gesneriaceae were collected. After conducting a comprehensive study of the literature and herbarium specimens, *Primulina davidioides* and *P. hiemalis* are recognized as two species new to science, and described and illustrated here. *P. davidioides* is morphologically close to *P. lunglinensis* based on the shape of the leaf and flower, but it can be easily distinguished by the shape of the bracts, corolla and stigma, indumentum of peduncles, pedicels and pistil, number of staminodes. *P. hiemalis* is closely relate to *P. luzhaiensis* in vegetative appearance, but differs in the shape of the calyx and stigma, and number of bracts and staminodes, indumentum of the leaf blade and peduncle, and position of stamens in the corolla tube. The conservation statuses of *P. davidioides*, and *P. hiemalis* were assessed as "Critically Endangered" (CR) and "Near Threatened" (NT) according to the IUCN Red List Category and Criteria, respectively.

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#### 18 ABSTRACT

The limestone karst area of South China is a major biodiversity hotspot of global terrestrial 19 biomes. During extensive field work on the Guangxi limestone formations, two unknown species 20 of Gesneriaceae were collected. After conducting a comprehensive study of the literature and 21 herbarium specimens, Primulina davidioides and P. hiemalis are recognized as two species new 22 to science, and described and illustrated here. P. davidioides is morphologically close to P. 23 lunglinensis based on the shape of the leaf and flower, but it can be easily distinguished by the 24 shape of the bracts, corolla and stigma, indumentum of peduncles, pedicels and pistil, number of 25 staminodes. P. hiemalis is closely relate to P. luzhaiensis in vegetative appearance, but differs in 26 the shape of the calyx and stigma, and number of bracts and staminodes, indumentum of the leaf 27 blade and peduncle, and position of stamens in the corolla tube. The conservation statuses of P. 28 davidioides, and P. hiemalis were assessed as "Critically Endangered" (CR) and "Near 29 Threatened" (NT) according to the IUCN Red List Category and Criteria, respectively. 30



#### INTRODUCTION

The tropical and subtropical karst landforms of southern and southwestern China are renowned 33 because of their unrivalled biodiversity and high endemism among the tropical and subtropical 34 floras of the world (Myers et al. 2000; Clements et al. 2006; Hou et al. 2010). Karst areas in 35 South China offer a multitude of ecological niches for plant diversification and speciation (Ai et 36 al., 2015), with about half of all the endemic genera of flowering plants in China (Ying & Zhang 37 1994). Among these, the Gesneriaceae form the most abundant with 28 genera amounting to 38 90% of all endemic genera of the family in China (Wang et al. 1990, 1998; Li & Wang 2004; 39 Möller et al. 2016). At the same time, China is a significant centre of diversity of Old World 40 Gesneriaceae, which consists of 52 genera (Möller et al., 2016), with 75% of all species endemic 41 to this region (Gao et al., 2015). 42 A great number of new species were described in the genus *Primulina* (Gesneriaceae) in recent 43 years (Yang et al., 2018), and it is becoming one of the most interesting genera of the Old World 44 Gesneriaceae, comprising more than 170 species (Wang et al. 2011; Möller et al., 2011; Möller 45 et al. 2016). This group shows high levels of endemism and ecological specialisation (Hao et al., 46 2015), with narrow, island distributions (Wang et al., 1998, Li & Wang, 2004, Wei et al., 2010), 47 i.e., only in karst towers and caves (Ai et al., 2015). The limestone regions of southern and 48 southwestern China and northern Vietnam possess the highest biodiversity of *Primulina* with 49 about 80% of species endemic here (Wei et al. 2010). Many Primulina species pairs can 50 successfully interbreed through artificial experiments (Wen 2008; Zhang et al., 2017), suggesting 51 that *Primulina* is probably a genus under recent or ongoing speciation and differentiation (Gao et 52



53 al., 2015).

During our continuous floristic surveys of limestone karsts flora in 2009 and 2010, we 54 have revealed an additional two species of *Primulina* not previously known. Further fieldwork 55 was conducted at the same locality, and flowering specimens were collected. Available 56 information suggested that these two species are rare and usually occur only at one or two 57 localities. After a comprehensive analysis of the literature (Wang et al. 1990, 1998, Wang & Li 58 2004, Wei et al. 2010), as well as herbarium specimens of E, GH, HITBC, HN, IBK, IBSC, K, 59 MO, KUN, PE and US (herbarium acronyms according to Index Herbariorum; Thiers 2017), the 60 specimens were identified as two new taxa of *Primulina* based on results of detailed examination 61 of morphological anatomical features, which we hereby describe and illustrate. 62

#### MATERIALS AND METHODS

#### **Ethics statement**

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All the collecting locations of the new species reported in this study are not in any natural conservation area and no specific permissions were required for these locations. Since the species are currently undescribed, inevitably, they are not currently included in the China Species Red List (Wang & Xie 2004). Our field studies did not involve any endangered or protected species. No specific permits were required for the present study.

#### Nomenclature

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(ICN), hence the new names contained in the electronic version are effectively published under
that Code from the electronic edition alone. In addition, new names contained in this work which
have been issued with identifiers by IPNI will eventually be made available to the Global Names
Index. The IPNI can be accessed and the associated information contained in this publication
viewed through any standard web browser using the web address "http://ipni.org/". The online
version of this work is archived and available from the following digital repositories: PeerJ,
PubMed Central, and CLOCKSS.

#### **Material collection**

These two species were collected and examined during the floristic field surveys. In addition, the plants were monitored in the field and nursery in the Gesneriad Conservation Center of China (GCCC) by the authors over the past eight years. We collected leaf materials of these possible new species, using silica gel to dry them in the field for DNA extraction. We also cultivated about ten young plants from leaf cuttings of each new species for ex situ conservation in the Genseriad Conservation Center of China (GCCC) so that we can preserve the germ plasma resource of this rare species.

#### Morphological observations and specimens examined

An overview of the genus *Primulina* from southern China and adjacent areas of northern Vietnam was prepared. All available specimens of *Primulina* stored in the following herbaria were carefully examined: E, GH, HITBC, HN, IBK, IBSC, K, MO, KUN, PE and US. The images of type specimens were also obtained from Tropicos (http://www.tropicos.org) and JSTOR Global Plants (http://plants.jstor.org). Morphological description of the new species was



- based on examination of fresh and pressed specimens. All morphological characters, particularly
- 95 the details of the flowers, were studied under a stereomicroscope (MD-90, Olympus, Tokyo,
- Japan), and are described using the terminology presented by Wang et al. (1998). The
- 97 morphological comparison with other species was based on study of live plants in the field and in
- 98 cultivation in GCCC, herbarium specimens, and also information gathered in the literature
- 99 searches.
- 100 RESULTS
- 101 *Primulina davidioides* F. Wen & Xin Hong sp. nov. (Figs 1, 2)
- 102 **IPNI**:
- 103 Type. China. Guangxi: Donglan County, Donglan Town, Dayou village, growing in rocky
- crevices and tufa surface on the bottom of a limestone hill, elevation ca. 350 m, 05 May 2012
- 105 (fl.), F. Wen & Xin Hong 201205005 (holotype: IBK; isotype: AHU).
- 106 Paratypes. China: Guangxi: Donglan County, Donglan Town, Dawen Village, ca. 350 m, 12
- 107 May 2009, F. Wen 0905012 (IBK); ibid., 350 m, 28 May 2011, Xin Hong 201209030 (AHU).
- 108 **Diagnosis.** Primulina davidioides is morphologically close to P. lunglinensis based on elliptical
- to broadly ovate leaf blade, purple corolla and cylindrical tube, but it can be easily distinguished
- by puberulent peduncles (vs. glandular puberulent); bracts cordate to suborbicular, base
- subtruncate,  $4-6 \times 4-5$  cm (vs. elliptic to broadly ovate, base attenuate,  $0.6-3.8 \times 0.4-2.7$  cm);
- pedicels glandular and eglandular–pubescent (vs. glandular puberulent); corolla ca. 6 cm long (vs.
- 3–3.8 cm long), staminodes 3 (vs. 2), pistil ca. 3.7 cm long, glandular and eglandular pubescent
- 114 (vs. ca. 2.7 cm long, puberulent), stigma narrowly obtrapeziform (vs. cuneate) (see Table 1).



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**Description.** Perennial. Rhizome terete, 2–6 cm long, 1–2 cm in diameter. Leaves in basal rosette, 4–10, opposite; pachyphyllous, rigid and coriaceous when dry, densely pubescent on both surfaces; petiole flattened, 1–2 cm long, 6–10 mm wide, densely pubescent; blade elliptical to broadly ovate, (5–) 11–12.5 cm, 5–10 cm wide, apex somewhat obtuse, base obliquely cuneate, margin shallowly serrate; lateral veins 3-5 on each side, impressed adaxially and prominent abaxially. Cymes 1-3, 5-9 (11)-flowered; peduncles 5-9 (12) cm, 4-8 mm in diameter, pubescent; pedicels ca. 1.4 cm long, glandular and eglandular-pubescent; bracts 2, opposite, free, cordate to suborbicular, thickly chartaceous, slightly fleshy, leathery when dry, white when flowering, with crinkled and virescent margins, 4–6 cm long, 4–5 cm wide, base subtruncate, apex acute, outside densely puberulent and sparsely strigillose, inner surface glandularpuberulous. Calyx membranous, white to subtranslucent, ca. 7 mm, 5-sect; segments equal, triangular, 2–3 mm wide, margin obscurely serrated from the middle, apex acute, outside densely 126 puberulent. Corolla ca. 6 cm long, purple, with dark purple lines inside, outside glandular and eglandular-pubescent, inside pubescent near base; tube cylindrical; ca. 4.5 cm long, ca. 1 cm diameter at mouth, ca. 0.5 cm in diameter at base; limb distinctly 2-lipped, adaxial lip 2-parted to the base, lobes ovate, ca.  $6 \times 5$  mm, 3-lobed from near the middle, lobes oblong,  $10-15 \times 4-6$ 130 mm. Stamens 2, abaxial, adnate to ca. 1.7 cm above corolla base; filaments white, ca. 1.1 cm, strongly geniculate near the base, ca. 1 mm wide, sparsely glandular-puberulous; anthers fused 132 along their entire adaxial surfaces, reniform—oblong, ca. 3 mm long, ca. 2 mm wide, pale vellow, bearded on the back. Staminodes 3, adaxial, linear, apex capitellate, lateral ones ca. 7 mm long, adnate to ca. 1.5 cm above corolla base, central one ca. 4.5 mm long, adnate to ca. 0.9 cm above



136	corolla base, sparsely glandular-puberulous. Disc ring-snaped, indistinctly lobed, 0.5–0.8 mm
137	high, glabrous. Pistil linear, ca. 3.7 cm long, green, densely puberulent with both glandular and
138	eglandular hairs; ovary ca. 2.0 cm long, ca. 1.5 mm wide. Lower lip of stigma narrowly
139	obtrapeziform, apex retuse, translucent to green, ca. 3 mm long, recurved. Capsule ca. 5 cm long,
140	brownish.
141	Distribution, Habitat and Ecology. Primulina davidioides is rare, only found in the type
142	locality, i.e. an unnamed limestone hill ca. 20 km west of Donglan Town, Donglan County, in
143	northern Guangxi province of southern China. It grows in rocky crevices and tufa surface on the
144	bottom of a limestone hill at an elevation of 350 m a.s.l. The average temperature of Donglan
145	County is 18.7°C, the average annual precipitation has been calculated at ca. 1660 mm. The
146	forest where P. davidioides occurs is subtropical evergreen broad-leaf forest. Flowering in May,
147	fruiting in September.
148	Etymology. The specific epithet is derived from its cordate to suborbicular bracts with
149	subtruncate base. The shape of the bracts looks similar to the bracts of <i>Davidia involucrate</i> Baill.,
150	native to China.

- 152 *Primulina hiemalis* Xin Hong & F. Wen sp. nov. (Figs 3, 4)
- 153 Type: China, Guangxi, Yongfu county, Baishou Town, Chuanyan village, on the moist rock face
- at the entrance of limestone caves, 526 m a.s.l., 09 December 2010, F. Wen & L.F. Fu
- 155 WFBCJT101209-01 (holotype: IBK; isotype: AHU).
- 156 Additional collections. China, Guangxi, Yongfu county, Baishou Town, Baishouyan, on the



moist rock face at the bottom of limestone hills, 511 m a.s.l., 15 December 2011, F. Wen 157 WFBCJT111215-01 (IBK) 158 **Diagnosis.** Morphologically, *Primulina hiemalis* resembles *P. luzhaiensis* since both species 159 having thick chartaceous leaves, obliquely ovate or oblong leaf blade, purple corolla, 4-6 cm, 160 tubular tube. But it can be readily distinguished from P. luzhaiensis by the indumentum of the 161 leaf blade and peduncle (puberulent vs. villous); bracts 3 (vs. 2), lateral ones ca. 2 cm long (vs. 162 0.2–0.8 mm long); longer calyx, ca. 1.5 cm long (vs. 0.5–1 cm long); stamens adnate to ca. 2 cm 163 (vs. 1.4–1.7 mm) above the corolla tube base; staminodes 2 (vs. 3), stigma ligulate, apex 2-lobed 164 to middle (vs. obtrapeziform, apex retuse) (shown in Table 2). 165 **Description.** Perennial. Rhizome subterete, ca.  $1.0-2.5 \times 0.5-2.0$  cm wide, internodes 166 inconspicuous. Leaves in basal rosette, 6–8, opposite; petiole  $0.5-3.5 \times 0.4-0.6$  cm; leaf blade 167 thickly chartaceous, markedly obliquely ovate or oblong, 5.5–9.5 × 3.5–5.0 cm, apex obtuse, 168 base obviously oblique, cuneate-attenuate, margin crenate, obviously serrated from the base; 169 densely puberulent on both surfaces, lateral veins 4-5 on wider side of midrib, lateral veins 170 inconspicuous, 3–4 on narrower side of midrib. Cymes 2–4, 1–3-flowered; peduncle 1.5–3.0 cm, 171 densely puberulent; bracts 3, free, narrowly lanceolate, lateral ones ca. 20 × 2-2.5 mm, the 172 central one ca.  $5 \times 0.8-1$  mm, puberulent, margin entire, apex acute. Pedicel 0.5-1.0 cm, densely 173 puberulent. Calyx 5-sect from base; segments equal, lanceolate-ovate, ca. 15 × 6 mm, puberulent, 174 margin entire, apex acute. Corolla purple, 4-6 cm, outside puberulent, inside glabrous; tube 175 tubular, 2.5-4.8 cm long, base ca. 3-5 mm in diameter, top 12-16 mm in diameter; limb 176 distinctly 2-lipped, adaxial lip 2-parted to the base, lobes ovate, ca. 10 × 8 mm, 3-lobed over the 177



middle, lateral ones ovate, central one oblong, obtuse to truncate at apex, 12–15 × 8–10 mm. 178 Stamens 2, adnate to ca. 2 cm above the corolla tube base, filaments ca. 1 cm long, geniculate 179 near the base, glabrous; anthers fused by their entire adaxial surfaces, elliptic, ca. 2.5 mm long, 180 glabrous; staminodes 2, linear, 6-7 mm, glabrous. Pistil ca. 3 cm, densely puberulent with both 181 glandular and eglandular hairs; ovary ca. 2.0 cm, glandular-puberulent. Stigma ligulate, lobes 182 linear, 2–2.5 mm long. Capsule 2.5–3.0 cm long. 183 Distribution, Habitat and Ecology. Primulina hiemalis is locally abundant, narrowly endemic 184 and only known from the type locality, i.e. Chuanyan village, Baishou Town, Yongfu county, 185 Guangxi province. It grows on moist, shady cliffs at the entrance of a limestone cave, at an 186 elevation of 530 m a.s.l. The average temperature is 18.8°C, the average annual precipitation has 187 been calculated at ca. 2,000 mm. The forest where P. hiemalis occurs is subtropical evergreen 188 broad-leaf forest. Flowering from November to January, fruiting in April. 189 **Etymology.** The specific epithet is derived from the species' winter flowering time. The Latin 190 word "hiemalis" is an adjective, meaning "to belong to winter," and hints at the flowering time 191 of the new taxon (November to January). 192

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#### **DISCUSSION**

In contrast to its high species diversity, the morphological variation of *Primulina* is relatively limited compared to other genera (Möller et al. 2016). The corolla morphology, especially, is relatively uniform, and most species possess straightly infundibuliform corollas, with only the salverform (e.g. *Primulina tabacum*), campanulate (e.g. *P. dichroantha*, *P. mollifolia*, *P.* 



hezhouensis, and P. renifolia), and P. curvituba having a strongly curved tube shape. In addition, Primulina displays a wide range of diversity of involucral bracts. The two opposite bracts are brightly white when flowering and serve the function of attracting pollinators as in Primulina eburnea, P. lutea, P. xiziae, P. lungzhouensis, P. lunglinensis, P. beiliuensis var. beiliuensis, P. beiliuensis var. fimbribracteata and the new species, P. davidioides. The development of cymes in these species shows that the two lateral paraclades of the pair-flowered cyme are reduced, so all flowers are clustered together. While flowers of these species always blossom for a short duration (Hong 2016), the large white bracts and central flower cluster make the inflorescence look superficially like a single flower, and attract pollinators.

#### CONSERVATION ASPECTS

These two new species belong to a group of stenochoric plants which only grow in limestone areas. Currently there is insufficient information concerning the distribution and population status of this new species. Obviously, further field study is needed in northwestern Guangxi as its geographic range may well be more extensive than presently known. Based on IUCN red list categories (IUCN, 2016), Primulina davidioides can be assessed as "Critically Endangered, CR Alabed+C2a (i, ii)" because the number of mature individuals known is less than fifty. Primulina hiemalis can be assessed as "Near Threatened" (NT) because its localised and patchy distribution, and also on facing the risk of over-collecting by tourists and local people because of its beautiful flowers. 



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During our continuous floristic surveys of limestone karsts in recent years, two unknown species of Gesneriaceae were collected. After conducting a comprehensive study of the literature and herbarium specimens, *Primulina davidioides* and *P. hiemalis* are recognized as two species new to science based on results of detailed examination of morphological anatomical features. Available information suggested that these two species are rare and usually occur only at one or two localities. The conservation statuses of *P. davidioides* and *P. hiemalis* were assessed as "Critically Endangered" (CR) and "Near Threatened" (NT), respectively, according to the IUCN Red List Category and Criteria.

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#### Table 1(on next page)

Table 1. Diagnostic character differences between *Primulina davidioides* sp. nov. and its close relatives *P. lunglinensis*.



1 **Table 1**. Diagnostic character differences between *Primulina davidioides* sp. nov. and its close relatives *P*.

#### 2 lunglinensis.

Characters	P. davidioides	P. lunglinensis		
Indumentum of peduncles	puberulent	glandular puberulent		
Bracts	cordate to suborbicular, base	elliptic to broadly ovate, base		
	subtruncate, $4-6 \times 4-5$ cm	attenuate, $0.6-3.8 \times 0.4-2.7$		
		cm		
Indumentum of Pedicel	glandular and eglandular pubescent	glandular puberulent		
Corolla size	ca. 6 cm long	3–3.8 cm long		
Number of staminodes	3	2		
Pistil	ca. 3.7 cm long, glandular and	ca. 2.7 cm long, puberulent		
	eglandular pubescent			
Shape of Stigma	narrowly obtrapeziform	cuneate		

3

4



### Table 2(on next page)

Table 2. Diagnostic character differences between *Primulina hiemalis* sp. nov. and its close relatives *P. luzhaiensis*.



1 Table 2. Diagnostic character differences between Primulina hiemalis sp. nov. and its close relatives P.

#### 2 luzhaiensis.

Characters	P. hiemalis	P. luzhaiensis
Indumentum of of leaf blade	puberulent on both surfaces	villous and pubescent adaxilly
Indumentum of peduncle	densely puberulent	densely villous
Bracts	3, lateral ones ca. 2 cm long,	2, 0.2–0.8 mm long
	the central one ca. 0.5 cm long	
Calyx size	ca. 1.5 cm long	0.5-1 cm long
The location of stamens	adnate to ca. 2 cm above the	adnate to 1.4–1.7 mm above the
	corolla tube base	corolla tube base
Number of staminodes	2	3
Shape of stigma	ligulate, apex 2-lobed to middle	obtrapeziform, apex retuse



Figure 1. Illustration of *Primulina davidioides* sp. nov.

(A) habit in flowering period; (B) dissection of a flower showing stamens and staminodes; (C) anthers, showing beard; (D) pistil; (E) stigma; (F) ovary and calyx; (G) calyx (Image credit: Xiao-Ming Xu, drawn from the holotype).

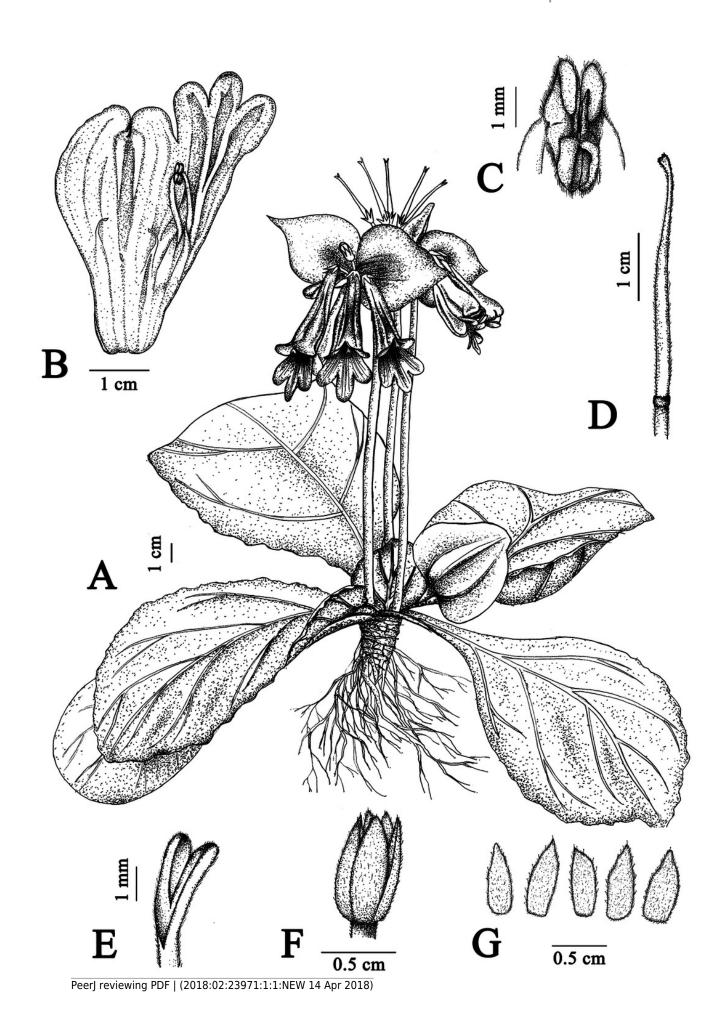




Figure 2. Photographs of *Primulina davidioides* sp. nov.

(A) habitat; (B) population; (C) the flower bud; (D) flowering plant with cymes; E—H: cymes: (E) showing adaxial bracts; (F) showing abaxial bracts; (G) frontal view of cymes; (H) top view of cymes); (I) lateral view of corolla; (J) young fruit; (K) top view of corolla; (L) upward view of corolla; (N) opened corolla, pistils without corolla and calyx lobes; (O) dissection of a flower showing staminodes; P—Q: stamens (P) the reverse side, showing beard; (Q) the frontal side, showing the anthers; (R) capsules (Photo credit: Fang Wen and Xin Hong).

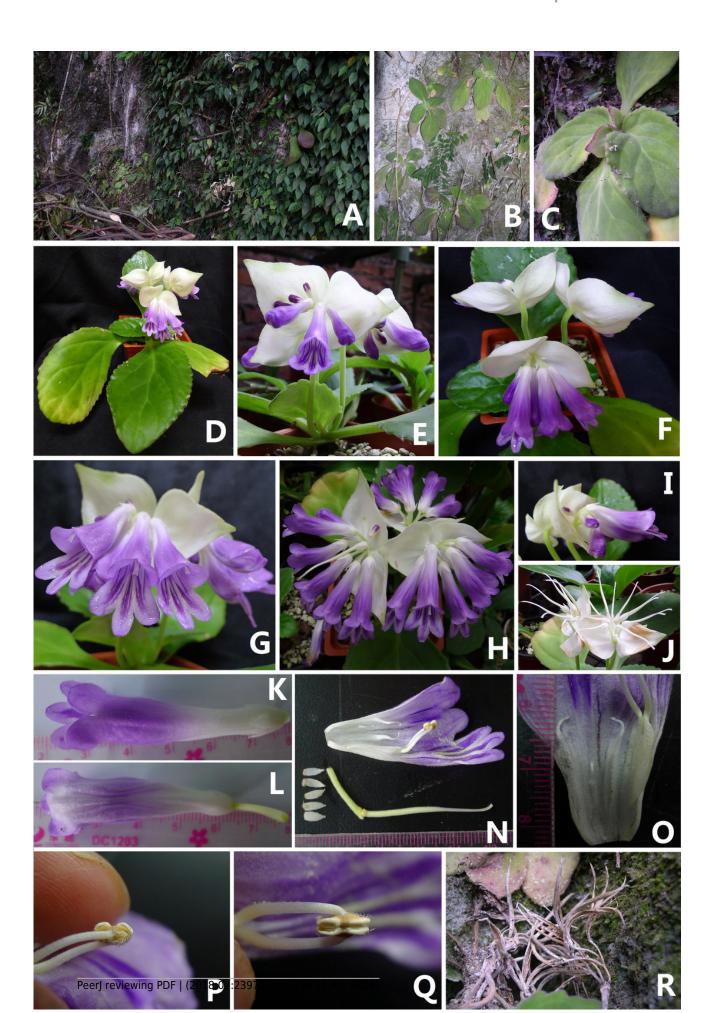




Figure 3. Illustration of *Primulina hiemalis* sp. nov.

(A) habit in flowering period; (B) dissection of a flower showing stamens and staminodes; (C) calyx and pistil; (D) calyx, showing puberulence (Wen Ma, drawn from the holotype).

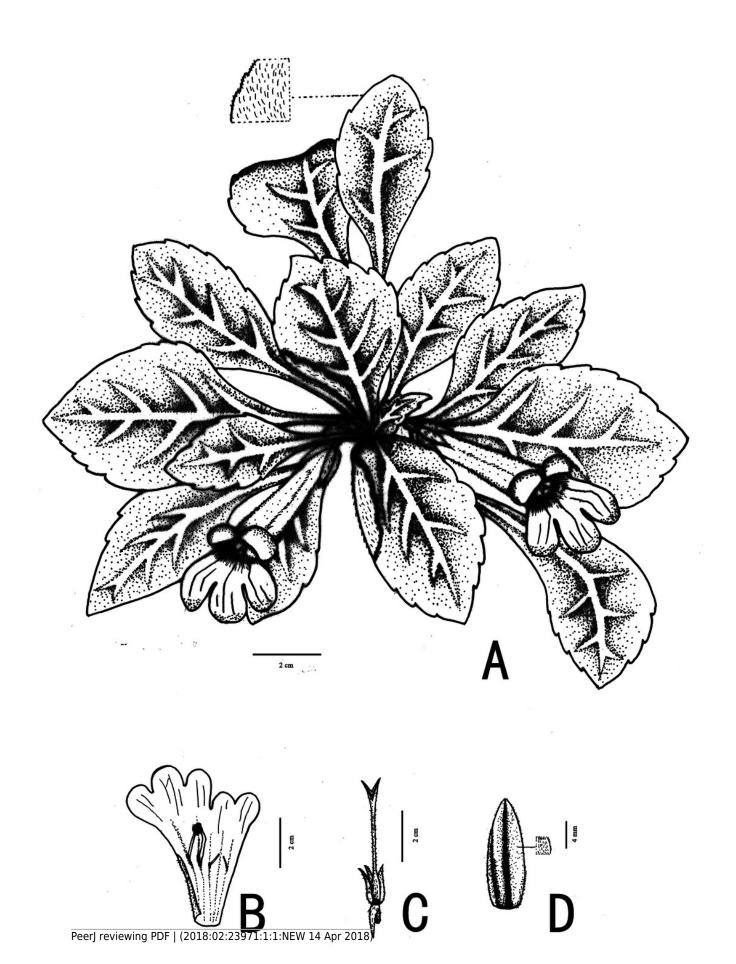




Figure 4. Photographs of *Primulina hiemalis* sp. nov.

(A) habitat; (B) plant; C—F: corolla: (C) frontal view; (D) right side view; (E) top view; (F) left side view; (G) calyx; (H) pistils with calyx lobes, showing the stigma; (I) opened corolla (Photo credit: Fang Wen).

