Primulina anisocymosa (Gesneriaceae), a new species with a unique inflorescence structure from Guangdong, China

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22 **ABSTRACT**

23 A new *Primulina* species from Guangdong, China with an unusual inflorescence is
24 described here. *Primulina anisocymosa* is most similar to *P. bobaiensis*. It can be
25 distinguished from all species within *Primulina* morphologically by its unique zigzag
26 monochasial cyme and infructescence. Two samples from different populations
27 identified as *Primulina anisocymosa* are monophyletic and were nested in a
28 monophyletic clade within *Primulina* with high branch support. The somatic
29 chromosome number of the new species is also reported ($2n = 36$), supporting its
30 placement in the genus.

31

32 **INTRODUCTION**

33 *Primulina* Hance (Didymocarpinae: Gesneriaceae) was originally described as a
34 monotypic genus with *P. tabacum* Hance the only known species (Wang et al. 1998;
35 Li and Wang 2004; Zheng and Xia 2005; Wei et al. 2010). *Primulina* was recently
36 expanded to include other Asian genera making the genus one of the largest within
37 Gesneriaceae. The genus is distributed from southern China southward into central
38 Vietnam (Möller et al. 2016) and comprises more than 174 recognized species. Thus
39 far, only 19 species are reported from Vietnam, the remainder occur in China (Hộ
40 2000; Weber et al. 2011a; Möller et al. 2016).

41 Some recent studies have suggested that the vast majority of *Primulina* species
42 grow on limestone in tropical and subtropical Karst areas (Xu 1993; Ai et al. 2015;
43 Kang et al. 2014). Most of the known species from China and Vietnam share a similar

44 inflorescence structure (dichotomous cyme). This inflorescence is highly variable
45 between species. *Primulina diffusa* Xin Hong, Fang Wen, & S.B. Zhou for example
46 has a single-flowered cyme, which is an extreme reduction of the dichotomous cyme
47 (Li and Wang 2004; Wang et al. 1998; Wei et al. 2010). The significant variation in
48 inflorescence structure and corolla morphologies suggests a high degree of
49 evolutionary lability, presumably driven by adaptation to different pollinators (Weber
50 et al. 2011b). Due to the wide variation in characteristics within the Asian genera it is
51 difficult to ascertain a single synapomorphy for the genus (Chen et al. 2014). Thus, it
52 is necessary to have molecular evidence to effectively place unique species in the
53 right genus.

54 In 2009, we found a population of *Primulina* with only the previous year's fruit
55 in Yangchun city, Guangdong province. Then the following year, Mr. Wei-Jun Wu
56 (WJW) found the same purported species on another limestone hill near Gaozhou city,
57 Guangdong province. We observed the species closely and noted that it was an
58 undocumented species of *Primulina*, based on morphology. The species looked quite
59 different from other *Primulina* species based on the infructescence which looked
60 similar to a cincinnus, a determinate cymose inflorescence with a zigzag rachis. The
61 following year, FW and WJW collected some specimens with flowers from the same
62 locality for study. They observed the development of the flowers and the unusual
63 zigzag monochasial inflorescence and infructescence. To confirm the phylogenetic
64 relationships and generic placement of this species, chromosome and DNA sequence
65 data were collected and analysed. After consulting the relevant literature (Wang et al.

1998; Hộ 2000; Li and Wang 2004; Wei et al. 2010) along with the chromosome characteristics and molecular analysis, we concluded that this new species was assignable to *Primulina* (Weber et al. 2011a). We provide a description and illustration of the new species here.

MATERIALS AND METHODS

Ethics statement

All the collecting locations of the new species reported in this study are outside any natural conservation area and no specific permissions were required for these locations. Since the species are currently undescribed, 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

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants (ICN), and 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 LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix

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"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

The new species has been monitored in the field and nursery of The Gesneriad Conservation Center of China (GCCC) by the authors since the plants were collected. We collected leaf materials of this possible new species, using silica gel to dry them in the field for DNA extraction.

Morphological observations and specimens examined

A study of the genus *Primulina* from S and SW China and adjacent areas of Vietnam was undertaken. All available specimens of *Primulina* stored in the following herbaria from China, Vietnam, United States and the United Kingdom were examined: E, GH, HN, IBK, IBSC, K, MO, KUN, PE, US and VMN ((herbarium acronyms according to Index Herbariorum; Thiers 2017). All morphological characters were studied using a dissecting microscope (SZX16, Olympus, Tokyo, Japan). Characters were described using the terminology presented by Wang et al. (1998) were applicable. The morphological comparison with other species was based on study of live plants in the field and in cultivation in GCCC, herbarium specimens, and also information gathered in the literature searches.

DNA Extraction and PCR

The DNA was extracted using a DNeasy plant mini kit (Qiagen, Crawley, UK) following the manufacturer's protocol. The extractions were checked for quality and quantity using a RapidHIT200 (IntegenX, Pleasanton, CA, USA). Total genomic

DNA was used as the template for polymerase chain reactions (PCR). The PCR conditions and cycle sequencing reactions followed Möller et al. (2009, 2011).

Phylogenetic Analyses

For the phylogenetic analyses, sequences were newly acquired for each population of this new species and also the morphologically similar species *P. bobaiensis* Li, Pan and Zhang (Table 1), were added to a reduced Old World Gesneriaceae matrix of Middleton et al. (2014, 2015) and the matrices realigned manually. The resulting matrix contained 185 samples (177 species) with 26 species of *Primulina* included, covering all 39 genera currently recognized in the advanced Asiatic and Malesian Gesneriaceae at present (Möller et al. 2009, 2011; Weber et al. 2013; Middleton et al. 2014, 2015). The tree was rooted with the outgroup taxon *Tetraphyllum* (Möller et al. 2009). Data for the phylogenetic analyses were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Species, voucher information, and NCBI accession numbers are listed in Appendix 1.

Combinability of the ITS and *trn*LF datasets were investigated by the incongruence length difference (ILD) test (Farris et al., 1995a, b), implemented in PAUP* 4.0a146 (Swofford, 2002) as a partition-homogeneity test (PHT). Phylogenetic analyses were performed using Bayesian inference (BI) following Möller et al. (2011) and Weber et al. (2011a). The model GTR + I + G was selected as the optimal model for both DNA regions based on the the Akaike Information Criterion (AIC; Akaike, 1974) in MrModeltest version 2.3 (Nylander 2004). Bayesian inference analyses were carried out in MrBayes version 3.2.2 (Ronquist and Huelsenbeck, 2003). Two independent

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analyses of 10,000,000 generations each were run with four Markov chain Monte Carlo (MCMC) chains and a tree sampled at every 1000th generation. The first 10 percent of the generations were discarded as burn-in and Bayesian Posterior probabilities (BPP) obtained from the analysis were used to indicate the support for various branches.

Chromosome Preparations

Seeds for chromosome analysis were collected from the two wild populations in 2012. Voucher specimens (vouchered as: *Fang Wen 201090406-1*, *SLY160515-01*) have been deposited in IBK. Root tips were collected from leaf-cuttings started in vermiculite in the culture room at GCCC. Usable roots were gathered and pretreated in 2 mM 8-hydroxyquinoline at 15–18 °C for about 6 h, then fixed overnight in an ethanol-acetic acid solution (3:1) at 4°C. The root tips were macerated with 1 N hydrochloric acid (10:1). The root tips were then stained and squashed in 2% acetic orcein. Metaphase plates were photographed using an Olympus BX51 microscope with Olympus DP71 camera attachment (Olympus, Tokyo, Japan). The chromosome numbers were determined in at least 20 cells with well-spread chromosomes of 10 different root tips from the two known populations.

RESULTS

Molecular phylogenetic studies

The data matrix was assembled and included 1381 characters, including 608 for ITS and 773 for *trn*L-F. The results did not indicate incongruent phylogenetic signals

(P = 0.36) and the two matrices were analysed together. The phylogenetic tree (Fig. 1) was highly resolved with a topology consistent with previous phylogenetic analyses by Middleton et al. (2014, 2015). The new species was nested in a clade within *Primulina* with high branch support (PP = 1.00). Two samples from different populations identified as *Primulina anisocymosa* are monophyletic (PP = 1.00). The new species was strongly supported in a clade (PP = 1.00) comprised of *Primulina gemella* (D. Wood) Y.Z. Wang and its morphologically most similar congener *P. bobaiensis*.

Chromosome characteristics

The chromosome size of *Primulina* was measured and noted as small with a range from 0.6–2.4 μm (Lima-de-faria, 1980; Yang et al. 2012). The somatic chromosomes at metaphase of *P. anisocymosa* were photographed (Fig. 2) and determined to be diploid with $2n = 36$.

Taxonomic treatment

Primulina anisocymosa F. Wen, Xin Hong & Z.J. Qiu, sp. nov. (Figs 3, 4)

IPNI:

Type. China. Guangdong: Gaozhou city, rocky crevices in moist shady cliffs on a red sedimentary rock hill, elevation ca. 120 m, 20 Nov. 2012, *F. Wen 20121120* (holotype IBK!; isotype ANU!).

Additional collections. China. Guangdong: Yangchun, ca. 107 m, 06 April 2009, *Fang Wen 201090406-1* (IBK!; ANU!); China. Guangxi, cultivated in nursery of GCCC, Guilin Botanical Garden, introduced from Gaozhou Ceity, growing on rocky

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crevices in moist shady cliffs on a red sedimentary rock hill, elevation ca. 120 m, *SLY160515-01* (IBK!).

Diagnosis. *Primulina anisocymosa* differs from other congeners by the presence of a zigzag monochasial cyme.

Description. Perennial herb, acaulescent. Rhizome subterete, 1–3 cm long, 1.5–2 cm diam, glabrous. Leaves 6–9 or more, 3-whorled, basal; sessile or subsessile. Leaf blade slightly fleshy, dried papery, oblong-rhombic, or elliptic-oblong, 3–9(–12) × 2.5–6(–8) cm, apex obtuse or sometimes rounded, base cuneate to attenuate, and decurrent into slightly broad wings of extremely inconspicuous petiole, or nearly sessile, margin distinctly serrate with 6–8 triangular serration on each side; densely pubescent intermixed with sparsely villous trichomes on both surfaces, lateral veins 4–7 on each side, impressed adaxially and prominent abaxially. Cyme monochasial, reduced with 4–6 (–10) or more cymules alternating along rachis (appearing zigzag), 3–6 flowers per cymule. Peduncles 15–20 cm long, ca. 2 mm in diameter, pubescent intermixed sparsely with glandular trichomes. The inflorescence has only one of the two lateral paraclades of the pair-flowered cyme extended. The others are reduced to the point of pedicel attachment and form the swollen nodules at the base of 2–3(–5) flowers. All flowers of each cymule clustered and each cluster facing away from the rachis. Bracts variable, usually 1–2, occasionally 3, sometimes withered when flowering, free, narrowly triangular, 2–3 × ca. 1 mm, outside puberulent, inside verrucose and glabrous, margin entire, apex acute, aligned on one side of the rachis; bracteoles three, subulate, 1 × ca. 0.2 mm, not-paired, aligned on one side at the base

of pedicel. Pedicels extended only on one side, 18–20 mm long, ca. 1.0 mm in diameter, densely glandular-puberulent. Calyx 5 lobed from base; segments equal, lanceolate-oblong, 5–8 × 4–5 mm, outside densely puberulent, inside verrucose and glabrous, margin entire, apex acute. Corolla ca. 1.5 cm long, purpe, upper part of the interior of the corolla with two dark brown short stripes; mouth 0.6–0.8 cm diam, outside glandular-pubescent, inside glabrous; tube nearly infundibuliform, ca. 1.3 cm, limb distinctly 2-lipped, adaxial lip 2-parted for over half its length, lobes obliquely ovate, ca. 0.5 cm long, 0.3–0.4 cm in diameter at base, abaxial lip 3-parted to the base, central lobe ovate oblong, 0.5–0.6 cm long, 0.25–0.35 cm in diameter, lateral ones obliquely oblong. Stamens 2, adnate to ca. 6 mm above the corolla base; filaments ca. 6 mm long, glandular-pubescent, geniculate at the base, anthers fused by their entire adaxial surfaces, elliptic, ca. 2 mm long, yellowish brown, glabrous; staminodes two, linear, ca. 3 mm long, glabrous, adnate to ca. 7 mm above the corolla base; capitate at apex. Disc annular, ca. 1 mm high, margin entire. Ovary linear, 0.9–1.1 cm long, ca. 2 mm in diameter, densely glandular- and eglandular-puberulent; style green, ca. 6 mm long, ca. 0.8 mm in diameter, glandular-puberulent. Stigma translucent to green, obtrapeziform, apex retuse, ca. 1.2 mm long. Capsule linear, 4–4.5 cm long, ca. 2 mm in diameter, glandular- and eglandular-puberulent. Seeds long-ellipsoidal, dark brown, mammillate on the surface.

Distribution. The new species is known from two locations: Gaozhou City and Yangchun City, Guangdong Province, southern China. This area is in the transitional zone between the tropics and subtropics.

Habitat and Ecology. *Primulina anisocymosa* is locally abundant in Yangchun, although very rare in Gaozhou, Guangdong. The total number of this species in Gaozhou does not exceed 100 individuals. It grows in rocky crevices of moist shady cliffs on a red sedimentary rock hill, at an elevation of 50 m a.s.l, in Gaozhou City. The species also grows on the moist rock surface of limestone cave entrances in Yangchun. The average annual temperature of the two localities is similar (ca. 21° C) and the average annual precipitation is around 2380 mm. *P. anisocymosa* occurs in subtropical evergreen broad-leaved forest. Flowering from September to October and fruiting from October to December.

Etymology. The scientific name is derived from its unusual zigzag monochasial cyme. The latin prefix, "*aniso*-", means different, uneven or asymmetrical; "*cyma*" refers to the predominant inflorescence type seen in *Primulina* a pair-flowered cyme.

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DISCUSSION

The size of *P. anisocymosa* chromosomes is within the above-mentioned range for the genus. At present, almost all known chromosome numbers in this genus appear to represent a diploid number with a basic number of $x = 18$, except the polyploid *P. longgangensis* (Wang) Liu and Wang with $2n = 72$ (Christie *et al.* 2012, Möller *et al.* 2002 onwards). Our chromosome counts of ($2n = 36$) for *P. anisocymosa* corresponds with known chromosome number ($x = 18$) supporting its generic placement in *Primulina*.

The pair-flowered cyme is the basic type of inflorescence in the Old World

Gesneriaceae (Weber 1978, 1982). The inflorescences in *Primulina* are usually dichotomous cymes with few to many flowers or simplified single-flowered cymes (Li and Wang 2004; Wang et al. 1998; Chen et al. 2008; Wei et al. 2010). The development of pair-flowered cymes shows that the main axis of the inflorescence has a true terminal flower and a front flower. Two lateral paraclades arise in the axils of two opposite lateral bracts below the terminal flower, the following branches continuously repeat the same pattern (Weber 1978, 1982, 2013; Wang and Li 2002; Li and Wang 2004). This new species has an inflorescence morphology unlike any other described *Primulina* species to date.

The branching morphology of the inflorescences of this new species is a zigzag monochasial branching structure. The inflorescence has only one of the two lateral paraclades of the pair-flowered cyme extended. The others are reduced to the point of pedicel attachment and form the swollen nodules at the base of 2–3(–5) flowers. Each flower cluster whorl turns in the direction of the main axis and flowering continues upward in an acropetal sequence. Frthermore, the markedly special character of inflorescence in two known populations is very stable, as long as the plants grow normally. This is morphologically distinctive from all known *Primulina* species.

The zigzag monochasial inflorescence and infructescence of *Primulina anisocymosa* is so peculiar that we can easily distinguish it from other known species in the genus. It morphologically resembles *P. bobaiensis* (see Fig. 5) in having similar corolla shape and color. Both species are found in southern China (Guangdong and Guangxi), but *Primulina anisocymosa* can be easily distinguished by the inflorescence

architecture.

CONCLUSIONS

A new species of Gesneriaceae with an unusual inflorescence was collected and examined during our ongoing floristic field surveys of Southern China. To confirm the phylogenetic relationships and generic placement of this species, not only morphological anatomical features but also chromosome and DNA sequence data were examined and analysed. *Primulina anisocymosa* can be distinguished from other species within *Primulina* morphologically by its unique zigzag monochasial cyme and infructescence. This species is vegetatively similar to *P. bobaiensis*. The somatic chromosome number of the new species is also reported ($2n = 36$).

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