RESEARCH ARTICLE



Begonia parvibracteata, a new species in Begonia sect. Platycentrum (Begoniaceae) from Guangxi of China, based on morphological and molecular evidence

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Abstract

The previously reported begonias in a limestone forest of Guangxi mainly belong to *Begonia* sect. *Coelocentrum* Irmscher. In this article, we described and illustrated a new species in sect. *Platycentrum* (Klotzsch) A.DC., *Begonia parvibracteata* X.X.Feng, R.K.Li & Z.X.Liu, which was discovered in a karst forest of south-western Guangxi. The begonia shows high morphological similarity to *B. subhowii* S.H. Huang and *B. psilophylla* Irmscher, but differs from the latter two in its narrower oblique-ovate asymmetric leaf blade, 4 (occasionally 6) tepals of pistillate flower and smaller membranous inflorescence bracts. Molecular phylogenetic analysis, based on ITS sequence data, supports the new species as monophyletic and distinct from *B. subhowii* and *B. psilophylla*. Considering its narrow distribution and the disturbance of human activities, the conservation status of new taxon is evaluated as "Vulnerable" (VU B1, B2 ab (i, iv, v), D2) according to the IUCN Red List Categories and Criteria.

Keywords

ITS, morphology, new taxon, southern China, taxonomy

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Introduction

According to the latest report, China's wild begonias have already increased to 239 species (iBegonia 2021). The total number of species could reach 300 in the coming years, meeting the prediction of Tian et al. (2018). Consisting of 122 species, Sect. *Platycentrum* (Klotzsch) A.DC. represents the largest section of *Begonia* in China, with 66 species mainly distributed in Yunnan. Eighty-seven species of *Begonia* have so far been found in Guangxi (iBegonia 2021), most of which (60 species) are distributed in the karst forest and belong to *B*. sect. *Coelocentrum* Irmscher.

In Guangxi, only 11 species belong to *B.* sect. *Platycentrum* (Klotzsch) A.DC., and six of which are widespread in south and southeast China: *B. circumlobata* Hance, *B. edulis* Lévl., *B. handelii* Irmsch., *B. hemsleyana* Hook.f., *B. longiciliata* C.Y.Wu and *B. longifolia* Blume. Only five species of *B.* sect. *Platycentrum* are endemic to Guangxi, including *B. tsoongii* C.Y.Wu (Wu and Ku 1995), *B. longanensis* C.Y.Wu (Wu and Ku 1997), *B. auror*a C.I Peng, Yan Liu & W.B. Xu (Liu et al. 2020), *B. scorpiuroloba* D.K.Tian & Q.Tian (Tian et al. 2021) and *B. pseudoedulis* D.K.Tian, X.X.Feng & R.K.Li (Feng et al. 2021).

An unknown *Begonia* taxon with reproductive organs was collected from Guangxi during our field survey and plant collection in May 2020 and October 2021, respectively. The begonia definitely belongs to *B.* sect. *Platycentrum*, but differs markedly from the reported 11 begonias of the same section in Guangxi. After further detailed morphological observation, morphological comparison with similar species and molecular analysis, it is confirmed as a new species in *Begonia* sect. *Platycentrum*.

Materials and methods

Taxonomic observation

Morphological characters were observed and measured from fresh samples in the field. Morphological comparisons with similar taxa were undertaken by consulting the literature, examining herbarium (IBK and IBSC) specimens and observing living collections cultivated in the nursery of Enshi Dongsheng Plant Development Co. Ltd. The specimens were deposited at the Herbarium of South China Botanical Garden (**IBSC**), CAS and Chenshan Herbarium (**CSH**) of Shanghai Chenshan Botanical Garden.

DNA sequencing and molecular analysis

The fresh leaves of the putative new species and the morphologically similar allied species, *B. subhowiii* and *B. psilophylla*, were collected in the field and the nursery, respectively. Total DNA was extracted from nine individuals of the three species (*B. parvibracteata*, *B. subhowiii* and *B. psilophylla*) with the CTAB method (Doyle and Doyle 1987). The internal transcribed spacers 1 and 2 (ITS) and the 5.8S gene were amplified using the primers 17SE and 26SE (Forrest and Hollingsworth 2003).

PCR amplification and Sanger sequencing were performed according to Fan et al. (2014), with an annealing temperature of 53 °C. The ITS sequences of the three species were uploaded to GenBank (http://www.ncbi.nlm.nih.gov/) with the accession numbers OL892048, OL892049 and OL892050 for *B. parvibracteata* sp. nov., OL871361, OL871362 and OL871363 for *B. subhowii* and OL851701, OL851702 and OL851703 for *B. psilophylla* (Table 1).

We chose 17 species from sect. *Platycentrum* and 8 species from other sections of *Begonia* native to mainland China to place the new species in a phylogenetic context (Table 1). *Begonia socotrana* Hook.f in sect. *Peltaugustia* (Warb.) Barkley from Socotra (for the coast of Africa) was selected as an out-group in the phylogenetic analysis (Moonlight et al. 2018). Except for the nine individuals of the putative new species and its two allied species, the additional ITS sequences for 23 *Begonia* species were downloaded from the NR database of NCBI. All these sequences were aligned using BioEdit v.7.2.5 (Hall 1999) and a phylogenetic analysis using Bayesian Inference (BI) was undertaken in MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003). The GTR+G model was chosen as the optimal model of nucleotide substitution according to the Akaike Information Criterion (AIC; Burnham and Anderson 2002) as implemented in MrModeltest 2.3 (Nylander 2004). The Markov chains were run for 1,000,000 generations and sampled at each 100 generations, with the first 25% discarded as burn-in.

Taxon	Origin	GenBank	Section	Collector, voucher
		accession no.		(Herbarium)
Begonia acetosella Craib.	Mengla, Yunnan, China	MW690106	Platycentrum	Wang, W.G., WWG005
				(HITBC)
<i>Begonia biflora</i> Ku	Malipo, Yunnan, China	JF975965	Coelocentrum	Shui, Y.M. et al. 20484
				(KUN)
Begonia chingii Irmsch.	Napo, Guangxi, China	KP710820	Reichenheimia	Tian, D.K., Li, C.
				TDK785 (CSH)
Begonia circumlobata Hance	Xinyi, Guangdong,	KP710815	Platycentrum	Tian, D.K., Li, X.P.
	China			TDK866 (CSH)
Begonia cucurbitifolia C. Y. Wu	Yunnan,China	JF975969	Platycentrum	Y,M,Shui et al.GBOWS1284
				(KUN)
	-	JF975968		_
Begonia edulis Lévl.	Bama, Guangxi, China	KP710813	Platycentrum	Tian, D.K., Li, C.
				TDK757 (CSH)
<i>Begonia grandis</i> Dry.	Yongshun, Huhan, China	KP710828	Diploclinium	Li, X.P. Li, X.J. LXJ022 (CSH)
Begonia handelii Irmsch.	Fengshan, Guangxi,	KP710818	Platycentrum	Tian, D.K., Li, C.
	China			TDK763 (CSH)
Begonia hatacoa BuchHam. ex	-	AF485111	Platycentrum	-
D. Don				
<i>Begonia hemsleyana</i> Hook. f.	-	KP710806	Platycentrum	_
<i>Begonia henryi</i> Hemsl.	Leshan, Sichuan, China	KP710822	Reichenheimia	Tian, D.K., Tian, L.Z.
				TDK2249 (CSH)
<i>Begonia huangii</i> Y. M. Shui &	Gejiu, Yunnan, China	JF976001	Coelocentrum	Shui, Y.M. et al. 40782
W. H. Chen				(KUN)

Table 1. *Begonia* species and populations included in the phylogenetic analysis (Sectional placement follows Moonlight et al. 2018).

Taxon	Origin	GenBank accession no.	Section	Collector, voucher (Herbarium)
Begonia jinyunensis C. I Peng, B.	Jinyunshan, Chongqing,	MZ145345	Platycentrum	-
Ding & Q. Wang	China			
Begonia labordei Lévl.	Yunnan, China	KF636452	Diploclinium	Peng 20520 (HAST)
Begonia longifolia Blume	Mengla, Yunnan, China	MW690102	Platycentrum	Wang, W.G., WWG001 (HITBC)
<i>Begonia longistyla</i> Y. M. Shui & W. H. Chen	Hekou, Yunnan, China	JF976018	Coelocentrum	Shui, Y.M. et al. 40778 (KUN)
<i>Begonia megalophyllaria</i> C. Y. Wu	Yunnan, China	JF976026	Platycentrum	Y,M,Shui et al.D-33(KUN)
<i>Begonia multangula</i> Blume	Jawa, Lesser Sunda Is.	MN453434	Platycentrum	-
<i>Begonia ornithophylla</i> Irmsch.	Guangxi, China	JF976032	Coelocentrum	Y,M,Shui et al. B2005- 061(KUN)
Begonia parvibracteata	Longzhou, Guangxi,	OL892048	Platycentrum	Xin Xin Feng, et al.
X.X.Feng, R.K.Li & Z.X.Liu	China	OL892049		835307 (IBSC)
		OL892050		
Begonia pedatifida Lévl.	Tianlin, Guangxi, China	KP710810	Platycentrum	Tian, D.K., Li, C.
				TDK774 (CSH)
Begonia psilophylla Irmsch.	Hekou, Yunnan, China	OL851701	Platycentrum	-
		OL851702		-
		OL851703		-
Begonia pulchrifolia D.K.Tian	Meinvfeng, Leshan,	KP710811	Platycentrum	Tian, D.K., et al.
& C.H.Li	Sichuan, China			TDK2243 (CSH)
Begonia scorpiuroloba D.K.Tian	Fanchenggang, Guangxi,	MZ145351	Platycentrum	Tian, D.K., et
& Q.Tian	China			al.TDK2269(CSH)
Begonia socotrana Hook.f.	Socotra	AF469121	Peltaugustia	-
Begonia subhowii S. H. Huang	Malipo, Yunnan, China	OL871361	Platycentrum	-
		OL871362		-
		OL871363		_

Taxonomy

Begonia parvibracteata X.X.Feng, R.K.Li & Z.X.Liu, sp. nov. urn:lsid:ipni.org:names:77308553-1 Figs 1, 2 Chinese name: 小苞秋海棠

Type. CHINA Guangxi, Longzhou County (龙州县), Zhubu Town (逐卜乡), Bannong Village (板弄屯), Yinghuagu Tourist Resort, 22°33'51"N, 106°57'03"E, (Fig. 3), 263 m alt., in shaded environment of limestone forest, October, 2021, *Xin-Xin Feng, Ren-Kun Li & Zhi-Xian Liu* (holotype: 835307, IBSC!; isotype: SYS!). Longzhou County (龙州县), Zhubu Town (逐卜乡), Pona Village (坡 那屯), Nonggang National Nature Reserve, 22°39'03"N, 106°57'18"E, 190 m alt., on rock under limestone forest, 18 May 2020, *Dai-Ke Tian & Jinye Zhou* TDK4119 (CSH!).

Diagnosis. *Begonia parvibracteata* morphologically resembles *B. subhowii* and *B. psilophylla* in rhizome and leaf characters. However, it has narrowly oblique-ovate asymmetric leaf blades, 4 (rarely 6) tepals in pistillate flowers and small (6–8 × 3–5 mm)

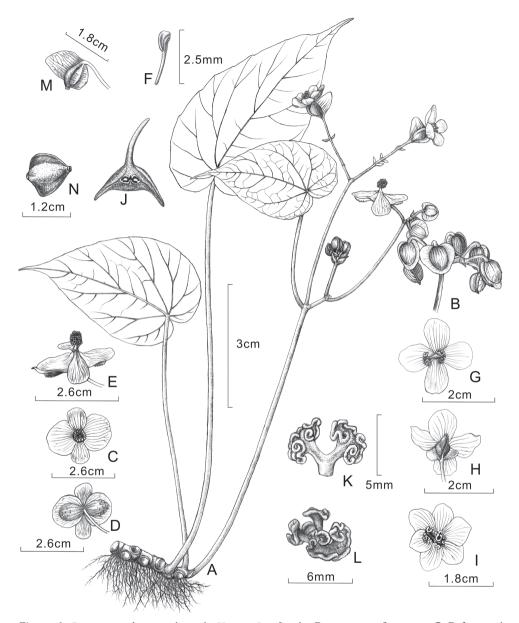


Figure 1. *Begonia parvibracteata* drawn by Yunxiao Liu **A** xabit **B** staminate inflorescence **C**, **D** front and back views of staminate flower **E** lateral view of staminate flower **F** stamen **G**, **H** front and back views of pistillate flower with 4 tepals **I** front view of pistillate flower with 6 tepals **J** cross section of ovary **K** styles and stigmas **L** stigmas **M** abaxial view of capsule showing wings **N** lateral view of capsule showing two shorter wings.

bracts in inflorescences. These characters differ from the widely ovate leaf blades, 5 (rarely 6) tepals of pistillate flowers, and distinctly large bracts in inflorescences of *B. subhowii. B. parvibracteata* is dissimilar to *B. psilophylla* in its 4 (rarely 6) tepals in pistillate flowers and asymmetric, narrowly oblique-ovate leaf blade.

Begonia parvibracteata forms a monophyletic group clustered with *B. cucurbitifolia* in the phylogenic tree, but the latter has nearly symmetric, 3–4 lobed leaf blade, 5-tepaled pistillate flower, definitely differing from the new species.

Description. Perennial evergreen herb, monoecious, 15-30 cm tall. Rhizome creeping, ca. 8.5–12 cm long and 6–10 mm thick, internode short or not obvious; erect stem only seen at anthesis, ca. 7.5-10 cm long, internodes 1-2 (3), green to reddish-green, glabrous. Stipules brownish-red, translucent, ovate-triangular, ca. 8–10 × 4-5 mm, glabrous. Leaves 3-6 basal and 2-3 aerial, petiole yellowish-green, 18-22 cm long, glabrous; blade asymmetric, ovate to narrowly ovate, $14-16 \times 7.5-9$ cm; apex acuminate to caudate, base oblique-cordate; leaf margin usually almost entire or occasionally crenate; venation palmate with 6-8 primary veins, adaxially slightly concave, abaxially convex; leaf blade fleshy, abaxially pale-green, glabrous; adaxially green, glabrous. Inflorescences arising from erect stem; dichasial cymes branching one to two times, peduncle 10-12 cm long, glabrous; flowers unisexual, 3-7 flowers per inflorescences; bracts membranous, triangular to widely ovate, brownish-red, $6-8 \times 3-5$ mm, glabrous. *Staminate flower:* pedicels pale green, ca. 2–2.3 cm long, glabrous; tepals 4, outer 2 tepals ovate to circular, 13-21 × 12-20 mm, adaxially concave, pinkish-white, abaxially convex, pinkish-white, red hispid; inner 2 obovate, 10–18 × 8–12 mm, margin irregularly entire, white, glabrous; androecium cylindrical, ca. 6.6-7.5 mm across; stamens numerous, ca. 2-2.5 mm long, anthers yellow, clavate, base cuneate, ca. 1.5 mm long. Pistillate flower: pedicels pale green, ca. 2-2.6 cm long, glabrous; tepals 4, occasionally 6, white, irregularly ovate, obovate or cuneate, sub-equal, $18-20 \times 10-15$ mm, glabrous; ovary yellowish-green, trigonous-ellipsoid, $11-12 \times 5-6$ mm (wings excluded), glabrous; 2-loculed, placentae axillary, placentae bifid per locule; styles 2, fused at base, yellow, ca. 5-6 mm long, apically Y-shaped, stigma U-shaped, spirally twisted. Capsules nodding, trigonousellipsoid, ca. 11-13 × 5-6.5 mm (wings excluded), yellowish-green, glabrous, unequally 3-winged, abaxial wing triangular to ligulate, ca. 10-13 mm long; lateral wings lunate, 3-6 mm long.

Distribution and habitat. Currently known from four localities in Longzhou County and Daxin Countym Guangxi, China. It usually grows on rocks or rock cracks in limestone under forest.

Phenology. Flowering September-October, fruiting October-December.

Etymology. The specific epithet "*parvibracteata*" refers to the short small bracts of the new species. The Chinese name is given as "小苞秋海棠" (Begonia with small inflorescence bracts).

Conservation status. There are three populations with approximately 1000 individuals found in Longzhou County. Another one population with approximately 500 individuals is distributed in the Encheng National Nature Reserve, Daxin County. Some plants of this begonia are over-collected and sold in the local medicinal herb market of Longzhou County. According to the IUCN Red List Categories and Criteria (IUCN 2022), *B. parvibracteata* should be assessed as "Vulnerable (VU B1, B2 ab (i, iv, v), D2)" due to its narrow distribution and the disturbance by human activities.



Figure 2. Habitat and morphology of *Begonia parvibracteata* **A** habitat **B** flowering plant **C** creeping rhizome **D** shoot top with stipule **E** leaf blade (adaxial) **F**, **G** leaf blade (abaxial) **H** erect stem with inflorescence **I**, **J** young inflorescence and bract; **K**, **L** front and back views of staminate flower with 4 tepals **M** lateral view of staminate flower **N** stamens **O**, **P** front and back views of pistillate flower with 4 tepals **Q** front view of pistillate flower with 6 tepals **R** cross section of ovary **S** stigmas **T**, **U** immature capsule **V** dried mature capsule (Photos by Z.X. Liu).

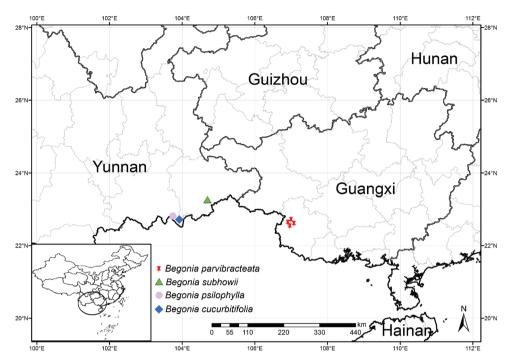


Figure 3. Distribution of B. parvibracteata, B. subhowii, B. psilophylla and B. cucurbitifolia.

Molecular analysis

The aligned matrix of the ITS sequence data was 727 bp long. The result of Bayesian Inference analysis is shown in Fig. 4. *Begonia* sect. *Platycentrum* appears monophyletic with a high Bayesian posterior probability (bpp = 1) (Fig. 4). The three samples of the putative new species form a monophyletic group clustered with *B. cucurbitifolia* (bpp = 0.89). *Begonia subhowii* with the highest morphological similarity to *B. parvibracteata* formed another subclade.

The nodes with bpp < 0.50 have been collapsed. Sectional placement of taxa is indicated by the following abbreviations: *Coe (Coelocentrum)*, *Dip (Diploclinium)*, *Pla (Platycentrum)*, *Rei (Reichenheimia)* and *Pel (Peltaugustia)*. The numbers after the species names indicate different populations. The samples of the new species are marked with stars.

Discussion

The new begonia with 2-loculed ovary, axillary placentation and bifid placentae can be clearly assigned to *B.* sect. *Platycentrum* (Gu et al. 2007). Within this largest section for *Begonia* in China, *B. parvibracteata* shows high morphological resemblance to *B. subhowii* and *B. psilophylla*, both of which are distributed in south-eastern Yunnan (Shui and Huang 1999; Shui and Chen 2018), including creeping rhizome, glabrous plants with both basal and cauline leaves at anthesis, usually almost entire leaf margin, palmate venation, fleshy green leaf blade and 4 tepals of staminate flower (Table 2).

In *B. subhowii*, there are 5 (occasionally 6) tepals in the pistillate flower and the bracts of inflorescence is distinctly long and large $(5 \times 3 \text{ cm})$ (Fig. 5). These characters differ from 4 (occasionally 6) tepals and triangular to widely ovate $(6-8 \times 3-5 \text{ mm})$ bracts in *B. parvibracteata*. In addition, the leaf blade is wider ovate in *B. subhowii*, compared with the narrower oblique-ovate shape of *B. parvibracteata*.

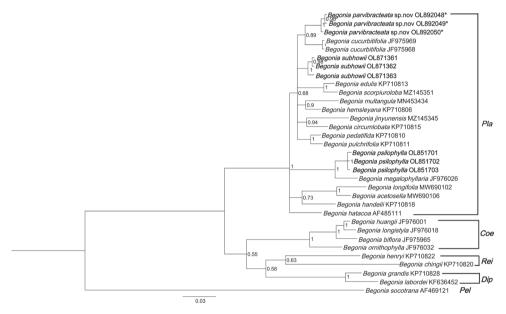


Figure 4. Bayesian Inference of the phylogenetic position of the newly-described *B. parvibracteata* within sect. *Platycentrum*, based on nuclear ITS sequences.

Character	B. subhowii	B. psilophylla	B. cucurbitifolia	B. parvibracteata
Leaf blade shape	asymmetric, widely	nearly symmetric,	nearly symmetric,	asymmetric, narrowly
	ovate	widely ovate to cordate	orbicular, 3–4 lobed	oblique-ovate
Leaf blade base	asymmetric, oblique-	nearly symmetric,	nearly symmetric,	asymmetric, oblique-
	cordate	obtuse	cordate	cordate
Leaf margin	irregularly serrulate	minutely serrulate	minutely serrulate	usually almost entire or
				occasionally crenate
Inflorescence bracts	ovate-oblong, 50 ×	elliptic to ovate-oblong,	subglabrous, caduceus	triangular, 6–8 ×
	30 mm, glabrous, apex	7–9 × 4–5 mm,		3–5 mm, glabrous
	acuminate	glabrous		
Tepal number of	usually 5, rarely 6	usually 5, rarely 6	5	usually 4, rarely 6
pistillate flowers				
Phenology	April-May; May-July	February-March;	July-August; August-	September-October;
(flowering; fruiting)		March-May	October	October-December
Habitat	700–1500 m alt.,	100–700 m alt.,	430 m alt., limestone,	263 m alt., limestone,
	limestone, Yunnan;	limestone, Yunnan	Yunnan	Guangxi
	Vietnam			-

Table 2. Morphological comparison of *B. parvibracteata* and relevant taxa.



Figure 5. *B. subhowii* **A–I** *B. psilophylla* **J–L** *B. cucurbitifolia* **M**, **N** showing similar features comparable to *B. parvibracteata* **A** habitat **B** young leaf blade (adaxial) **C** leaf blade (abaxial) **D** inflorescence **E** bracts **F**, **G** front and back views of staminate flower **H** front view of pistillate flower with 5 tepals **I** lateral view of pistillate flower **J** flowering plant **K** symmetric leaf blade **L** front view of flower **M** leaf blade of juvenile plant **N** mature leaf blade (Photos **A–I** by R.K. Li **J–L** by Z.X. Liu **M**, **N** by D.K. Tian).

In *B. psilophylla*, the leaf blade is nearly symmetric, widely ovate with symmetric cordate base, in contrast with the narrower ovate leaf blade and oblique-cordate base of the new species. Furthermore, there are 5 (occasionally 6) tepals in the pistillate flower of *B. psilophylla*, being different from the 4 (occasionally 6) tepals of *B. parvibracteata*.

According to the phylogenetic tree, based on ITS sequences, the closest relative of *B. parvibracteata* is *B. cucurbitifolia* which is also distributed in south-eastern Yunnan.

However, *B. cucurbitifolia* is remarkably distinct from *B. parvibracteata* in morphology, including nearly symmetric, 3–4 lobed leaf blade and 5-tepaled pistillate flower.

For the flowering time, *B. parvibracteata* blooms later compared with its three allied species mentioned above.

Acknowledgements

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