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Begonia leipingensis (Begoniaceae), a new compound-leaved species with unique petiolule pattern from Guangxi of China

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Abstract

Begonia leipingensis D. K. Tian, L. H. Yang & C. Li (2n = 30), a new species in *Begonia* sect. *Coelocentrum* from the limestone area of Guangxi, China. *B. leipingensis*is is easily distinguished from any other compound-leaved species in *Begonia* by its large variation in petiolule number and its unique spirally-arranged petiolule pattern, which has never been seen in *Begoniaceae* before and rarely seen even in other angiosperm taxa. Besides having non-overlapping flowering periods, it is clearly different from *B. fangii*, the most morphologically similar species in the same section and with the same chromosome number. In addition to its unique petiolule pattern, *B. leipingensis* has longer abaxial wings, shorter internodes, and usually larger leaves, leaflets and habit. Molecular phylogenetic analysis showed that *B. leipingensis* formed an independent lineage belonging to Sect. *Coelocentrum*. Morphological and phylogenetic evidence strongly supports this species as a new taxon in Sect. *Coelocentrum* of *Begonia*. *B. leipingensis* was assessed to be critically endangered based on criterion outlined by IUCN Red List Categories and Criteria.

Introduction

Begonia L. is widely distributed in Asia, Africa and America (William *et al*, 2010). The latitude extends from the equator to 40°40'N (Lingyuan of Liaoning, China) (Li *et al*, 2014). Extensive distribution range, variable habitats and long evolutionary history make *Begonia* a mega-diverse genus and the sixth largest angiosperm genus encompassing approximately 1600 species (Frodin, 2004). Leaf morphology of *Begonia* exhibits great diversity, from simple leaves with entire margins to crenate margins, to deeply divided and real compound leaves. In China, about 191 species have been recorded according to *Flora of China* (Gu *et al.* 2007) and subsequent publications (Ding *et al.* 2014, Ku *et al*. 2008, Li *et al*. 2008, Liu *et al*. 2007, Ma *et al*. 2006, Peng *et al*. 2008a,b, 2009, 2010, 2012, 2013, 2014a,b, Shui 2007, Tian *et al.* 2014, 2015, Wei *et al*. 2007). Of which, most have simple leaves with only four having real compound leaves, *B. coptidifolia* H. G. Ye *et al.* (2004: 259), *B. fangii* Y. M. Shui & C.-I Peng (2005: 83), *B. hemsleyana* J. D. Hooker (1899: 125), and *B. jinyunensis* C.-I Peng, B. Ding & Q. Wang (2014: 62). In April 2013, Li-hua Yang posted pictures of an unknown *Begonia* species with compound leaves on website of Chinese Field Herbarium (CFH). Dr. Dai-ke Tian identified it as a potentially new species. To understand more about this putative new taxon, the authors conducted field surveys in its native habitat (Daxin of Guangxi, China) in July 2014 and March 2015, respectively.

Through further morphological comparison between this putative new taxon and its similar species, review of literature and herbarium specimens, and phylogenetic analysis using internal transcribed spacers (ITS) and rpl16, the status of this undocumented begonia as a new species was strongly supported and confirmed. It was named as *Begonia leipingensis* D. K. Tian, L. H. Yang & C. Li, and is described and illustrated here for the first time.

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Materials and Methods

Leaflet count

During survey in July 2014, a large variation was roughly observed in leaflet number of *B. leipingensis*. To better understand leaflet number variation between mature plants or in a single plant, in March 2015, the leaves were randomly sampled from different mature plants along the pathsides in the field for counting the number of leaflets, except of considering the possibly extreme number of leaflets in the investigated population. In total, 181 leaves were sampled for statistical analysis.

Taxon sampling

After further morphological observation, *B. leipingensis* is assigned to *Begonia* sect. *Coelocentrum* Irmscher. This new species, along with its two most similarly related species, *B. fangii* and *B. hemsleyana*, and 33 other species from mainland China were selected to reconstruct the phylogenetic relationships. *B. dregei* Otto & Dietr. (1836: 357) from Africa was used as outgroup in the phylogenetic analysis. The data set represented 37 species and four main sections in China (18 sp. of sect. *Coelocentrum*, 4 sp. of sect. *Diploclinium* (Lindl.) A. DC., 12 sp. of sect. *Platycentrum* (Klotzsch) A. DC., and 2 sp. of sect. *Reichenheimia* (Klotzsch) A. DC.) and one section (sect. *Augustia* (Klotzsch) A. DC.) from Africa according to Doorenbos and Shui's treatment (Doorenbos *et al.*, 1998; Shui *et al.*, 2002). Species information of two newly obtained sequences from phylogenetic analysis was listed in Table 1 and the data on the remaining species were based on Chung *et al*. (2014). No materials of new sequences were either collected from nature reserve or involved in the endangered or protected species. No specific permits were required for field studies and sampling.

TABLE 1. Species information of two newly obtained sequences used for phylogenetic analysis.

Taxon	Origin	Section	Voucher	ITS	*rpL16*
B. fangii Y. M. Shui & C. I Peng	Daxin, Guangxi	Coelocentrum	130420-4(CSH)	KR704275-KR704282	KR709171
B. leipingensis D. K. Tian, L. H. Yang & C. Li	Longzhou, Guangxi	Coelocentrum	TDK-2270(CSH)	KR704283-KR704286	KR709170

CSH: Herbarium of Shanghai Chenshan Botanical Garden.

Molecular analysis

The methods for DNA extraction, amplification and data analysis were adopted from Tian *et al*. (2014, 2015). Both ITS and rpl16 were amplified with primers from Chung *et al.* 2014. PCR products of rpl16 were directly sent to Sangon Biotech (Shanghai, China) to be sequenced. The PCR products of ITS need to clone before sequencing. PCR products were purified using GenClean kit (Generay Biotech, Shanghai, China), then were cloned using pEASY-T1 Cloning Kit (Transgen Biotech, Beijing, China). Four to eight clones per individual were sequenced.

The phylogenetic relationship was reconstructed with the combined dataset of ITS and rpl16 gene fragments partitioned using two methods: Maximum Likelihood (ML) and Bayesian Inference (BI) using RAxML (Stamatakis, 2014) and MrBayes (Ronquist & Huelsenbeck, 2003) at the CIPRES Science Gateway (Miller*et al.*, 2010). The program jModeltest (Posada, 2008) was used to determine the optimal model of nucleotide substitution using the Akaike Information Criterion (AIC) (Burnham & Anderson, 2002). For ML analysis, the clade supports were assessed via a bootstrap analysis of 1,000 replicates using the GTRCAT model. For Bayesian inference, two independent MrBayes analyses were performed with four Markove chains and five million generations. Trees were sampled every 100 generations, resulting in 50,000 trees for each run, with the first 25% discarded as burn-in.

Chromosome count

Chromosome analysis was performed on type material (TDK-2270). Root tips were collected from cuttings of rooting leaves and were pretreated with 0.002 mol/L 8-hydroxyquinoline solution at room temperature for about 4 h and fixed overnight in ethanol-acetic acid (3:1), followed by hydrolyzation in 1 mol/L HCl at 60°C for 8 min. Material was washed 3–4 times and then stained in Carbol Fuchsine for 5 min. Five cells in metaphase were observed for counting chromosome number using a Olympus BX43 microscope. Voucher specimens are deposited in CSH.

Results

Petiolule pattern and leaflet number

Besides a large variation in leaflet (petiolule) number, *Begonia leipingensis* has a unique petiolule pattern, in which, the petiolules are spirally arranged with the smallest leaflet on the top (Fig. 1). This petiolule pattern has never been seen in *Begoniaceae* before and rarely seen even in other angiosperm taxa. According to its unique petiolule pattern (Fig. 1M), *B. leipingensis* can be easily distinguished from the morphologically similar species with the compound leaves, such as *B. fangii* and *B. hemsleyana*, in which the petiolules are palmately or radiationally arranged (Fig. 1N, Fig. 8AC).

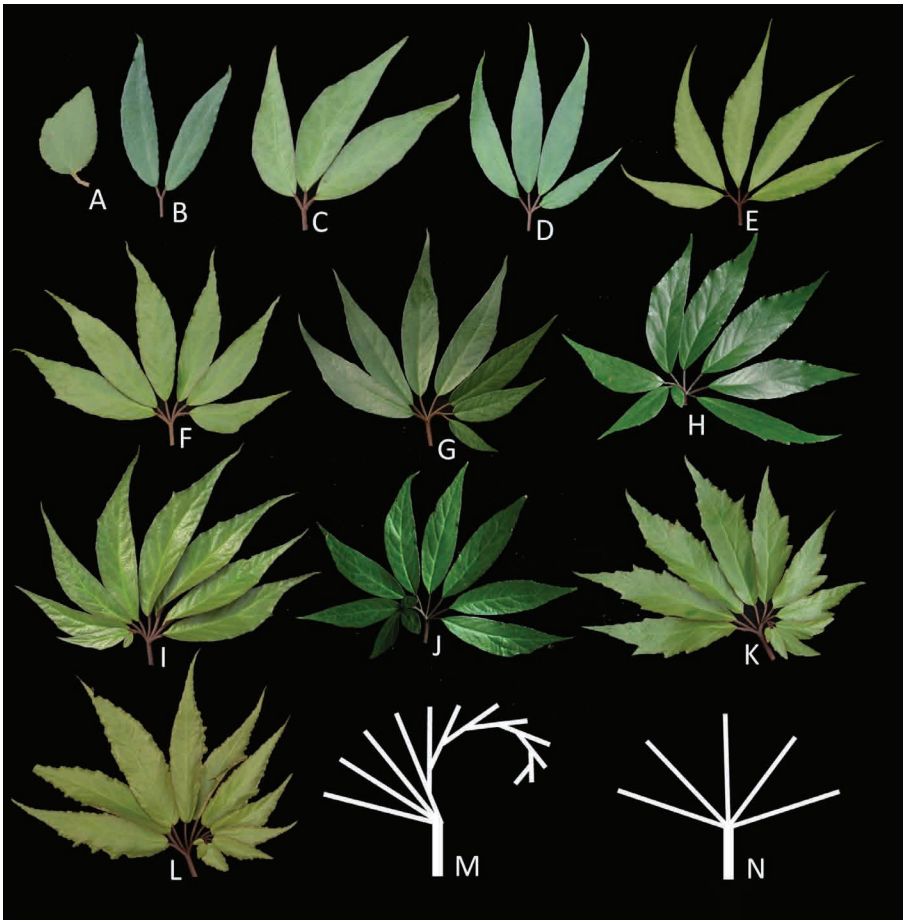

FIGURE 1. Foliage of *B. leipingensis* and comparison of its petiolule pattern and that of *B. fangii* and *B. hemleyana.* **A–L.**Young and mature leaves of *B. leipingensis*: **A.** Simple leaf (transition status) from low position of rhizome in small plants; **B–L.** Compound leaves with 2 to 12 leaflets; **M.** Line drawing of unique petiolule pattern of *B. leipingensis,* in which the petiolules (leaf L with 12 leaflets for exmaple) are spirally arranged; **N.** Line drawn petiolule pattern of *B. fangii* and *B. hemsleyana,* in which the petiolules (5 leaflets for example) develop from the same position.

Based on the data of randomly sampled 181 leaves from mature individuals in the field, large variation in leaflet number shows both between plants and in a single plant. The number of leaflets or petiolules ranges from 2 to 12, but 3–7 leaflets per leaf are more often seen (Fig.2). Also, the leaves at middle and higher position of rhizome have more leaflets than those at lower position (a mature plant as example, Fig. 5B).

Phylogenetic analysis

Twelve new clone sequences of ITS, eight from *B. leipingensis* and four from *B. fangii*, and two new sequences of rpl16 were generated in our lab. The combined dataset contained 47 terminals representing 37 species, four main sections of *Begonia* in China and one section from Africa. The aligned matrix of the combined dataset for ITS and rpl16 gene was 1978 bp (ITS: 868 bp and rpl16: 1110 bp). There was no difference in topographies between the two methods. Topology from Bayesian Inference with bootstrap support values of Maximum Likelihood (BS) and the posterior probabilities (PP) are shown in Fig.3.

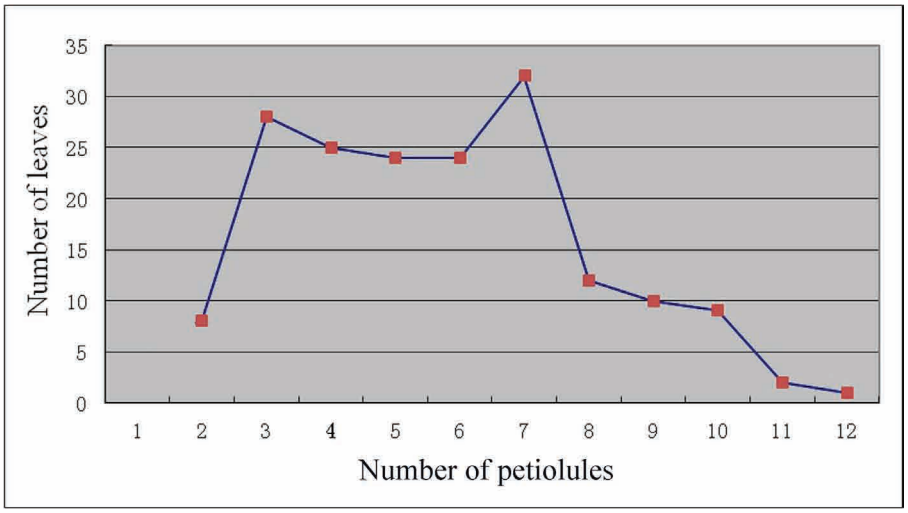

FIGURE 2. Number of leaflets per leaf of *Begonia leipingensis* (N = 181 leaves). The 3 to 7 leaflets are the most commonly seen in mature plants, and 12 is the maximum number of leaflets ever observed during the survey.

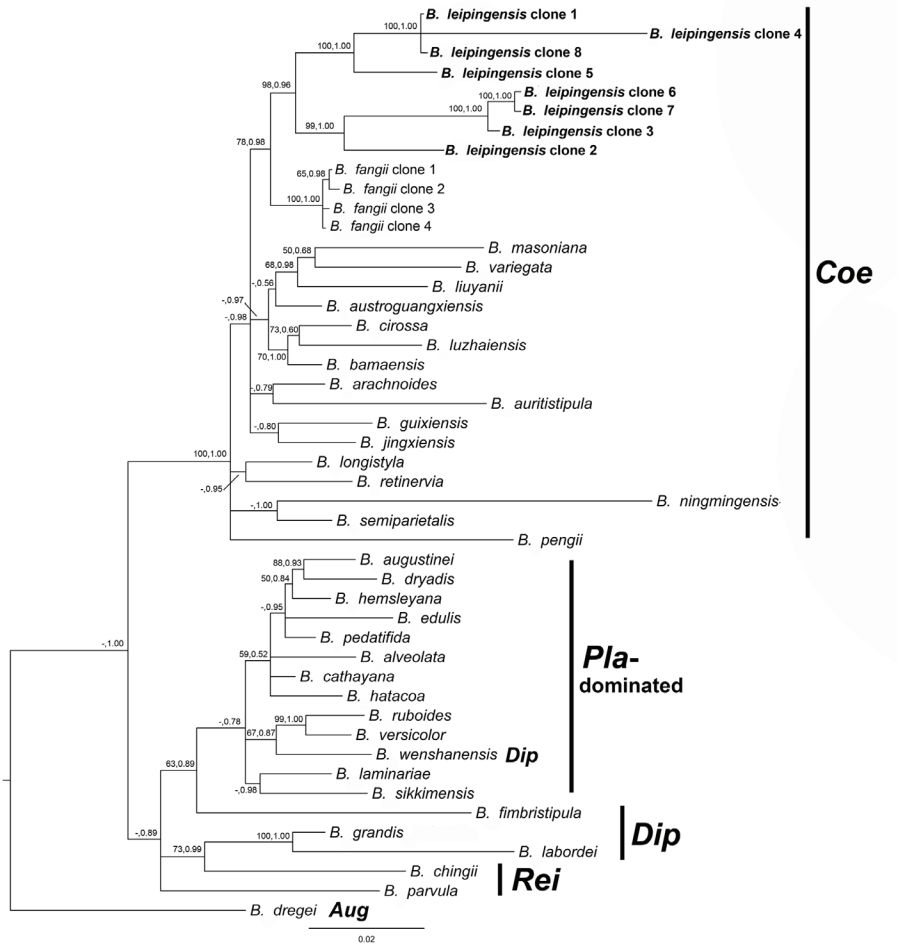

FIGURE 3. Phylogenetic tree inferred using Bayesian Inference. Bootstrap support values of Maximum Likelihood (BS) and the posterior probabilities (PP) are shown on the branches. Sectional placement of taxa is indicated by the following abbreviations: *Aug*: *Augustia*, *Coe*: *Coelocentrum*, *Dip*: *Diploclinium*, *Pla*: *Platycentrum*, and *Rei*: *Reichenheimia*. The putative new species is indicated in bold.

Chromosome count

Five cells in metaphase were observed and the chromosome number at metaphase of *B. leipingensis* was counted to be 2n = 30 (Fig.4), the same number as its morphologically close species, *B. fangii* (2n = 30) (Peng, Liu, Ku, & Ku, 2005), and eight chromosomes more than that of foliage similar species, *B. hemsleyana* (2n = 22) (Nakata *et al.*, 2003; Tian *et al.*, 2001). The chromosomes are very small, 1.2–1.8 μm long.

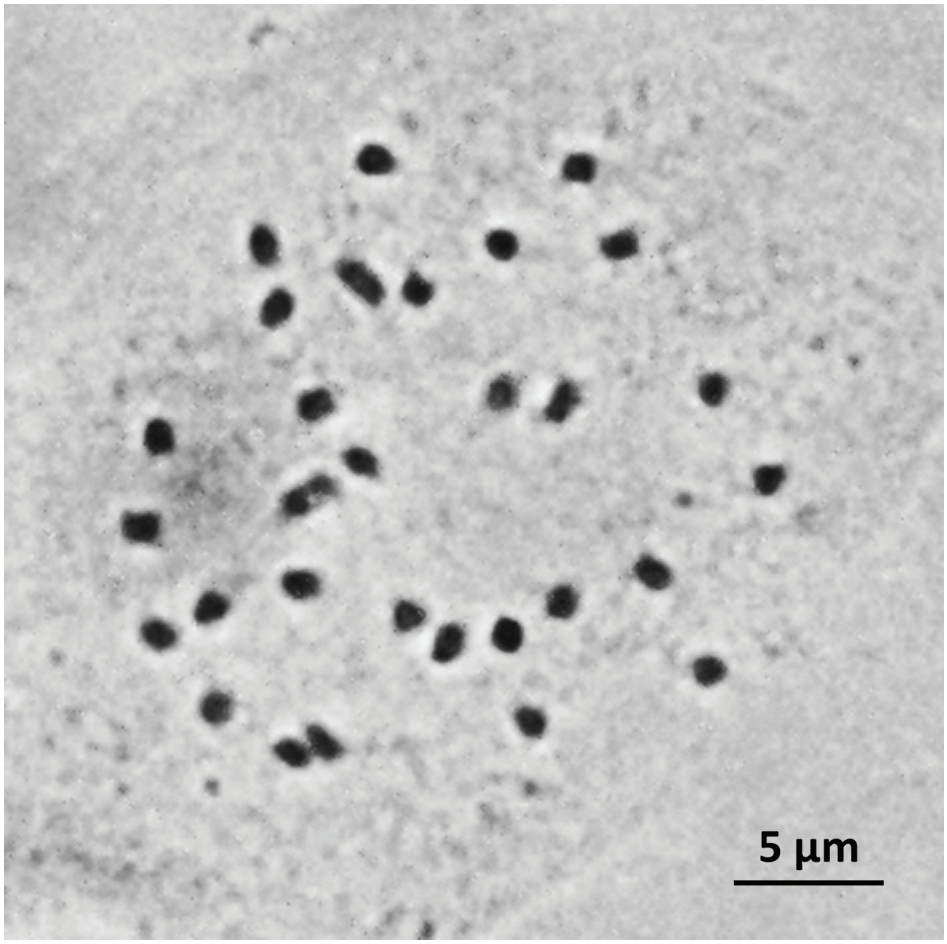

FIGURE 4. Somatic chromosomes at metaphase of *Begonia leipingensis* (2n = 30, TDK-2270, CSH).

Taxonomy

Begonia leipingensis **D. K. Tian, L. H. Yang & C. Li, *sp. nov*. (Fig. 5–6). Type:—**CHINA. Guangxi Zhuang Autonomous Region: Daxin County, Leiping Town, moist rocks under forest, 22°38'34.69''N, 107°04'27.89''E, elevation 264 m, 23 September 2014 (fl. and fr.), TDK-2270 (CSH0085839, holotype CSH!).

Plant perennial and evergreen without erect stems, 10–45 cm high. Rhizome succulent, creeping, often branched, 5–30 cm long, 5–25 mm thick, internodes short, 5–18 mm long. Stipules persistent, reddish, triangular to narrowly triangular, 10–15 mm long and 6–8 mm wide, apex obtuse. Leaves on rhizomes, palmately compound, asymmetric, up to 30 cm long, 35 cm wide. Petioles and petiolules terete, brownish with sparse white spots, hairs whitish initially and turning brownish; petiole 3–42 cm long, 1–10 mm thick. Leaflets 2–12 and more often 3–7 (Fig.2), lanceolate, 2–23 cm long, 0.7–7.3 cm wide, adaxial surface deep green and subglabrous, abaxially light green and hairy along veins, base cuneate, apex tapering, margins usually entire, few undulate, irregularly serrate to sparsely lobed; petiolule 0.3–4 cm long, 2–2.5 mm thick, spirally arranged (Fig.1), middle 1–2 leaflets nearly symmetric, lateral ones slightly asymmetric; net-veined, 4–12 lateral veins each side, midrib adaxially depressed and abaxially protruded. Inflorescences 2–11, often 1–2 axillay on each rhizome branch, dichasial, 6–20 cm long, flowers 5–29 per inflorescence. Primary peduncle red-brown, 4.2–16 cm long, 1.5–3.5 mm thick, sparsely pubescent. Bracts persistent, light green and upper pinkish, triangular to narrowly triangular, apex obtuse, margin hairy, 4–7.5 mm long, 2.5–4 mm wide. Male flower: pedicel white or slightly pinkish, 11.2–27.8 mm long, 0.6–1.2 mm thick, sparsely pubescent; corolla 16–31 × 17–24 mm; tepals 4, glabrous, outer 2 larger, 9–20 × 9–17 mm, white or slightly pinkish, nearly round or wide oval, apex rounded, radial stripes not obvious on adaxial surface; inner 2 smaller, 7.5–12.5 × 3.5–5.2 mm, white, oblanceolate, apex obtuse; androecium 3.5–4.5 mm long, 5–6.5 mm in diameter, stamens 36–64; filaments free, 2 mm long, 0.2 mm thick; anthers yellow, 2 mm long, 1 mm wide, oval, apex often retuse. Female flower: pedicel white to light pink, 10–20 mm long, about 1 mm thick, sparsely pubescent; corolla 12–22 × 9–19.5 mm; tepals 3, glabrous, outer 2 larger, 8–14 × 8.5–16 mm, white to pale pink, nearly

round or wide oval, apex round, radial stripes not obvious on adaxial surface; inner 1 smaller, 5–11.5 × 2.5–5 mm, white, oblanceolate; styles 3, 2–3.5 mm long, fused at base; stigma U-shaped, spirally twisted about 1.5 circles on each side; ovary sparsely pilose or subglabrous; 1-loculed with parietal placentae, bifid. Carpopodium pinkish, glabrous, 11–21 mm long, 0.8–1.2 mm in thick. Capsule nodding, ovoid, 9.5–13.5 mm long, 6.5–9.5 mm wide, unequally 3-winged, abaxial wing nearly semicircular to lunate, 6.5–11 × 10.5–16 mm, lateral wings falcate, 3–7 × 11–15.5 mm. Seeds numerous. 2n = 30 (Fig.4).

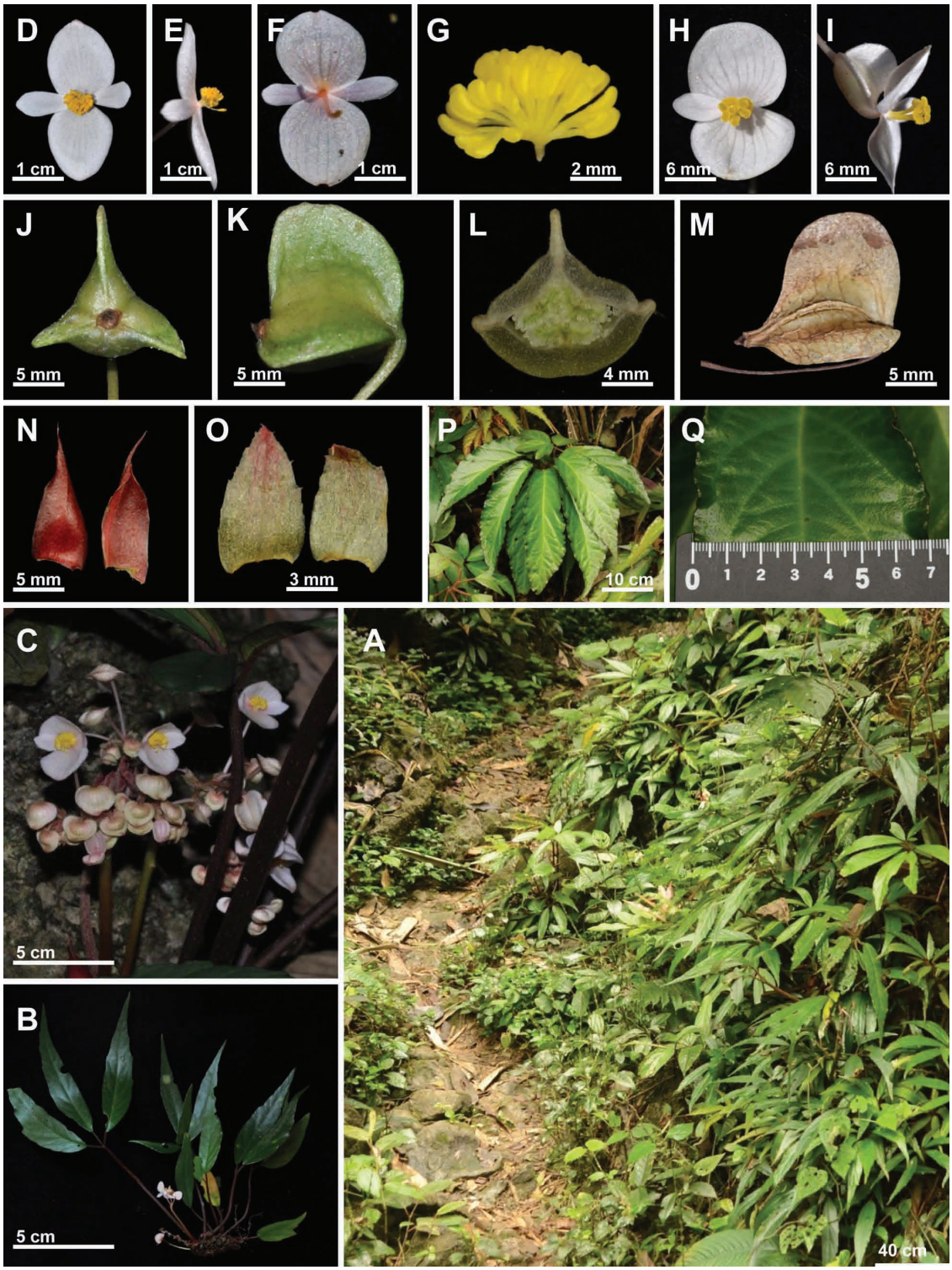

FIGURE 5. Habitat and morphology of *Begonia leipingensis* D. K. Tian, L. H. Yang & C. Li. **A.** Natural habitat; **B.** Small-medium individual with flowers. **C.** Inflorescence and flowers. **D–F.** Male flower (the frontal, side and back view). **G.** Androecium. **H–I.** Female flower (the frontal and side view). **J–K.** Young capsule (the frontal and side view). **L.** Cross-section of capsule with three parietal placentae. **M.** Dried mature capsule. **N.** Stipule (left: abaxial view, right: adaxial view). **O.** Bract (left: abaxial view, right: adaxial view). **P.** Large young leaf with eleven leaflets. **Q.** The widest leaflet ever seen in population.

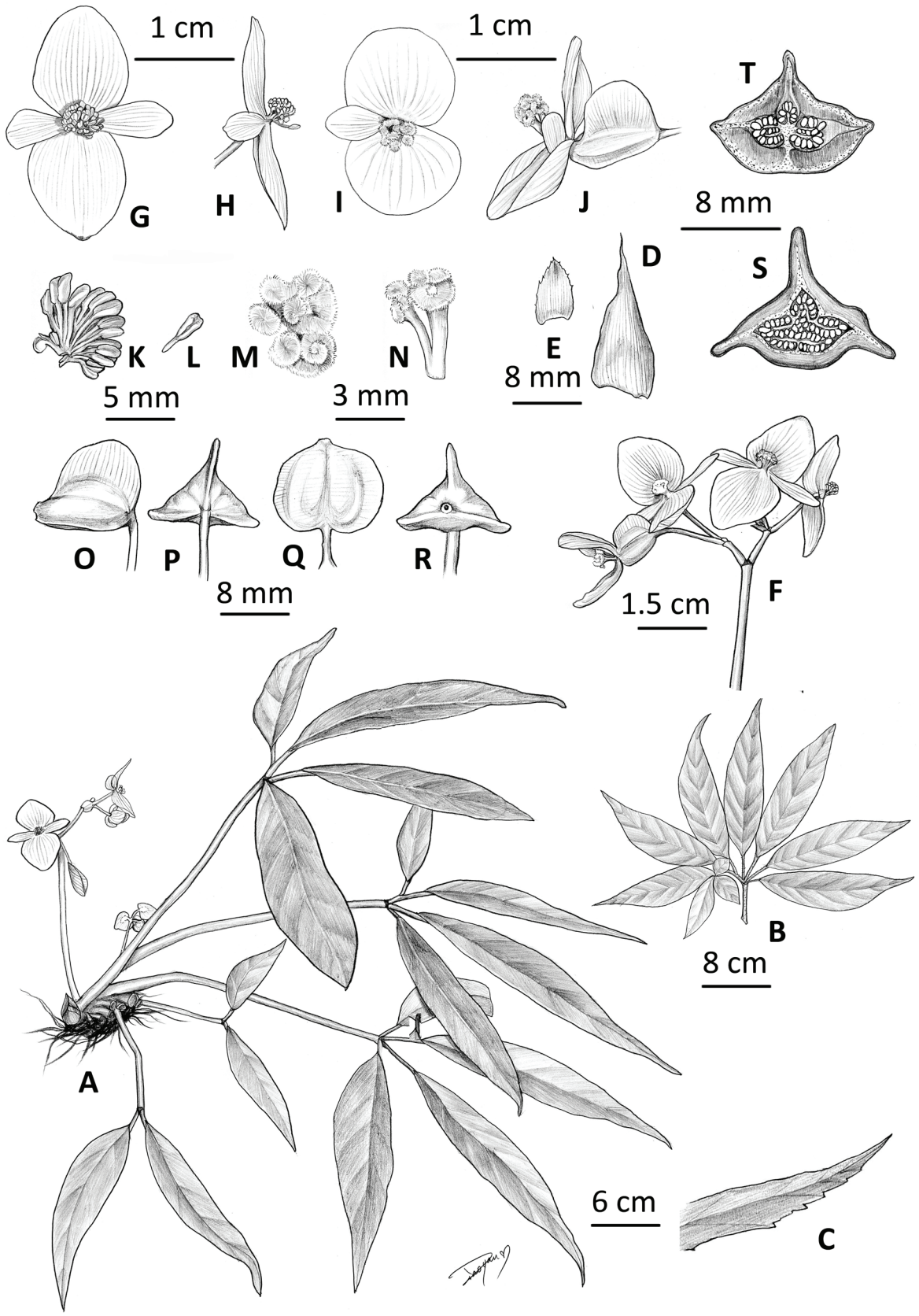

FIGURE 6. *Begonia leipingensis* D. K. Tian, L. H. Yang & C. Li. **A.** Habit (small-medium plant). **B.** Leaf with ten leaflets (adaxial view). **C.** Leaflet with serrate margin in partial plants. **D.** Stipule. **E.** Bract. **F.** Inflorescence and flowers. **G–H.** Male flower (the frontal and side view).**I–J.** Female flower (the frontal and side view). **K.** Androecium. **L.** Filament. **M–N.** Three styles and stigmas (the frontal and side view). **O–R.** Young capsule (the side, back, adaxial and frontal view). **S–T.** Cross-sections of capsule (the middle, upper position).

Etymology:—This new species is named after the name of the town, Leiping, where it was discovered in Guangxi, southwest China.

Distribution and Habitat.:—Only one population of this new species was discovered at Xinkang Village of Leiping Town, Daxin County, Guangxi, China (Fig. 7). The plants grow on moist rocks under the small trees and shrubs along shady pathsides on a limestone hill at 260–270 m elevation.

Phenology:—Flowering August to October, fruiting September to December.

Conservation Status:—Only one population, including approximately 500 individuals, was observed along about 100 m long path and nearby on a Karst hill. Although the distribution site is only four kilometers away from the town of Leiping, the habit is relatively undisturbed by human activities due to a sparse and decreasing number of local residents. According to the IUCN red list categories and criteria (IUCN 2012), *B. leipingensis* should be assessed as Critically Endangered (CR) based on the present situation: restricted geographic range of less than 1 km^2 with only one single location (CR:B2a) and about 300 mature individuals by estimation.

FIGURE 7. Distribution of *Begonia leipingensis* in Southwest Guangxi of China.

Conclusion

Begonia leipingensis is most similar to *B. fangii* (Sect. *Coelocentrum*) based on plant shape and leaf morphology, but significantly differs by its unique petiolule pattern (spirally-arranged) and large variation of leaflet number. It has thicker rhizome, shorter internodes, larger size of leaves and leaflets, often expanded petiole base, white to pale-pink flowers and longer abaxial wing (Table 2 and Fig. 8). Additionally, the flowering periods of these two species are completely separate (Table 2). *B. leipingensis* is also close to *B. hemsleyana* (Sect. *Platycentrum*) in foliage, but it can be easily distinguished from the latter, which has erect stems, pink to red flowers, 5-petaled male flowers, 2-loculed axile placenta and a terrestrial habitat (Table 2 and Fig. 8).

In the phylogenetic tree, all species from mainland China formed a well-supported clade (1.00 PP) with one African

species as outgroup. All species from Sect. *Coelocentrum* including the new species formed a strongly supported clade (100% BS and 1.00 PP). Eight clones of *B. leipingensis* formed one solid independent clade (98% BS and 0.96 PP) consisting of two strongly supported subclades (>99% BS and 1.00 PP). Two kinds of haplotypes from those two subclades were distinguished by a 29 bp indel. Four clones of *B. fangii* also formed an independent clade with high support (100% BS and 1.00 PP), and then was sister with *B. leipingensis* in a well-supported clade (78% BS and 0.98 PP). Combining evidences from both morphologic and molecular phylogenetic analyses, *B. leipingensis* was identified as having an independent lineage and was placed in Sect. *Coelocentrum* in *Begonia* phylogeny.

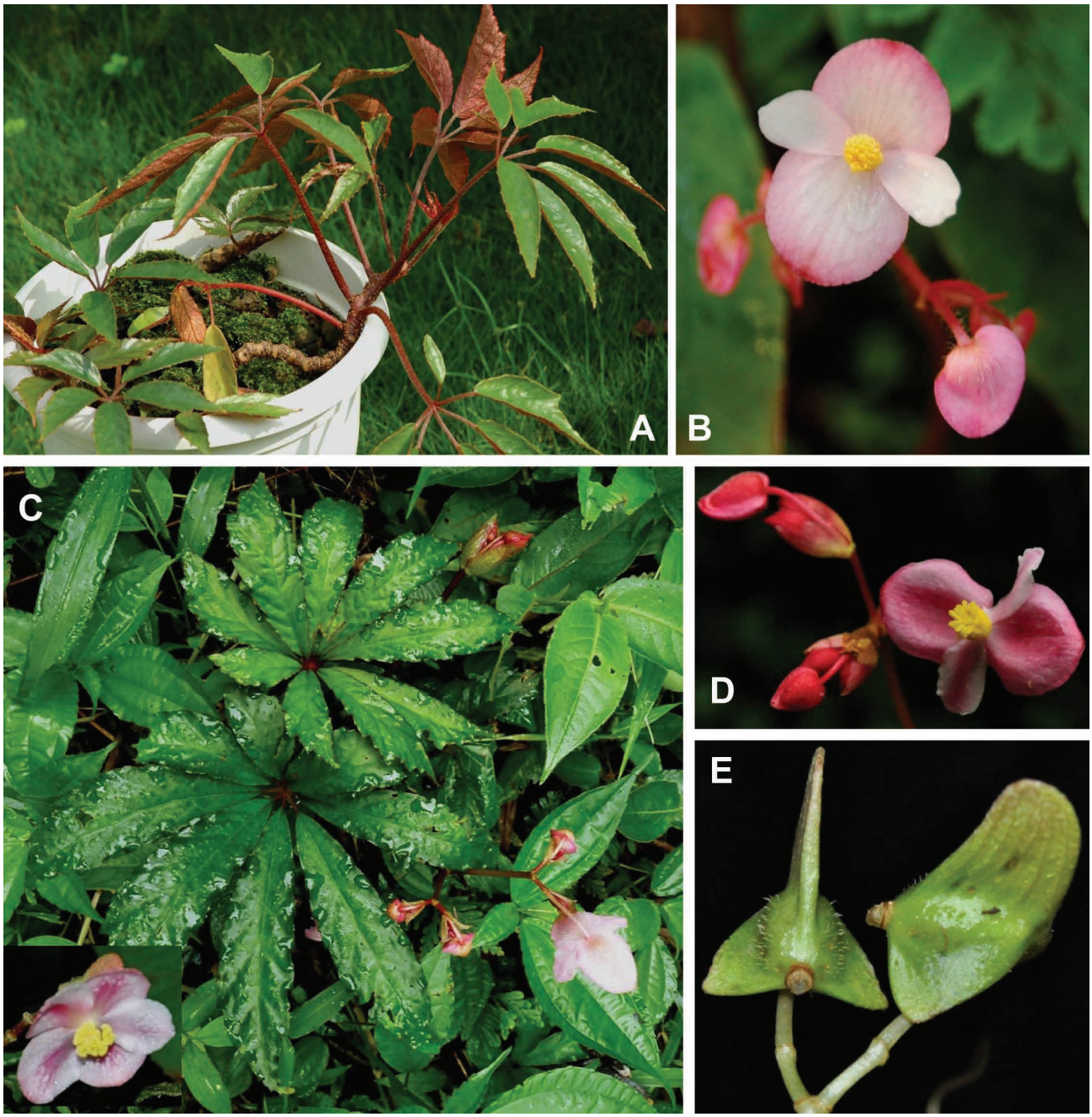

FIGURE 8. The two species most similar to *Begonia leipingensis* in morphology. **A–B.** *Begonia fangii*: **A.** Introduced plant showing petiolule pattern of compound leaf. **B.** Inflorescence and male flowers. **C–E.** *B. hemsleyana*: **C.** The individual with pink flowers (left bottom-corner: female flower) and the same petiolule pattern as *B. fangii*. **D.** Red flower type. **E.** Young fruits.

Author Contributions

Field works: CL, LHY, DKT. Conceived and designed the experiments: CL, DKT. Performed the experiments: CL, DKT, LHY, YC, RJW, NFF. Analyzed the data: CL, DKT, LHY. Wrote the paper: CL, DKT, LHY. Taken photographs: DKT, CL, LHY, RJW.

TABLE 2. Comparison of *Begonia leipingensis* with *B. fangii* and *B. hemsleyana*.

	Begonia leipingensis	*Begonia fangii*	*Begonia hemsleyana*
Section	*Coelocentrum*	*Coelocentrum*	*Platycentrum*
Habitat	Epipetric, on moist limestone rock	Epipetric, on limestone rock	Terrestrial
Rhizome	5–30 cm long; internode shorter	10–60 cm long; internode long	Very short and stout
Erect stem	Absent	Absent	30–80 cm
Leaf	Subcoriaceous	Subcoriaceous	Herbaceous
Type	Basal, palmately compound with spirally arranged petiolules	Basal, palmately compound	Basal and cauline, palmately compound
Petiole length (cm)	3–42, base often expanded	6–20, base unexpanded	5–14, base unexpanded
Leaflet number	2–12	3–7	5–11
Petiolule pattern	Spiral-like arrangement	Radial	Radial
Abaxial blade color	Light green	Red or reddish	Light green to reddish
Bract	Persistent	Caducous	Persistent
Female flower			
Tepal	3	3	5
Styles	3	3	2
Placentation	Parietal, locule 1	Parietal, locule 1	Axile, locule 2
Fruit			
Abaxial wing shape and length	Nearly semicircular to lunate, 6–11 mm	Lunate, 4–6 mm long	Nearly oblong to triangular, 15–21 mm
Flowering time	August–October	December–March	July–October
Chromosome Number	30	30	22

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