



Begonia intermedia, a new species of Begoniaceae from Hainan, China

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Abstract

Begonia intermedia D.K. Tian & Y.H. Yan, a new species in *Begonia* sect. *Diploclinium* (Wright) A. DC (Begoniaceae) from Hainan, China, is described and illustrated. It differs from the morphologically similar *B. fimbristipula* Hance by its orchid-root-like rhizomes, later flowering and distinctive hairy bracts. Also, based on a molecular phylogenetic analysis, *B. intermedia* is distinct from *B. fimbristipula*.

Key words: China, Hainan, *Begonia*, new species

Introduction

Begonia is one of the most diverse plant taxa and is considered the sixth largest genus of vascular plants in the world (Hoover *et al.* 2004). Nearly 1600 species have been named so far (Sands 2001, Aitawade *et al.* 2012). China, after Brazil, has the second largest number of *Begonia* species, with 173 species recorded in *Flora of China* and 141 endemic in China (Gu *et al.* 2007). After publication of *Flora of China*, 14 new *Begonia* species, all endemic in China, are described and illustrated (Ku *et al.* 2008, Li *et al.* 2008, Liu *et al.* 2007, Ma *et al.* 2006, Peng *et al.* 2008a, 2008b, 2009, 2010, 2012, 2013, Shui 2007, Wei *et al.* 2007). There are still many potential new taxa under investigation, therefore, the total number of *Begonia* species in China could easily reach over 200.

During fieldwork on Yinggeling National Natural Reserve, Qiongzong, Hainan, in June 2012, we collected several specimens and living plants from an interesting species of *Begonia*. This species is very similar to *Begonia fimbristipula* Hance (1883: 202) but it has orchid-root-like rhizome without nodes and distinctive hairy bracts, and blooms late. After a further field investigation in September 2012 when the plants were in full bloom, we confirmed that this species should be recognized as a new taxon. In order to obtain more evidence for its taxonomic placement in distinctiveness, a molecular phylogenetic analysis based on the chloroplast *ndhA* intron region was conducted with 48 terminals representing a total of 44 species and eight sections of *Begonia* delimited in China based on Shui's treatment (Shui *et al.* 2007).

Begonia intermedia D.K. Tian & Y.H. Yan was mistreated as *B. fimbristipula* by the author of *The Coloured Illustrative Plates of Wild Plants in Diaoluoshan Hainan China* (Qin 2013).

Materials and methods

Taxonomic sampling

To position new species within the phylogeny of the *Begonia*, the sequence data of all sections except sect. *Leprosae* (T.C. Ku) Y.M. Shui delimited in Chinese *Begonia* were used for analysis. Partial data were sequenced by us including all species except *B. howii* Merrill & Chun (1940: 138) distributed in Hainan and three morphologically similar species outside Hainan (*B. fimbristipula*, *B. labordei* Lévl. (1904: 323), *B. augustinei* Hemsl. (1900: 286)). The others were downloaded from NCBI to ensure at least three species from each section of Chinese *Begonia*, except one species for sect. *Alicida* C.B. Clarke. Three species from Africa were chosen as outgroup based on molecular phylogenetic studies. The related information of all species used in phylogenetic analysis is listed in Table 1.

TABLE 1. Taxon, origin, Genbank accession number, section, collector and voucher included in the phylogenetic analysis.

Taxon	Origin	Genbank accession no.	Section	Collector, Voucher (Herbarium)
<i>Begonia aceroides</i> Irmsch.	Thailand	JF756385	<i>Diploclinium</i>	Phutthai, T. 243 (BKF, PSU)
<i>Begonia acetosella</i> Craib	Vietnam	JF756367	<i>Sphenanthera</i>	Thomas, D. C. 08-105 (E)
<i>Begonia alicida</i> C.B.Clarke	Thailand	JF756388	<i>Alicida</i>	Phutthai, T. 139 (BKF, PSU)
<i>Begonia augustinei</i> Hemsley	Menghai, Yunnan, China	KF521900	<i>Platycentrum</i>	Tian, D.K., Mo, H.B., Li, X.P. TDK209 (CSH)
<i>Begonia burbidgei</i> Stapf	Borneo, Malaysia,	JF756396	<i>Petermannia</i>	Thomas, D. C. 07-26 (E)
<i>Begonia chloroneura</i> P.Wilkie & Sands	Luzon Island, Philippines,	JF756394	<i>Diploclinium</i>	Forrest, L.L. 128 (E)
<i>Begonia cleopatrae</i> Coyle	Palawan, Philippines,	JF756390	<i>Diploclinium</i>	Wilkie, P., Mendum, M., Argent, G. C. G., Cronk, Q., Middleton, D. J., Fuentes, R. & Chavez, R. V. 25373 (E)
<i>Begonia elisabethae</i> Kiew	Thailand	JF756381	<i>Parvibegonia</i>	Phutthai, T. 239 (PSU)
<i>Begonia fangii</i> Y. M. Shui & C. I Peng	Longzhou, Guangxi, China	KF521898	<i>Coelocentrum</i>	Tian, D.K. KIB12 (CSH)
<i>Begonia fenicis</i> Merr.	The Philippines	JF756392	<i>Diploclinium</i>	Thomas, D. C. 08-119 (E)
<i>Begonia fimbristipula</i> Hance	Yongxing, Hunan, China	KF521901	<i>Diploclinium</i>	Tian, D.K., Liu, K.M., Gu, J.Z., Li, X.J. TDK540-2 (CSH)
<i>Begonia fimbristipula</i> Hance	Ningyuan, Hunan, China	KF521910	<i>Diploclinium</i>	Tian, D.K., Liu, K.M., Gu, J.Z., Li, X.J. TDK551-2 (CSH)
<i>Begonia fimbristipula</i> Hance	Ziyuan, Guangxi, China	KF521906	<i>Diploclinium</i>	Tian, D.K., Gu, J.Z. TDK584-1 (CSH)
<i>Begonia flagellaris</i> Hara	Nepal	JF756353	<i>Diploclinium</i>	Rajbhandary, S. & Bista, S. 54 (E)
<i>Begonia goegoensis</i> N.E.Br.	Sumatra, Indonesia,	JF756376	<i>Reichenheimia</i>	Thomas, D. C. & Ardi, W. H. 08-107 (E)
<i>Begonia grandis</i> Dryand.	China	JF756351	<i>Diploclinium</i>	Thomas, D. C. 08-145 (E)
<i>Begonia guttapila</i> D.C.Thomas & Ardi	Sulawesi	JF756405	<i>Petermannia</i>	Thomas, D. C. & Ardi, W. H. 08-81 (E)
<i>Begonia hainanensis</i> Chun & F. Chun	Lingshui, Hainan, China	KF521903	<i>Petermannia</i>	Tian, D.K., Li, C. TDK725-7 (CSH)
<i>Begonia handelii</i> Irmscher	Wuzhishan, Hainan, China	KF521897	<i>Sphenanthera</i>	Tian, D.K., Li, C. TDK713 (CSH)
<i>Begonia hatacoa</i> Buch.-Ham.	Nepal	JF756354	<i>Platycentrum</i>	Thomas, D. C. 08-110 (E)
<i>Begonia hernandioides</i> Merr.	Philippines	JF756393	<i>Diploclinium</i>	Forrest, L.L. 129 (E),
<i>Begonia intermedia</i> D.K.Tian & Y.H.Yan	Qiongzong, Hainan, China	KF521908	<i>Diploclinium</i>	Li, X.P. LXP022-1 (CSH)
<i>Begonia intermedia</i> D.K.Tian & Y.H.Yan	Qiongzong, Hainan, China	KF521909	<i>Diploclinium</i>	Li, X.P. LXP022-2 (CSH)
<i>Begonia intermedia</i> D.K.Tian & Y.H.Yan	Qiongzong, Hainan, China	KF521907	<i>Diploclinium</i>	Tian, D.K., Li, C. TDK710-11 (CSH)
<i>Begonia koordersii</i> Warb. ex L.B.Sm. et Wassh.	Indonesia, Sulawesi	JF756407	<i>Petermannia</i>	Thomas, D. C. & Ardi, W. H. 08-62
<i>Begonia labordei</i> H. Léveillé	Lancang, Yunnan, China	KF521899	<i>Diploclinium</i>	Tian, D.K., Mo, H.B., Li, X.P. TDK196 (CSH)
<i>Begonia longifolia</i> Blume	Qiongzong, Hainan,	KF521912	<i>Sphenanthera</i>	Tian, D.K., Li, C. TDK712-1 (CSH)
<i>Begonia masoniana</i> Irmsch. ex Ziesenh.	China	JF756372	<i>Coelocentrum</i>	Thomas, D. C. 07-24 (E)
<i>Begonia morsei</i> Irmsch.	China	JF756373	<i>Coelocentrum</i>	No voucher available
<i>Begonia muricata</i> Blume	Java, Indonesia,	JF756378	<i>Reichenheimia</i>	Ardi, W. H. & Thomas, D. C. 27 (E)
<i>Begonia nigritarum</i> Steud.	Luzon Island, Philippines	JF756391	<i>Diploclinium</i>	Thomas, D. C. 07-25 (E)
<i>Begonia oxyloba</i> Welw. ex Hook.f.	Tanzania, Africa,	JF756335	<i>Meziera</i>	Thomas, D. C. 08-141 (E)
<i>Begonia palmata</i> D. Don	Lingshui, Hainan, China	KF521904	<i>Platycentrum</i>	Tian, D.K., Li, C. TDK727-1 (CSH)
<i>Begonia peltatifolia</i> H. L. Li	Qiongzong, Hainan,	KF521902	<i>Diploclinium</i>	Tian, D.K., Li, C. TDK694-1 (CSH)
<i>Begonia pendula</i> Ridl.	Borneo, Malaysia	JF756395	<i>Petermannia</i>	Thomas, D. C. 09-03 (E)
<i>Begonia poculifera</i> Hook.f.	Cameroon, Africa	JF756348	<i>Squamibegonia</i>	Forrest, L. L. 234 (E)
<i>Begonia polygonoides</i> Hook.f.	Ivory Coast, Africa	JF756336	<i>Tetraphila</i>	van der Burg, W. J. 244 (WAG)
<i>Begonia pseudolateralis</i> Warb.	The Philippines	JF756408	<i>Petermannia</i>	Hughes, M. 1448 (E)
<i>Begonia puttii</i> Craib	Thailand	JF756387	<i>Diploclinium</i>	Suddee, S. 3375
<i>Begonia rabilii</i> Craib	Thailand	JF756383	<i>Diploclinium</i>	Suddee, S. 3371 (PSU)
<i>Begonia siccaudata</i> J.Door.	Sulawesi, Indonesia	JF756418	<i>Petermannia</i>	Thomas, D. C. & Ardi, W. H. 09-60 (E)
<i>Begonia sikkimensis</i> A.DC.	India	JF756359	<i>Platycentrum</i>	Thomas, D. C. 08-144 (E)
<i>Begonia</i> sp. China 1	China	JF756352	<i>Diploclinium</i>	Möller, M. 01-156B (E)
<i>Begonia</i> sp. Thailand 1	Thailand	JF756380	<i>Parvibegonia</i>	Phutthai, T. 195 (PSU)
<i>Begonia sublongipes</i> Y. M. Shui	Baoting, Hainan, China	KF521905	<i>Petermannia</i>	Tian, D.K. KIB10 (CSH)
<i>Begonia sudjanae</i> Jansson	Sumatra, Indonesia	JF756377	<i>Reichenheimia</i>	Thomas, D. C. 08-109 (E)
<i>Begonia tenuifolia</i> Dryand.	Bali, Indonesia	JF756349	<i>Parvibegonia</i>	Thomas, D. C. & Ardi, W. H. 08-86 (E)
<i>Begonia watuwilensis</i> Girm.	Sulawesi, Indonesia	JF756406	<i>Petermannia</i>	Thomas, D. C. & Ardi, W. H. 09-55 (E)

DNA sequencing and molecular analyses

Total genomic DNA was extracted from silica-gel dried leaves using DNasecure Plant Kit (Tiagen Biotech, Beijing, China), following to the manufacturer's protocols. For each individual, the chloroplast *ndhA* intron region was amplified using the primers *ndhAx1* and *ndhAx2* (Shaw *et al.* 2007, Thomas *et al.* 2011). Each 20 μ L PCR contained 11.0 μ L of ddH₂O, 4.0 μ L of 5 \times Fast HiFidelity PCR buffer, 1.0 μ L of 20 \times Fast PCR Enhancer, 0.8 μ L of each forward and reverse primer (10 μ M), 0.4 μ L of Fast HiFidelity Polymerase (Tiagen Biotech), 2.0 μ L of DNA template, following the thermocycler conditions: an initial pre-denaturation at 94°C for 2 min, followed by 35 cycles including 15 s at 94°C, 10 s at 60°C and 30 s at 68°C, and a final extension step at 68°C for 5 min. Sequencing was conducted on an ABI PRISM 3730xl DNA Analyzer (Applied Biosystems, Invitrogen, Foster City, CA, USA).

Each sequence was assembled with SeqMan (DNASTar Inc., Madison, WI, USA), alignment using Clustal X version 2.0 (Thompson *et al.* 1997), and manually adjusted using BioEdit version 7.2.0 (Hall 1999). All indels were treated as missing data in the *ndhA* dataset. All sequences generated for the present study are available through GenBank (Table 1).

Phylogenetic relationships were reconstructed using maximum parsimony (MP). Maximum parsimony analyses were conducted with PAUP*4.0b10 (Swofford 2003) with heuristic searches of 1,000 replicates, with random stepwise sequence addition, tree bisection reconnection (TBR) branch swapping. Bootstrap values of the internal nodes were obtained with 1,000 replicates (Felsenstein 1985). In each replicate, 10 random sequence additions were performed, followed by TBR swapping, keeping no more than 1,000 trees per replicate.

Results

Molecular analyses

The *ndhA* matrix contained 48 sequences, including three accessions each for *B. intermedia* and *B. fimbristipula*. The alignment was 1281 bp in length. Results of MP analyses are shown in Figure 4, displaying a 50% majority rule consensus tree. The phylogenetic analysis showed that all species of Asian *Begonia*, were clustered in a solid clade (BS = 99). All species of sect. *Platycentrum* (Klotzsch) A. DC., sect. *Sphenanthera* (Hassk.) Warb. and the partial species of sect. *Diploclinium* formed a weakly supported clade (BS = 53). In this clade, the three accessions of *B. intermedia* (BS = 100) and *B. fimbristipula* (BS = 99) appear as monophyletic, indicating that *B. intermedia* was a lineage independent from *B. fimbristipula* and other taxa. In our phylogenetic tree, sect. *Diploclinium* is polyphyletic, that is consistent with the results from Thomas *et al.* (2011). *B. intermedia* falls into a grade that is mostly composed of species from sect. *Diploclinium*, although this relationship is only poorly supported (BS = 53).

Taxonomy

Begonia intermedia D.K. Tian & Y.H. Yan, *sp. nov.* (Fig. 1 & 2). Type:—China. Yinggeling National Natural Reserve, Qiongzong, Hainan, 19°01'14"N, 109°34'25"E, 28 September 2012, TDK710 (holotype CSH!; isotype CSH!).

Begonia intermedia is most similar to *B. fimbristipula* in general morphology, but it differs from the latter in late flowering (August to October), instinctive hairy and upper-clefted bracts, and the very rare orchid-root-like rhizomes. This species is endemic to Hainan Island in South China.

Deciduous herb, up to 20 cm high (up to 55 cm including inflorescence), basal, or short stem with 1–2 small leaves occasionally on large plants. Rhizomes without nodes, short, 4–15 cm long and 4 mm in diameter, creeping, orchid-root like, not highly branching. Leaves 2–3 (occasionally 4–5 seen in larger plants). Petiole green or reddish, 1.6–19 cm long and 1.5–5 mm in diameter, with gray curly hairs. Lamina heart-shaped, 4–19 cm long and 3.6–19 cm wide, subequal in size and slightly asymmetric, unlobed, margin unequally serrulate and hairy, apex acute, basal lobes not overlapping; both sides gray pubescent, hairs longer on abaxial veins; adaxial surface green, venation

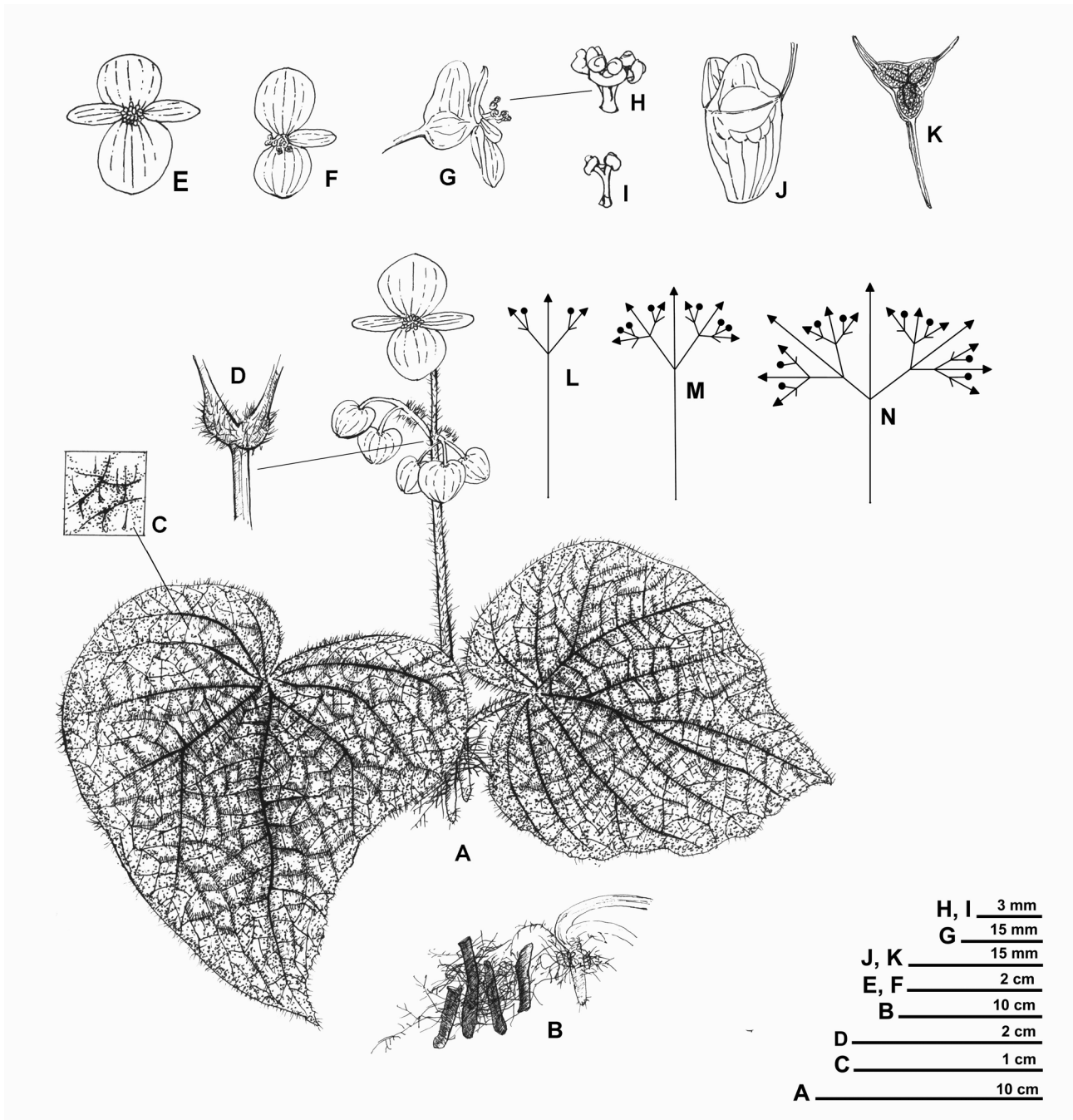


FIGURE 1. A–N *Begonia intermedia* (TDK 710, CSH). **A.** Habit. **B.** Rhizome. **C.** Magnification of leaf adaxial surface. **D.** Floral bract with hairs. **E.** Male flower. **F.** Female flower. **G.** Side view of female flower. **H–I.** Style and stigma (H: Whole; I: individual). **J.** Capsule. **K.** Cross-section of capsule. **L–N.** Sketch of inflorescences (more male flowers ▲ than female flowers ●, short bars without ▲ and ● mean failure in development of flowers).

palmate, 8–12 veined, impressed; abaxial veins purple-red, distinctly prominent. Inflorescences terminal, subdichasial, 1–2, 6–35 cm long, occasionally with 1–2 small leaves. Primary peduncle green or reddish, 4–28 cm long and 2–4 mm in diameter, sparsely pubescent. Bracts ovate, hairy, apex usually 3-clefted. Flowers 5–23 per inflorescence, male flowers open first at the same node, and usually more than female in number due to failure in development of some female flower buds at an early stage. Flower colors variable among individuals, pink flowers more common, white or red flowers rare. Staminate flowers: pedicels pink or red, glabrous, 1.5–2.7 cm long and up to 1.0 mm in diameter, corollas 1.7–2.2 × 1.5–1.9 cm, tepals 4, very rarely 6. Outer 2 largest, broadly ovate or oval, 8–11 × 8–11 mm, equal or subequal in length and width, distinct radial stripes on adaxial surface, sparse hairs on lower half of abaxial surface; inner 2 smaller, lighter in color, elliptic-lanceolate or oblanceolate, 6–10 × 3–4 mm,

no distinct stripes. Androecium 4 mm long; stamens 20–33, filaments free, 1.5–2.5 mm long, anthers nearly 0.8 mm long, subequal in length and width, apex obtuse. Pistillate flowers: pedicels pink or red, glabrous, 1–2 cm long, 1 mm in diameter. Flower ca. 1.4×0.9 – 1.3 cm in diameter, tepals 3, very rarely 4. Outer 2 larger, broadly ovate, glabrous, 6 – 7×8 – 11 mm, ca. 9 distinct radial stripes on adaxial surface; inner 1, rarely 2, smaller, lighter in color, oblanceolate, 5 – 6×2.5 – 3 mm wide, longitudinal stripes at base. Styles 3, 3 mm long, stigmatic surface U-shaped, spiralled 1.5 times. Ovary 3-loculed, placentae axile, bifid. Capsule nodding, ovoid, ca. 10 mm long, unequally 3-winged, abaxial wing nearly triangular, 14×9 mm, short wings 7×10 mm.

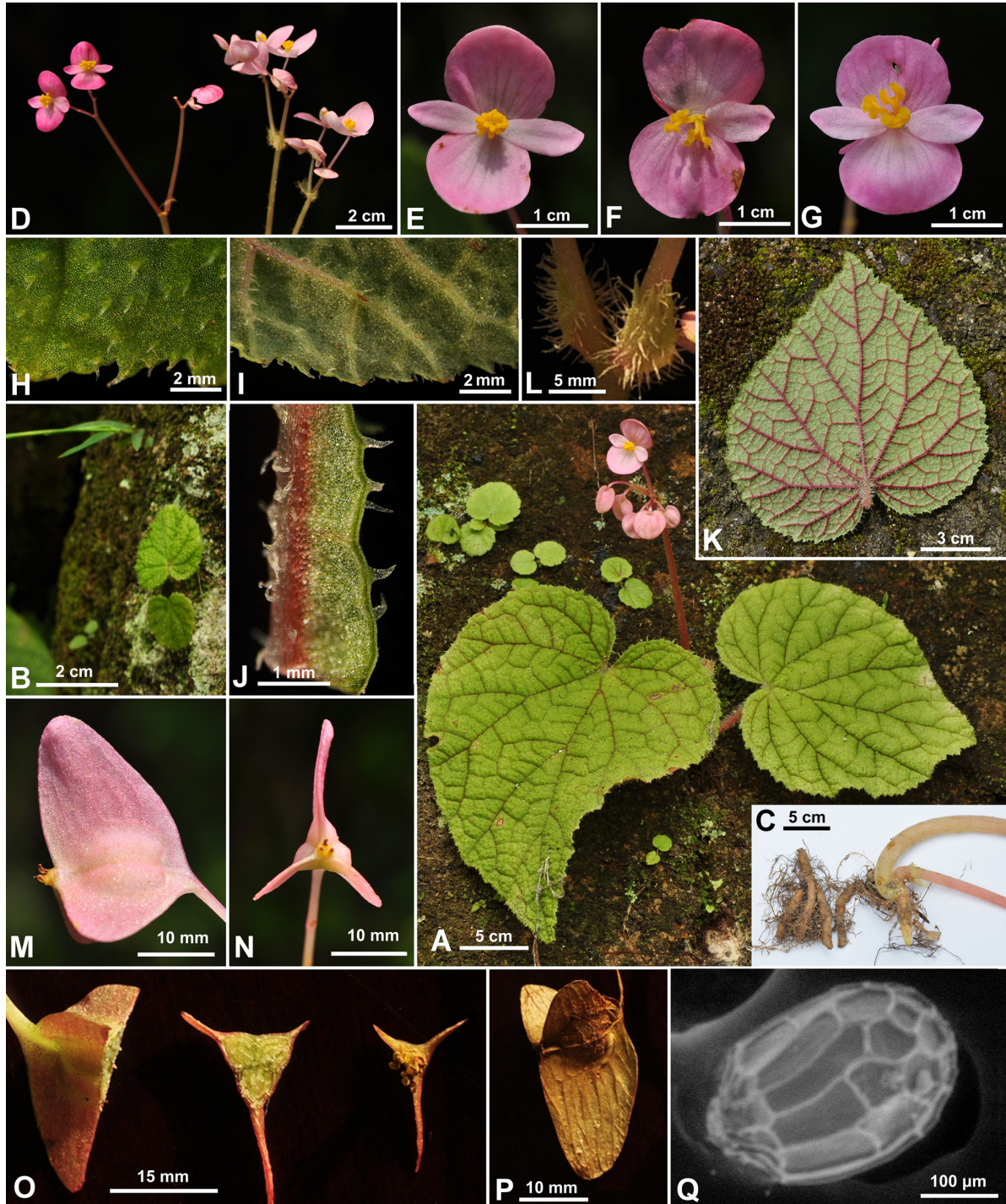


FIGURE 2. A–Q. *Begonia intermedia* (TDK 710, Photos: Daike Tian). A–B. Habit and seedlings. C. Rhizome. D. Inflorescence and flowers in different colors. E. Male flower. F. Female flower normally with three petals. G. Female flower rarely with four petals. H. Margin and adaxial surface of leaf section. I. Margin and abaxial surface of leaf section. J. Leaf cross-section. K. Leaf adaxial surface. L. Floral bract. M. Side view of young capsule. N. Frontal view of young capsule. O. Cross-sections of capsule. P. Dried mature capsule. Q. Seed.

This species flowers from August to October, and fruits from October to December.

This species grows either sparsely or densely in clusters of up to 100 plants per square meter, on roadside rocky cliffs with moss or between rock gaps, or among weeds on mountain slopes under thin forest, where the place is a little bit dry, pooled with *Ageratina adenophora*, *Melastoma sanguineum*, *Alocasia odora*, Urticaceae, Poaceae, ferns. Only three small populations were found along a kilometer long roadside. The holotype TDK710 (28 September, 2012) was collected from a site (19°01'14"N, 109°34'25"E, 502 m) 3.5 kilometers away from an Administration Substation of Yinggeling National Nature Reserve, Qiongzong County, Hainan Province, China, and paratype LXP022 (22 June, 2012), was also collected from the same site. Both types are stored in the Herbarium of Chenshan Botanic Garden, Shanghai (CSH).

Begonia intermedia is morphologically similar to *B. fimbristipula* and *B. chingii* Immsch. (1939: 519), but is easily separated from the later two species by its orchid-root-like rhizome without nodes (presenting an unusual intermediate state between rhizome and tuber), later flowering, and hairy bracts. This species is endemic to Hainan Province where the later two species have not been found so far. No herbarium specimens of this species have been ever found before our collection in any herbaria either in China or abroad. In a recently published monograph, *The Coloured Illustrative Plates of Wild Plants in Diaoluoshan Hainan China*, *B. intermedia* was mistakenly treated by authors as *B. fimbristipula* in page 89 (Qin 2013). Therefore this species is distributed in both Yinggeling and Diaoluoshan, Hainan, China (Fig. 3).

Etymology:—Named for its intermediate morphology of orchid-root-like rhizome between rhizomatous and tuberous types of *Begonia* species.

Distribution and habitat:—In Yinggeling National Natural Reserve, Qiongzong of Hainan, China, as well as in Diaoluoshan National Nature Reserve nearby (Fig. 3). Three close populations grow with moss on rock of roadside under thin forest with a stream nearby, at 502 m elevation in Yinggeling.

Additional specimens examined (paratypes):—The same site of holotype, China. Yinggeling National Natural Reserve, Qiongzong, Hainan, 19°01'14"N, 109°34'25"E, 22 June 2012, LXP022 (CSH).

IUCN Conservation assessment:—EN. Based on the restricted geographic range, small populations and less number of the individuals, *B. intermedia* should be considered endangered (EN) according to the IUCN red list criteria (IUCN 2008).

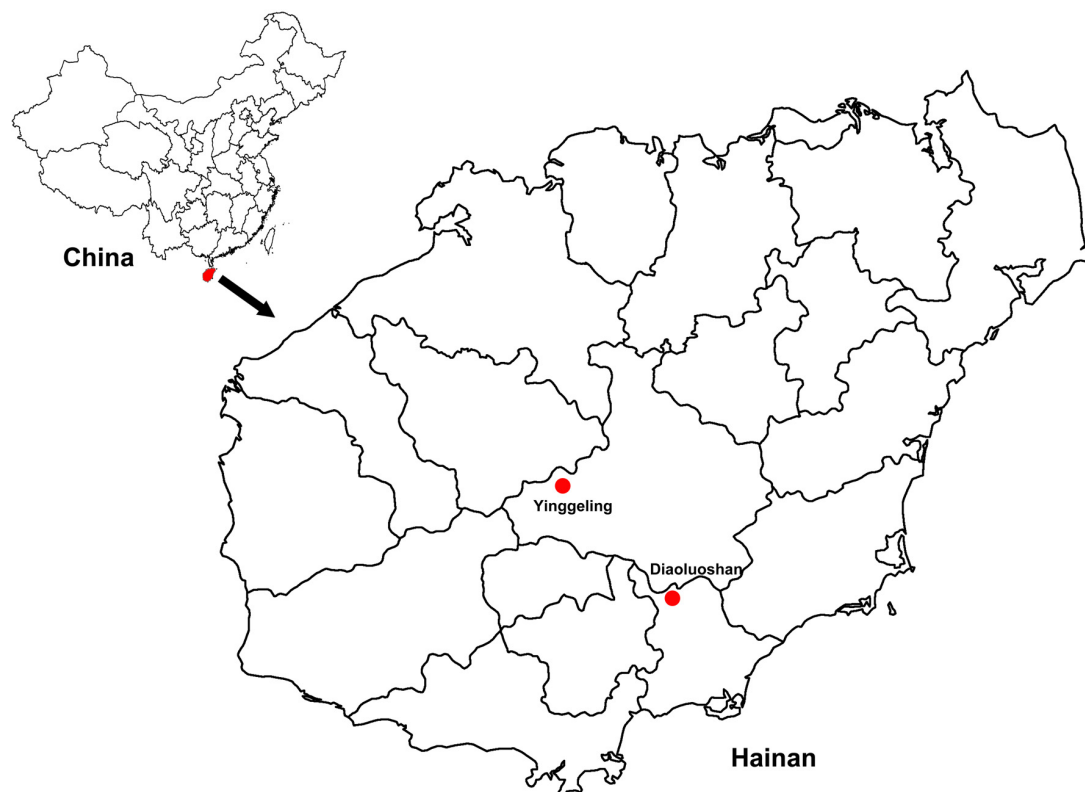


FIGURE 3. Distribution of *Begonia intermedia* in Hainan Province, China.

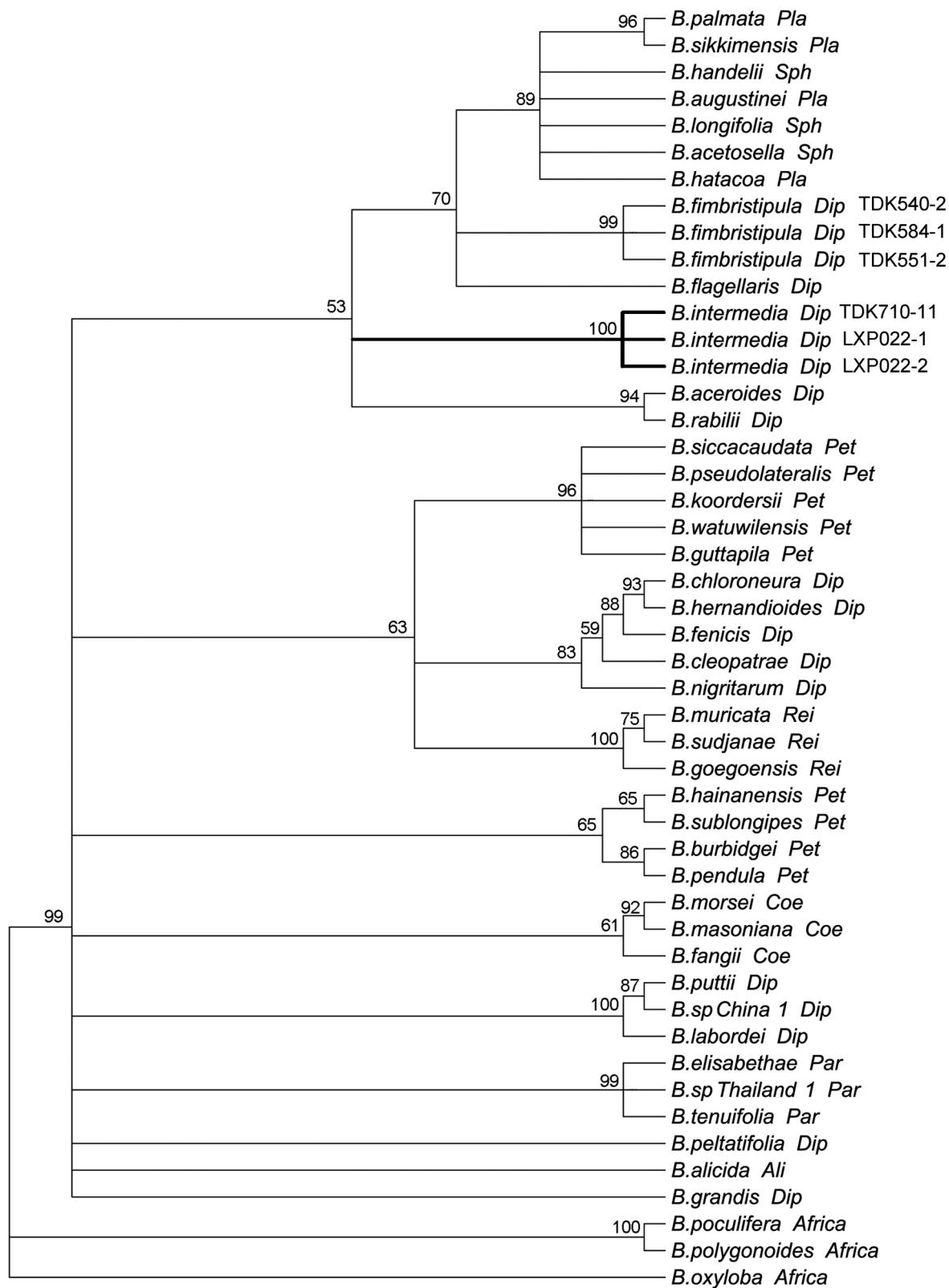


FIGURE 4. Strict consensus tree derived from parsimony analysis of the *ndhA* dataset of *Begonia intermedia* and related taxa; bootstrap values are indicated above branches. Sectional placement of taxa is indicated by the following abbreviations: Ali: *Alicida*, Coe: *Coelocentrum*, Dip: *Diploclinium*, Par: *Parvibegonia*, Pet: *Petermannia*, Pla: *Platycentrum*, Rei: *Reichenheimia*, Sph: *Sphenanthera*. Numbers after the taxon names indicates a different population or individual. The *B. intermedia* lineage is indicated in bold.

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