

Begonia barosma, a new species in *Begonia* sect. *Coelocentrum* (Begoniaceae) from limestone karsts across Sino-Vietnamese Border

Xin-Xin FENG^{1,#,*}, Yan-Man CHEN^{2,#}, Jian-Xun LIU¹, Yu-Ni HUANG¹, Zhi-Xian LIU¹, Ren-Kun LI³, Tong ZHAO⁴, Wei GUO^{2,*}

1. Dongguan Botanical Garden, Dongguan 523086, Guangdong, China. **2.** Department of Horticulture and Landscape Architecture, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, China. **3.** Enshi Dongsheng Plant Development Co. Ltd., Enshi 445000, Hubei, China. **4.** Guangdong Eco-engineering Polytechnic, Guangzhou 510520, China. #contributed equally. *Corresponding authors' e-mails: XXF: xinxin8715@163.com; WG:gwei717@163.com

(Manuscript received 20 February 2023; Accepted 11 August 2023; Online published 2 September 2023)

ABSTRACT: *Begonia barosma*, a new species in *Begonia* sect. *Coelocentrum* Irmscher (Begoniaceae) from limestone karsts across Sino-Vietnamese border is described in detail. It shows high similarities to the adjacently distributed *B. lanternaria* Irmscher, but differs from it in fleshy, relatively flat leaf blade with different pattern of variegation, sparsely pilose on abaxial surface of outer tepals. The molecular phylogenetic analysis based on ITS supports that the new species is a monophyletic lineage, separating from *B. lanternaria* Irmscher. Considering its small population size, narrow distribution, and disturbance by human activities, its conservation status is categorized as "Vulnerable (VU B1, B2 ab (iv, v), C2 a (i), D2)" according to the IUCN Red List Categories and Criteria.

KEY WORDS: Begonia lanternaria, China, Guangxi, ITS, limestone karst, morphology, new taxon, Sino-Vietnamese Border.

INTRODUCTION

As a pan-tropically distributed genus, *Begonia* L. increased considerably in specific diversity. 2116 species of 70 sections have been accepted around the world (Hughes *et al.*, 2015–present). Based on the latest report of *Begonia* diversity in China (IBegonia, 2022), *Begonia* has increased to 277 taxa including 253 species, 7 hybrids, 3 subspecies, and 14 varieties. With many new species under investigated and described, the number in China is expected to reach 300 soon (Tian *et al.*, 2018).

The area stretching from Southeastern Yunnan to Southwestern Guangxi and Northern Vietnam is characterized by typically limestone karst landform and the lowland with less than 1200 m elevation (Fang et al., 1994; Averyanov et al., 2003; Chen et al., 2018; Liu et al., 2020). Many plant species have been reported in past decade, in which Begonia, particularly sect. Coelocentrum occupy the dominant taxa (Chung et al., 2014; Peng et al., 2013, 2014, 2015a, b; Chen et al., 2018). Peng et al. (2015a) estimated the actual number of begonias in eastern Indochina to be 180-200 and new taxa belonging to Begonia sect. Coelocentrum will be reported continuously.

In August, 2019, we conducted fieldwork in southwestern Guangxi close to the Sino-Vietnamese border and collected the begonia with distinctive leaves. The begonia shows high resemblance to *B. lanternaria* Irmscher but differs from the latter in leaf coloration. Both detailed morphological comparisons and molecular evidence lead us to assign the species as a new one belonging to the section *Coelocentrum* Irmscher.

MATERIALS AND METHODS

Morphological observation

Living plants of the new species collected in the field were cultivated in nursery of Dongguan Botanical Garden. Morphological characters were observed and recorded both in the field and from specimens. Similar species were checked and examined in the Herbarium of Guangxi Institute of Botany (IBK). Type specimens were deposited at the Herbarium of South China Botanical Garden (IBSC), CAS and Herbarium of Sun Yat-sen University (SYS), respectively.

DNA sequencing and molecular analysis

Fresh leaves of the new species, B. lanternaria, B. filiformis and B. ornithophylla of the sect. Coelocentrum were collected in the field. Total DNA was extracted from 10 individuals of the 4 species 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 4 species uploaded to GenBank were (http://www.ncbi.nlm.nih.gov/) with the accession numbers OL998713-OL998716, OL913110-OL913112, OM003688 and OL913114–OL913115.

15 species from sect. *Coelocentrum* and 7 species from other sections of *Begonia* native to mainland China were selected to place the new species in a phylogenetic context. *Begonia poculifera* Hook.f in sect. *Squamibegonia* Warb.



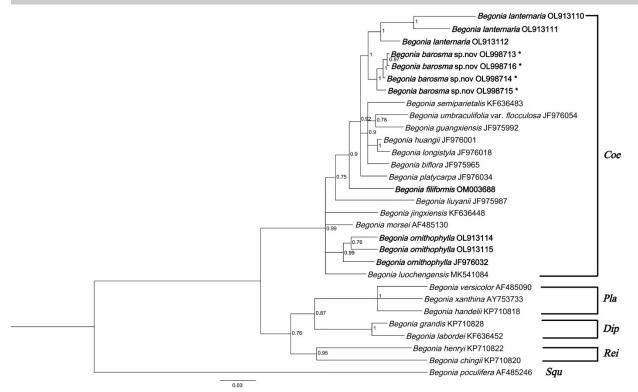


Fig.1. Bayesian inference of the phylogenetic position of the newly described **Begonia barosma** within sect. **Coelocentrum** based on nuclear ITS sequences. Nodes with bpp<0.50 have been collapsed. Sectional placement of taxa is indicated by the following abbreviations: **Coe**: Coelocentrum, **Pla**: Platycentrum, **Dip**: Diploclinium, **Rei**: Reichenheimia, **Squ**: Squamibegonia. The numbers after the species names indicate different individuals. The samples of new species are marked with black stars.

from Africa was selected as an outgroup in the phylogenetic analysis (Moonlight *et al.*, 2018). Besides the above 10 individuals of the 4 species, additional ITS sequences for 19 *Begonia* species and *B. ornithophylla* (JF976032) were downloaded from the NR database of NCBI.

All these sequences were aligned using BioEdit v7.2.5 (Hall, 1999), and a phylogenetic analysis using Bayesian Inference (BI) was undertaken in MrBayes v3.1.2 (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 1,500,000 generations and sampled at each 100 generations, with the first 25% discarded as burn-in.

RESULTS AND DISCUSSION

Molecular analyses

The aligned matrix of the ITS sequence data is 780 bp long. The result of Bayesian inference analysis was shown in Fig. 1. The sect. *Coelocentrum* appears monophyletic with a high Bayesian posterior probability (bpp=0.99) (Fig. 1). Within *B.* sect. *Coelocentrum*, four samples of the new species form a monophyletic group clustered with *B. lanternaria* (bpp=1), which shows the highest morphological similarity to *B. barosma*. Four individuals representing three populations of *B. barosma* and three individuals representing one population of *B. lanternaria* form two distinctive clades (bpp=1) in the phylogenetic tree.

Morphological comparisons

The species is characterized by 1-loculed ovary, 3 parietal placenta, 2 lobes each placenta, enabling us to assign it to the sect. *Coelocentrum* Irmscher, which is widely distributed in the karst forest in Guangxi and Northern Vietnam. Within the largest section in Guangxi begonias, *B. barosma* shows high morphological resemblance to closely distributed *B. lanternaria* (Fig. 2) in stem and flower morphology (Shui and Chen, 2018), including: creeping cylindrical rhizome, basal leaves, asymmetric widely ovate leaf blade, almost entire leaf margin, palmate venation (Table 1).

However, there are distinctive differences in leaf blade coloration, and flower tepals between them (Figs. 3, 4, S1). In *B. lanternaria* (Fig. S1), the leaf blade is leathery and wavy, adaxially greenish-black along palmate veins and abaxially greenish-red along palmate veins. On the contrary, the leaf blade of *B. barosma* is fleshy and almost relatively flat, adaxially blackish-green along main veins, randomly silver white between the veins, abaxially purple-red and densely pilose along main



Characters	B. lanternaria	B. barosma
Leaf texture	leathery and slightly bullate	fleshy and relatively flat
Adaxial leaf blade	greenish-black along palmate veins	blackish-green along main veins, randomly silver white between the veins
Abaxial leaf blade	greenish-red along palmate veins	purple-red and densely pilose along main veins, pale green between the veins
Abaxial surface of outer tepals	glabrous	abaxially sparsely pilose

Table 1. Morphological comparisons of Begonia lanternaria and B. barosma.

veins, pale green between the veins. Furthermore, outer flower tepals of *B. lanternaria* are glabrous, being dissimilar with the abaxially sparsely pilose flower tepals of *B. barosma*.

In conclusion, both the molecular phylogenetic analysis and the detailed morphological comparisons support that the begonia represents a new species in *Begonia* sect. *Coelocentrum* (Begoniaceae).

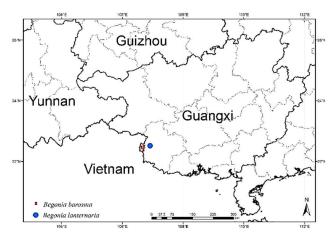


Fig. 2. Geographic distribution of *Begonia barosma* and *B. lanternaria*.

TAXONOMIC TREATMENT

Begonia barosma X.X. Feng, Y.N. Huang & Z.X. Liu, sp. nov. Figs. 3-4

Type: CHINA. Guangxi, Longzhou County (龙州县), Xiadong Town (下冻镇), Buju Village (布局村), 22°26'31"N, 106°34'43"E, (Fig. 2), 334 m a.s.l., in shaded environment of karst forest, October, 2019, X. X. Feng, Z. X. Liu & Y. N. Huang (holotype: IBSC 835308, IBSC!; isotype: SYS!).

Diagnosis: Begonia barosma morphologically resembles *B. lanternaria* in rhizome and flower characters. However, it differs from the latter in having fleshy, relatively flat leaf blade which is adaxially blackish-green along main veins, randomly silver white between the veins, abaxailly purple-red and densely pilose along main veins, pale green between the veins. In addition, the new species also can be distinguished from *B. lanternaria* in sparsely pilose on abaxial surface of outer tepals.

Description: perennial evergreen herb, monoecious, 20-35 cm tall. Rhizome stout and creeping, 10-15 cm long and 15-25 mm in diameter, internodes obvious and crowded. Stipules reddish-brown, ovate-triangular, 8-13 × 9-15 mm, pilose. Leaves all basal, petiole reddishbrown, 8-12 cm long, densely pilose with pale green lenticles; blade asymmetric, widely ovate, $19-20 \times 12-14$ cm; apex caudate, base oblique-cordate; margin subentire; venation palmate with 6-9 primary veins, adaxially slightly concave, abaxially convex; leaf blade fleshy and relatively flat, adaxially blackish-green along main veins, randomly silver white between the veins, sparsely puberulous, abaxially purplish-red and densely pilose along main veins, pale green and subgrabrous between the veins. Inflorescences arising directly from rhizome; dichasial cymes branching two or three times, peduncle 13-18 cm long, glabrous; flowers unisexual, 10-12 flowers per inflorescence; bracts membranous, widely ovate to subcircular, pinkish-yellow, $4-10 \times 3-8$ mm, margin puberulous. Staminate flower: pedicels pale green, 20-23 mm long, glabrous; tepals 4, outer 2 whitish-pink, widely ovate to circular, margin entire, 20- $23 \times 16-26$ mm, adaxially glabrous, abaxially redly pilose; inner 2 pinkish-white, lanceolate, margin entire, $15-18 \times 7-8$ mm, glabrous; and roccium zygomorphic, 5.6×7.5 mm; stamens numerous, 5.0 mm long, filaments fused at base, anthers golden yellow, clavate, base cuneate, apex retuse. Pistillate flower: pedicels pink, 13-20 mm long, glabrous; tepals 3, whitish-pink, outer 2 widely ovate to subcircular, margin entire, $16-18 \times 15-$ 16 mm, adaxially glabrous, abaxially sparsely pilose or glabrous; inner 1 obovate, 10-12 × 5-6 mm, glabrous; ovary pink, trigonous-ellipsoid, 11-18 × 4.6-8 mm (wings excluded), sparsely redly pilose; 1-lobuled, placentation parietal; placentae 3, each 2-branched; styles 3, fused at base, golden yellow, 3.8-5 mm long, stigma spirally U-shaped twisted. Capsule nodding, trigonousellipsoid, $18-20 \times 5-6$ mm (wings excluded), pinkishgreen, glabrous, unequally 3-winged, lateral wings 2, crescent-shaped, $14-16 \times 2-3$ mm, abaxial wing semilune-shaped, $18-20 \times 7-9$ mm.

Distribution and habitat: currently known from 3 populations along the Sino-Vietnamese border with the elevation from 220 m to 334 m. It grows in a shaded environment of the limestone karst forest.

Phenology: Flowering September–October, Fruiting October–December.



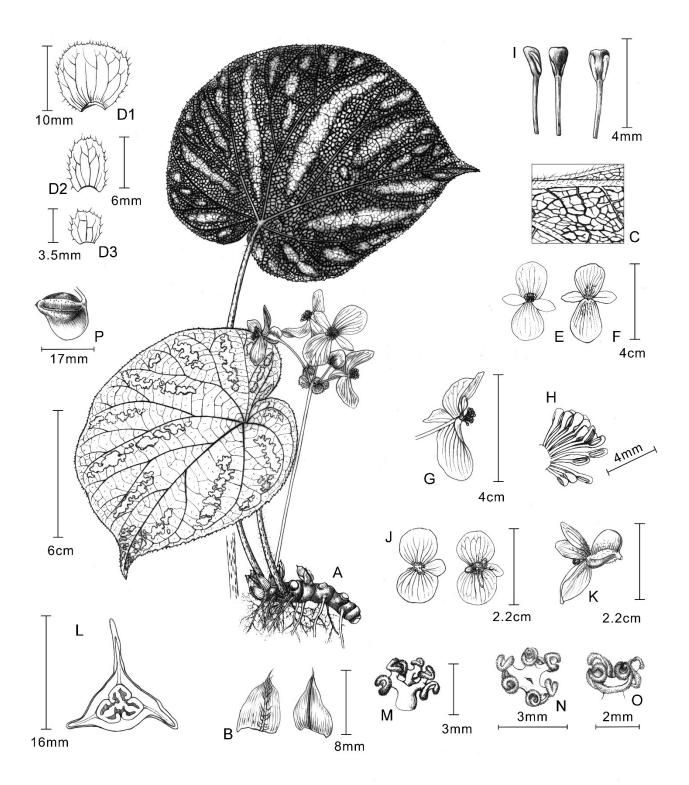


Fig. 3. *Begonia barosma* drawn by Yun Xiao Liu. A. Habitat; B. Stipules; C. Abaxial leaf blade; D1-D3. Bracts; E. Front view of staminate flower showing 4 tepals; F. Back view of staminate flower showing 4 tepals; G. Lateral view of staminate flower; H. Androecium; I. Stamen; J. Front and back views of pistillate flower; K. Lateral view of pistillate flower; L. Cross section of ovary; M. Style; N, O. Stigma; P. Capsule.

352



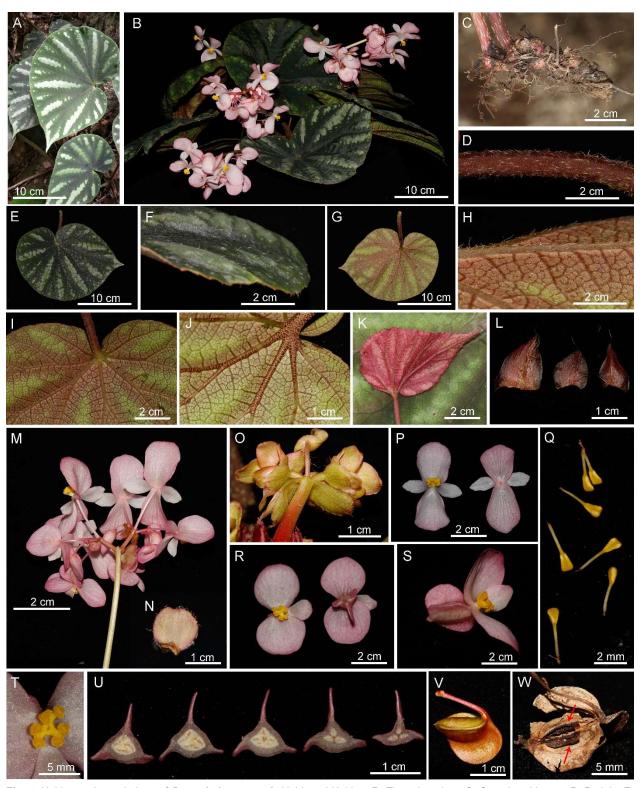


Fig. 4. Habitat and morphology of *Begonia barosma*. A. Habit and Habitat; B. Flowering plant; C. Creeping rhizome; D. Petiole; E. Adaxial leaf blade; F. Lateral leaf blade; G-J. Abaxial leaf blade showing relatively flat surface; K. Juvenile leaf; L. Stipules; M. Inflorescence; N,O. Bracts; P. Front and back views of staminate flower; Q. Stamens; R. Front and back views of pistillate flower; S. Lateral view of pistillate flower; T. Style and stigma; U. Cross sections of ovary; V. Mature capsule; W. Dry capsule showing placenta (Photos: Z.X. Liu).



Etymology: The specific epithet "*barosma*" refers to the extreme sweet smell of the flower during blooming season.

Vernacular name: 醉香秋海棠

Conservation status: 3 populations with no more than 1000 individuals have been found along the National Defense Road of China. The population size is prone to decrease by over collection for medicinal and ornamental uses. Considering its small population size, restricted distribution, and disturbance by human activities, *B. barosma* is currently assessed as "Vulnerable (VU B1, B2 ab (iv, v), C2 a (i), D2)" according to the IUCN Red List Categories and Criteria (IUCN, 2022).

ACKNOWLEDGMENTS

This study was supported by the Guangdong Rural Revitalization Strategic Foundation (20211800400042), the Dongguan Sci-tech Commissioner Program (Grant No. 20221800500042), the Dongguan Municipal Project for Social Science Improvement of and Technology (2020507101165), the Guangzhou Science and Technology Program (201903010076) and the Guangdong Basic and Applied Basic Research Foundation (Grant No. 2021A1515110529). We are also grateful to the editor and the reviewers for reviewing our manuscript.

LITERATURE CITED

- Averyanov, L., Cribb, P., Phan, K.L., Nguyen, T.H. 2003 Slipper orchids of Vietnam: with an introduction to the Flora of Vietnam. Royal Botanic Gardens, UK, 317 pp.
- Burnham, K.P., Anderson, D.R. 2002 Model Selection and Multimodel Inference: a practical information-theoretic approach. Springer, New York, USA, 488 pp.
- Chen, W.-H., Radbouchoom, S., Nguyen, H.-Q., Nguyen, H.-T., Nguyen, K.-S., Shui, Y.-M. 2018 Seven new species of *Begonia* (Begoniaceae) in Northern Vietnam and Southern China. Phytokeys 94: 65–85.
- Chung, K.-F., Leong, W.-C., Rubite, R.-R., Repin R., Kiew, R., Liu, Y., Peng, C.-I 2014 Phylogenetic analyses of *Begonia* sect. *Coelocentrum* and allied limestone species of China shed light on the evolution of Sino-Vietnamese karst flora. Bot. Stud. 55:e1.
- **Doyle, J.J., Doyle, J.L.** 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19**: 11–15.
- Fan, Q., Chen, S.-F., Li, M.-W., Guo, W., Jing, H.-J., Wu, W., Zhou, R.-C., Liao, W.-B. 2014 Molecular evidence for natural hybridization between wild loquat (*Eriobotrya japonica*) and its relative *E. prinoides*. BMC Plant Biol. 14(1): 275.
- Fang, R.-Z., Bai, P.-Y., Huang G.-B., Wei, Y.-G. 1994 A floristic study on the seed plants from tropics and subtropics of Dian-Qian-Gui. Acta Bot. Yunnanica Supp. VII: 111– 150.
- Forrest, L.L., Hollingsworth, P.M. 2003 A recircumscription of *Begonia* based on nuclear ribosomal sequences. Plant Syst. Evol. 241(3-4): 193–211.

- Hall, T.A. 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.
- Hughes, M., Moonlight, P.W., Jara-Muñoz, A., Tebbitt, M.C., Wilson, H.P., Zhong, K. Pullan, M. 2015–Begonia Resource Centre. Online database available from http://padme.rbge.org.uk/begonia/.(updated: 13 July 2023)
- IBegonia 2022 The 2022 report of *Begonia* Diversity in China (In Chinese).
- https://mp.weixin.qq.com/s/OAyd1xWQKHBrIjDf2vTo4w. IUCN. 2022 Guidelines for Using the IUCN Red List Categories and Criteria. Version 15. Prepared by the Standards and Petitions Committee of the IUCN Species Survival Commission. Available from: https://www.iucnredlist.org/documents/RedListGuidelines. pdf (accessed 12 February 2023).
- Liu, Y., Tseng, Y.-H, Yang, H.-A., Hu, A.-Q., Xu, W.-B., Lin, C.-W., Kono, Y., Chang, C.-C., Peng, C.-I, Chung, K.-F. 2020 Six new species of *Begonia* from Guangxi, China. Bot. Stud. 61: 21.
- Moonlight, P.W., Ardi, W.H., Padilla, L.A., Chung, K.F., Fuller, D., Girmansyah, D., Hollands, R., Jara-Munoz, A., Kiew, R., Leong, W.C., Liu, Y., Mahardika, A., Marasinghe, L.D.K., O'Connor, M., Peng, C.-I, Perez, A.J., Phutthai, T., Pullan, M., Rajbhandary, S., Reynel, C., Rubite, R.R., Sang, J., Scherberich, D., Shui, Y.-M., Tebbitt, M.C., Thomas, D.C., Wilson, H.P., Zaini, N.H., Hughes M. 2018 Dividing and conquering the fastestgrowing genus: Towards a natural sectional classification of the mega-diverse genus *Begonia* (Begoniaceae). Taxon 67(2): 267–323.
- Nylander, J.A.A. 2004 MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. Available from: http://www.softpedia.com/get/Science-
 - CAD/MrModeltest.shtml (accessed 28 November 2022).
- Peng, C.-I, Lin, C.-W., Yang, H.-A., Kono, Y., Nguyen, H.-Q. 2015a Six new species of *Begonia* (Begoniaceae) from limestone areas in Northern Vietnam. Bot. Stud. 56: e9.
- Peng, C.-I, Ku, S.-M., Yang, H.-A., Leong, W.-C., Liu, Y., Nguyen, T.-H., Yoshiko, K., Chung, K.-F. 2014 Two new species of *Begonia* sect. *Coelocentrum*, *B. guixiensis* and *B. longa*, from Sino-Vietnamese limestone karsts. Bot. Stud. 55: e52.
- Peng, C.-I, Yang, H.-A., Kono, Y., Chung, K.-F., Huang, Y.-S., Wu, W.-H., Liu, Y. 2013 Novelties in *Begonia* sect. *Coelocentrum: B. longgangensis* and *B. ferox* from limestone areas in Guangxi, China. Bot. Stud. 54: e44.
- Peng, C.-I, Yang, H.-A., Yoshiko, K., Jung, M.-J., Nguyen, T.-H. 2015b Four new species of *Begonia* (Begoniaceae) from Vietnam: *B. abbreviata*, *B. calciphila*, *B. sphenantheroides* and *B. tamdaoensis*. Phytotaxa 222(2): 83–99.
- Ronquist, F., Huelsenbeck, J.P. 2003 MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574.
- Shui, Y.-M., Chen, W.-H. 2018 *Begonia* of China. Yunnan Science and Technology Press, Kunming, China, 285 pp.
- Tian D.-K., Xiao, Y., Tong, Y., Fu, N.-F., Liu, Q.-Q., Li, C. 2018 Diversity and conservation of Chinese wild Begonias. Plant Diver. 40(3): 75–90.

Supplementary materials are available from Journal Website