



Begonia markiana (Begoniaceae), a new species from Lower Dibang Valley of Arunachal Pradesh, Northeast India

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ABSTRACT: The new species *Begonia markiana* is described and illustrated. It is similar to *B. cathcartii* but can easily be distinguished by a lamina with a dark central spot and a densely pilose upper surface in combination with a short dorsal capsule wing. A phylogenetic analysis based on three chloroplast regions placed the new species together with other continental species in section *Platycentrum*. Both morphology and molecular data suggest the new species as a member of the section *Platycentrum*.

KEY WORDS: *Begonia annulata*, *Begonia cathcartii*, chloroplast DNA, *ndhA*, new species, *ndhF-rpl32*, phylogeny, *Platycentrum*.

INTRODUCTION

Begonia L. (Begoniaceae) with over 2000 accepted species is one of the largest and fastest growing genera (Hughes *et al.*, 2015, continuously updated). The north-eastern region of India with Myanmar is a hotspot for the genus *Begonia*, from where several species are described recently and many are under review (e.g., Borah *et al.*, 2021abcd; Wahlsteen, 2018, 2019; 2021; Taram *et al.*, 2020, 2021). The region is situated within the transition zone of three biogeographic regions (India, Indo-Malaya and Indo-China) comprising lowlands, and highlands which corresponds to several microhabitats for endemism (Roy and Joshi, 2002). It is so far represented by a total of 42 species of *Begonia*, falling under four different sections (*Begonia* sect. *Diploclinium* (Lindl.) A.DC., sect. *Parvibegonia* A.DC., sect. *Platycentrum* (Klotzsch) A.DC. and sect. *Monophyllon* A.DC.) (Camfield and Hughes, 2018; Taram *et al.*, 2020; Krishna *et al.*, 2021; Borah *et al.*, 2021c).

Based on ITS sequences, the phylogeographic origin of the *Begonia* species in the region are assumed to have two different sources. One is characterized by tuberous, deciduous species (i.e., section *Diploclinium* and section *Monophyllon*) with endemic radiations in the Himalayan region beginning circa 7.4 Ma; the other is a group of evergreen, rhizomatous species with a probable origin in China, which immigrated to the Himalayas circa 5.1 Ma (Rajbhandary *et al.* 2011). The species discussed in the present paper belong to the latter group. In the present article, chloroplast DNA regions have been used to put the new species in a phylogenetic context. The main reason is that the available data from GenBank is superior to that for ITS in terms of species number, and in ITS many base positions in the spacers are saturated with substitutions. The disadvantage to plastid data is that chloroplast captures can mislead the interpretation of the

results. That the genus *Begonia* is prone to chloroplast exchange is exemplified by Hughes *et al.* (2018) in section *Baryandra*.

In total 80.3 % of Arunachal Pradesh is covered by forests (Saikia *et al.*, 2017). However, most of the primary forests of Dibang river valley are replaced with secondary forests and partly lumbered for firewood or road improvement. Typical refuges and reminiscences of a once rich flora are the lush stream valleys crossing the road with dripping wet rock walls covered by ferns, gesneriads and begonias. The low elevation areas in the south experience a sub-tropical humid climate during the summer monsoon period and dry mild winters (Deka *et al.*, 2009) and are characterized by evergreen broadleaved subtropical forests.

On a recent expedition to Lower Dibang Valley of Arunachal Pradesh (Fig. 1), the authors collected an interesting specimen of *Begonia*, which on perusal of relevant literature was found to be an undescribed species.



Fig. 1. Distribution of *Begonia markiana* Taram, Wahlsteen & D.Borah in Arunachal Pradesh, north east India.

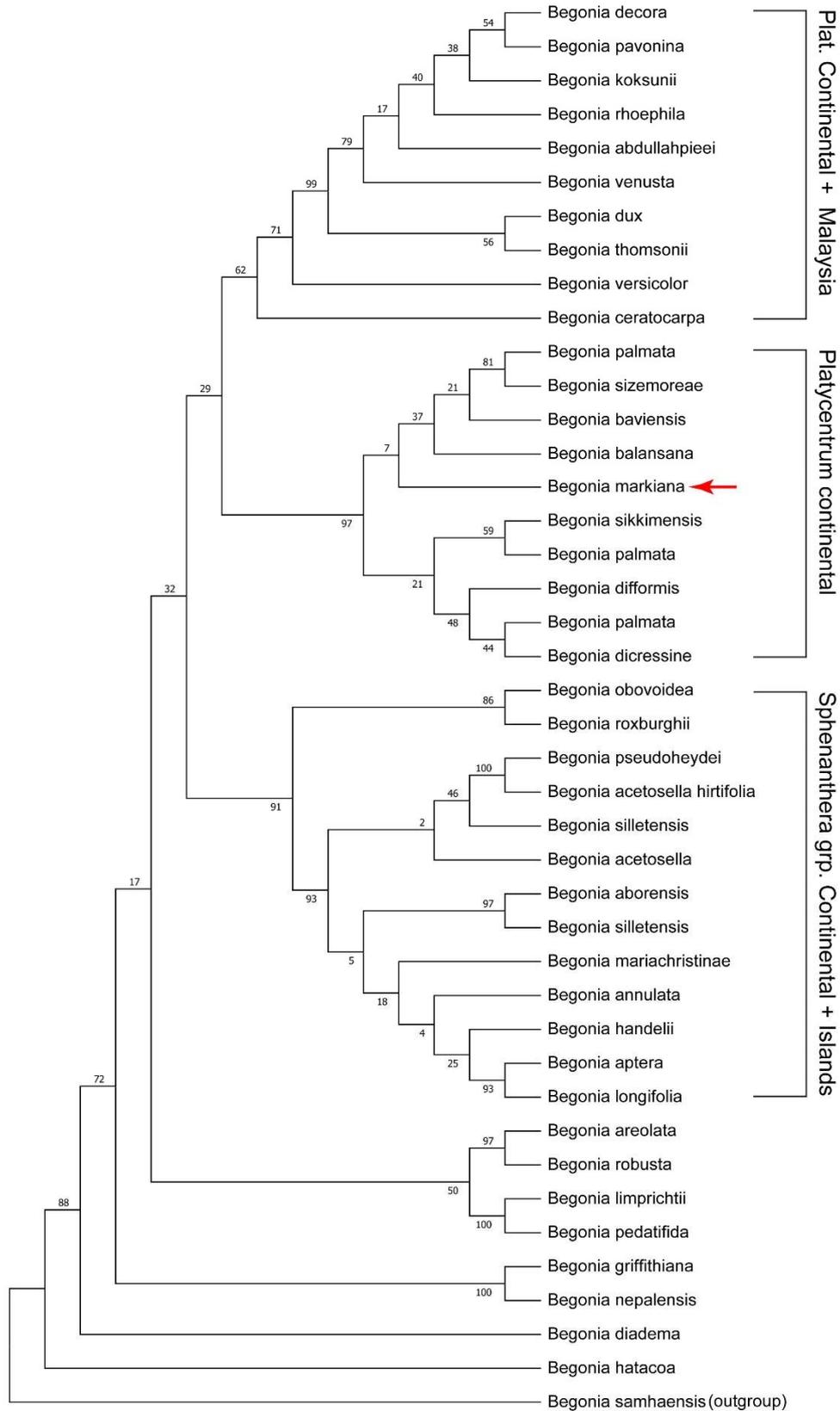


Fig. 2. The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model. The phylogeny based on chloroplast DNA sequence data showing the relationships of the new species in *Begonia* sect. *Platycentrum*. Number at the nodes are bootstrap values.



Fig. 3. *Begonia cathcartii* Hook.f. & Thomson in Arunachal Pradesh, India.

MATERIALS AND METHODS

Key morphological characters to establish the taxonomic status of the included samples were obtained from Doorenbos *et al.* (1998) for the section *Platycentrum*. Living and pressed material of the new species were compared to herbarium specimens and digital images from CAL, ASSAM, E, K, PE and P herbaria. Key references include Camfield & Hughes (2018) for Northeast India, Gu *et al.* (2007) for China and Grierson (1991) for Bhutan.

The phylogenetic analysis comprised sequences from 41 *Begonia* species from section *Platycentrum* and one species from section *Peltaugustia* (outgroup) downloaded from GenBank (Appendix 1) earlier prepared by Moonlight *et al.* (2018) in combination with one accession newly generated from this study.

Total genomic DNA was extracted using Qiagen DNeasy Plant Mini Kits in accordance with the manufacturer's protocols. Three DNA regions were chosen for amplification: *ndhA* intron and *rpl32-trnL* spacer using universal primers by Shaw *et al.* (2007), and *ndhF-rpl32* spacer, using the specific internal primers described by Thomas *et al.* (2011). The three regions were amplified in 20 μ l reactions containing 1.0 μ l of

template, (100ng/ μ l), 0.75 μ l of both forward and reverse primers (10 μ M), 0.2 μ l of Phusion polymerase, 11.7 μ l of mQ H₂O, 4 μ l of 5 \times reaction buffer, 0.4 μ l of dNTPs (10mM), and 0.2 μ l of DMSO. The PCR temperature profile included: template denaturation at 96°C for 4 min; 34 cycles of denaturation at 98°C for 30 s, primer annealing at 58°C for 30 s, primer extension at 72°C for 30 s; and a final stage primer extension at 72°C for 10 min. PCR products were purified by incubating 5 μ l aliquots with 2U / μ l Exonuclease I, and 0.2U / μ l Shrimp Alkaline Phosphatase for 20m at 37°C followed by 15 m at 80°C. Sequencing PCRs used the same primers as for amplification, in 10 μ l reactions containing purified PCR products diluted to 0.85ng/ μ l, using BigDye 3.1 cycle sequencing kits. Samples were sequenced using an Applied Biosystems 3730XL DNA sequencer equipped with a 50 cm 96 capillary array. Sequences were submitted to GenBank (www.ncbi.nlm.nih.gov) with accession numbers OK624680 (*ndhA*), OK624682 (*rpl32-trnL*) and OK624681 (*ndhF-rpl32*).

The new sequences were aligned together with sequences downloaded from GenBank with the MAFFT algorithm (Kuraku *et al.*, 2019). The alignments were trimmed at the ends and the three regions were combined prior to further analysis. The substitution model for maximum likelihood analyses was determined using the integrated model test in MEGAx (Kumar *et al.*, 2018) with default settings, suggesting the GTR+ Γ substitution model as best fitting. Maximum likelihood analyses were performed with 1000 replicates in a bootstrap test.

RESULTS

The concatenated matrix with trimmed ends and gaps comprised 3065 characters, of which 465 (15%) were variable and 191 (6%) parsimony informative. The maximum likelihood consensus tree with 50% bootstrap replicates, revealed three highly supported clades (Fig. 2). One consisted of species with fleshy fruits (formerly section *Sphenanthera*) mainly distributed in continental Asia, but also three species in Malaysia and Indonesia. A second clade included species with dry capsules (*Platycentrum* proper) distributed mainly in Malaysia with a few species in southern continental Asia. The new species was placed in a third clade including species from continental Asia belonging to the section *Platycentrum* proper. The results are congruent with earlier phylogenies (Moonlight *et al.*, 2018; Rajbhandary *et al.*, 2011) why serious impact from chloroplast capture would be dismissed.

The morphological characters of the new species revealed most similarity to *B. cathcartii* (Fig. 3 and in Taram *et al.* 2021) but it also shares similarities with *B. annulata* K.Koch and *B. thomsonii* A.DC. (all within the geographical range of the new species). Significant key characteristics of the new species are the prostrate habit,

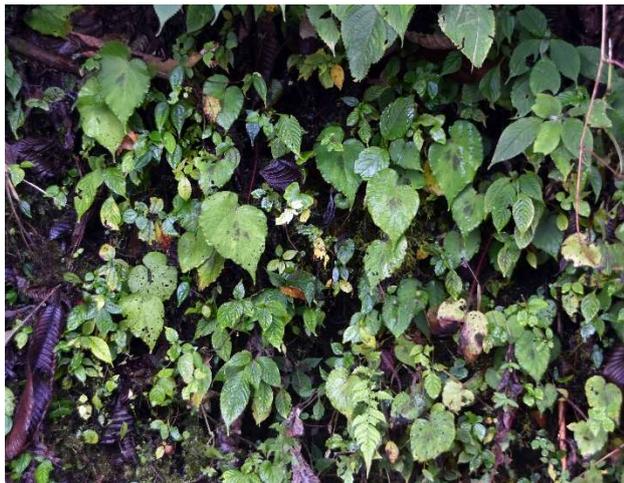


Fig. 4. A vertical cliff makes the habitat for *Begonia markiana* Taram, Wahlsteen & D.Borah. in the Mayodia area of Lower Dibang Valley District, Arunachal Pradesh.

the dark central spot of the lamina, two styles and a rather short upper capsule wing. It belongs to the section *Platycentrum* characterized by male flowers with four tepals, anthers with side slits and extended connectives, female flowers with two styles and locules and three unequal wings. The sectional placement is confirmed by the phylogeny where the new species is found in a rather recently evolved clade.

TAXONOMIC TREATMENT

Begonia markiana Taram, Wahlsteen & D.Borah, *sp. nov.* **Figs 4 & 5**

Type: INDIA. Arunachal Pradesh, Lower Dibang Valley District, Mayodia, 2000 m, 09 September 2021, *M. Taram* & *O. Taku* 5442 (holotype: CAL; Isotype: CAL).

Diagnosis. *Begonia markiana* is similar to *B. cathcartii* Hook.f. & Thomson (Fig. 3) but can easily be distinguished by the lamina with a dark central spot (versus unicolored) and the densely pilose upper surface (versus sparsely strigose), and a short dorsal capsule wing (circa 10 mm versus 15–24 mm).

Description. Rhizomatous, erect to creeping, monoecious herb, 30–60 cm high. Rhizome 1–3 cm × 0.6–1 cm, densely pilose. Stem stout, 0.4–0.8 cm wide, white pilose to tomentose, internodes 6–20 cm long, indumentum white hyaline. Stipules ovate, 1–1.5 cm × 0.6–1 cm, margin entire, apex acuminate, pilose abaxially, glabrous adaxially, semi-persistent. Leaves alternate, petiole 5–13 cm long, white pilose; lamina ovate to broadly ovate, basifixed, base cordate with lobes overlapping (rarely non-overlapping) 6–10 cm × 4–0 cm, asymmetric, adaxial surface dark green with dark red patches on the central part of lamina, densely puberulent pilose by long white hairs, abaxially pale green, pilose more prominent on veins, venation palmate, veins 7–9;

margin crenate to serrate, ciliate; apex acuminate. Inflorescence axillary, cymose, sometimes subtended with a pair of leaves, few; peduncles 3–11 cm × 0.2–0.3 cm, pilose, with 1–3 female flowers and 1–3 male flowers; bracts 1–1.3 × 0.5–0.7 cm, lanceolate to ovate-lanceolate, reflexed. Male flower: pedicel 1.5–2 cm long, pilose; tepals 4, inner 2.1–2.5 × 0.8–1.3 cm, outer 2.2–2.6 × 1.6–2.4 cm, inner and outer light pink to dark pink, pilose adaxially, glabrous abaxially; outer tepals orbicular to ovate; inner tepals lanceolate-ovate; androecium with 70–80 stamens, symmetric; filaments c. 0.1 cm, anther oblong elliptic-obovate, c. 0.2 cm long, connective slightly extended. Female flower: pedicel 1.5–2 cm long, pilose; tepals 5, imbricate unequal, orbicular-ovate, inner 2.1–2.5 × 0.8–1.3 cm, outer 2.2–2.6 × 1.6–2.4 cm, inner and outer light pink to dark pink (white), pilose adaxially, glabrous abaxially; ovary 2-locular, placentae bifid, styles 2, c. 0.2 cm long; stigma yellow coiling. Capsule: ellipsoid, 1–1.5 cm long, densely pilose, with one long round oblong wing and two short triangular wings; longest wing 0.5–1 cm long; side wings 0.2–0.5 cm long; wings extending along the pedicel slightly.

Phenology: *B. markiana* flowers during late August to September and fruits during late September to November.

Etymology: The species epithet honors Mark Hughes (UK) who is one of the leading scholars untangling the knots of the genus *Begonia* in the Old World and his contribution to the knowledge of the genus in northeast India, especially.

Distribution and ecology: So far, *B. markiana* is only known from the Mayodia area in the Lower Dibang River District in Arunachal Pradesh, India. It grows by stream sides in large patches, hanging from vertical rocks.

Additional specimens examined: INDIA. Arunachal Pradesh, Lower Dibang Valley District, Mayodia, 2000 m, 24 November 2018, *D. Borah* 18096 (ASSAM).

Conservation status: Vulnerable (VU) (IUCN 2019). The species was only found on Mayodia pass along the road and seems not to be present in any herbaria. North of Mayodia the road is poor, and several road constructions are ongoing with devastating results for the environment.

Notes: *Begonia markiana* shares similarities with *B. annulata* but the latter has variegation by banding parallel with the margin, shorter and denser hairs on the lamina and peduncle and larger flowers. Another similar species is *Begonia thomsonii* with a unicoloured lamina with long, red strigose hairs. All three species belong to section *Platycentrum* and have their distribution in northeast India.

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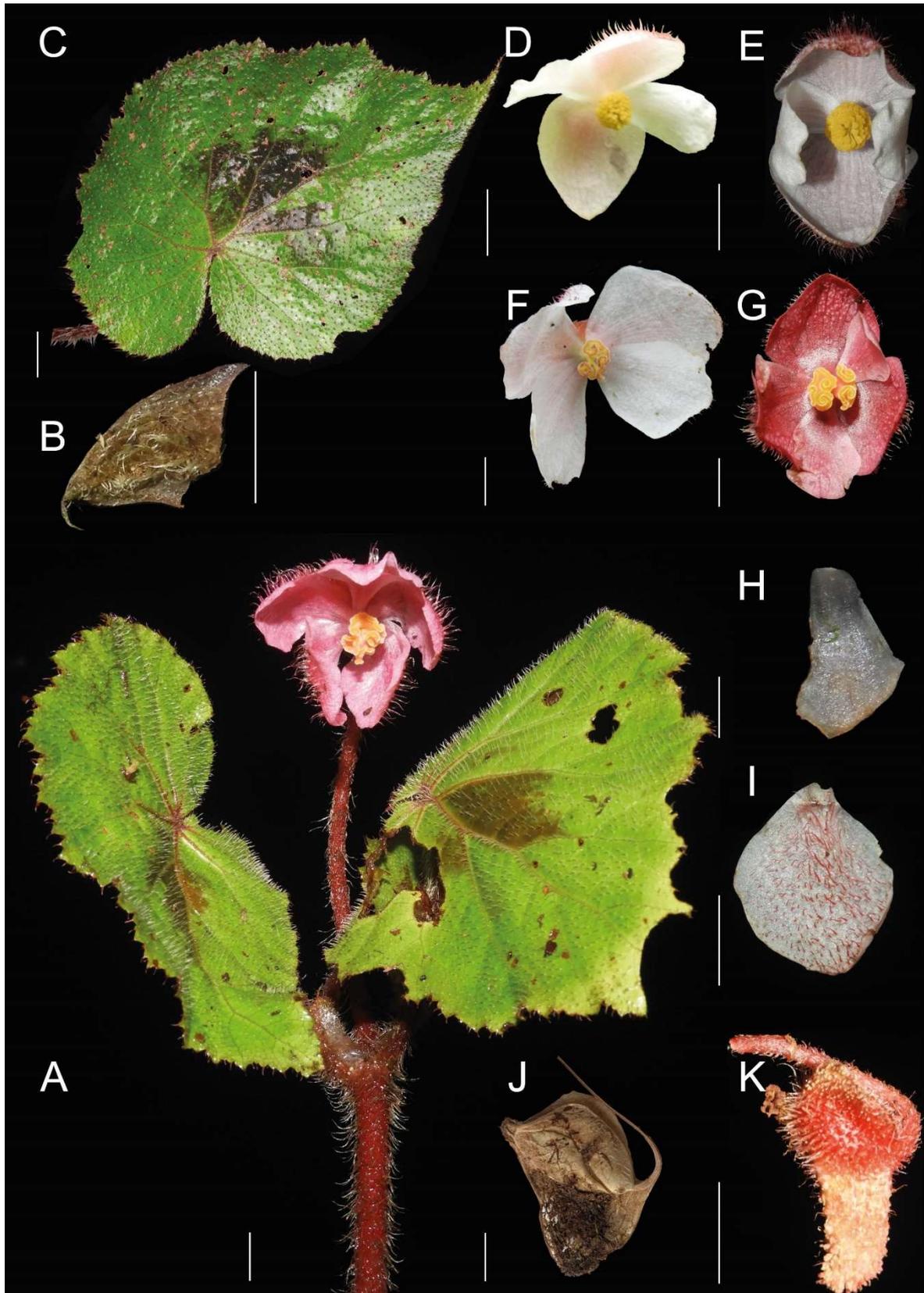


Fig. 5. *Begonia markiana* Taram, Wahlsteen & D.Borah. **A.** Habit. **B.** Stipule. **C.** Leaf. **D & E.** Male flowers. **F & G.** Female flowers. **H.** Inner tepal. **I.** Outer tepal. **J.** Dry capsule. **K.** Fresh capsule. All scale bars indicate 1 cm. From *M. Taram & O. Taku 5442*.



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Appendix 1. GenBank accession numbers of downloaded sequences (*ndhA* intron, *ndhF-rpl32* spacer, *rpl32-trnL* spacer)

Begonia abdullahpieei MH207018, MH207427, MH207848; *B. aborensis* MH207019, MH207428, MH207849; *B. acetosella* JF756367, JF756451, JF756535; *B. acetosella* var. *hirtifolia* MW476744, MW476768, MW476792; *B. annulata* MW476747, MW476771, MW476795; *B. aptera* JF756369, JF75645, JF756537; *B. areolata* JF756366, JF756450, JF756534; *B. balansana* MH207051, MH207460, MH207876; *B. baviensis* MH20705, MH207464, MH207880; *B. cathcartii* MH207089, MH207499, MH207915; *B. ceratocarpa* MH207090, MH207500, MH207916; *B. decora* JF756355, JF756439, JF756523; *B. diadema* MH207129, MH207539, MH207952; *B. dicressine* MW476750, MW476774, MW476798; *B. difformis* MW476751, MW476775, MW476799; *B. dux* MH207137, MH207546, MH207960; *B. griffithiana* MH207172, MH207582, MH207998; *B. handelii* MH207176, MH207586, MH208002; *B. hatacoa* JF756354, JF756438, JF756522; *B. koksunii* MH207214, MH207624, MH208038; *B. limprichtii* MH207223, MH207633, MH208047; *B. longifolia* JF756368, JF756452, JF756536; *B. mariachristinae* MW476753, MW476777, MW476801; *B. markiana* OK624680, OK624681, OK624682; *B. nepalensis* MH207257, MH207669, MH208079; *B. obovoidea* JF756386, JF756470, JF756554; *B. palmata* India MW476754, MW476778, MW476802; Myanmar MW476755, MW476779, MW476803; China JF756360, JF756444, JF756528; *B. pavonina* JF756356, JF756440, JF756524; *B. pedatifida* MH207288, MH207700, MH208105; *B. perakensis* MH207291, MH207704, MH208108; *B. pseudoheydei* MW476764, MW476788, MW476812; *B. rhoephila* MH207331, MH207745, MH208143; *B. robusta* JF756363, JF756447, JF756531; *B. roxburghii* JF756371, JF756455, JF756539; *B. samhaensis* JF756339, JF756423, JF756507; *B. sikkimensis* JF756359, JF756443, JF756527; *B. silletensis* India MW476759, MW476783, MW476807, China JF756370, JF756454, JF756538; *B. sizemoreae* JF756361, JF756445, JF756529; *B. thomsonii* MH207398, MH207818, MH208210; *B. venusta* JF756357, JF756441, JF756525; *B. versicolor* JF756358, JF756442, JF756526.