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Three new species of *Impatiens* (Balsaminaceae) from Myanmar

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Abstract

Three new species of *Impatiens* (Balsaminaceae) from Myanmar are here described: *I. decurva* Ruchis. & S.B. Janssens, *I. hartnolliae* Hook. f. ex Ruchis. & Suksathan, and *I. oblongata* Ruchis. &Van der Niet. The 5-lobed short fusiform fruit of all three species suggests that they are members of subgen. *Impatiens* sect. *Uniflorae* Hook. f. & Thomson. For *I. decurva* and *I. oblongata*, subgenus membership was corroborated by phylogenetic analyses of a combined dataset of nuclear ITS and plastid *atpB-rbcL* intergenic spacer DNA sequences. This was not possible for *I. hartnolliae*, which is only known from a single herbarium specimen.

Key words: *Impatiens*, Myanmar, Southeast Asia, taxonomy, *Uniflorae*

Introduction

Impatiens L. (Linnaeus 1753: 937), comprising almost the entire family of Balsaminaceae, is a highly diverse genus with more than 1000 species distributed in tropical and subtropical Africa and Eurasia (Grey-Wilson 1980). A recent infrageneric classification based on molecular and morphological characters supports a subdivision of the genus into two subgenera: subgen. *Clavicarpa* S.X. Yu ex S.X. Yu & Wei Wang (Yu *et al*. 2015: 13) and subgen. *Impatiens* L. (Yu *et al*. 2015: 13). The latter is further divided into seven sections (Yu *et al*. 2015). Of these, sect. *Uniflorae* Hook. f. & Thomson (1860: 113) is characterized by a 5-locular ovary and a short fusiform capsule (Yu *et al*. 2015).

The known diversity of *Impatiens* is strongly influenced by efforts resulting from taxonomic studies using dried and fresh specimens. Consequently, some geographic regions are better explored than others. Myanmar is the largest country in continental Southeast Asia and recent explorations have revealed a relatively rich biodiversity (Kress *et al*. 2003). Currently 50 *Impatiens* species are known from the country (Hooker 1905, Toppin 1920, Comber 1934, Grey-Wilson 1989, Kress *et al*. 2003, Tanaka *et al*. 2015, Ruchisansakun *et al*. 2017, Yang *et al*. 2017). As part of an ongoing project to revise the Balsaminaceae of Myanmar, a field expedition in the country conducted between July and December 2015 as well as analyses of herbarium specimens and relevant literature was conducted. Based on these studies, we discovered three new species, all of which are described here. We place the species within the infrageneric classification of Yu *et al*. (2015) by using comparative morphology and molecular phylogenetics.

Methods

Comparative morphology

Morphological characters of *Impatiens decurva* and *I. oblongata* were examined from living material in the field. The morphology of *I. hartnolliae* was studied from the single known herbarium specimen (at K). A set of morphologically

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similar species was selected for comparative analyses. These species were studied from herbarium specimens from AAU, BR, BK, BKF, BM, C, E, K, L, MAND, P, QBG, RAF, and RANG, and from their descriptions in the literature (Wight & Arnott 1834, Hooker & Thomson 1860, Hooker 1875, 1905, Ridley 1914, Toppin 1920). Terminology of morphological characters follows that of Grey-Wilson (1980).

Phylogenetic analyses

To determine the phylogenetic relationships of *Impatiens decurva* and *I. oblongata*, DNA sequence data were obtained and analyzed under the Bayesian Inference methodology. A combined dataset, consisting of nuclear ITS and plastid *atpB-rbcL* sequences was analysed. *Impatiens decurva* (*S. Ruchisansakun* & *Makino BG Exped. 734* (L), ITS: GenBank accession MF979085, *atpB-rbcL*: MF979082), *I. oblongata* (*S. Ruchisansakun* & *Makino BG Exped. 735* (L), MF979086, *atpB-rbcL*: MF979083), and *I. florulenta* (*S. Ruchisansakun* & *Makino BG Exped. 736* (L), ITS: MF979087, *atpB-rbcL*: MF979084) were added to the dataset of Ruchisansakun *et al*. (2017). DNA extraction and PCR amplification were conducted as in Janssens *et al.* (2006). Sequences were added to the matrix and manually aligned. Bayesian phylogenetic analyses were conducted in accordance with the protocols provided in Ruchisansakun *et al*. (2017). Bayesian analysis of the combined dataset was run for 10,000,000 generations, starting from different random trees and sampled every 500 generations. The initial 25% of sampled trees were discarded as burn-in. A majority-rule consensus tree was constructed in MrBayes 3.2.2 on XSEDE on the Cipres portal (Ronquist *et al*. 2001).

Results and Discussion

Taxonomy

1. *Impatiens decurva* Ruchis. & S.B. Janssens, *sp. nov.* (Figs. 1, 2)

Impatiens decurva Ruchis. & S.B. Janssens is similar to *I. pendula* B. Heyne ex Wight & Arn. (1834: 136) but differs in having congested leaves towards the stem apex, a pilose midrib on the dorsal petal, pink lateral united petals with a white base, an unequally bilobed apex of the lower lateral united petals, and a strongly decurved pedicel in fruiting stage.

Type:—MYANMAR. Shan State: Kalaw, 20°39’21.92”N 96°34’55.89”E, 27 Sep. 2015, *S. Ruchisansakun* & *Makino BG Exped.734* (holotype L!, isotypes L!, RAF!, RANG!).

Lithophytic annual *herb* 6–30 cm tall. *Stem* erect, 1–3 mm in diam., cylindrical, simple to moderately branched, red, glabrous. *Leaves* spirally arranged, mostly crowded towards the apex of stem. *Petiole* absent or to 7 mm long, ca. 1 mm in diam., pale green to green to pink, glabrous. *Lamina* 10–40 × 5–15 mm, ovate to elliptic to obovate, apex acute to acuminate, base cuneate to attenuate, margin shallowly serrate, adaxial surface green, margin pilose, abaxial surface pale green and glabrous, with 3–5 long red hairs along the margin near the base; lateral veins 3–5 pairs, adaxially pilose. *Flowers* solitary, axillary, erect, 10–11 × 8–10 mm, 6–8 mm deep, pale pink and white, with two yellow spots at center. *Bracts* <1 × <1 mm, linear to narrowly lanceolate, the apex acute and mucronate, base cuneate, green with red apex, persistent. *Pedicel* 10–15 mm long, <1 mm in diam., pale green, pilose. *Lateral sepals* 2, <1 × 1 mm, free, ovate to lanceolate, apex acute, base obtuse, pale green with red tip, glabrous. *Lower sepal* 4–5 × 2.5–3 mm, ca. 2 mm deep, navicular, apex acuminate and mucronate, white with red tip, pilose outside, spurless. *Dorsal petal* 4–5 × 5–5.5 mm, broadly ovate, cucullate, apex round and slightly mucronate, base truncate to shallowly cordate, white, mostly glabrous but pilose on midrib, abaxial midvein simple or with narrow crest, ca. 1 mm wide, green. *Lateral united petals* 7–9 mm long, free: upper petals 4.5–5 × 2.5–3 mm, ovate, apex round, base cuneate, white to pale pink; the lower petals 6–7 × 3.5–4 mm, free, elliptic to obovate, apex unequally bilobed, pink, each with a yellow spot at base. *Stamens* 5: filaments ca. 2.5 mm long, white to pale pink; anthers pale pink. *Ovary* 2 mm long, <1 mm in diam., 5-carpellate, green, pilose. *Fruits* 8–10 mm long, 2.5–3 mm in diam., short fusiform, 5–lobed, green, pilose; pedicel strongly decurved in middle during fruiting stage. *Seeds* ca. 2 mm long, ovoid, 9–11 per fruit, brown.

Phenology:—Flowering from September to October; fruiting in October.

Distribution:—Endemic to Myanmar (Shan State).

Ecology:—Growing in limestone soils on a mountain summit in open, fragmented evergreen forest, 1500–1600 m elevation.

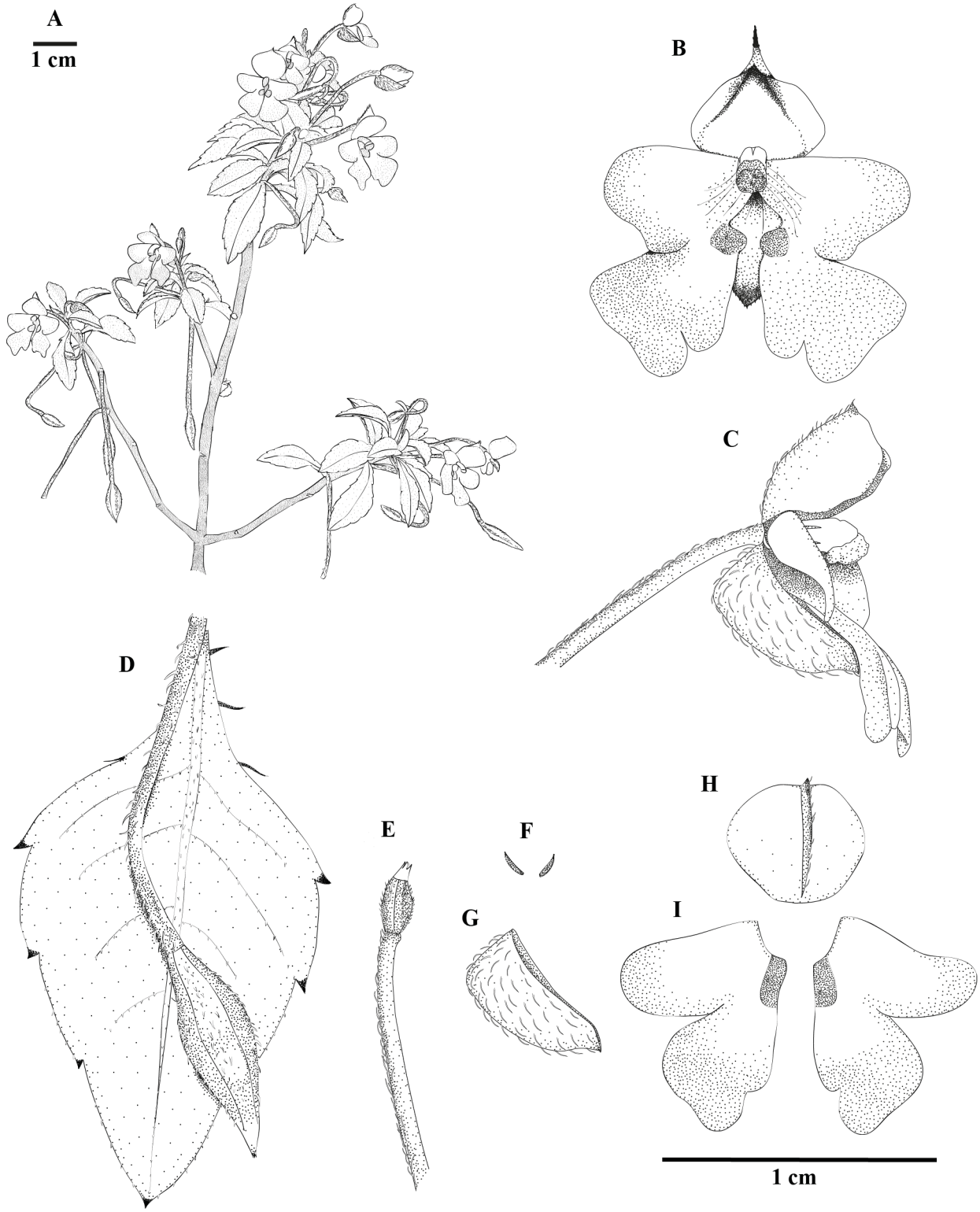

FIGURE 1. *Impatiens decurva*. **A.** Habit; **B.** Front view of flower; **C.** Lateral view of flower; **D.** Fruit; **E.** Pedicel and ovary; **F.** Lateral sepals; **G.** Lower sepal; **H.** Dorsal petal; **I.** Lateral united petals. Drawn by Saroj Ruchisansakun.

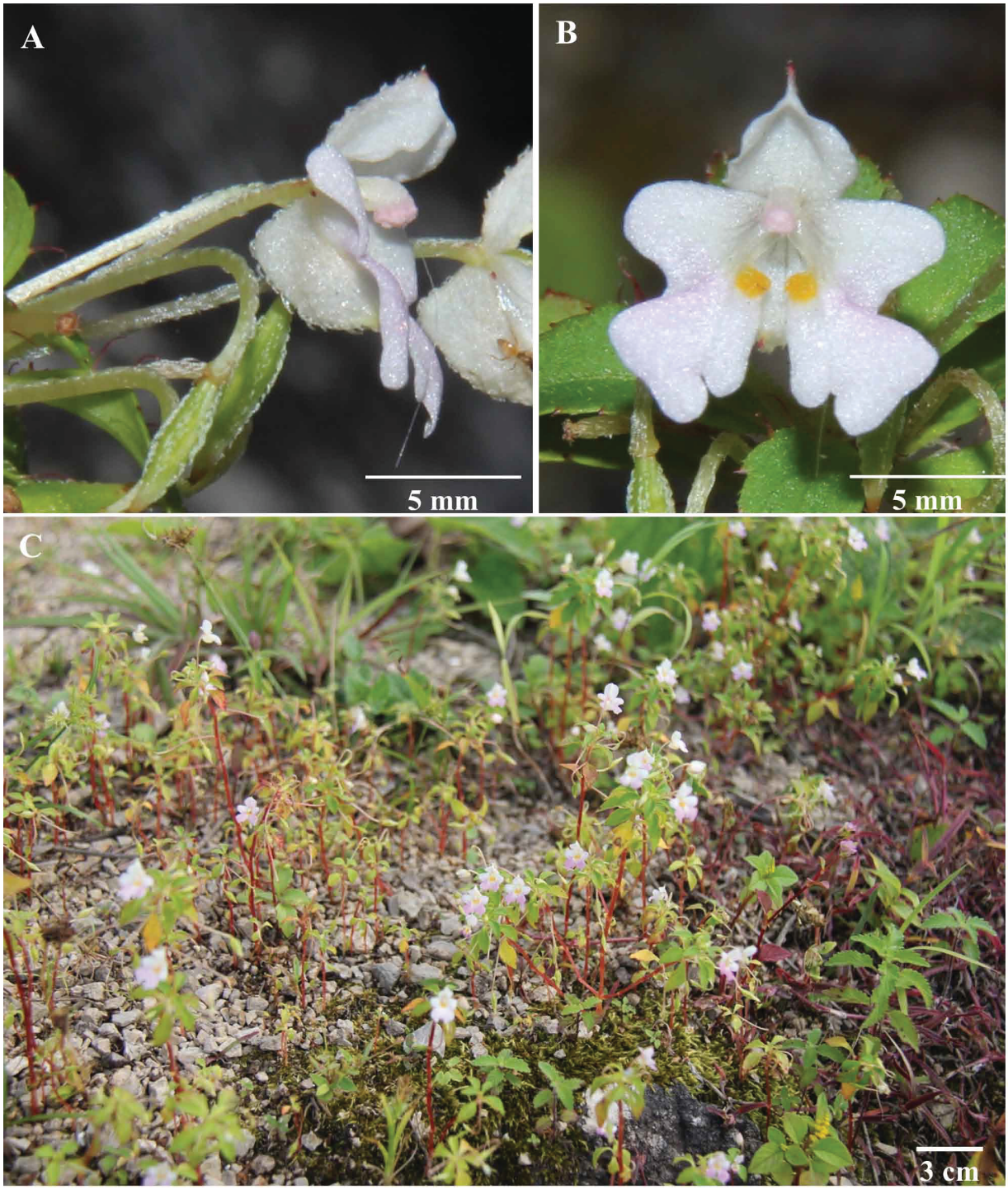

FIGURE 2. *Impatiens decurva*. **A.** Lateral view of flower; **B.** Front view of flower; **C.** Habit *in situ*. Photographs by Saroj Ruchisansakun.

Proposed IUCN conservation assessment:—Critically Endangered B1ab (i, ii, iii) + 2ab (i, ii, iii). This species is only known from the type locality. The extent of occurrence is estimated as < 5 km, where it occurs as a small population (IUCN 2012).

Etymology:—The specific epithet refers to the decurved pedicel at the early fruiting stage.

Note:—*Impatiens decurva* Ruchis. & S.B. Janssens is the only spurless species in Myanmar with solitary flowers and spirally arranged leaves. Although it morphologically resembles *I. pendula*, *I. decurva* possesses a distinct characters: the leaves clustered towards the stem apex, a pilose midrib on the dorsal petal, the apex of the lower lateral united petals unequally bilobed, and a pedicel that is strongly decurved from the middle in the early fruiting stage. *Impatiens decurva* resembles *I. muscicola* Craib (1926: 162) in morphology. *Impatiens muscicola* is a species endemic

to northern Thailand, and differs from *I. decurva* in having lower lateral petals with an unequally bilobed apex and a pedicel that is strongly decurved in the middle during the fruiting stage.

Phylogenetic analysis:—Bayesian phylogenetic analyses of the combined ITS and *atpB-rbcL* dataset confirm its position within subgen. *Impatiens* sect. *Uniflorae* Hook. f. & Thomson (1860: 113) (Yu *et al*. 2015) (Fig. 3). Despite the morphological similarity with *I. muscicola* Craib, *I. decurva* appears more closely related to the Myanmar species *I. florulenta* Hook. f. (1905: 25 & 32), a long-spurred species distributed in the same area.

Pollination ecology:—We predict that *I. decurva* relies on autonomous self-pollination. This prediction is based on the strong similarity in floral features, including flower size, between *I. decurva* and *I. muscicola*. For the latter species autonomous self-pollination was experimentally confirmed (Ruchisansakun *et al*. 2016).

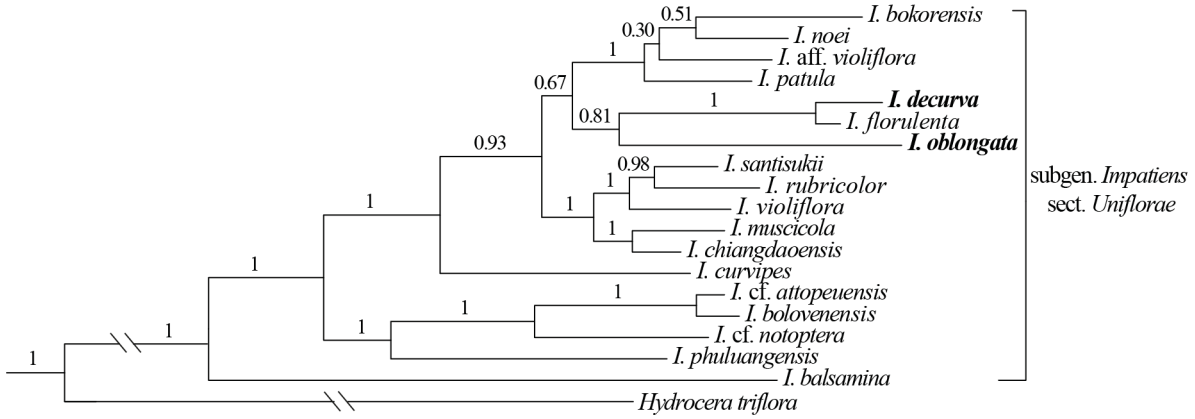

FIGURE 3. Majority-rule consensus tree from Bayesian phylogenetic analyses of a combined dataset of nuclear ITS and plastid *atpB-rbcL* DNA sequences. *Impatiens decurva and I. oblongata* are highlighted in bold font. Bayesian posterior probabilities are indicated at each node.

TABLE 1. Comparison of morphological characters in *Impatiens decurva*, *I. muscicola*, and *I. pendula.*

Characters	*I. decurva*	*I. pendula*	*I. muscicola*
Leaves	congested at stem apex	scattered along the stem for most of its length	congested at stem apex
Dorsal petal	glabrous but pilose along the midrib	glabrous	densely pilose
Lateral united petals	pink with white base	white with red base	white with red base
Apex of lower lateral united petals	unequally bilobed	round	round to unequally bilobed
Anthers	pink	white	white
Pedicels at fruiting stage	strongly decurved at middle when young	deflexed at base, further portion straight	straight to slightly curved

2. *Impatiens hartnolliae* Hook. f. ex Ruchis. & Suksathan, *sp. nov.* (Figs. 4, 5)

Impatiens hartnolliae Hook. f. ex Ruchis. & Suksathan is most similar to *I. allanii* Hook. f. (Ridley 1914: 325) but can be distinguished by its possession of a large orbicular sepal and large upper lateral petals.

Type:—MYANMAR. Rakhine State (Arakan): Akyab, Urittaung Pagoda, 5 Sep. 1907, *H.S. Hartnoll* s.n. (holotype K000694663!).

Terrestrial annual *herb* to 36 cm tall. *Stem* erect, ca 12 mm in diam., richly branched, glabrous. *Leaves* spirally arranged. *Petiole* 6–12 mm long, ca. 1 mm in diam., glabrous. *Lamina* 110–120 × 45–50 mm, ovate to elliptic, the apex acute to acuminate, base round to obtuse to cuneate, margin crenate to serrate, adaxial surface glabrous to sparsely pilose, abaxial surface glabrous with 2–3 pairs of long hairs along the margin near base; lateral veins 8–9 pairs. *Inflorescence* subterminal, erect, 6–7-flowered racemes. *Peduncle* 10–15 mm long, ca. 1 mm in diam., glabrous. *Rachis* 7–14 mm long, ca. 1 mm in diam. *Flowers* ca. 18 mm long, ca. 15 mm wide, ca. 23 mm deep. *Pedicel* 9–10 mm long, ca. 1 mm in diam., glabrous. Bracts 2.5–3.5 × 1.5–2 mm, ovate, apex acute to mucronate, base round, margin entire, glabrous, persistent. *Lateral sepals* 2, 6.2–6.6 × 7.5–8 mm, free, orbicular to broadly elliptic, apex round to mucronate, base round, glabrous. *Lower sepal* ca. 10 mm long, ca. 8 mm deep, navicular, apex acuminate to mucronate, glabrous, distal part gradually tapering into a straight or curved spur, 15–17 mm long. *Dorsal petal* ca. 6.5 × 8 mm, broadly obovate, the apex emarginate, base obtuse, truncate, glabrous, abaxial midvein simple or with a narrow crest. *Lateral united petals* ca. 15.5 mm long, free: upper petals 9–10 × 5–5.5 mm, obliquely broadly oblong, apex truncate and slightly emarginate, base cuneate; the lower petals 8.5–9× 3.5–4 mm, free, oblong, apex truncate, without auricle. *Stamens* 5: filaments 4–5 mm long; anthers obtuse. *Ovary* ca. 3.5 mm long, ca. 1 mm in diam., glabrous. *Fruits* short fusiform, glabrous, 5-lobed. *Seeds* ellipsoid, ca. 1.6–2 mm long, pilose.

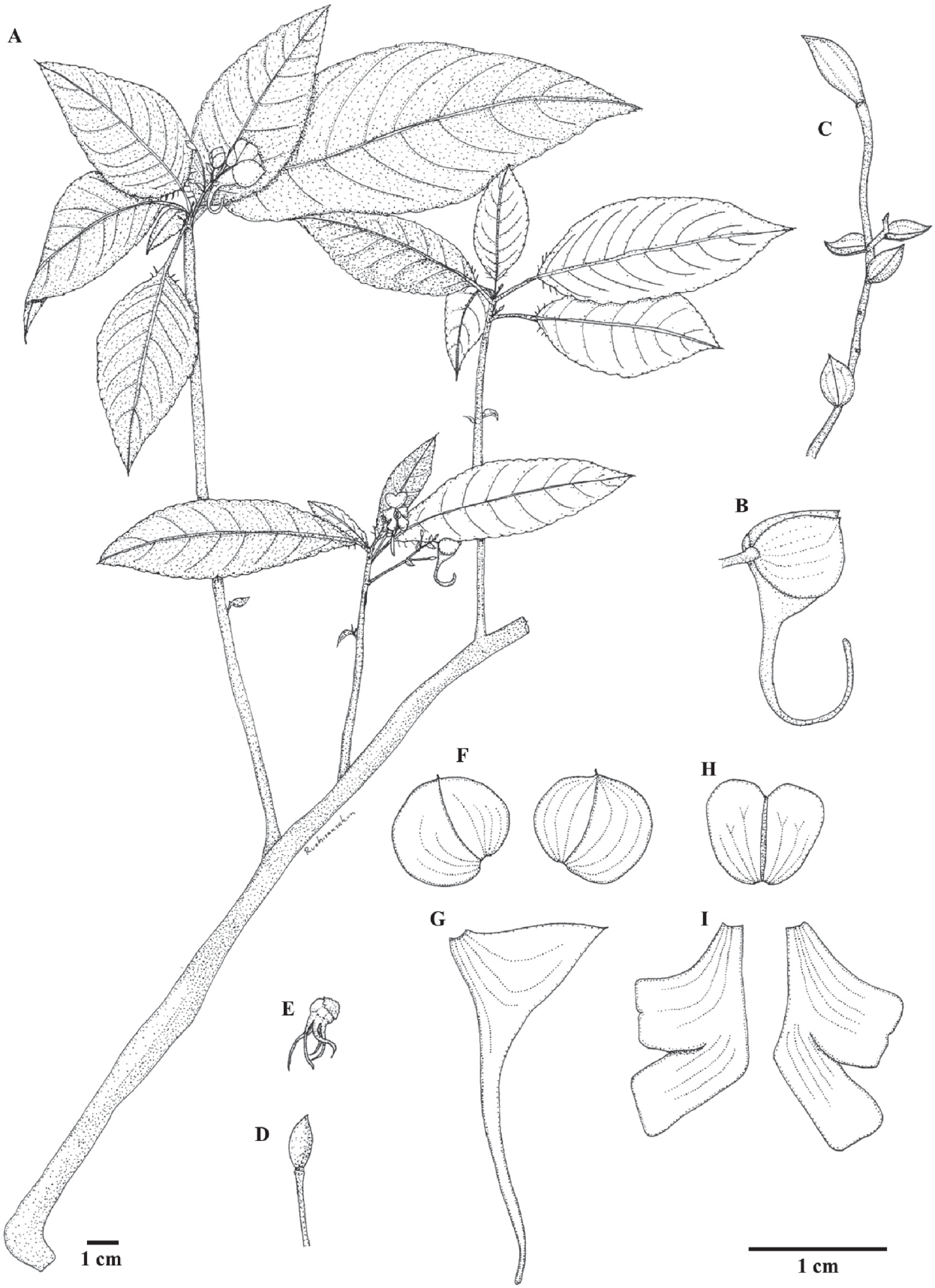

FIGURE 4. *Impatiens hartnolliae.* A. Habit; B. Immature flower; C. Immature fruit; D. Ovary; E. Stamen; F. Lateral sepals; G. Lower sepals; H. Dorsal petal; I. Lateral united petals. Drawn by Saroj Ruchisansakun.

FIGURE 5. The type specimen of *Impatiens hartnolliae*. http://specimens.kew.org/herbarium/K001097654.

Phenology:—Flowering and fruiting in September.

Distribution:—Endemic to Myanmar (Rakhine state).

Ecology:—Growing in limestone soils.

Proposed IUCN conservation assessment:—Critically Endangered B1ab (i, ii, iii) + 2ab (i, ii, iii). This species is known from only one specimen from the type locality (IUCN 2012).

Etymology:—The specific epithet is derived from the collector name, H.S. Hartnoll.

Notes:—*Impatiens hartnolliae* was written by J.D. Hooker on a single specimen sheet kept at Kew but was not validly published as according to Art. 30.1 (McNeill *et al.* 2012). To recognize Hooker's work on *Impatiens*, we used the initial name provided by him to name this new species. The species can be easily distinguished from other species in having a racemose inflorescence, truncate lateral united petals, an emarginate dorsal petal, and a fusiform fruit.

Pollination ecology:—We have not observed living plants of this species. However, based on the possession of a long, broad spur, we predict that this species is pollinated by both bees and butterflies (cf. Ruchisansakun *et al*. 2016).

TABLE 2. Comparison of morphological characters of *Impatiens hartnolliae* and *I. allanii*.

Characters	*I. hartnolliae*	*I. allanii*
Leaf arrangement	spiral	opposite, decussate
Lateral sepals	orbicular	elliptic to ovate
Upper lateral united petals	9–10 × 5–5.5 mm, obliquely broadly oblong	1.5–6.5 × 1–4 mm, falcate to obliquely ovate
Lower lateral united petals	8.5–9× 3.5–4 mm, oblong	24–32 × 14–20 mm, ovate to elliptic

3. ***Impatiens oblongata*** Ruchis. & Van der Niet, *sp. nov.* (Figs. 6, 7)

Impatiens oblongata Ruchis. & Van der Niet is most similar to *I. patula* Craib (1926: 164) but can be distinguished by a distinctly shorter spur, broadly oblong upper lateral united petals with a truncate to slightly emarginated apex, and the apex of the lower lateral united petals truncate to slightly bilobed.

Type:—MYANMAR. Shan State: Kalaw, 20°39'24"N, 96°34'96"E, 1569 m elevation, 27 Sep. 2015, *S. Ruchisansakun & Makino BG Exped. 735* (holotype L2071128!, isotypes L2071129, L2071130, L2071131!, RAF!, RANG!).

Terrestrial annual *herb* 30–50 cm tall. *Stem* erect, 1–4 mm in diam., angular, simple, or moderately to richly branched, red, mostly glabrous except sparsely pilose towards apex. *Leaves* spirally arranged. *Petiole* 3–10 mm long, ca. 1 mm in diam., pale green to pink, pilose. *Lamina* 50–75 × 10–20 mm, lanceolate to narrowly ovate, apex acute, base cuneate to attenuate, margin serrate, adaxial green, abaxial pale green, pilose on both sides, with 3–5 long red hairs along the margin near base; lateral veins 6–7 pairs. *Flowers* solitary, axillary, erect, 19–20 × 16–18 mm, 14–17 mm deep, pink, with two small dark pink dots and two small yellow dots at center. *Bracts* ca. 2 mm × <1 mm, linear, apex acute, base cuneate, green with red apex, pilose, persistent. *Pedicel* 18–20 mm long, less 1 mm in diam., pink, pilose. *Lateral sepals* 2-4: upper pair ca. 2 × <1 mm, sometimes absent, linear to oblong, apex acute, base cuneate, pale green with red tip, pilose; lower pair 1.5–2 mm × 1.5–2 mm, ovate, apex acuminate, base obtuse, pink, glabrous. *Lower sepal* 5–6 × 3–4 mm, 3–4 mm deep, navicular, apex acuminate and mucronate, pale pink with dark pink mark near base, pilose outside, distal part abruptly constricted into a straight or curved spur, 8–12 mm long, pink with dark pink tip. *Dorsal petal* 5–6 × 6–9 mm, broadly obovate, flat, apex truncate and stipitate, to 2 mm long, base truncate, pink with green stipitate tip, glabrous with pilose midrib and tip, abaxial midvein with an acute appendage, <1 mm tall, pink or green. *Lateral united petals* 12–14 mm long, free: upper petals 7–8 × 4–5 mm, broadly oblong, apex truncate to slightly emarginate, base cuneate, pink; lower petals 10–11 × 4–5 mm, free, elliptic to obovate, apex truncate to slightly bilobed, pink, each with a yellow dot and dark pink dot at base. *Stamens* 5: filaments ca. 2.5 mm long, pale pink; anthers pale pink. *Ovary* ca. 2 mm long, ca. 1 mm in diam., 5–carpellate, green, pilose. *Fruits* 15–20 mm long, 4–5 mm in diam., short fusiform, 5–lobed, green, pilose. *Seeds* 6–7, ca. 3 mm long, ovoid, brown.

Phenology:—Flowering from September to October; fruiting from September to October.

Distribution:—Endemic to northeastern Myanmar (Shan State), where it is known only from the type locality.

Ecology:—Growing in shady areas on a mountain summit in open fragmented evergreen forest, 1500–1600 m elevation.

Proposed IUCN conservation assessment:—Critically Endangered B1ab (i, ii, iii). This species is only known from a small population from a single locality. The extent of occurrence is estimated to be < 5 km (IUCN 2012).

Etymology:—The specific epithet is derived from its broadly oblong upper lateral petals.

Note:—This species usually has four lateral sepals, rarely two, in contrast to other similar species which have only two sepals, e.g., *I. patula*, *I. violiflora* Hook. f. (Hooker 1875: 457), *I. curvipes* Hook. f. (Hooker 1905: 25 & 32), and *I. florulenta*.

Phylogenetic analysis:—The results suggest that this species is part of *Impatiens* subgen. *Impatiens* sect. *Uniflorae* (Yu *et al*. 2015) (Fig. 7) and closely related to the Burmese species *I. florulenta* and *I. decurva* rather than to the species it resembles most closely in morphological characters, i.e., *I. patula* from Thailand.

Pollination ecology:—We did not observe any animals visiting the flowers of the species. However, based on the possession of a long, broad spur, we predict that this species is pollinated by both bees and butterflies (cf. Ruchisansakun *et al*. 2016).

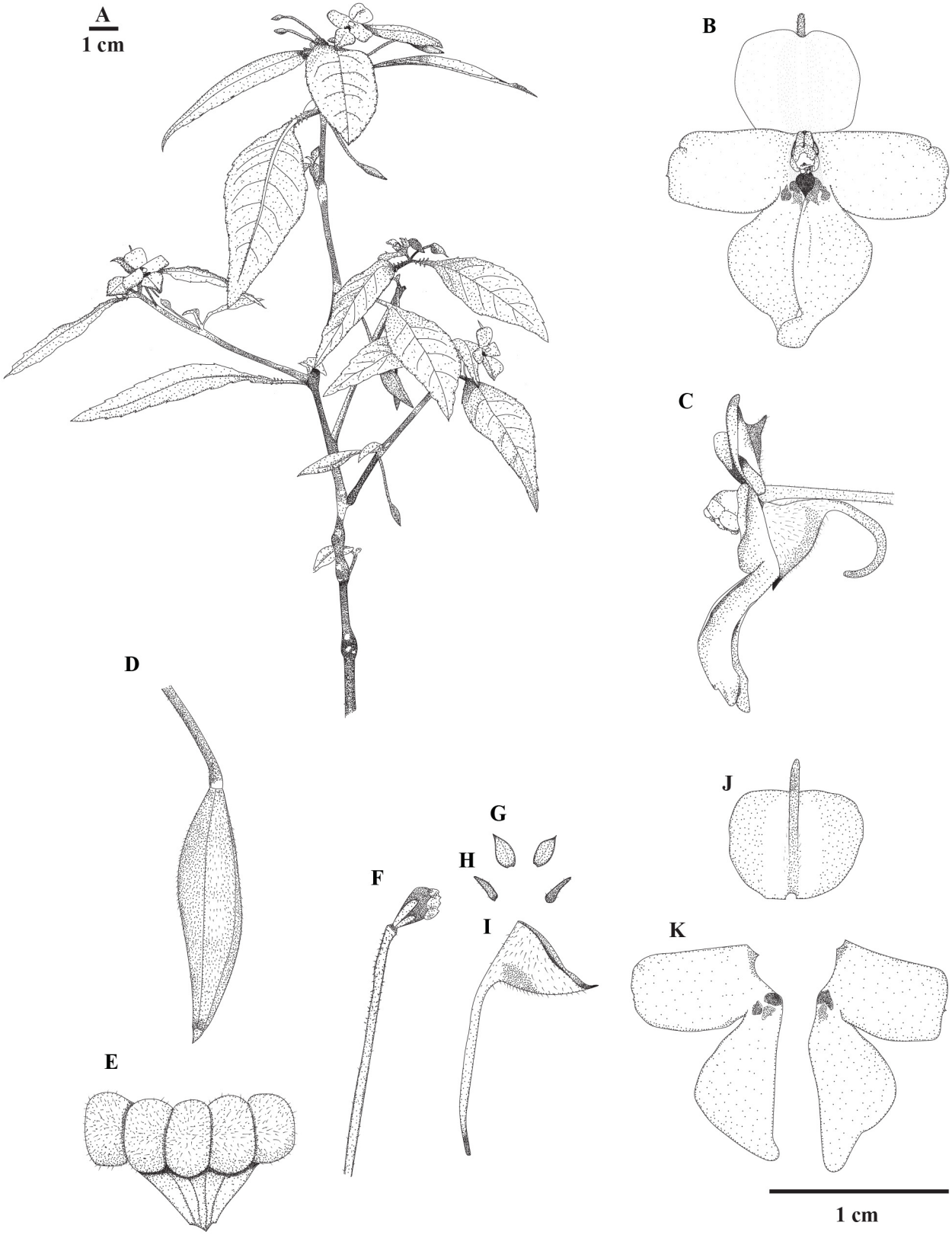

FIGURE 6. *Impatiens oblongata*. A. Habit; B. Front view of flower; C. Lateral view of flower; D, E. Fruit; F. Pedicel and Stamens; G. Upper lateral sepals; H. Lower lateral sepals; I. Lower sepal; J. Dorsal petal; K. Lateral united petals. Drawn by Saroj Ruchisansakun.

FIGURE 7. *Impatiens oblongata*. **A.** Lateral view of flower; **B.** Front view of flower; **C.** Habit *in situ*. Photographs by Saroj Ruchisansakun.

TABLE 3. Comparison of morphological characters of *I. oblongata* and *I. patula*.

Characters	*I. oblongata*	*I. patula*
Lateral sepals	(2) 4	2
Spur length	8–12 mm	20–30 mm
Upper lateral united petals	broadly oblong with truncate to slightly emarginate apex	falcate with obtuse apex
Lower lateral united petals	truncate to slightly bilobed at apex	distinctly asymmetric bilobed at apex

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