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CHAPTER 3

***Impatiens tanintharyiensis* (Balsaminaceae), a new species from Southern Myanmar**

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3.1. ABSTRACT

Impatiens tanintharyiensis Ruchisansakun, Suksathan & Saw-Lwin from the Tanintharyi region of Southern Myanmar is described and illustrated as a new species. The presence of connate lateral united petals and a four-locular ovary, as well as results of molecular phylogenetic analyses of nuclear ITS and plastid *atpB-rbcL* spacer DNA sequences, suggest that the new

species is a member of *Impatiens* section *Semeiocardium* (Zoll.) S.X. Yu & Wei Wang. The new species is morphologically most similar to *I. spectabilis* Triboun & Suksathan, but can be distinguished by its asymmetric flowers, saccate-bucciniform lower sepal, and shorter, slightly incurved spur. Floral traits, including the presence of a large floral chamber with a wide entrance, are consistent with the bee-pollination syndrome in *Impatiens*. Since *I. tanintharyiensis* is only known from two small populations, its conservation status is assessed as Endangered.

3.2. INTRODUCTION

With more than 1000 species, *Impatiens* Linnaeus (1753: 937) is a species-rich genus in angiosperms. The genus is distributed in tropical and subtropical Africa and Eurasia (Grey-Wilson 1980). Within its range, five hotspots of diversity are recognized: tropical Africa, Madagascar, Southeast Asia, Southern India, and the Sino-Himalayan region (Yuan *et al.*, 2004).

Taxonomic research on *Impatiens* is ongoing and has resulted in an updated infrageneric classification (Yu *et al.*, 2016) and the discovery of many new species in the last decade (e.g. Fischer & Rahelivololona, 2007a, 2007b; Suksathan & Triboun, 2009; Pusalkar & Singh, 2010; Janssens *et al.*, 2010, 2011; Shui *et al.*, 2011; Bhaskar, 2012; Utami, 2012; Souvannakhoummane & Suksathan, 2015; Yu *et al.*, 2015). The availability of detailed regional taxonomic studies of *Impatiens* is unevenly distributed, and some areas have received remarkably little attention, despite their promising geography in relation to *Impatiens* diversity. For instance, studies on the *Impatiens* species of Myanmar, which is situated at the intersection of the Southeast Asian, Southern Indian, and Sino-Himalayan hotspots, are sparse. The most recent taxonomic treatments of *Impatiens* in Myanmar date back to Hooker (1904) and Toppin (1920). In comparison to the number of species that occur in neighbouring countries (i.e. India: ca. 202 species (Bhaskar, 2012;

Prabhukumaret *et al.*, 2015; Vivekananthan *et al.*, 1997), China: ca. 280 species (Guo *et al.*, 2016; Wang *et al.*, 2016), Thailand: ca. 100 species (Shimizu, 1970; 1977, 1991, 2000; Shimizu & Suksathan, 2004; Suksathan & Triboun, 2009; Suksathan pers. comm.; Ruchisansakun *et al.* 2014), the 47 currently recognized species in Myanmar is surprisingly low (Hooker, 1904; Toppin, 1920; Kress *et al.*, 2003; Tanaka *et al.*, 2015). During a field expedition by the first author in the Tanintharyi Nature Reserve, located in the Tenasserim Hills (Southern Myanmar), an unidentifiable *Impatiens* species was collected. Following studies of morphology and molecular phylogenetics, the results support the recognition of a new species, which is here described.

3.3. MATERIALS AND METHODS

3.3.1. Comparative morphology

The morphology of *Impatiens tanintharyiensis* was examined from living material in the field. Morphological characters of other species were studied from living plants, herbarium specimens deposited in several herbaria (AAU, BR, BK, BKF, BM, C, E, K, L, P, QBG, RAF, RANG), and relevant literature (Hooker, 1875, 1904; Toppin, 1920; Shimizu, 1969, 1970, 1977, 1991, 2000; Shimizu & Suksathan, 2004; Chen *et al.*, 2007; Suksathan & Triboun, 2009; Gogoi & Borah, 2013, 2014, 2015a, 2015b; Ruchisansakun *et al.*, 2014). The terminology of morphological characters follows Grey-Wilson (1980).

3.3.2. Phylogenetic analyses

To infer the phylogenetic affinities of the new species, nuclear ITS and plastid *atpB-rbcL* DNA sequences of *I. tanintharyiensis* (Ruchisansakun & Thet Yu Nwe 707 (L), KX354389, KX470395) were added to the datasets of Ruchisansakun *et al.* (2015). DNA extraction, PCR amplification, and sequence alignment were carried out as described by Ruchisansakun *et al.* (2015). Sequences of the new taxon were added to the existing matrix and manually aligned with it. To infer phylogenetic relationships, we used Bayesian Inference according to the protocols described in Ruchisansakun *et al.* (2015). Due to previously discovered topological incongruence between nuclear and plastid data partitions, ITS and *atpB-rbcL* datasets were analyzed separately (cf. Ruchisansakun *et al.*, 2015). For each dataset, Modeltest 3.06 (Posada & Crandall, 1998) was used to determine the

best models of sequence evolution for each locus using the Akaike Information Criterion (ITS: GTR + I + G, *atpB-rbcL*: GTR + G). Two simultaneously independent analyses were 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. Bayesian majority rule consensus trees of separate datasets were constructed by MrBayes 3b4 (Huelsenbeck & Ronquist, 2001).

3.4. RESULTS AND DISCUSSION

Taxonomy

Impatiens tanintharyiensis Ruchisansakun, Suksathan & Saw-Lwin, *sp. nov.* (Figs. 3.1, 3.2)

Impatiens tanintharyiensis Ruchisansakun, Suksathan & Saw-Lwin is morphologically similar to *I. spectabilis* Triboun & Suksathan but can be distinguished by having asymmetric flowers due to anticlockwise distorted lateral united petals, a saccate-bucciniform lower sepal, and a shorter, slightly incurved spur.

Type:—MYANMAR. Tanintharyi Region: Dawei, Thet Kal Kwet Village, Hawang falls, ca. 146 m elevation, 17 August 2015, *Ruchisansakun & Thet Yu Nwe 707* (holotype L!, isotypes L!, RAF!, RANG!).

Terrestrial to lithophytic, annual, glabrous *herb*, 15–25 cm high. *Stems* erect, cylindrical with small ridges near the nodes, 3–7 mm in diam., simple to sparsely branched, upper part slightly zig-zagged, purplish red with dark purple dots. *Leaves* spirally arranged; petioles 10–30 mm long, 1–1.5 mm in diam., green to red with dark red dots; laminae 40–65 mm × 15–25 mm, elliptic to ovate to lanceolate, apex acute, base obtuse to attenuate, margin shallowly serrate, adaxial green, abaxial pale green; lateral veins 4–5 on each side of midrib; extra-floral nectaries present as stalked short glands on each side of the base of the leaf margin. *Flowers* axillary, solitary, highly asymmetric due to anticlockwise distorted lateral united petals, 35–40 mm, purplish pink, centre white with two yellow marks. *Bracts* minute, ca. 2 × 1 mm, narrowly triangular, apex acute, base cuneate, green. *Pedicels* slender, 20–30 mm long, ca. 1 mm in diam., the same colour as stems. *Sepals* 3. *Lateral sepals* 2, 5–7 × 5–6 mm, ovate to broadly ovate, sometimes slightly oblique, apex acute to acuminate and mucronate, base obtuse to cordate,

purplish pink with green tips. *Lower sepal* 13–16 mm long, 8–10 mm wide, 14–15 mm deep, saccate-bucciniform, purplish pink outside, whitish inside with dark pink veins and yellow marks at the throat, distal part gradually constricted into a long, slightly incurved spur, 19–22 mm long, pink with dark pink dots. *Petals* 5. *Dorsal petal* strongly reflexed, 12–13 × 15–17 mm, broadly ovate to broadly elliptic to broadly obovate, purplish pink with a green tip, apex cordate and mucronate, base cuneate with a basal triangular crest. *Lateral united petals* connate, asymmetric due to distorted lateral united petals; upper petals 12–15 × 18–22 mm, broadly depressed obovate, apex round to truncate, purplish pink with white base; lower petals connate, 23–30 × 9–13 mm, obovate in outline, anticlockwise distorted, apex round to shallowly bilobed, purplish pink, the base white with a yellow mark. *Stamens* 5; filaments ca. 4 mm long, flat, white; anthers white. *Ovary* ca. 4 mm long, 1 mm in diam., 4-carpellate, green, glabrous. *Fruits* loculicidal dehiscent capsules, 4-lobed, 15–17 mm long, ca. 3 mm in diam., clavate. *Seeds* ca. 2.5 mm long, 14–16 per capsule, brown.

Phenology:—Flowering period August–September.

Distribution:—Endemic to Southern Myanmar (Tanintharyi Region), only known from two localities.

Ecology:—Growing among decaying organic material on low granular metamorphic rock of granitic schist facies (Phongphat Prasong, pers. comm), along a waterfall at 146–155 m above sea level.

Common name:—Tanintharyi Dan Pan, Tanintharyi balsam.

Proposed IUCN conservation assessment:—Endangered (ENB2 ab(iii,v)) based on a preliminary risk of extinction assessment using the IUCN red list categories and criteria (IUCN, 2012). This species is only known from 2 localities, 2 km apart on the same mountain range. The Hawang Falls locality is under severe pressure from local tourism. Since there are fewer than 100 individuals at each locality, it is assumed that human activities in the region could lead them to rapid extinction (IUCN, 2012).

Etymology:— The specific epithet refers to its locality, the Tanintharyi region of Myanmar.

Additional specimens examined (paratypes):—MYANMAR. Tanintharyi division: Dawei, Thet Kal Kwet Village, Rachaung falls, 155 m above sea level, growing on rocks in a shady area along a waterfall, 17 August 2015, *Ruchisansakun & Thet Yu Nwe 708* (L, RAF, RANG).



Fig. 3.1. *Impatiens tanintharyiensis*. A. Lateral view of flower; B. Front view of flower; C. Habit in situ. Photographs by Saroj Ruchisansakun.

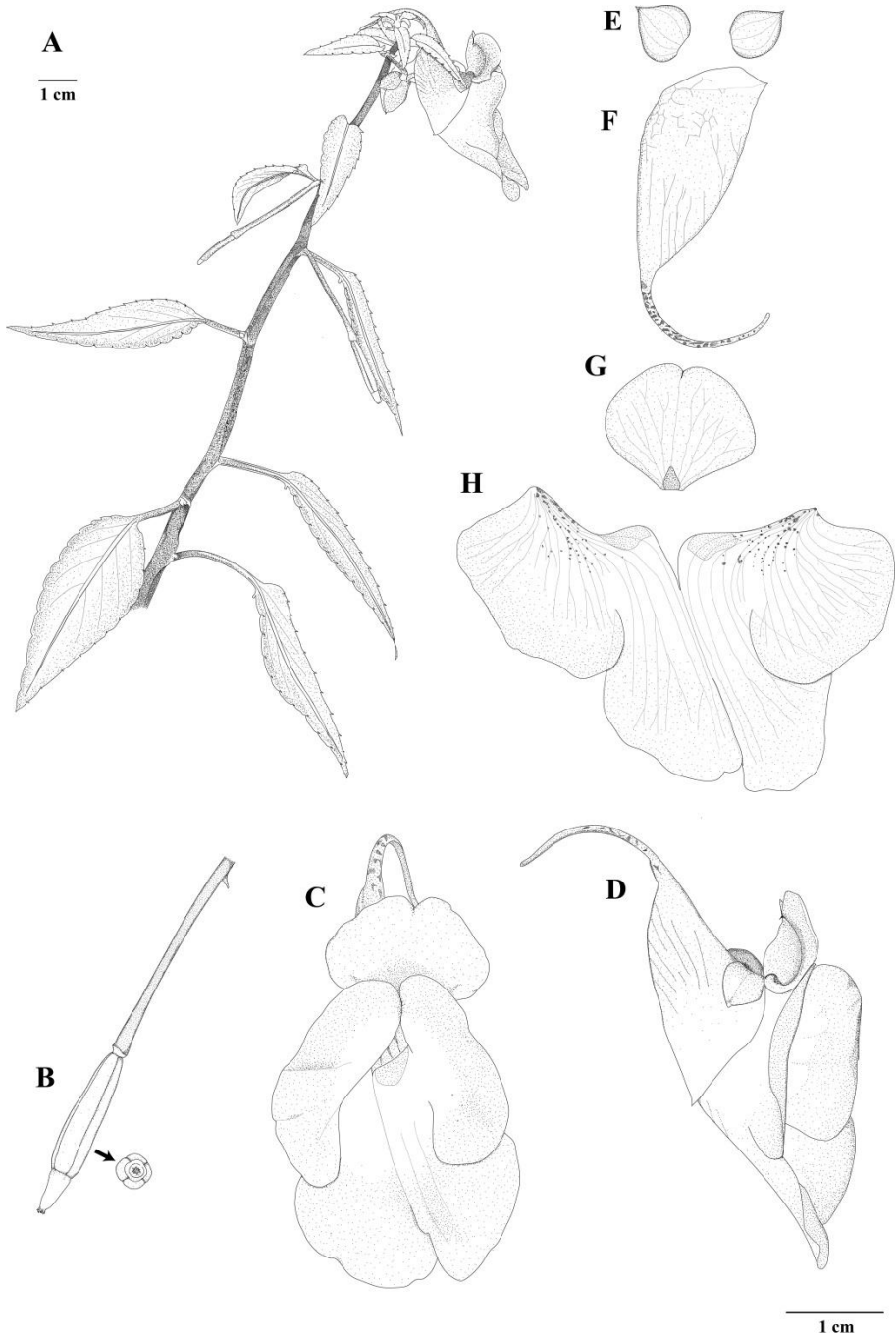


Fig. 3.2. *Impatiens tanintharyensis*. A. Habit; B. Fruit; C. Front view of flower; D. Lateral view of flower; E. Outer lateral sepals; F. Lower sepal; G. Dorsal petal; H. Lateral united petals. Drawn by Saroj Ruchisansakun.

Note:—Based on the presence of connate lateral united petals and a 4-locular ovary, *Impatiens tanintharyiensis* is a member of *Impatiens* sect. *Semeiocardium* (Zoll.) S.X. Yu & Wei Wang (2015: 13) (Ruchisansakun *et al.*, 2015; Yu *et al.*, 2015). The species can be distinguished from closely related species due to the presence of asymmetric flowers (caused by distorted lateral united petals), a saccate-bucciniform lower sepal, and a slightly incurved long spur. An additional comparison to its closest relatives is shown in Table 3.1.

Phylogenetic analysis:—Bayesian phylogenetic analyses of the ITS and *atpB-rbcL* datasets also recovered *I. tanintharyiensis* as part of *Impatiens* sect. *Semeiocardium* (Fig. 3.3), although some incongruence between the nuclear and plastid topologies was observed (cf. Janssens *et al.*, 2006; Ruchisansakun *et al.*, 2015). According to the majority rule consensus tree based on analysis of ITS sequences, the new species is strongly supported (Bayesian Posterior Probability (BPP): 1.0) as part of a clade in which it is sister to a clade containing *I. spectabilis* Triboun & Suksathan, *I. larsenii* T.Shimizu (1977: 33), *I. siamensis* T.Shimizu (1977: 34), *I. suksathanii* Ruchisansakun & Triboun (2014: 237), *I. nalampoontii* T.Shimizu

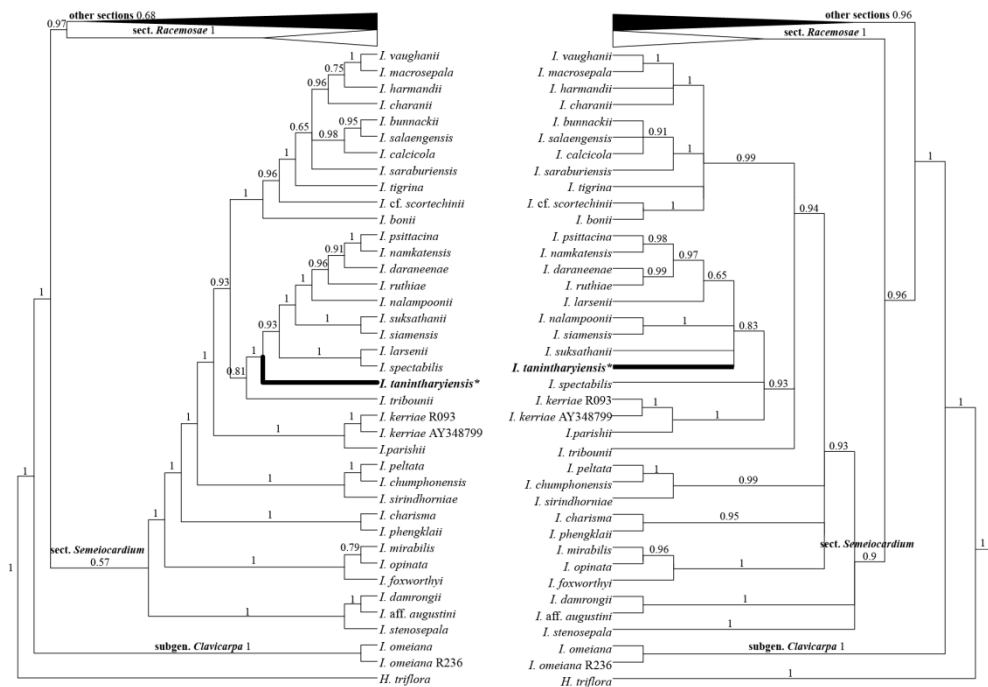


Fig. 3.3. Majority rule consensus trees from Bayesian analyses of nuclear ITS (left) and plastid *atpB-rbcL* (right) DNA sequences. *Impatiens tanintharyiensis* is highlighted in bold font and with an asterisk. Bayesian posterior probabilities are indicated at each node.

(1969: 39), *I. ruthiae* Suksathan & Triboun (2009: 172), *I. daraneenae* Suksathan & Triboun (2009: 164), *I. namkatensis* T.Shimizu (2000: 37), and *I. psittacina* Hook.f. (1901: 7809) (Fig. 3.3). In contrast, the majority rule consensus tree of the plastid sequences shows that *I. tanintharyiensis* is part of relatively weakly supported polytomy (BPP: 0.83) consisting of *I. suksathanii*, a clade containing *I. siamensis* and *I. nalampooinii*, and a clade containing *I. larsenii*, *I. ruthiae*, *I. daraneenae*, *I. namkatensis* and *I. psittacina*, but without *I. spectabilis* (Fig. 3.3). Topological incongruence between two gene trees, in particular those based on plastid versus nuclear loci, may be the result of ancient hybridization (Alvarez & Wendel, 2003; Wang *et al.*, 2016); yet, more data is required to understand the exact evolutionary history of the new species.

Pollination ecology:—A recent comparative study of floral morphology and pollination ecology demonstrated that the closely related and morphologically similar *I. daraneenae*, is pollinated by bees (Ruchisansakun *et al.*, 2016). The presence of a large floral chamber with a wide entrance are traits associated with bee pollination in Southeast Asian *Impatiens* (Ruchisansakun *et al.*, 2016). Based on the traits of the new species, we hypothesize that the new species is also bee-pollinated.

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Table 3.1. Comparison of floral morphological characters of *Impatiens tanintharyensis* to *I. spectabilis* and other closely related *Impatiens* species.

Characters	<i>I.tanintharyensis</i>	<i>I.spectabilis</i>	<i>I.daraveanae</i>	<i>I.larsenii</i>	<i>I.siamensis</i>	<i>I.saksathanii</i>	<i>I.nalampoontii</i>	<i>I.ruthiae</i>	<i>I.namkatensis</i>	<i>I.pstacina</i>
Plant height	15–25 cm	up to 40 cm	20–50 cm	ca. 2.5 cm	up to 3.5 cm	1.5–4.5 cm	30–40 cm	upto 50 cm	10–40 cm	20–30 cm
Floral symmetry	asymmetric	zygomorphic	asymmetric	asymmetric	zygomorphic	zygomorphic	zygomorphic	asymmetric	asymmetric	zygomorphic
Lateral sepal number	2	2(–4)	4	4	4	4	2	4	2	2
Lower sepal shape	saccate-bucciniform	shallowly navicular	saccate-bucciniform	saccate-bucciniform	shallowly navicular	saccate-bucciniform	saccate-bucciniform	saccate-bucciniform	saccate-bucciniform	saccate-bucciniform
Spur shape	long-slightly incurved	long-curved	incurved	upcurved	long-curved	incurved	incurved and coiled	short incurved	short incurved	incurved, hooked
Spur length	19–22 mm	45–48 mm	ca. 10 mm	2–3 mm	15–35 mm	3–5 mm	ca. 5 mm	ca. 5 mm	ca. 5 mm	ca. 5 mm
Upper petal shape	broadly obovate	obovate	obliquely ovate	elliptic to obovate	obovate	broadly triangular	obovate	obliquely ovate	obliquely ovate	broadly elliptic to broadly obovate
Lower petal apex	round to slightly bilobed	minutely apiculate	deeply bilobed	round	truncate	truncate to shallowly emarginate	round to slightly emarginate	emarginate	emarginate	truncate to emarginate