



Impatiens damingensis (Balsaminaceae), a new species from Guangxi, China

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Abstract

Impatiens damingensis S. X. Yu, C. Y. Xia & H. P. Deng, (Balsaminaceae) discovered in Guangxi, China, is described and illustrated here. This species is similar to *I. aquatilis* Hook.f., but differs from the latter by having blades that are ovate or ovate-lanceolate vs. lanceolate, ovate-lanceolate, or ovate-oblong; apices of bracts acute vs. glandular awned; apices of lateral sepals mucronulate vs. long mucronulate; and spur slender substraight or slightly incurved vs. incurved. Furthermore, molecular data as well as micro-morphological evidence under SEM (of pollens and seeds) also support the establishment of the new species.

Key words: Balsaminaceae, *Impatiens*, morphology, new species, phylogeny, SEM

Introduction

The genus *Impatiens* Linnaeus (1753: 937) (Balsaminaceae) is large, with over 1000 species distributed throughout much of tropical Africa, India, southwest Asia, southern China and Japan (Grey-Wilson, 1980; Fischer, 2004; Yu *et al.*, 2015). Although deemed ‘notorious’ for presenting difficulties in morphological differentiation (Hooker, 1908; Grey-Wilson, 1980), new species of *Impatiens* are discovered every year (e.g. Seong-Hyun Cho *et al.*, 2017; Tan *et al.*, 2015; Kuang *et al.*, 2014; Luo *et al.*, 2014; Hou *et al.*, 2011; Bi *et al.*, 2010; Yu *et al.*, 2009). Indeed, it appears that many species still remain to be discovered and cataloged in this prolific genus.

Five diversity hotspots for *Impatiens* have been recognized, namely tropical Africa, Madagascar, southern India and Sri Lanka, the eastern Himalayas, and southeast Asia (Song *et al.*, 2003; Yuan *et al.*, 2004). The latter two diversity centers, located in southwest China, account for more than 270 species of *Impatiens* (Yu, 2012; Chen, 2001; Chen *et al.*, 2007). Our lab has been involved in cataloging *Impatiens* species in China by conducting large numbers of field investigations in south and southwest China, resulting in the recording of several new species (Yu *et al.*, 2013; Guo *et al.*, 2016; Ding *et al.*, 2016). Here we describe another *Impatiens* species that we believe to be new to science, discovered during one such field trip in 2004 in the Guangxi Zhuang Autonomous Region (GZAR). After studying the micro-morphology of the pollen grains and seed coat of these specimens, and investigating the evolutionary relationship with known *Impatiens* species using molecular phylogenetics, we can say with confidence that these specimens belong to a species that is new to science.

Materials and Methods

Gross plant traits

Various morphological characters, such as leaf size and shape, inflorescence type, flower color, etc., were carefully observed and measured in the field. The specimens collected were then meticulously examined for line drawing upon returning to the lab. All these traits as well as the field records and photographs taken during the expedition were compared with those of similar species, based on FAA-fixed material and dried specimens from PE (Chinese National Herbarium, Institute of Botany, Chinese Academy of Sciences).

Pollen grains and seeds

Mature whole pollen grains and seeds collected from the field were observed directly and measured under an anatomical lens. They were then mounted on double-sided adhesive tape and coated with a layer of gold before being photographed by a camera-fitted HITACHI S-570 SEM. Morphological characters were described following Wang & Wang (1983) and Lu (1991) for pollen grains, and Liu *et al.* (2004), Lu & Chen (1991), and Song *et al.* (2005) for seeds. Expectancy values of seed size, including the maximum and minimum values, were calculated based on 20 pollen grains and 20 seeds. Samples of the new specimens were obtained from voucher specimens that were collected in our 2004 field exploration and conserved in PE, which was also the source for the sample from the related species (*I. aquatilis*).

Taxon sampling and DNA sequencing

DNA sequences for the ITS were obtained from GenBank for 148 species of *Impatiens* and three outgroup species: [*Hydrocera triflora*] (L.) Wight & Arnott (1834: 140) (Balsaminaceae), and [*Marcgravia umbellata*] Linnaeus (1753: 503) and [*Norantea guianensis*] Aublet (1775: 554) (Marcgraviaceae), based on the results of Yuan *et al.* (2004), Janssen *et al.* (2006) and Yu *et al.* (2015), were obtained from GenBank. The corresponding sequences for the proposed new species were generated in this study. Species and GenBank accession numbers are listed in Table S1.

DNA was extracted from silica gel-dried leaves using the CTAB protocol of Doyle and Doyle (1987), with some modifications. The primers and PCR protocol for the three components of the ITS were obtained from White *et al.* (1990). The PCR products were purified using a GFXTMPCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Sequencing reactions were carried out using an ABI Prism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), and the products were analyzed on an ABI3730xl automated DNA sequencer.

Phylogenetic analysis

Sequences were aligned using the default parameters in Clustal X v.1.83 (Thompson *et al.*, 1997) and further adjusted manually in BioEdit v.7.0 (Hall, 1999). Bayesian phylogenetic inference (BI) was done, using MrBayes v.3.0b4 (Ronquist & Huelsenbeck, 2003). The region (ITS) was assigned its own model of nucleotide substitution, as determined by the Akaike information criterion (AIC) in Modeltest v.3.06 (Posada & Crandall, 1998).

Results

Taxonomic treatment

Impatiens damingensis S. X. Yu, C. Y. Xia & H. P. Deng, *sp. nov.* Figs. 1, 2

Type. China. Guangxi: Wuming, Damingshan; shade and damp places, Alt. 1220m, 2004-09-02, S. X. Yu 3227 (Holotype, PE; Isotype, IBK), same place, 2002-07-09, Y-G Wei 0205 (IBK).

Diagnosis. Similar to *I. aquatilis* Hook.f. in having racemose inflorescences, two lateral sepals and ellipsoid seeds, but is different by way of having ovate or ovate-lanceolate blades, acute apices of bracts, mucronulate apices of lateral sepals, and a slender and slightly incurved spur. SEM results also reveal distinct differences in seedcoat and pollen grain micro-morphology when compared to *I. aquatilis*.



FIGURE 1. *Impatiens damingensis* S. X. Yu, C. Y. Xia & H. P. Deng. A. plant; B. bract; C. lateral sepal; D. vexillum; E. wings; F. labellum; G. filaments and anthers; H. ovary; I. capsule. Drawn by Y. B. Sun from S. X. Yu 3227.

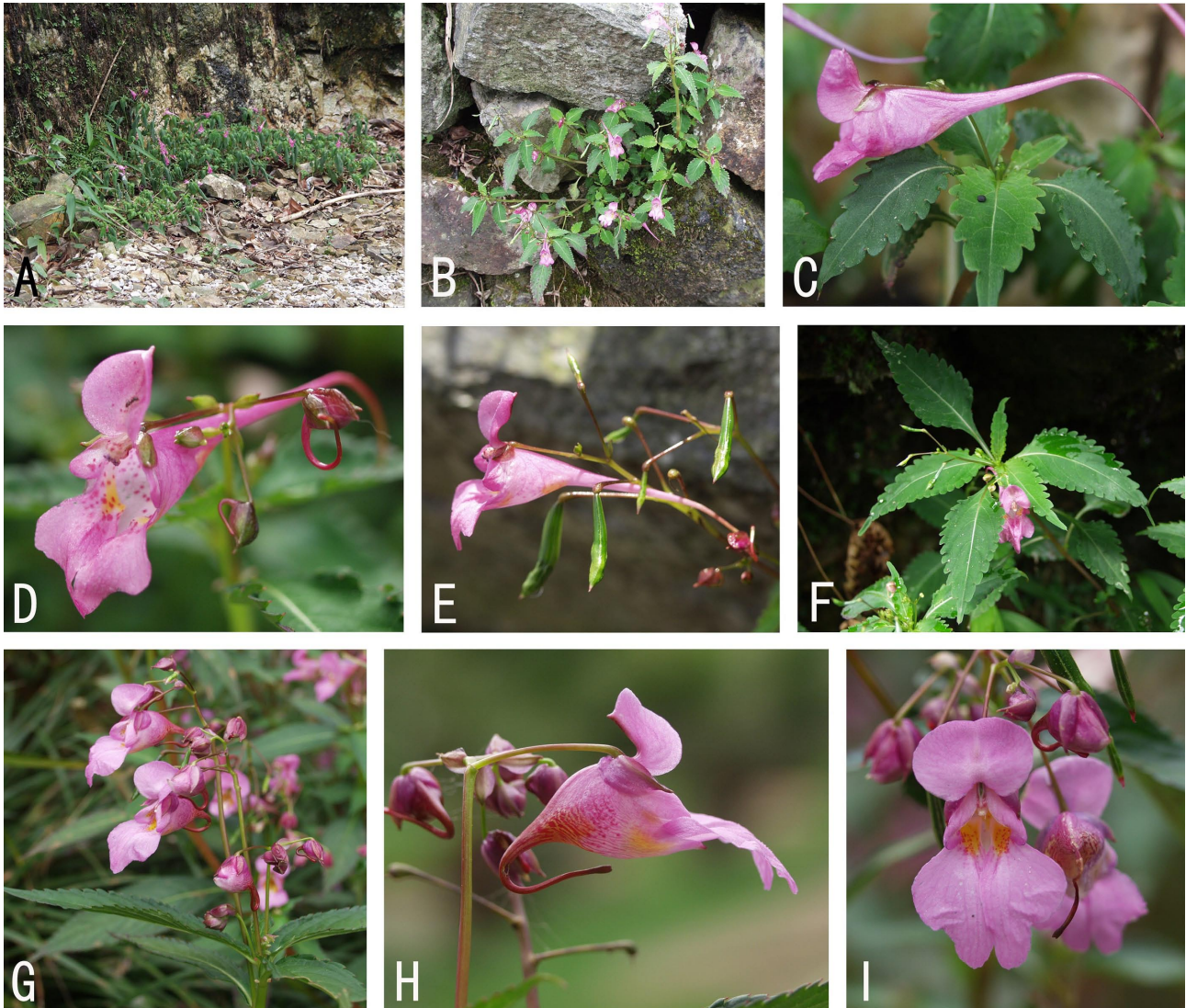


FIGURE 2. A–F: *Impatiens damingensis* S. X. Yu, C. Y. Xia & H. P. Deng. A. Habitat; B. plants; C. flower, lateral view; D. flower, front view; E. flower and capsule; F. flower branch. G–I: *Impatiens aquatilis* Hook. f. G. plants; H. flower, lateral view; I. flower, front view.

Annual herb, 25–50 cm tall, glabrous, stems fleshy, erect or ascendant, well branched, basal nodes swollen with adventitious roots. Leaves alternate, often aggregated above, blades 2–4 (–6) cm long, 1.5–2.5 cm wide, ovate or ovate-lanceolate, apex acuminate, margin deep crenate, fimbriated towards the base, base attenuate, petiole 0.5–1.5 cm long, base with 2 petiole glands, midvein obvious beneath, lateral veins 4–6 pairs, arcuate, deep green above, pale green beneath. Inflorescences racemose, 5–9 flowered, solitary in leaf axil, peduncle 5–8 cm long, glabrous, obviously longer than leaves. Flowers 4–4.5 cm long expanded, rose color. Bracts 3–4 mm long, membranous, ovate-elliptic, acuminate, persistent. Pedicels 10–15 mm long, slender. Sepals 3, lateral sepals 2, 4–5 mm long, 2–2.5 mm wide, oblique ovate, inequilateral, apex apiculate. Lip (lower sepal) 10–15 mm long, caliciform or infundibuliform, oral area slightly oblique above, constricted into a 3–3.5 mm long, curved, apex bifid, filiform spur. Standard (dorsal petal) 7–8 mm long, 11–12 mm wide, base truncate, oblong, dorsally with a narrower crest terminating, beak-like. Alae (united lateral petals) sessile, 2–2.3 mm long, bilobed, base lobe 7–9 mm long, oblong; distal lobe 1.2–1.5 mm long, slightly concave. Stamens 5, filament linear, 2–3 mm long, anther obtuse at apex, Ovary glabrous, erect. Fruit 1.7–1.8 mm long, coryneform, apex beaked, polyspermy. Seeds obovoid, brown, 2.0×1.3 mm, L/W = 1.54, seed coat tuberculate (Fig. 3:A). Fl. Jul.–Sep., Fr. Aug.–Oct.

Etymology

The specific epithet ‘damingensis’ refers to the locality of the type specimen, Daming Mountain, Wuming County, Guangxi, China.

Micromorphological observations

Seeds: Ovate, with a size of 2.0×1.3 mm, L (long) / W (wide) = 1.54, under SEM (Fig. 3: A). The surface contains coarse tubercular ornamentation, is brown in color, some epidermal cells irregularly arachnoid under SEM (Fig. 3: B, C). Seeds of *I. aquatilis* are ovoid in shape, with a size of 2.4×1.8 mm, L(long)/W(wide) = 1.33, under SEM (Fig. 3: D). The surface of these seeds also contains coarse tubercular ornamentation, and is brown in color, but the epidermal cells without irregular arachnoid under SEM (Fig. 3: E, F).

Pollen grains: The pollen grains of the proposed new species are long-elliptic, irregular reticulated, with average size of $E_1 \times E_2 = 35.48$ (34.10–36.72) \times 15.31 (14.50–15.73) μm (Fig. 3: G, H), while those of *I. aquatilis* are subelliptic, constricted in the middle, $E_1 \times E_2 = 34.61$ (34.15–34.92) \times 16.24 (15.20–16.68) μm in average size (Fig. 3: I, J). The surface of the pollen grains under SEM (Fig. 3: H, J) of the two species is more or less similar.

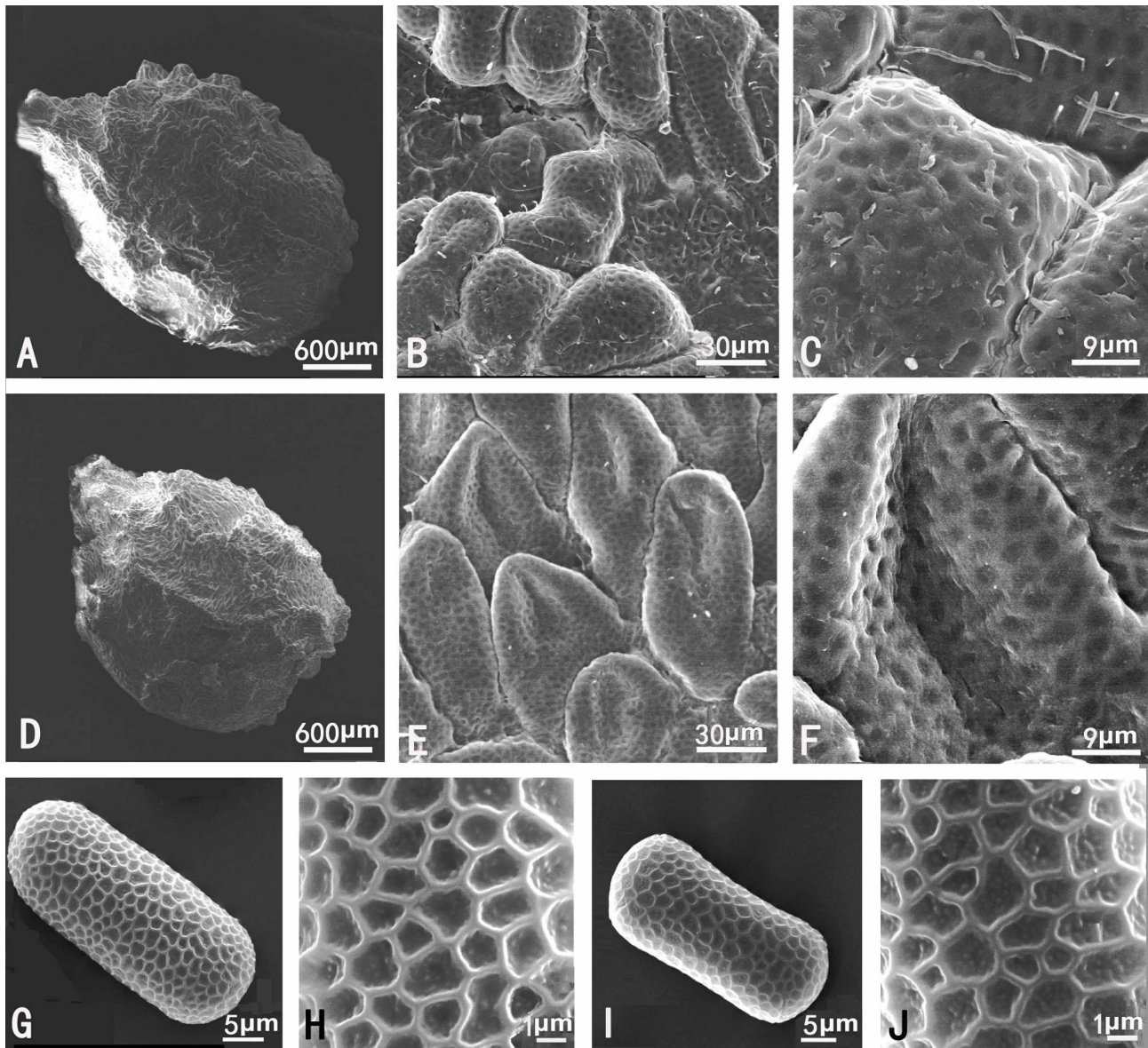


FIGURE 3. A–C, G–H: *Impatiens damingensis* S. X. Yu, C. Y. Xia & H. P. Deng. A–C. SEM images of seeds. A. whole view; B. partial view; C. partial view; G–H. SEM images of pollen grains. G. polar view; H. partial view. D–F, I–J: *Impatiens aquatilis* Hook.f. D–F. SEM images of seeds: D. whole view; E. partial view; F. partial view; I–J. SEM images of pollen grains: I. polar view; J. partial view.

Molecular phylogenetic analysis

The topology of the phylogenetic tree reconstructed in this study is congruent with those of previous studies (Yu *et al.*, 2016, Janssens *et al.*, 2006, Yuan *et al.*, 2004). Eight major clades that represent one subgenus and seven sections are recognized based on the concatenated data set in this study, with each clade highly supported. As seen in Fig. 4, the proposed new species is nested within subg. *Impatiens* sect. *Racemosae*, and forms a small clade with *I. uliginosa*, *I. aquatilis* and *I. cyanantha* with high bootstrap support (1.00 PP; Fig. 4 and Fig. S1). However the inner relationship of these four species remains ambiguous due to lack of resolution and support.

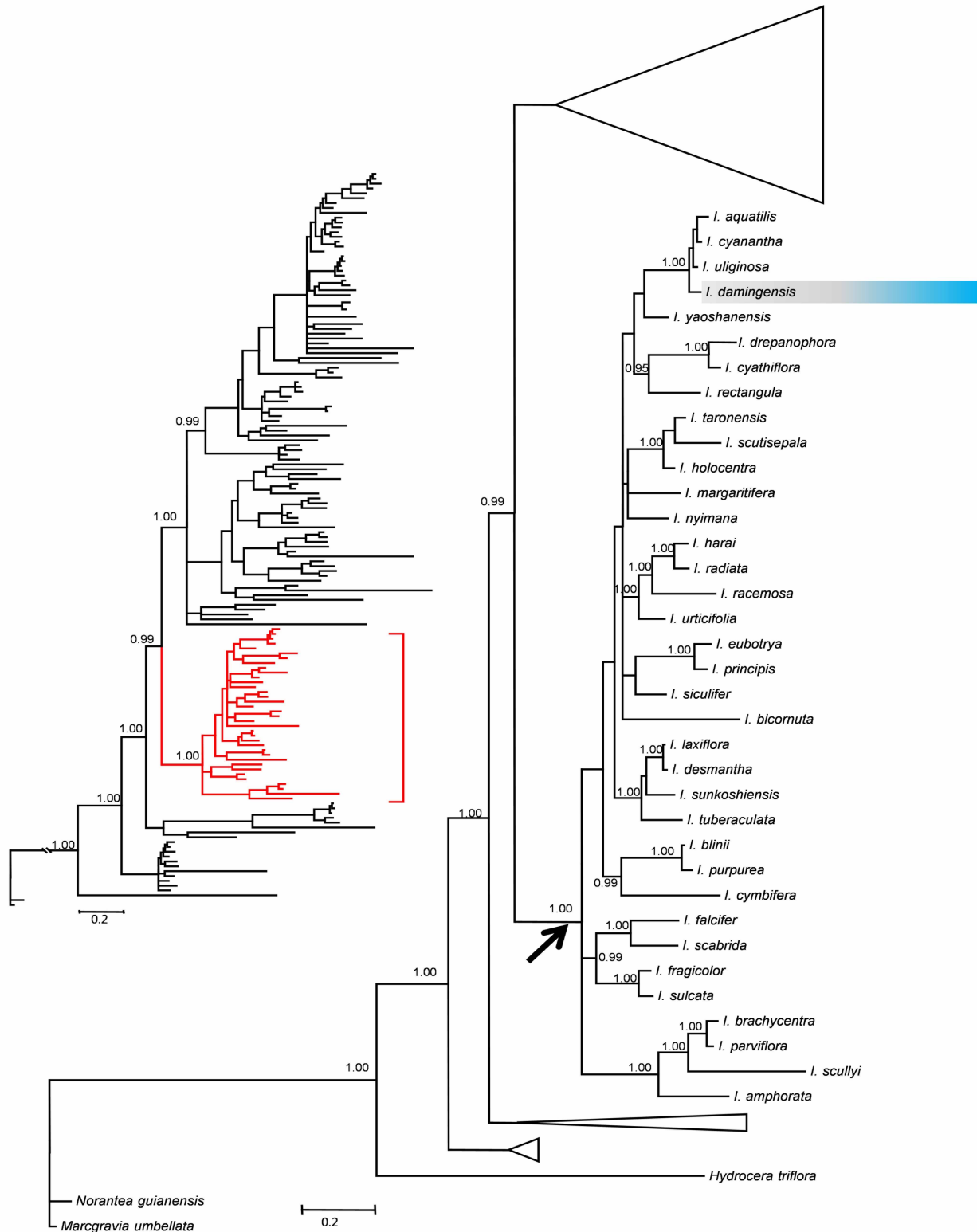


FIGURE 4. Partial Bayesian consensus phylogram based on the branch lengths of the ITS data. Numbers above branches are Bayesian posterior probabilities (> 0.95).

Thus, based on comparative gross and micro-morphology, and its placement in the well-resolved phylogeny whose topology is corroborated by previous studies, we conclude that the specimens under consideration in this study belong to a species new to science, which we name *Impatiens damingensis* S. X. Yu, C. Y. Xia & H. P. Deng.

Discussion

We have shown here that the specimens collected by our lab during a field trip in 2004 qualifies to be a new species, which we name *I. damingensis* S. X. Yu, C. Y. Xia & H. P. Deng. The proposed species exhibited unique gross morphological traits and micro morphological traits when compared with those of a closely related species, *I. aquatilis* (Fig. 4 and Fig. S1), and showed a similar morphology, with the difference that the leaves are ovate or ovate-lanceolate, and the apex and lateral apical apex do not have long glandular tips, with slender distances, and the top of the fruit has sputum. *I. damingensis* and *I. uliginosa* are closely related in the phylogenetic tree. Both flowers are red in shape, the fruit is rod-shaped, and the top is scorpion. *I. aquatilis* and *I. cyanantha* are closely related to the phylogenetic tree. In terms of morphology, both leaves are serrated with bristles between the teeth. Comparisons among *I. damingensis*, *I. uliginosa*, *I. aquatilis* and *I. cyanantha* are listed in table 1.

This new species is similar to *I. aquatilis* in habit and size, but distinguished by the leaves ovate or ovate-lanceolate, the apex of bracts acute, the apex of lateral sepals mucronulate, slender slightly incurved spur, and apex of capsule beaked; pollen grains long-elliptic, irregular reticulated (Fig. 2: G, H) and the ovoid seeds with irregular arachnoid on surface under SEM (Fig. 2: B, C); obviously distinguished from the control species: the subelliptic pollen with irregular reticulate, constricted in the middle (Fig. 2: I, J) and the seedcoat glabrous under SEM. (Fig. 2: E, F). The distribution of the two species is also different: the new species limited in centre of Guangxi province, Wuming county; however, the control species covered centre and north parts of Yunnan province. This may be because the geographical distribution of *Impatiens* is extremely regional and peculiar. Most of the species are endemic species distributed in China or a certain province. This peculiar phenomenon is more pronounced in limestone areas in Yunnan, Sichuan, Guizhou, and Guangxi.

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