



## *Impatiens occultans* (Balsaminaceae), a newly recorded species from Xizang, China, and its phylogenetic position

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### Abstract

*Impatiens occultans* Hook. f. (Balsaminaceae) is newly recorded for China, from Gyirong County, Xizang Province. A morphological description and notes on its distribution and ecology are provided. A phylogenetic analysis yields a placement of the species that it is sister to *I. tuberculata* (sect. *Racemosae*) with which it agrees in having a navicular lower sepal without a spur and 4-colpate pollen grains, but differs in elliptic leaves, 1-flowered racemes, 4 lateral sepals and glabrous capsules.

**Keywords:** Balsaminaceae, China, *Impatiens*, morphology, phylogeny

### Introduction

The Qinghai-Tibetan Plateau is a region with high biodiversity (Myers *et al.* 2000), but because of challenging field conditions it continues to be poorly investigated. *Impatiens* Linnaeus (1753: 937) species, with their perishable semi-succulent stems, fleshy leaves and delicate complex flowers, are generally difficult to preserve, the more so when collection conditions are difficult. Therefore the knowledge of *Impatiens* from this area has been particularly inadequate.

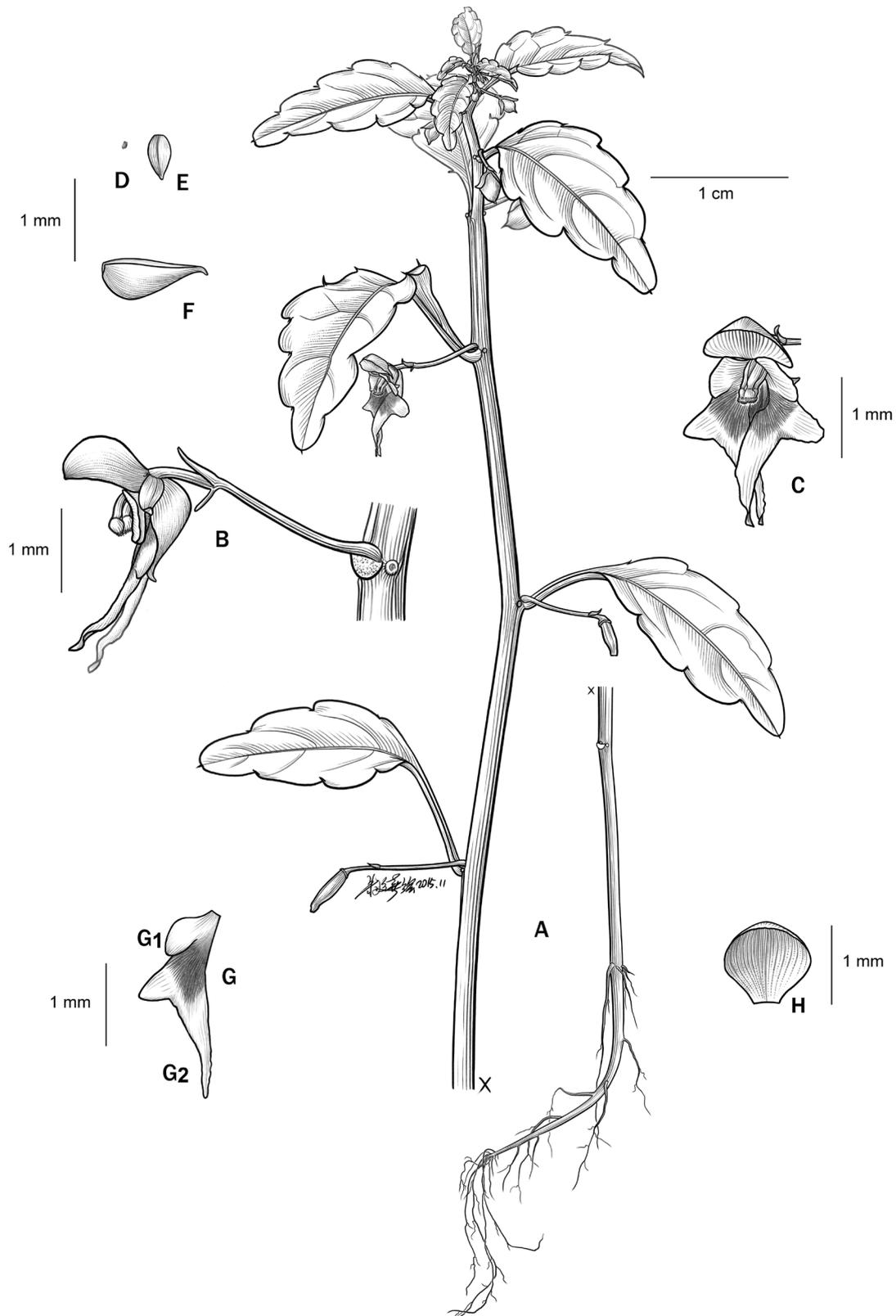
Owing to the *Flora of Pan-Himalaya* project (Hong 2015), several botanical expeditions have been launched to this region in the last few years. Many significant specimens have been collected during these expeditions, including an interesting *Impatiens* from Gyirong (Xizang), collected in 2013, that could be attributed to *I.* sect. *Racemosae*, a section comprising 50–70 species distributed on the southern slope of the Himalayas, with a few species in southern China and Europe (Yu *et al.* 2016). On critical examination, we determined the species to be *I. occultans* Hook. f. (1905: 17&22), a new record for flora of China. Complementary material was collected by three of the authors (LW, JCH, YFD) in Gyirong in 2016. On the basis of morphological and molecular evidence, the taxonomy and phylogenetic position of *I. occultans* are discussed.

### Materials and Methods

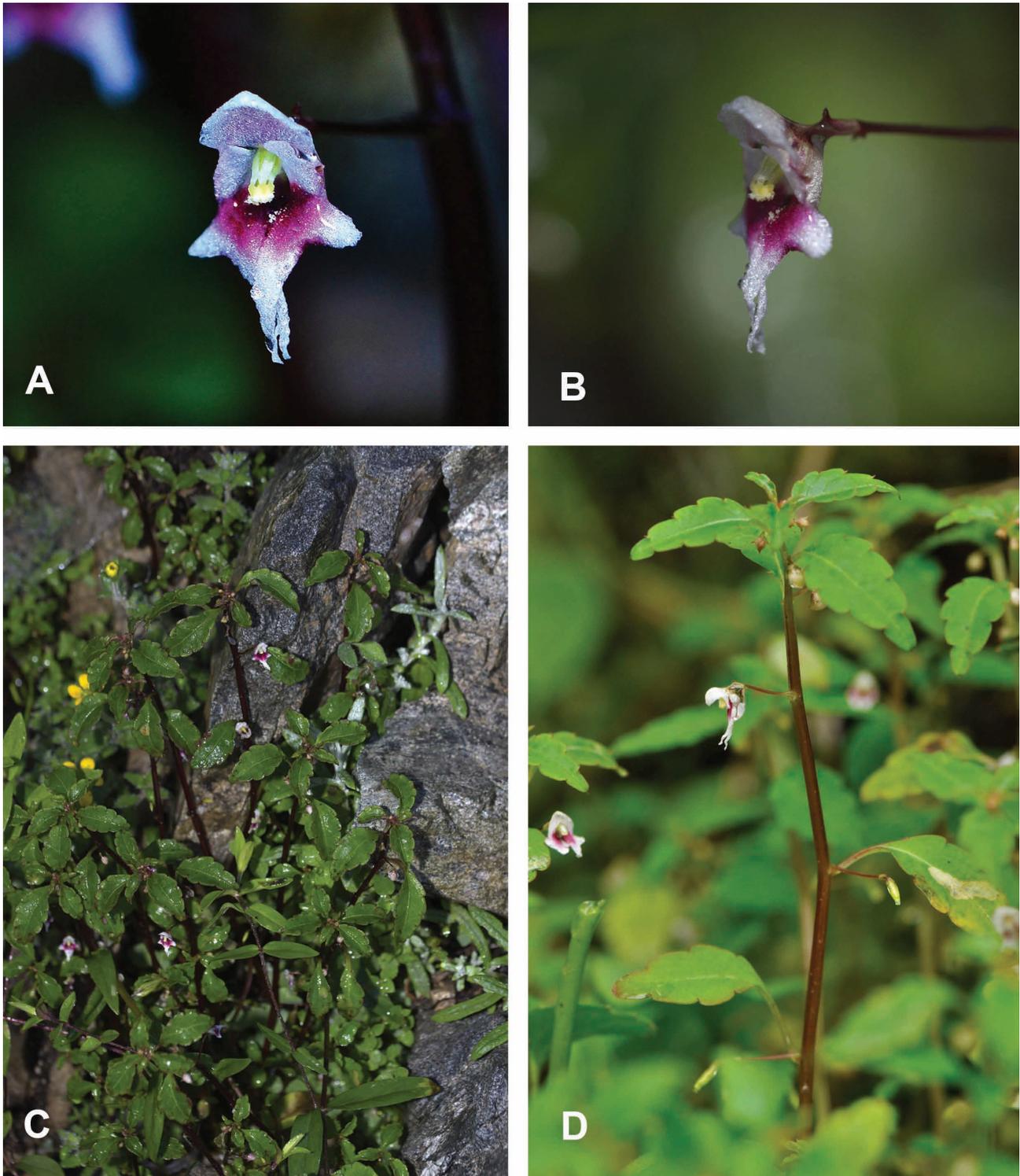
**Molecular methods:**—We included DNA sequences of 151 species of *Impatiens*. As the outgroup we used *Hydrocera triflora* (L.) Wight & Arnott (1834:140) (Balsaminaceae), *Marcgravia umbellata* Linnaeus (1753:503) (Marcgraviaceae) and *Norantea guianensis* Aublet (1775:554) (Marcgraviaceae) based on the results of Yuan *et al.* (2004), Janssen *et al.* (2006) and Yu *et al.* (2016). All sequences were downloaded from GenBank, except *I. occultans* which was generated for this study. Vouchers and GenBank accession numbers are listed in Table S1.

Three regions were used for DNA sequencing: ITS, *atpB-rbcL* and *trnL-F*. Total DNA was extracted from silica gel-dried leaves with a modified CTAB protocol (Doyle and Doyle 1987). Primers and PCR protocols for ITS, *atpB-rbcL* and *trnL-F* were taken from White *et al.* (1990), Janssens *et al.* (2006) and Taberlet *et al.* (1991), respectively.

PCR products were purified with a GFX™PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Sequencing reactions were carried out with an ABI Prism Bigdye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Products were analyzed on an ABI3730xl automated DNA sequencer.



**FIGURE 1.** *Impatiens occultans* A. habit; B. flower (lateral view); C. flower (front view); D. upper lateral sepal; E. lower lateral sepal; F. lower sepal; G. lateral united petals; G1. upper petal; G2. lower petal; H. dorsal petal. (All from *Lai wei & Jia-Chen Hao 15397* (PE) and drawn by Yun-Xi Zhu).



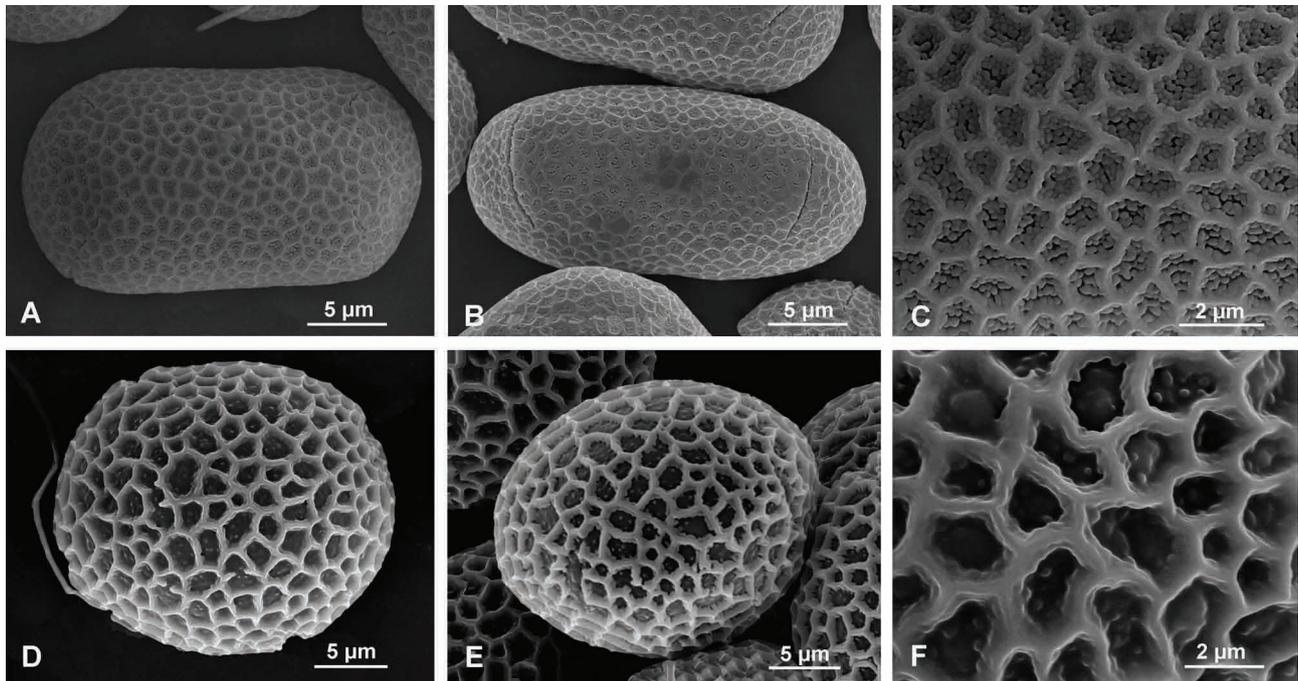
**FIGURE 2.** *Impatiens occultans* A. flower (front view); B. flower (lateral view); C. habitat; D. habit (photographed by Lai Wei, Jiachen Hao and Yufen Du).

Sequences were aligned with the default parameters in Clustal X v.1.83 (Thompson *et al.* 1997) and further adjusted manually in BioEdit v.7.0 (Hall 1999). Four difficult-to-align regions in *trnL-F* (encompassing 73 sites) and one difficult-to-align region in *atpB-rbcL* (encompassing 42 sites) were excluded from the analyses.

Maximum parsimony (MP) and Bayesian inference (BI) were used to analyze the ITS and plastid data sets. The MP analyses were carried out in PAUP\* v.4.0b10 (Swofford 2003). Heuristic searches were conducted with 1000 replicates of random addition, one tree held at each step during stepwise addition, tree-bisection-reconnection (TBR) branch swapping, MulTrees in effect, and steepest descent off. Bootstrapping was conducted with 1000 replicates, each

with 10 random taxon additions, and heuristic search options. The BI analyses were carried out in MrBayes v.3.0b4 (Ronquist and Huelsenbeck 2003). Each of the three regions (ITS, *atpB-rbcL*, and *trnL-F*) was assigned its own model of nucleotide substitution, as determined by the Akaike information criterion (AIC) in Modeltest v.3.06 (Posada and Crandall 1998).

**Morphology:**—Morphological studies were carried out with conventional methods as in Chen *et al.* (2001).



**FIGURE 3.** Pollen micromorphology under SEM. A–C: *Impatiens occultans*. A. polar view; B. equatorial view; C. partial view. D–F: *I. tuberculata*. D. polar view; E. equatorial view; F. partial view. (Voucher specimen of *I. occultans*, Lai Wei & Jia-Chen Hao 15397, deposited in PE; *I. tuberculata*, S. X. Yu 4036, deposited in PE).

## Results and discussion

*Impatiens occultans* Hook.f., Rec. Bot. Surv. Ind. 4: 17 (1905). Type:—INDIA. Sikkim: Tankra Mts, 13500 ft, 2 Aug 1891, G. A. Gammie 414 (holotype K!).

Herbs perennial, 15–20 cm tall, glabrous. Stems simple, erect, fleshy; inferior nodes slightly swollen. Leaves alternate; blade elliptic, 2–3 × 1–1.2 cm, apex acuminate, base cuneate, deep green above, pale green beneath, margin deeply and sparingly crenate with conspicuous sinus bristles, lateral veins distinct; petioles ca. 0.5 cm. Flower solitary in upper axils; peduncle 2–3 cm long; bracts lanceolate, ca. 5 mm long, acute; pedicels thin, 3–5 mm long. Flowers pale purplish blue with purplish red patches in mouth. Lateral sepals 4; anterior small, 0.8–1.0 × 1.2–1.5 mm, glandiform; posterior large, ovate to lanceolate, 2- or 3- veined, yellowish green, base rounded, apex acuminate to caudate, 2.5–3.6 × 1.5–2.1 mm. Lower sepal 1.2–1.5 cm long without spur, navicular. Dorsal petal 4–6 × 3–5 mm, apex rounded, base broadly cuneate, midrib not distinct. Lateral united petals 8–12 mm long, lower lobes 8–10 × 3–5 mm, oblong, the upper 3–5 × 2–3 mm, elliptic, inner margin without appendage. Stamens 5; filaments linear, 2–3 mm long; anthers obtuse. Ovary narrowly fusiform. Capsule short, fusiform, middle part inflated.

**Phenology:** Flowering and fruiting from July to August.

**Ecology:** *Impatiens occultans* was found growing near rocks in the pass between Gyirong to Sale at an altitude of over 4000 m in an alpine meadow.

**Distribution:** *Impatiens occultans* was first discovered in northern Sikkim, India, by G. A. Gammie (Hooker 1905), and later found in central and eastern Nepal (Hara 1979). The species occurs in China (Xizang: Gyirong), Nepal (Beni Kharka, Chhurchathanga, Choarma, Chodo Gyabo, DudhKund, Koshing Kharka, Lamni Nama, Namikhil, Neju, Pike Dongshar, Sarkari Pati, Sete, Taktor, Thare Og, Thosa Kharka, Thudam, and Topke Gola), and India (Sikkim, Tankra) (Akiyama *et al.* 1991).

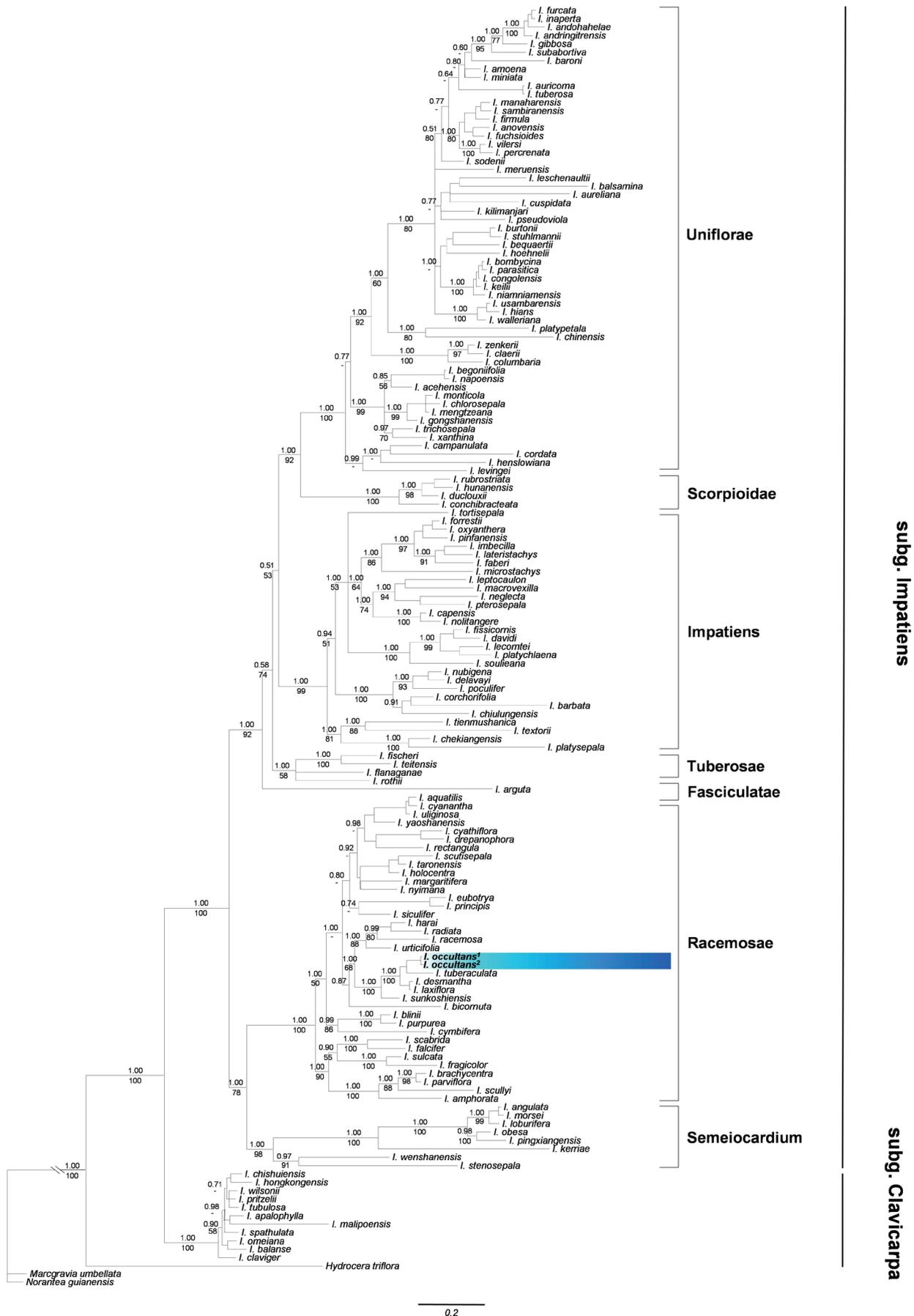


FIGURE 4. Bayesian consensus phylogram based on the branch length of the combined data. Numbers above and below branches are Bayesian posterior probabilities (> 0.5) and bootstrap percentages (> 50%), respectively. “-” indicates nodes not supported.

**Palynology:** In *Impatiens occultans*, the pollen size (P×E1×E2: length of polar axis × length of long equatorial axis × length of short equatorial axis) is (21.3–21.6) × (47.1–47.4) × (23.3–23.8) μm. The pollen is tetracolpate, oblong in polar view, the colpi are thin, and the exine possesses reticulate ornamentation and with dense granules in the lumina. The lumina are smaller close to the colpi (Fig 3 A–C). The pollen size (P×E1×E2) of *I. tuberculata* Hook. f. et Thoms. (1860: 155) is (12.1–12.6) × (26.9–27.5) × (17.1–17.8) μm. The pollen is tetracolpate, elliptic in polar view, the colpi are thin, and the exine possesses reticulate ornamentation with dense granules in the lumina (Fig 3 D–F). From the results of pollen grains observation, *I. occultans* is similar with the latter in shape, superficial ornamentations and second sculptures, but the former is larger than the latter in size.

*Additional specimens examined:*—**CHINA. Xizang:** Gyirong, 28°23' N, 85°24' E, 4004 m, 28 July 2015, *Lai Wei & Jia-Chen Hao 15397* (PE); 28°23' N, 85°21' E, 2800–3970 m, 23 July 2013, *PE-Xizang Botanical Expedition 3992* (PE). **NEPAL.** Rolwaling Khola, 3600–4050 m, 4 September 1983, *H. Ohba et al. 8320622* (TI); Khare Khola, 4000 m, 12 September 1983, *H. Ohba et al. 8331975* (TI); Chhurchathanga, 3200–3600 m, 10 August 1977, *H. Ohashi et al. 770637, 772219 & 773661* (TI); Janakpur Zone, 22 July 1985, *H. Ohba et al. 8570653* (TI); Sagarmatha Zone, 19 August 1985, *H. Ohba et al. 8520278 & 8571790* (TI); 9 September 1985, *H. Ohba et al. 8531381* (TI).

**TABLE 1.** Comparison among *I. occultans*, *I. tuberculata*, *I. laxiflora* and *I. radiata*.

Characters	<i>I. occultans</i>	<i>I. tuberculata</i>	<i>I. laxiflora</i>	<i>I. radiata</i>
Leaf shape	Lanceolate to narrowly ovate	Elliptic to elliptic-ovate	Ovate-lanceolate	Lanceolate
Leaf size	5–25 × 2–12 mm	1.5–3 × 1–1.5 cm	3–7 × 1.5–2.5 cm	6–12 × 2–3 cm
Inflorescence	1-flowered	4- to 10-flowered	5- to 9-flowered	10- to 16-flowered
Floral color	White with red patches	White with reddish patches	Yellow	White to pink
Outer lateral sepals	Oblong	Falciform	Obvate to ovate-subulate	Oovate-lanceolate
Inner lateral sepals	Scale-like	Absent	Black glandular	Absent
Lower sepal	Without patches	With reddish patches	Without patches	Without patches
Spur (mm)	Spurless	5–7	15–18	10–12
Upper lobe length of united petals (mm)	1.5–2	4–6	3–5	3–5
Upper lobe shape of united petals	Broadly oblong-ovate	Oblong	Oblong	Oblong
Lower lobe length of united petals (mm)	3.5–5	5–8	5–7	5–7
Lower lobe color of united petals	White, with purplish red patches	White, with reddish patches	Uniformly yellow	White
Dorsal petal size (mm)	3 × 2.5	7 × 5	6 × 5	6 × 5
Dorsal petal shape	Cucullate	Cucullate	Circular	Subcircular
Capsule shape	Fusiform to clavate	Short clavate	Linear	Linear
Capsule surface	Glabrous	Tuberculate along ribs	Glabrous	Glabrous

**Discussion:** The topologies of the ITS and *atpB-rbcL* + *trnL-F* trees are congruent with each other, and also agree with those from Yu *et al.* (2016). They yield a sister relationship between *Impatiens occultans* and *I. tuberculata*, and is included in a clade of sect. *Racemosae*, subg. *Impatiens* (Fig 4, S1, S2). Morphological data (Fig 4, S1, S2) confirm this relationship. *Impatiens occultans* is similar to *I. tuberculata*, but differs in its elliptic (vs oblanceolate) blade,

1-flowered (vs 4- to 10-flowered) raceme, 4 (vs 2) lateral sepals and glabrous (vs tuberculate) capsule. In Table 1, *Impatiens occultans* is compared with related and/or similar species (*I. tuberculata*, *I. laxiflora* Edgew. (1851: 39) and *I. radiata* Hook. f. (1875: 476)).

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