Phytotaxa 227 (3): 253–260
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.227.3.4

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

LEI ZENG[1, 2], YAN-NAN LIU[2], RAJIB GOGOI[3], LIN-JING ZHANG[1,*] & SHENG-XIANG YU[2,*]

[1]*College of Life Sciences, Shanxi Normal University, Linfen 041004, China*
[2]*State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences, Beijing 100093, China.*
[3]*Botanical Survey of India, Arunachal Pradesh Regional Centre, Senki View, Pin-791 111 Itanagar, India.*
**Correspondence: yushengxiang@ibcas.ac.cn; linjingzh@aliyun.com*

Abstract

Impatiens tianlinensis S. X. Yu & L. J. Zhang, a new species of the Balsaminaceae from Cenwang Laoshan Mountain, Tianlin County, Guangxi Region, is described and illustrated. This species closely resembles *I. apalophylla* and *I. clavigera* var. *auriculata* in having racemose inflorescences, 4 lateral sepals, hammer-shaped capsules and ellipsoid seeds, but differs in having sessile glanduliferous petioles, few-flowered inflorescences, incurved spur, yellow lower sepal without reddish patches, yellowish petals and lower sepal, and acuminate dorsal petal apex. The molecular data, from nuclear ribosomal and plastid genes, as well as pollen characters also support that the species is new to science.

Key words: Balsaminaceae, molecular data, new species, phylogeny, pollen character

Introduction

The family Balsaminaceae contains two genera currently recognized, the small *Hydrocera* Wight & Arnott (1834: 140) and *Impatiens* Linnaeus (1753: 937) with over 1000 species (Grey-Wilson 1980, Fischer 2004). *Impatiens* is distributed throughout tropical Africa, India, South-west Asia, South China and Japan, with a few species spreading into the north temperate zone of Europe, Russia, China and North America (Grey-Wilson 1980).

There are about 270 species known in China (Chen 2001, Chen *et al*. 2007, Yu 2012), and their majority are restricted to southwest China: Yunnan, Sichuan, Guizhou, Xizang (Tibet) and Guangxi. As in many other genera, several new species are discovered within these regions each year, such as *Begonia* of Begoniaceae (Ku *et al*. 2008, Peng *et al*. 2012, 2013), *Lagarosolen* of Geserinaceae (Xu *et al*. 2008), *Pararuellia* of Acanthaceae (Chen *et al*. 2009), *Aspidistra* of Asparagaceae (Lin *et al*. 2013) and *Codonopsis* of Campanulaceae (Wang & Hong, 2014).

During a taxonomic revision of *Impatiens* in Guangxi Region, the first author encountered several specimens of a species thought to be new to science. Further studies confirmed that it can be readily distinguished from related taxa, based on morphological (including palynological) and molecular characters.

Materials and Methods

Molecular methods:—In total, 152 species of *Impatiens* were sampled to represent different evolutionary patterns of the genus. Three species: *Hydrocera triflora* (L.) Wight & Arnott (1834: 140) (Balsaminaceae), and *Marcgravia umbellata* Linnaeus (1753: 503) (Marcgraviaceae) and *Norantea guianensis* Aublet (1775: 554) (Marcgraviaceae) were included as outgroups based on the results of Yuan *et al*. (2004), Janssen *et al*. (2006) and Yu *et al*. (2015). All sequences were downloaded from GenBank except *I. clavigera* Hook. f. (1908: 2863) var. *auriculata* Huang S.H. (2003: 277) and *I. tianlinensis*, which were newly generated for this study with accession numbers KT321312 for ITS, KT321311 for *atpB-rbcL* and KT321313 for *trnL-F*, respectively. Vouchers and GenBank accession numbers are listed in Table S1.

Three molecular markers were used: ITS (ITS-1, 5.8S, and ITS-2), *atpB-rbcL and trnL-F* region (*trnL* intron, and

Accepted by Hai He: 21 Aug. 2015; published: 25 Sept. 2015

trnL [UAA] 3' exon-*trnF* [GAA] intergenic spacer). Total genomic DNA was extracted from silica gel-dried leaves with a modified CTAB protocol (Doyle & Doyle, 1987). Primers and PCR protocols for ITS, *atpB-rbcL* and *trnL-F* are taken from White *et al.* (1990), Janssens *et al.* (2006) and Taberlet *et al.* (1991) respectively. PCR products were purified using a GFX™PCR 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). Products were analyzed on an ABI3730xl automated DNA sequencer.

Sequences were aligned using the default parameters in Clustal X v.1.83 (Thompson *et al*., 1997) and then 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 datasets. The MP analyses were carried out in PAUP* v.4.0b10 (Swofford, 2003). Heuristic searches were conducted with 1000 replicates with random sequence addition, in which one tree was 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 with 10 random taxon additions and heuristic search options. The BI analyses were carried out in MrBayes v.3.0b4 (Ronquist & Huelsenbeck, 2003). Each of the three regions (ITS, *atpB-rbcL,* and *trnL-F*) was assigned its own model of nucleotide substitution determined by the Akaike information criterion (AIC) in Modeltest v.3.06 (Posada & Crandall, 1998).

Morphological methods:—Measurements of leaves, inflorescences, flowers and seeds were made on both dried herbarium specimens (GXMI, IBCS, IKB, and PE) and live plants in the field. Pollen samples for scanning electron microscope (SEM; Hitachi S-4800) study, were attached to specimen stubs with a conductive adhesive and sputter-coated with gold-palladium. Pollen characters were evaluated according to the literature (e.g., Lu, 1991; Lu & Chen, 1991; Chen, 2001; Song *et al*., 2005; Janssens *et al*., 2012). Pollen grains were treated with the methods described by Erdtman (1971) and Moore and Webb (1978).

Taxonomy

Impatiens tianlinensis S. X. Yu & L. J. Zhang*, sp. nov*. (Figs. 1–2)

This species is similar to *I. apalophylla* Hook. f. (1908: 243) and *I. clavigera* var. *auriculata* in having racemose inflorescences, 4 lateral sepals, hammer-shaped capsules and ellipsoid seeds, but differs in sessile glanduliferous petioles, few-flowered inflorescences, incurved spur, yellow lower sepal without reddish patches, yellowish petals and lower sepal, and acuminate dorsal petal apex.

Type:—CHINA . Guangxi: Tianlin county, Cenwang Laoshan Mountain, in a valley near the river, 24°24'04.7" N, 106°23'09.5" E, ca. 1260 m, 9 Oct 2007, *S. X. Yu 3731* (holotype PE!, isotype IBK!).

Herb, perennial, 50–80 cm tall, glabrous. Stems fleshy, erect, simple, robust; inferior nodes swollen. Leaves alternate, towards the stem apex aggregated; blade deep green above, pale green beneath, obovate to oblanceolate, 10–15 (–18) cm long, 5–8 cm wide, apex acuminate, base cuneate, margin crenate, with fimbriae between the teeth; lateral veins 7–9 pairs. Petioles (0.5–) 1–2 cm (or upper leaves subsessile) with several short clavate glands. Inflorescence racemose; racemes solitary in the upper axils, 10–15 cm, 3–5 (–7) flowered, bracteate. Pedicels thin, 15–20 mm. Bracts ovate, 3–5 mm, acute, deciduous. Flowers yellowish or cream. Lateral sepals 4, the outer 2 small, ovate, symmetric, 5-veined, yellowish-green, 5–8 × 3–5 mm; the inner 2 large, 7–8× 2–3 mm, sickle-shaped, inaequilateral, apex acuminate or caudate. Lower sepal 2.5–3.5 cm excluding spur, saccate, abruptly constricted into a 1–1.5 cm involute spur. Dorsal petal 10–12 × 7–9 mm, ovate, apex obtuse, base broadly cuneate, midrib obvious, with a slight dorsal crest. Lateral united petals yellow, 2–2.5 cm; lower lobe 10–15 mm, oblong; upper lobe 20–25 mm, elliptic, apex emarginate, middle of inner margin without appendage. Stamens 5, filaments linear, 2–3 mm, anthers obtuse. Ovary clavate, superior part inflated. Capsule hammer-shaped, 1.5–2cm, seed ellipsoid.

Palynology:—Pollen grains of *Impatiens tianlinensis*, *I. apalophylla* and *I. claviger* var. *auriculata* are all triangular in polar view, tricolpate with long, thin colpi, an exine with reticulate ornamentation and dense granules in lumina (Fig. 3A–C). Pollen grains of *I. tianlinensis* are 30.13 (29.62–30.47) × 12.95 (12.68–13.54) μm, whereas those of *I. apalophylla* are very much narrower, ca. 31.85 (30.96–32.73) × 5.57 (5.13–6.75) μm. Pollen grains of *I. clavigera* var. *auriculata* are 27.09 (26.58–28.63) × 12.78 (12.34–13.69) μm..

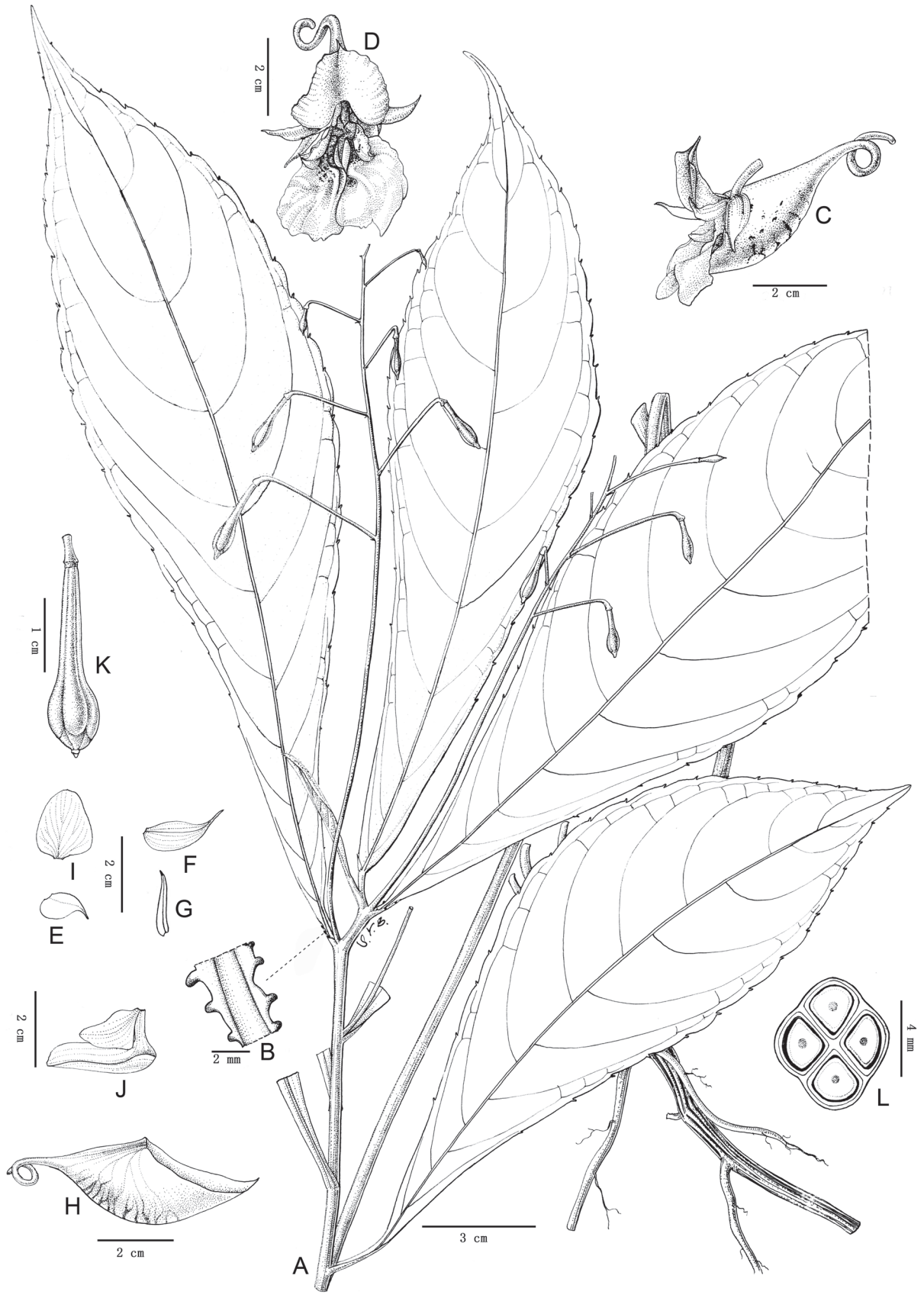

FIGURE 1. ***Impatiens tianlinensis***: A. habit; B. partial view of petiole; C. flower (laterial view); D. flower (front view); E. bractlet; F. outer lateral sepal; G. inner lateral sepal; H. lower sepal (lateral view); I. dorsal petal; J. lateral united petals; K. capsule; L. transection of ovary (Drawn by Y. B. Sun from S. X. Yu 3731).

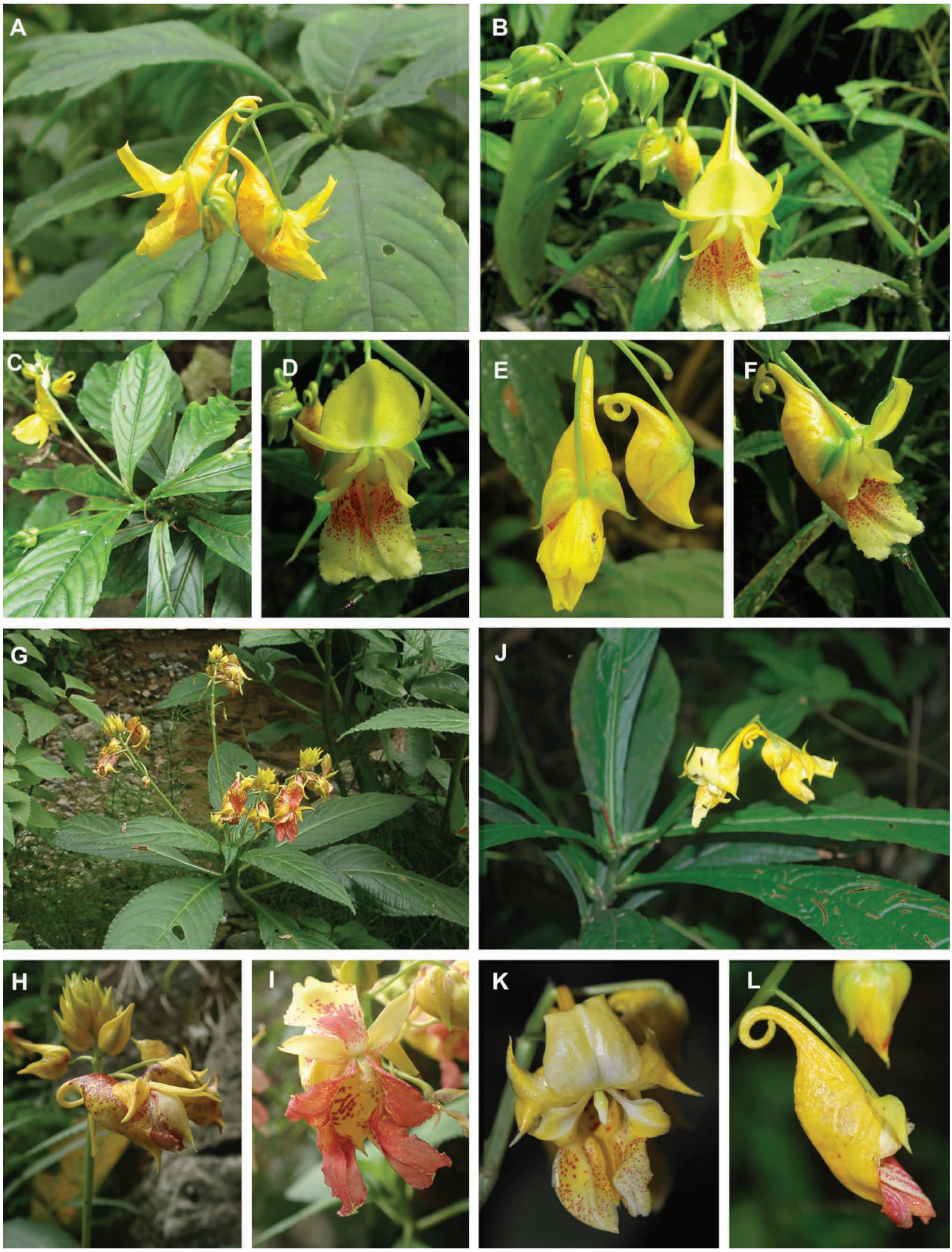

FIGURE 2. A–F: ***Impatiens tianlinensis***. A. flower branch; B. racemose inflorescence; C. upper stem; D. flower (front view); E. flower (dorsal view); F. flower (lateral view). G–I: ***I. apalophylla***. G. habitat; H. flower (lateral view); I. flower (front view). J–L: ***I. clavigera* var. *auriculata***. J. flower branch; K. flower (front view); L. flower (lateral view) (photographed by S. X. Yu).

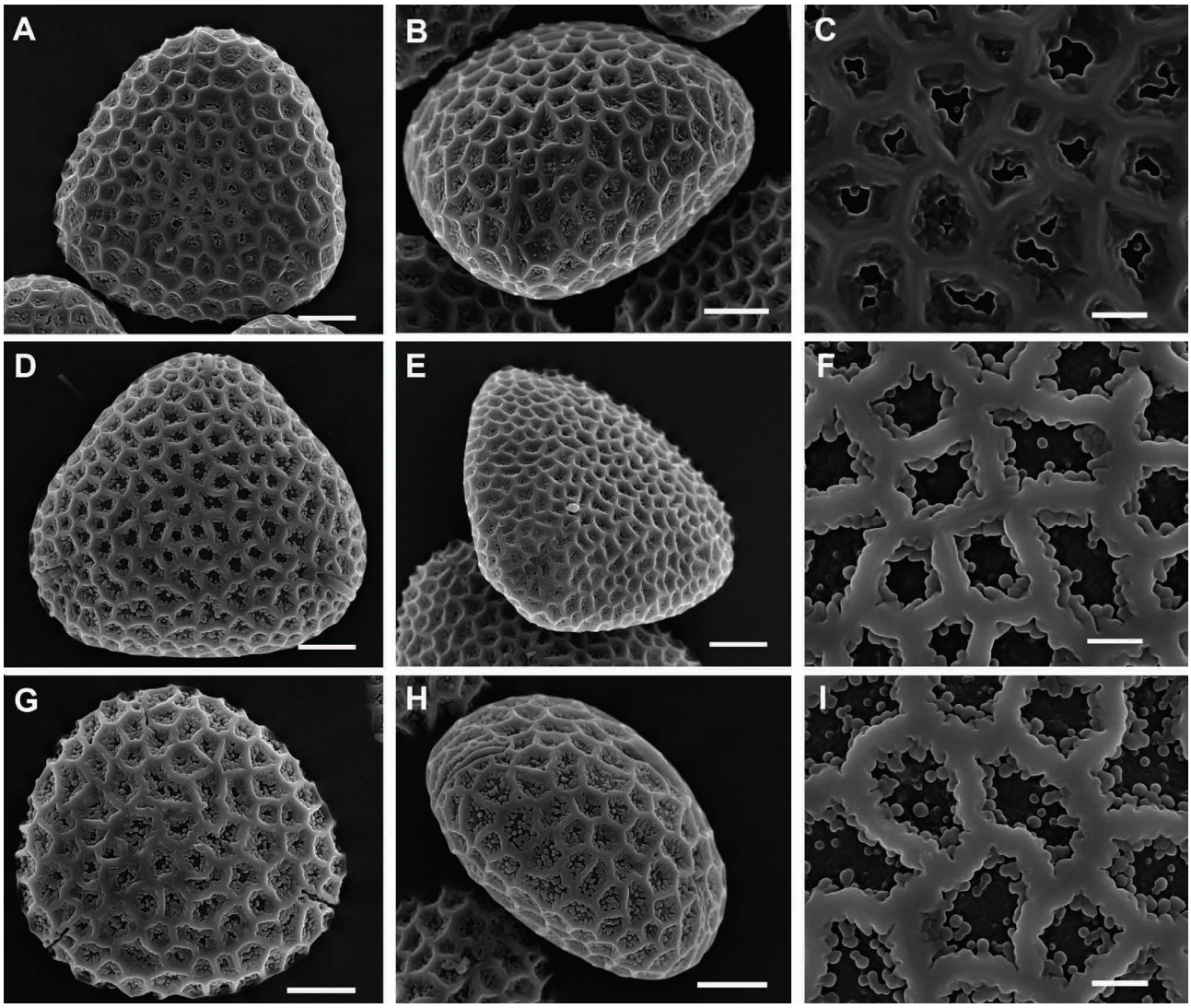

FIGURE 3. Pollen micromorphology under SEM. A–C: ***Impatiens tianlinensis***. A. polar view, bar = 6 μm; B. equatorial view, bar = 5 μm; C. partial view, bar = 2 μm. D–F: ***I. clavigera* var. *auriculata***. D. polar view, bar = 6 μm; E. equatorial view, bar = 6 μm; F. partial view, bar = 2 μm. (Voucher specimen of ***I. clavigera* var. *auriculata,*** S. X. Yu 4036, deposited in PE). G–I: ***I. apalophylla*.** G. polar view, bar = 5 μm; H. equatorial view, bar = 5 μm; I. partial view, bar = 2 μm (Voucher specimen of ***I. apalophylla*,** S. X. Yu 404*2*, deposited in PE).

Phenology:—Flowering and fruiting from September to November.

Ecology:—This new species grows in a valley at an elevation of 1100–1300 m. s. m., in a disturbed forest. The population is apparently small.

Distribution:—Except the type locality, *I. tianlinensis* is also known from Lingyun, Fengshan, Leye and Nandan of Guangxi.

Etymology:—The specific epithet '*tianlinensis*' refers to the type locality.

Additional examined materials:—China. Guangxi: Lingyun, Lingyun Expedition 3-6098 (GXMI); Fengshan, Fengshan Expedition 4-7-76 (GXMI); Leye, Leye Expedition 3-30133a (GXMI); Nandan, Nandan Expedition 4-5-113, 4-5-300, 4-5-472, 4-5-750 (GXMI).

Discussion

Our ITS and *atpB-rbcL* trees are congruent with those of previous studies (Yu *et al*. 2015). Two major subdivisions of *Impatiens* are recognized, with seven subclades (Figs. 4–5; S1–S2). Both ITS and *atpB-rbcL* indicate that *I. tianlinensis* is a member of subgenus *Clavicarpa* S.X. Yu & Wei Wang (2015: 13) (Figs. 4–5). In the ITS tree *I. tianlinensis* and *I. clavigera* var. *auriculata* form a clade (PP 1.00 and BS 66%), which is sister to *I. chishuiensis* Y. X. Xiong (1996: 98)

(PP 1.00 and BS 99%). In the *atpB-rbcL* tree *I. tianlinensis*, *I. clavigera* var. *auriculat*, *I. chishuiensis* and some other species form a large polytomy with moderate support (PP 0.91 and BS 99%), which is congruent with the ITS-tree. Also most morphological characters, such as perennial habit, racemose inflorescence, 4 lateral sepals, 4-carpellate ovary with one ovule per carpel, and 3-colpate pollen indicate that *I. tianlinensis* belong in subg. *Clavicarpa*.

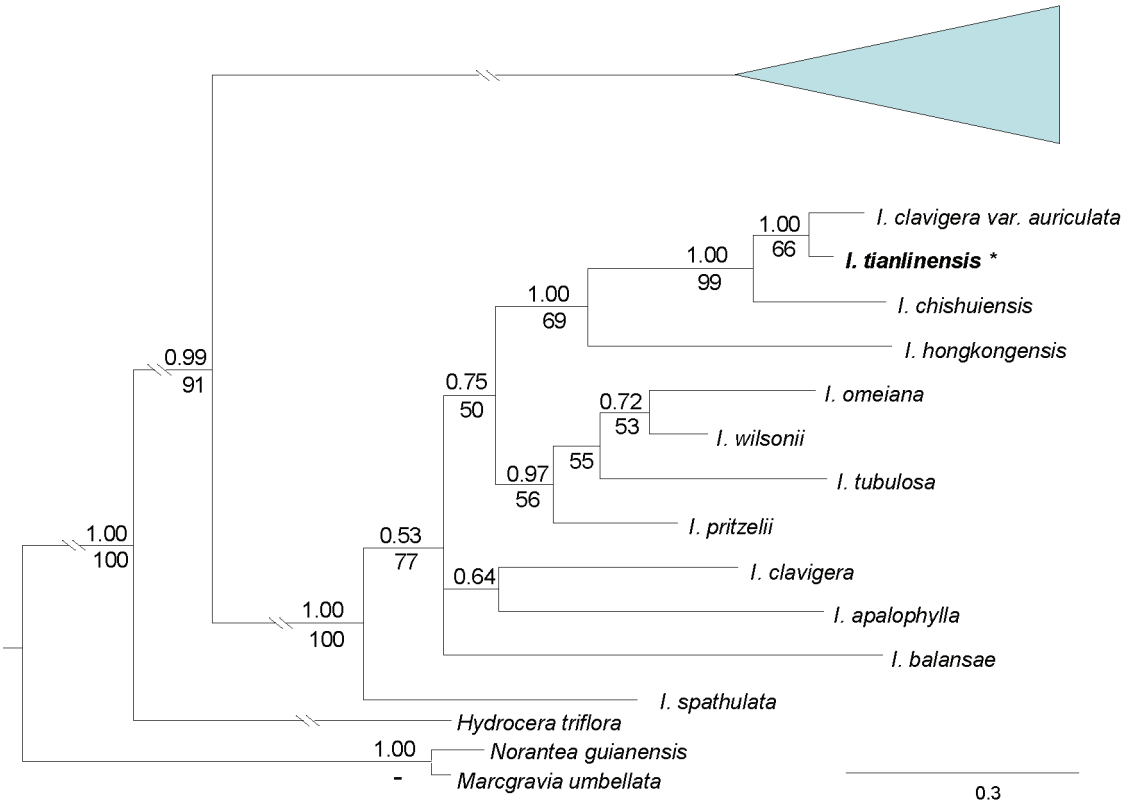

FIGURE 4. Bayesian consensus phylogram based on the branch length of the ITS data. Numbers above and below branches are Bayesian posterior probabilities (> 0.5) and bootstrap percentages (> 50%), respectively. Hyphen ("-") indicates node not supported.

I. tianlinensis is distinguished from its allies by the few-flowered raceme, the glanduliferous petiole and the involute spur. Pollen studies show that *I. tianlinensis* has obvious differences from the related species. The distinctions are the size, the murus morphology of the reticulate ornamentation and the lumina. The pollen grains of *I. tianlinensis* are slightly longer than those of *I. clavigera* var. *auriculata* in equatorial view. The muri of the reticulate ornamentation are much narrower in *I. tianlinensis*. The granules in lumina are evenly distributed in *I. tianlinensis* but restricted to the muri in *I. clavigera* var. *auriculata*. The nuclear ribosomal and plastid genes as well as morphology (including pollen characters) support the distinction of this new species.

Acknowledgements

The authors wish to express heartfelt thanks to the keepers of the herbaria of Paris (P) and Royal Botanic Garden, Kew (K) for loan of type specimens, and Ying-bao Sun for making the drawing. We are also grateful to Professor Magnus Lidén for polishing the language, and Eberhard Fischer for his critical review and comments. This work was supported by National Natural Science Foundation of China (31170177) and Main Direction Program of Knowledge Innovation of Chinese Academy of Sciences (grant no. KSCX2-EW-Z-1).

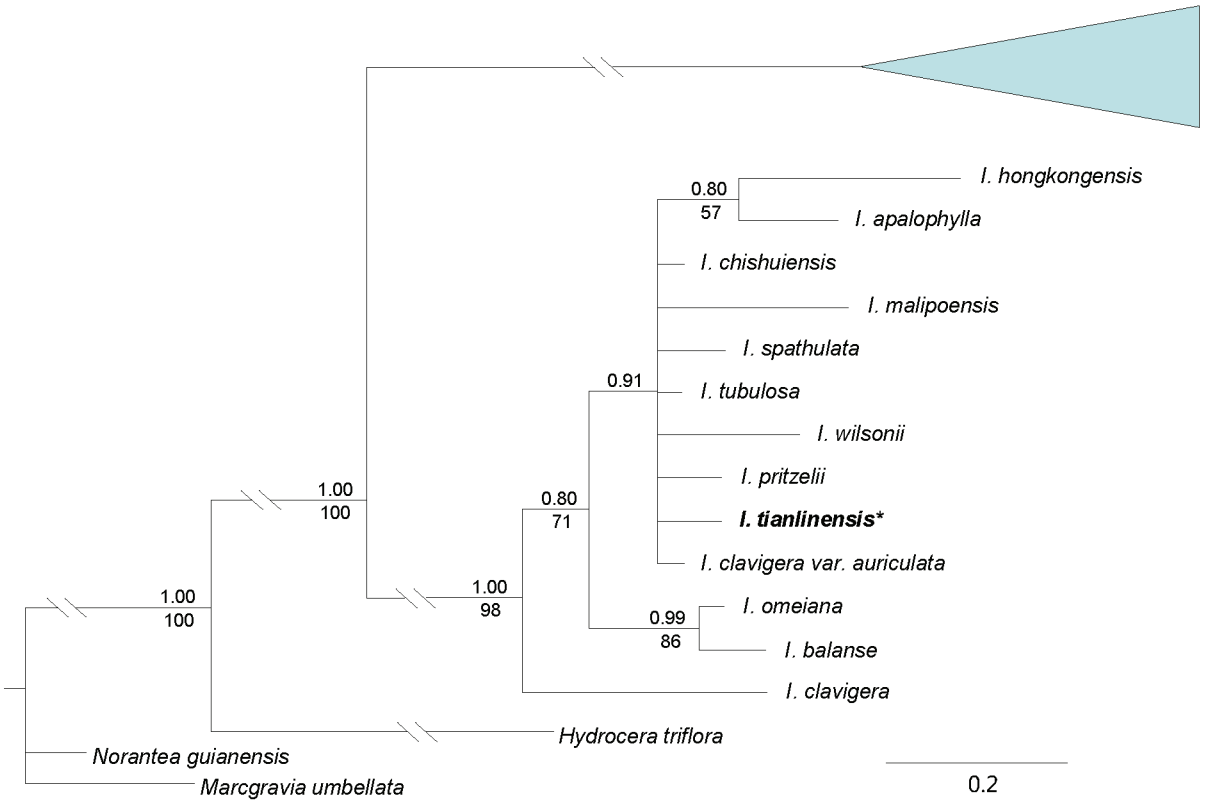

FIGURE 5. Bayesian consensus phylogram based on the branch length of the cpDNA data (*atpB-rbcL + trn L-F)*. Numbers above and below branches are Bayesian posterior probabilities (> 0.5) and bootstrap percentages (> 50%), respectively.

References

Aublet, J.B.C.F. (1775) *Histoire des plantes de la Guiane française*, vol. 2. Pierre-François Didot, London, 976 pp.

Chen, W.H., Shui, Y.M., Sima, Y.K., Zhang, R.M. & Wei, Z.D. (2009) *Pararuellia glomerata* (Acanthaceae), a new species from Yunnan, China. *Botanical Studies* 50: 261–267.

Chen,Y.L. (2001) Balsaminaceae. *In:* Anonymous (Eds.) *Flora Reipublicae Popularis Sinica* 47 (2). Science Press, Beijing, pp. 1–243.

Chen,Y.L., Akiyama, S. & Ohba, H. (2007) Balsaminaceae. *In*: Wu, Z.Y., Raven, P.H. & Hong, D.Y. (Eds.) *Flora of China*, vol. 12. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, pp. 43–113.

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Erdtman, G. (1971) *Pollen morphology and plant taxonomy: Angiosperms.* Hafner Publishing Company, New York, 553 pp.

Fischer, E. (2004) Balsaminaceae. *In:* Kubitzki, K. (Ed.) *The Families and Genera of Vascular Plants*, vol. 6. Springer Verlag, Berlin, pp. 20–25.
http://dx.doi.org/10.1007/978-3-662-07257-8_4

Grey-Wilson, C. (1980) *Impatiens of Africa*. Balkema, Rotterdam, 235 pp.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Window 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Hooker, J.D. (1908) *Hooker's icons plantarum*. Dulau & Co., Ltd. London, 2863 pp.

Huang, S.H., Shui, Y.M. & Chen, W.H. (2003) New Taxa of *Impatiens* from Yunnan. *Acta Botanica Yunnanica* 25 (3): 261–280.

Janssens, S., Geuten, K., Yuan, Y.M., Song, Y., Küpfer, P. & Smets, E. (2006) Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast *atpB-rbcL* spacer sequences. *Systematic Botany* 31: 171–180.
http://dx.doi.org/10.1600/036364406775971796

Janssens, S., Wilson, Y.S., Yuan, Y.M., Nagels, A., Smets, E.F. & Huysmans, S. (2012) A total evidence approach using palynological characters to infer the complex evolutionary history of the Asian *Impatiens* (Balsaminaceae). *Taxon* 61 (2): 355–367.

Ku, S.M., Kono, Y.S. & Liu, Y. (2008) *Begonia pengii* (sect. *Coelocentrum*, Begoniaceae), a new species from limestone areas in Guangxi,

China. *Botanical Studies* 49: 167–175.

Linnaeus, C. (1753) *Species Plantarum*. L. Salvius, Stockholm, 1200 pp.

Lin, C.R., Liu, Y., Nong, D.X., Kono, Y. & Peng, C.I. (2013) *Aspidistra crassifila* (Asparagaceae), a new species from Guangxi, China. *Botanical Studies* 54: 43.
http://dx.doi.org/10.1186/1999-3110-54-43

Lu, Y.Q. (1991) Pollen morphology of *Impatiens* L. (Balsaminaceae) and its taxonomic implications. *Acta Botanica Sinica* 29: 352–357.

Lu, Y.Q. & Chen, Y.L. (1991) Seed morphology of *Impatiens* L. (Balsaminaceae) and its taxonomic significance. *Acta Botanica Sinica* 29: 252–257.

Moore, P.D. & Webb, J.A. (1978) *An illustrated guide to pollen analysis.* Hodder and Stoughton, London, 192 pp.

Peng, C.I., Ku, S.M., Kono, Y. & Liu, Y. (2012) *Begonia chongzuoensis* (sect. *Coelocentrum*, Begoniaceae), a new calciphile from Guangxi, China. *Botanical Studies* 53: 283–290.

Peng, C.I., Yang, H.A., Kono, Y., Chung, K.F., Huang, Y.S., Wu, W.H. & Liu, Y. (2013) Novelties in *Begonia* sect. *Coelocentrum*: *B. longgangensis* and *B. ferox* from limestone areas in Guangxi, China. *Botanical Studies* 54: 44.
http://dx.doi.org/10.1186/1999-3110-54-44

Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
http://dx.doi.org/10.1093/bioinformatics/14.9.817

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
http://dx.doi.org/10.1093/bioinformatics/btg180

Song, Y., Yuan, Y.M. & Küpfer, P. (2005) Seedcoat micromorphology of *Impatiens* (Balsaminaceae) from China. *Botanical Journal of the Linnean Society* 149: 195–208.
http://dx.doi.org/10.1111/j.1095-8339.2005.00436.x

Swofford, D.L. (2003) *PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0 beta 10.* Sunderland, Massachusetts, Sinauer, 140 pp.

Taberlet, P., Gielly, L., Patou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
http://dx.doi.org/10.1007/BF00037152

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876–4882.
http://dx.doi.org/10.1093/nar/25.24.4876

Wang, Q. & Hong, D.Y. (2014) *Codonopsis gongshanica* (Campanulaceae), a new species from NW Yunnan base on morphology and molecular phylogenetic analysis. *Phytotaxa* 188 (3): 145–152.
http://dx.doi.org/10.11646/phytotaxa.188.3.3

Wight, R. & Arnott, G.A.W. (1834) *Prodromus Florae Peninsulae Indiae Orientalis*, vol. 1. Parbury, Allan & Co., London, 480 pp.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M., Gelfang, D., Sninsky, J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications.* Academic Press, San Diego California USA, pp. 315–322.
http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1

Xiong, Y.X. (1996) Two new species of *Impatiens* from Guizhou. *Acta Phytotaxonomica Sinica* 34 (1): 98–101.

Xu, W.B., Gao, H.S. & Liu, Y. (2008) *Lagarosolen jingxiensis* Yan. Liu, H. S. Gao & W. B. Xu, a new species of Gesneriaceae from Guangxi, China. *Journal of Tropical and Subtropical Botany* 16: 274–276.

Yu, S.X. (2012) *Balsaminaceae of China*. Peking University Press, Beijing, 215 pp.

Yu, S.X., Janssens, S.B., Zhu, X.Y., Lidén, M., Gao, T.G. & Wang, W. (2015) Phylogeny of *Impatiens* (Balsaminaceae): integrating molecular and morphological evidence into a new classification. *Cladistics* 1–19.

Yuan, Y.M., Song, Y., Geuten, K., Rahelivololona, E., Wohlhauser, S., Fischer, E., Smets, E. & Küpfer, P. (2004) Phylogeny and biogeography of Balsaminaceae inferred from ITS sequences. *Taxon* 53: 391–403.
http://dx.doi.org/10.2307/4135617