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Three New Species of *Impatiens* L. from China and Vietnam: Preparation of Flowers and Morphology of Pollen and Seeds

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Abstract*—**The genus *Impatiens* (Balsaminaceae) is one of the most difficult genera for making effective herbarium specimens because of its tender and complicated flowers. The preparation of flowers is always one of the most important steps in the collections of *Impatiens*. The present paper demonstrates a method to prepare flowering specimens of *Impatiens* in the field. Examples include three new species collected from the Sino-Vietnamese border, viz. ***Impatiens aconitoides, ***I. purpureifolia***, and ***I. rugata***. The related species *I. laojunshanensis*, *I. apalophylla,* and *I. clavigera* are also sampled using the same preparation method. As to the three new species, their pollen grains and seeds were observed by scanning electron microscopy. ***Impatiens aconitoides*** has 4-colpate pollen grains and ovoid seeds with protrusive-type of seed coat, whereas ***I. purpureifolia*** and ***I. rugata*** have 3-colpate grains and ellipsoidal seeds with reticulate-typed seed coat. Such an integrated approach to include detailed floral characters as well as pollen and seed characters is proposed when new taxa of *Impatiens* are described.

***Keywords*—**Flower structure, *Impatiens aconitoides*, *I. purpureifolia*, *I. rugata*, pollen, seed.

Balsaminaceae are well known for their horticultural value and include two genera and approximately 900 species (Chen 2001). *Hydrocera* Bl. is a monospecific genus, distributed in South India, China, Indo-China, and SE Asia. *Impatiens* L. has approximately 900 species. The genus flourishes in moist habitats in tropical mountains, and is mainly distributed in tropical and subtropical Asia, and Africa, with fewer species in temperate Asia, Europe, and North America. In China, *Impatiens* L. includes over 250 species and is mainly distributed in southwest China, especially in Yunnan (105 species), Sichuan (64), Tibet (Xizang) (31), Guizhou (26), and Guangxi (12) (Xiong and Luo 1988; Akiyama et al. 1995a, 1995b, 1996; Shui and Li 2000; Chen 2001; Jin and Ding 2002; Huang et al. 2003; Huang 2006; Chen et al. 2007b; Janssens et al. 2009a).

Careful preparation of flowers is necessary to determine useful character states of *Impatiens* species. Because the flowers are delicate and complicated, it is difficult to investigate the floral morphology of *Impatiens* in a dried state or identify *Impatiens* herbarium specimens. Careful preparation of flowers has thus been emphasized for over 150 yr in several studies of the genus (Hooker and Thompson 1859; Hooker 1874, 1908; Chen 1978; Grey-Wilson 1980a). However no standardized method has been reported so far. The need for a standardized method is important as some species descriptions (e.g. Léveillé 1916; Huang et al. 2003) are prepared from poorly preserved herbarium specimens.

Pollen and seed morphology have become important to the systematics of *Impatiens*. On the one hand, 4-colpate and 3-colpate pollen grains may have obvious implications to the systematics of *Impatiens* and Balsaminaceae (Huynh 1968a, b; Lu 1991; Janssens et al. 2005; Yu 2008). On the other hand, the seed morphology has not only confirmed the differentiation of numerous similar species but has also provided some systematic evidence in *Impatiens* (Lu and Chen 1991; Song et al. 2005; Chen et al. 2007a; Yu 2008). However, it is estimated that less than 10% of *Impatiens* species were examined in a seed or pollen morphological study (e.g. Perrier de la Bathie 1933; Coombe 1956; Huynh 1966, 1968a, b; Sohma 1971; Gupta and Sharma 1986; Nayar 1990; Lu 1991; Bhaskar 2006; Cai et al. 2007b; Yu 2008; Yu et al. 2009). We thus argue that pollen and seed characters should be described for all new taxa of *Impatiens*.

Materials and Methods

***Materials*—**The following three new species, *Impatiens aconitoides* Y. M. Shui & W. H. Chen sp. nov., *I. purpureifolia* S. H. Huang & Y. M. Shui sp. nov., and *I. rugata* S. H. Huang & Y. M. Shui sp. nov. were collected exclusively in the limestone region of the Sino-Vietnamese border between 1993 and 2009 (Fig. 1). The large and ovate dorsal petals without crestae and 2–3-dentate tips of distal lobes of lateral united petals show that *Impatiens aconitoides* is closely related to *I. laojunshanensis* S. H. Huang (Fig. 2C and F; Chen et al. 2007b). Perennial habit, numerous-flowered inflorescences, four lateral sepals and tricolpate pollen show that the latter two new species are grouped with *I. hongkongensis* C. Grey-Wilson, *I. balansae* Hook. f., *I. omeiana* Hook. f., and *I. wilsonii* Hook. f.(Chen et al. 2007b). Among them, *I. purpureifolia* is most similar to *I. apalophylla* Hook. f. in having ovate outer lateral sepals, bilobed upper lateral united petals, and recurved spurs (Fig. 2O and R), and different from *I. wilsonii* and *I. omeiana*. *Impatiens rugata* is most similar to *I. clavigera* Hook. f. as it is characterized by long bracts which are less than 1 cm long, bilobed lateral petals united near the base, and the presence of auricles (Fig. 2I and L), yet differs from *I. balansae*. All the above six species were sampled for preparation of flowers. A key was given to show the relationships among the *Impatiens* species from the Yunnan border with Vietnam.

***Preparation of Flowers*—**The detailed steps of preparation of flowers in the field are proposed below. First, in the field, images of the flowers must be obtained in front and lateral views to show the color, size and position of every part of the flower. Lateral united petals should be separated slightly to show their shape, especially the exterior shape of dorsal sepals, lateral sepals and spurs (Fig. 2A - B, D- E, G- H, J- K, M- N, P- Q). Second, all parts of a flower should be completely separated and photographed on a black or dark background, and must be examined in the field to make sure that there are seven or nine parts of flowers including two or four sheets of lateral sepals, one sheet of a dorsal petal, two sheets of lateral united petals and one group of stamens (Fig. 2C, F, I, O, R). Third, all of these flower parts should be pressed between soft paper until they are dried completely (Fig. 2L). If possible, additional flowers should be prepared and attached on each sheet of specimens.

***SEM Morphology of Pollen and Seeds*—**Mature dry pollen grains were collected from the specimens of all three newly described taxa. The pollen grains were washed in water using ultrasound, and then they were air-dried and fixed to aluminum stubs and sputter-coated with gold.

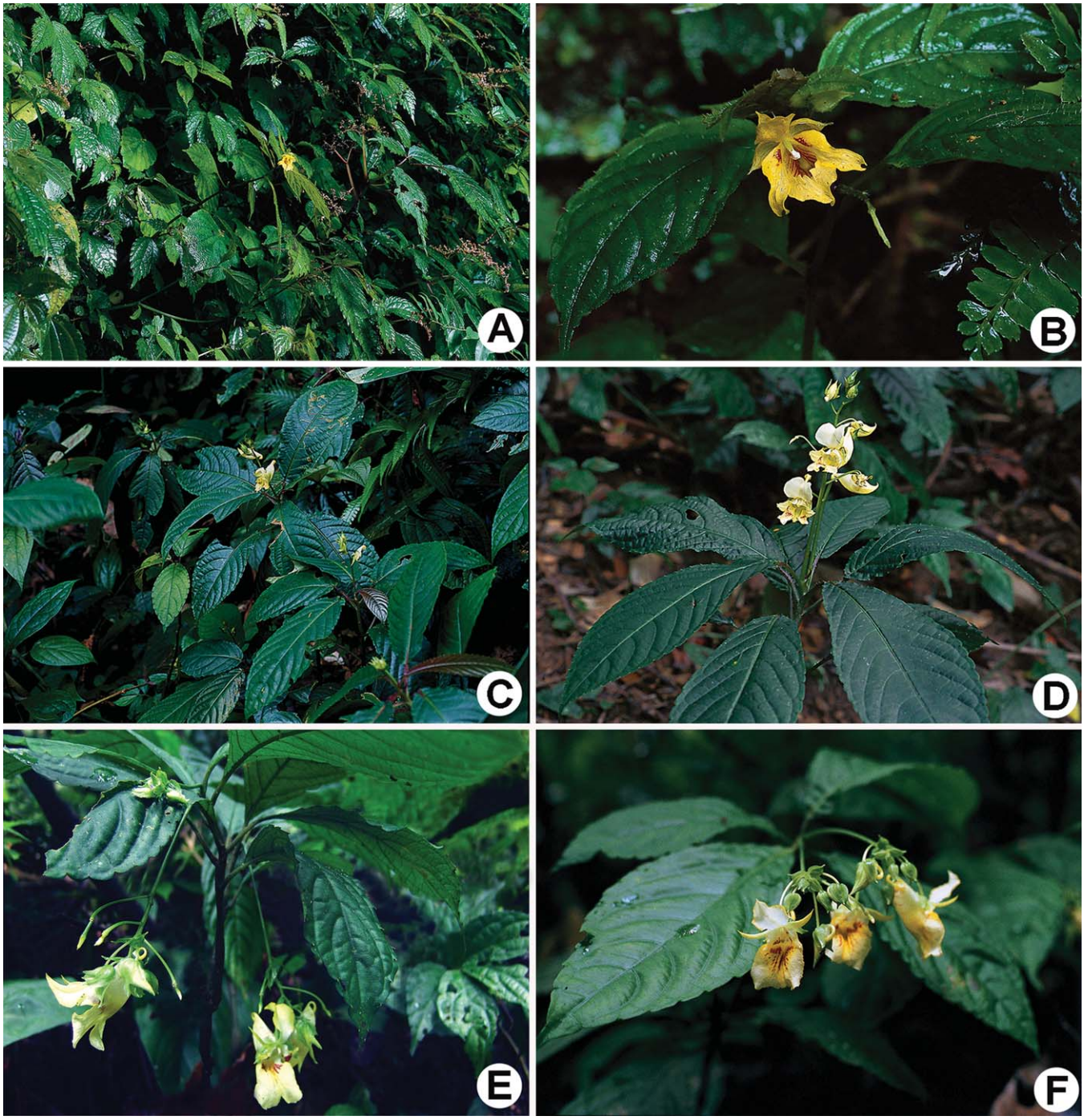

Fig. 1. Habitat and habit photographs of the three new *Impatiens* species. A. and B. *I. aconitoides* (*Y. M. Shui and W. H. Chen 32393*). C. and D. *I. purpureifolia* (*Y. M. Shui, W. H. Chen and J. S. Sheng 30583*). E. and F. *I. rugata* (E. from *C. I Peng et al. 17528*, F. from *Z. G. Yang 2009–01*). F. was photographed by Z. G. Yang, and all the others photographed by Y. M. Shui.

Morphological observations were made and micrographs were then taken with a Hitachi-S-3000N ESEM. Pollen grains and seeds were measured for over ten pollen grains under the microscope and described according to terminology of pollen grains and seeds (Erdtman 1952; Lu 1991; Song et al. 2005; Cai et al. 2007a, 2007b). The voucher specimens were either the holotypes or paratypes of these three new taxa, and deposited in the herbarium of the Kunming Institute of Botany, Chinese Academy of Science (KUN).

Results

Flowers of Three New Species—Figure 2 shows the structure of flowers of the new species and three related species. In *Impatiens aconitoides*, there are seven floral parts, including a pair of lateral sepals (Fig. 2A-C). *Impatiens laojunshanensis*, has a similar structure of flowers (Fig. 2D-F). Obviously, one of the most diagnostic characters comes from lateral united petals, viz. the shape of basal lobes. The former species is diagnosed by its emarginate oblong basal lobes of lateral united petals, while the latter species is characterized by its acute linear basal lobes of lateral united petals.

Impatiens purpureifolia is characterized by nine floral parts, including two pairs of lateral sepals (Fig. 2G-I). *Impatiens apalophylla* Hook. f. has similar floral parts except the spur

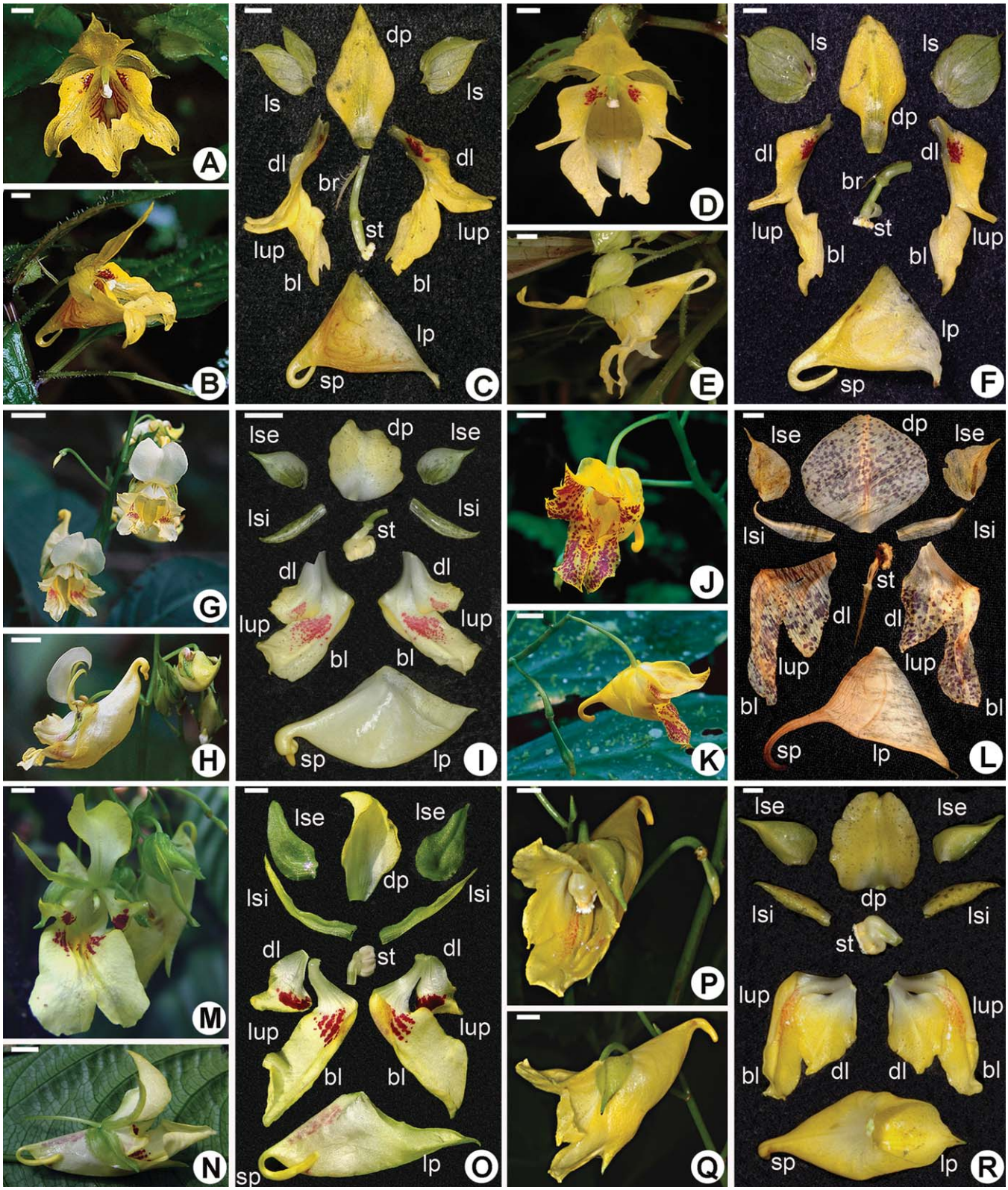

Fig. 2. The preparation of flowers in *Impatiens* L. A.-C. *I. aconitoides* (*Y. M. Shui & W. H. Chen 72055*). D.-F. *I. laojunshanensis* (*Y. M. Shui and W. H. Chen 72058*, KUN). G.-I. *I. purpureifolia* (*Y. M. Shui et al. 82529*). J.-L. *I. apalophylla* Hook.f. (*Y. M. Shui et al. 44020*, KUN). M.-O. *I. rugata* (*Z. G. Yang 2009–01*). P.-R. *I. clavigera* Hook. f. (*Y. M. Shui et al. 82688*, KUN). M.-O. photographed by Z. G. Yang, and all others photographed by Y. M. Shui. Notes: bl: basal lobes of lateral united petals; br: bracts; dl: distal lobes of lateral united petals; dp: dorsal petal; lp: lip; ls: lateral sepals; lse: exterior lateral sepals; lsi: interior lateral sepals; lup: lateral united petals; sp: spur of lip; st: stamens. All scale bars: 5 mm.

(Fig. 2J-L). The inflated end of the spur in *I. purpureifolia* is different from the claviform end of spur in *I. apalophylla* (Fig. 2I and L). Furthermore, the colorful patch is also pinkish on the lateral united petals in *I. purpureifolia*, but red on the lateral united petals and dorsal sepals in *I. apalophylla* (Fig. 2G, I, J, and L).

In the new species *Impatiens rugata*, there are also nine floral parts, including two pairs of lateral sepals (Fig. 2M-O). *Impatiens clavigera* Hook. f. has a similar floral structure, but differs from the above species in having laterally united petals and spurs (Fig. 2P-R). Furthermore, the basal lobes of its lateral united petals are larger, and its colorful spots on the lateral united petals are smaller and orange-pink instead of large and red as in *I. rugata* (Fig. 2M, O, P, and R). Finally, its spur is slightly bent and shortly claviform instead of recurved and long claviform as in *I. rugata* (Fig. 2N, O, and Q).

Pollen Morphology—Figure 3 shows the pollen size and shape of the three new species. *Impatiens aconitoides* has 4-colpate pollen grains, while the other two species have 3-colpate pollen grains. Furthermore, the latter two species differ in the shape of pollen grains and their lumen surface.

In *Impatiens aconitoides*, the polar view is nearly elliptic, 21.8–31.9 × 48.3–61.5 μm in size (Fig. 3: D), the equatorial view is oblong (Fig. 3A, B) or slightly obtuse (Fig. 3A, C), 22.5–28.4 μm thick; colpi four, narrowly shuttle-shaped, 7.9–8.7 μm (Fig. 3A, B-c, C-c and D-c); the entire surface is covered with numerous irregular lumens of 1.3–3.0 μm diam, almost sparely granulate and with large perforations (Fig. 3E-f and F-f); muri slightly straight, joint of muri obviously corniculate (Fig. 3E-e and F-e). Finally, some sticky substance was observed on the surface of pollen grains (Fig. 3B-d, C-d, D-d and F-d).

In *Impatiens purpureifolia*, the polar view is nearly rounded, 31.3–42.7 μm diam (Fig. 3G, H and I), the equatorial view elliptic, 19.7–28.0 μm thick (Fig. 3G and J); colpi three, linear, 10.5–12.2 μm (Fig. 3I-c and J-c); the whole surface is covered with numerous irregular lumens that are slightly shallow, 4.3–6.4 μm diam, densely granulate (Fig. 3K-f and L-f); muri substraight, joint of muri slightly corniculate (Fig. 3K-e and L-e). The pollen characteristics are different from those of *Impatiens aconitoides*.

In *Impatiens rugata*, the polar view is slightly triangular with three equal sides, each side 33.9–39.3 μm long (Figs. 3M, N and O), the equatorial view is elliptic, 24.9–30.2 μm thick, apex obtuse (Figs. 3M and P); colpi 3, linear, 11.4–13.1 μm (Fig. 3M, O-c, P-c and R-c); the entire surface is covered with numerous irregular lumens, lumens slightly deep, 2.9–5.7 μm diam, densely granulate (Fig. 3Q-f and R); muri obviously straight, joint of muri slightly corniculate (Fig. 3Q-e and R). These pollen characteristics are similar to those of *Impatiens purpureifolia*, but still differ from those of *Impatiens aconitoides* in having three colpi and nearly rounded or triangular equatorial view.

Seed Morphology—Figure 5 shows the seed morphology of the three new species. In *Impatiens aconitoides*, seeds are ovoid, brown, 3.08 × 1.53 mm in size (length/width = 2.01), base petiolate, ca. 0.45 mm long, top with a 0.15 mm long obtuse tip. The seed coat is basally protrusive and a composite of three types of epidermal cells. The first type is finger-like cells on the body of seeds that are thick and foveolate. The second is irregularly inflated cells with granulate walls. The two types of seed coat cells intercross with each other. The third is foveolate-walled cells restricted to the base and top of seeds (Fig. 5A, B, C).

In *I. purpureifolia*, seeds are ellipsoid, purple, ca. 6.56 × 4.81 mm in size (length/width = 1.36), base obtuse and top round or truncate (Fig. 5D, E). The seed coat is reticulate and a composite of one type of fine foveolate epidermal cells. The type is a composite of 4–6-angulate cell walls on the entire surface of seed coats and oblong cell walls on the dark middle strip of the abaxial surface of seed coats (Fig. 5F).

In *I. rugata*, seeds are ellipsoid, gray with brown lines, 3.12 × 1.70 mm in size (length/width = 1.84), base round and top round with a tip (Fig. 5G, H). The seed coat is basally reticulate and a composite of one type of finely foveolate lumens. The type is always a composite of slightly 5-angulate cell walls on the entire surface of the seed coat (Fig. 5I).

Taxonomic Treatment

Key to the Species of the Genus *Impatiens* from the Yunnan Border with Vietnam (Fig. 4)

1. Inflorescences more than 3- flowered; peduncle usually longer than leaves 2
   2. Lateral sepals 4 3
      3. Distal lobes of lateral united petals broad, less than 2 times as long as wide; lip usually broadly funnelform 4
         4. Lateral united petals bilobed at the middle, outer lateral sepals ovate, apex acute 5
            5. Petiole with 4–6-paired clavate glands; upper margin of lateral united petals emarginate; spur of lip 6–8 mm long, suddenly inflated and incurved near the tip, tip mucronate ..... *I. purpureifolia*
            5. Petiole eglandular or with 1–2-paired glands ..... *I. apalophylla*
         4. Lateral united petals bifid near base, outer lateral sepals broadly ovate, apex caudate 6
            6. Leaf base auriculate ..... *I. clavigera* var. *auriculata*
            6. Leaf base cuneate 7
               7. Leaf blade oblanceolate or obovate, base narrowly cuneate, obviously descending; distal lobes of lateral united petals with abaxial auricles 8
                  8. Stem and petiole cylindrical, hairs sparse, rectinerves lax and not conspicuous ..... *I. clavigera*
                  8. Stem and petiole angulate, rugate and glabrous, rectinerves dense and prominent ..... *I. rugata*
               7. Leaf blade round, elliptic or obtrapezoid, base rounded or broadly cuneate, never descending; distal lobes of lateral united petals without abaxial auricles ..... *I. balansae*
      3. Distal lobes of lateral united petals narrow, more than 2 times as long as wide; lip usually narrowly funnelform 9
         9. Flower purple ..... *I. porphyrea*
         9. Flower yellow or yellowish 10
            10. Bracts, lateral sepals and dorsal petals with glandular aristas ..... *I. clavicuspis*
            10. Bracts, lateral sepals and dorsal petals without glandular aristas 11

11. Flowers ca. 2.5 cm deep; leaf blade 2.5–3 cm wide ..... *I. luchunensis*
11. Flowers ca. 4 cm deep; leaf blade 4–6 cm wide ..... *I. maguanensis*
2. Lateral sepals 2 12
12. Spur broadly funnelform, more than 7 mm in diam. at mouth 13
13. Flowers purple or pink 14
14. Petiole without pairs of glands ..... *I. hancockii*
14. Petiole with 1 pair of glands 15
15. Lateral petals 8–10 mm long ..... *I. aquatilis*
15. Lateral petals less than 5 mm long ..... *I. uliginosa*
13. Flowers yellow ..... *I. racemosa*
12. Spur narrowly funnelform, less than 6 mm diam. at mouth 16
16. Flowers purple ..... *I. siculifer* var. *porphyrea*
16. Flowers yellow 17
17. Flowers 4–10, alternate on inflorescence axis; lateral united petals 3-lobed 18
18. Spur extremely curved or recurved 19
19. Leaves ovate; distal lobe of lateral united sepals about 2–3 times as long as basal lobes ..... *I. chapaensis*
19. Leaves ovate-lanceolate or elliptic-lanceolate; distal lobe of lateral united petals over 4 times as long as basal lobes ..... *I. siculifer*
18. Spur straight or slightly twisted 20
20. Lateral sepals ca. 2 mm long; spur slightly twisted ..... *I. ruiliensis*
20. Lateral sepals ca. 4 mm long; spur straight ..... *I. austroyunnanensis*
17. Flowers 20–37, verticillate on inflorescence axis; lateral united petals 2-lobed ..... *I. radiata*
1. Inflorescences 1- or 2 (3)-flowered; peduncle usually shorter than leaves (except *I. clavicuspis*) 21
21. Lateral sepals 4 27
27. Flowers white or yellow 28
28. Flowers white ..... *I. malipoensis*
28. Flowers yellow 29
29. Outer lateral sepals ca. 1.5 cm long 30
30. Spur ca. 8 mm long ..... *I. clavigeroides*
30. Spur ca. 5–6 mm long ..... *I. wenshanensis*
29. Outer lateral sepals ca. 0.7 cm long ..... *I. linearisepala*
27. Flowers blue ..... *I. arguta*
21. Lateral sepals 2 31
31. Adaxial costa of dorsal petal without appendage; end of distal lobes of lateral united petals 2–3-dentate 32
32. Stem unbranched; basal lobes of lateral united petals oblong, apex obviously emarginate, distal lobes furcated with the basal lobe ..... *I. aconitoides*
32. Stem ramose; basal lobe of lateral united petals broadly oblong, apex shortly caudate, distal lobes adherent to basal lobe ..... *I. laojunshanensis*
31. Adaxial costa of dorsal petal with appendage; end of distal lobes of lateral united petals entire or 2-lobed 33
33. Two lateral united petals joined along the inner margins 34
34. Lateral united petals blue; distal lobes of lateral united petals entire or apically retuse ..... *I. verrucifer*
34. Lateral united petals yellow, white, or pink, with orange punctas; distal lobes of lateral united petals 2-lobed, apically acute ..... *I. morsei*
33. Two lateral united petals free 38
35. Flowers white with pink striations, bracts broadly ovate ..... *I. rubrostriata*
35. Flowers yellow, orange or blue, bracts lanceolate to narrawly lanceolate 36
36. Flowers blue ..... *I. puberula*
36. Flowers yellow 37
37. Lateral sepals linear ..... *I. wuchengyihii*
37. Lateral sepals ovate or lanceolate 38
38. Leaves broadly elliptic to elliptic 39
39. Plants glabrous 40
40. Basal lobes of lateral united petals round, distal lobes elongate, oblong; anther obtuse ..... *I. mengtszeana*
40. Basal lobes of lateral united petals broadly obovate, distal lobes ovato-triangular; anther acute ..... *I. multiramea*
39. Plants more or less hairy 41
41. Flowers hairy 42
42. Lateral sepals lanceolate ..... *I. lancisepala*
42. Lateral sepals ovate ..... *I. kamtilongensis*
41. Flowers glabrous 43
43. Bracts broadly ovate ..... *I. duclouxii*
43. Bracts lanceolate ..... *I. lepida*
38. Leaves lanceolate to oblanceolate 44
44. Lateral sepals glabrous ..... *I. latipetala*
44. Lateral sepals hairy ..... *I. trichosepala*

Taxonomic Treatment

Impatiens aconitoides Y. M. Shui & W. H. Chen, sp. nov.—TYPE: CHINA. Yunnan: Malipo Co., Xinyan, 23°10′01″ N, 104°47′30″ E, alt. 1,800 m, on moist shady places on rocks in limestone cave, herb 0. 4 m tall, 14 Aug 2003, *Y. M. Shui & W. H. Chen 32393* (holotype: KUN!; isotypes: A!, MO!, NY!, PE!, US!, YUKU!).

Species haec habitu *Impatienti laojunshanensi* S. H. Huang similis, sed a qua caulibus simplicibus (nec ramosis) vexillis florum ovatis circ. 1.3 cm longis apice caudatis (nec ellipticis 1.0 cm longis apice acutis), costis vexillorum dorso villosis (nec glabris), lobis basalibus alorum oblongis apice emarginatis (in illa late oblongis apice longe caudatis) facile differt.

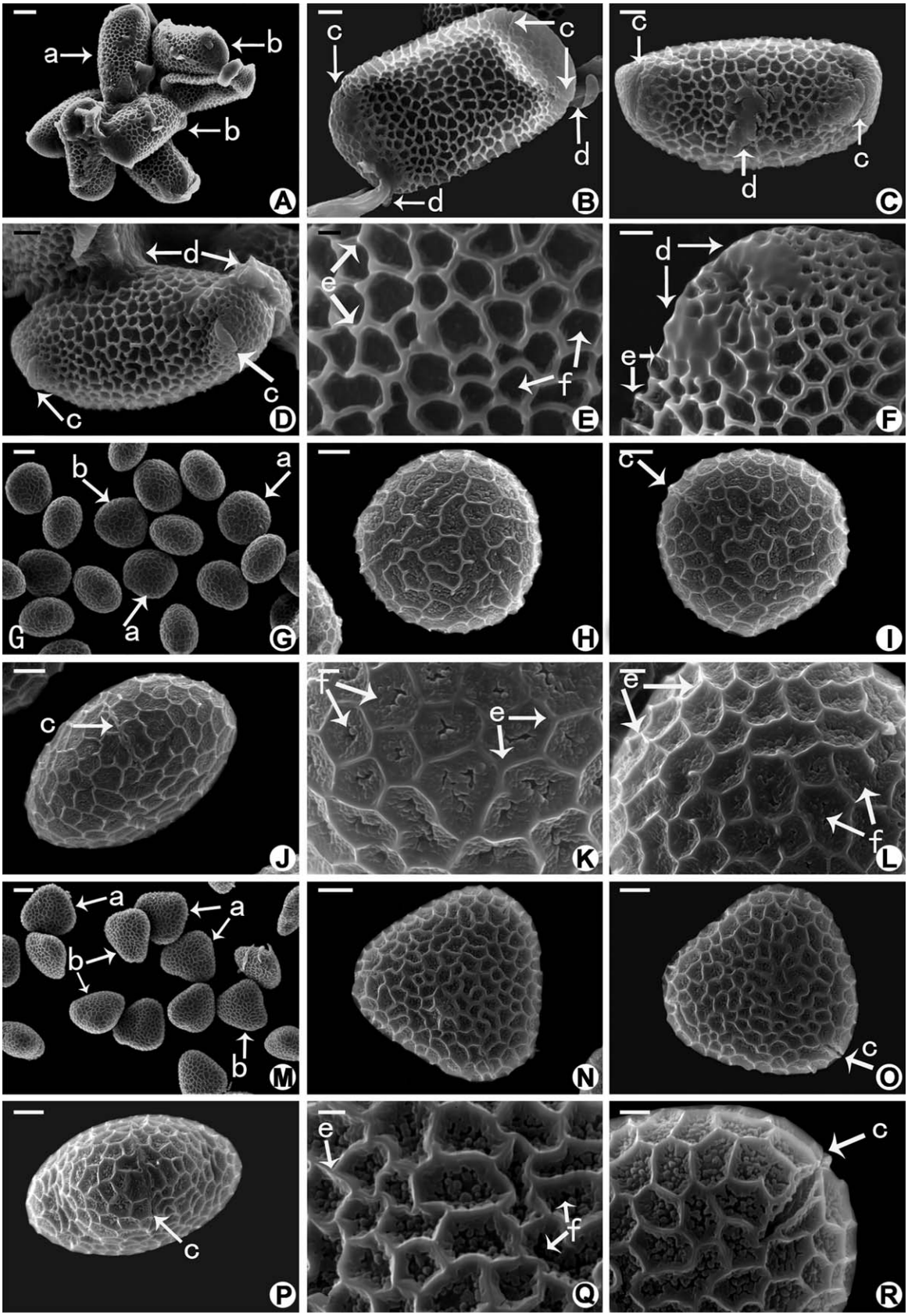

Fig. 3. Pollen grains of the three new species of *Impatiens* under SEM. A.-F. *I. aconitoides* (*Y. M. Shui & W. H. Chen 32393*) A. Group of pollen. B. Oblong pollen. C. Elliptic pollen. D. Polar view of elliptic pollen. E. Middle surface of pollen. F. Polar surface of pollen. G.-L. *I. purpureifolia* (*Y. M. Shui, W. H. Chen, & J. S. Sheng 30583*) G. Group of pollen. H. Face view of pollen. I. Lateral view of pollen showing colpi. J. Polar view of pollen showing colpi. K. Middle surface of pollen. L. Polar surface of pollen. M.-R. *I. rugata* (*C. I Peng et al. 17528*) M. Group of pollen. N. Face view of pollen. O. Lateral view of pollen showing colpi. P. Polar view of pollen. Q. Middle surface of pollen. R. Polar surface of pollen. References: a. Face view. b. Lateral view. c. Colpi. d. Sticky materials. e. Muri. f. Lumen. Scale bars: 1 μm- E., K., Q., R.; 2 μm- F., L.; 10 μm- A., G., M.; 20 μm- I., J.; 40 μm-B., C., D., H., N., O., P.

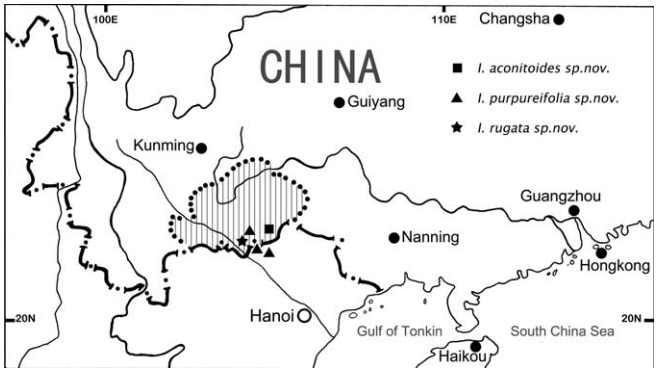

FIG. 4. The border between Yunnan and Vietnam and the localities for thee three new species of *Impatiens*.

Herb, annual, 30–50 cm tall. Stem erect, unbranched, glabrous, base with numerous fibrous roots. Leaves alternate, membranous, elliptic or ovate-elliptic, 5–10 × 3–3.8 cm, apex acuminate, base cuneate, with one pair of glands, margin crenato-serrate, adaxially green, with slight white pubescence or glabrescent, abaxially greenish, slightly puberulous along nerves, lateral nerves 4–6-paired; petioles 1.5–5 cm long. Peduncles solitary, arising from axils of leaves, puberulous, 1-flowered; pedicels, 1–1.5 cm long, above the middle with bracts; bracts linear, often deciduous, ca. 2 mm long, at the middle of pedicel. Flowers yellow, 1.5–1.8 cm long; lateral sepals 2, broadly ovate, oblique, ca. 5 × 3 mm, apex mucronate, base rotund, nerves numerous; dorsal petals ovate, ca. 1.3 × 0.6 cm, apex caudate, base attenuate and with red dots, abaxially with slightly thick and pilose costas; lateral united petals subsessile, ca.1.2 cm long, 2-lobed, basal lobes oblong, ca. 5 mm long, apex obviously emarginate, the distal oblong, ca. 5 mm long, 2–3-dentate; lips infundibular, ca. 1.2 × 1.1 cm, spurs short, ca. 3 mm long, incurved and attenuate; stamens ca. 3 mm long, filaments linear, 1.5–2 mm long, anthers obovoid, apex obtuse; ovaries linear, 4-loculed, with 5–7 ovules from the base to the top of locule. Capsules moniliform, 2–3 cm long. Seeds 5–7, ovate, 3–3.6 cm long, 2.8–3 mm tall, 1.8–1.9 mm in diam, with tip ca. 0.5 mm. Figures 1A, B, 2A-C, 6A-H.

Etymology—The epithetic "aconitoides" means that the dorsal petal is so conspicuous that it is like the flower of *Aconitum* Tourn. ex L. (Ranunculaceae).

Specimen Examined—CHINA. Yunnan: Malipo Co., Xinyan, alt. 2,000 m, on the moist cliff in the limestone cave, herb 0. 3–0.5 m tall, flowers yellow, 26 Aug 2007, *Y. M. Shui & W. H. Chen 72055* (IBSC, KUN, NY, PE, US).

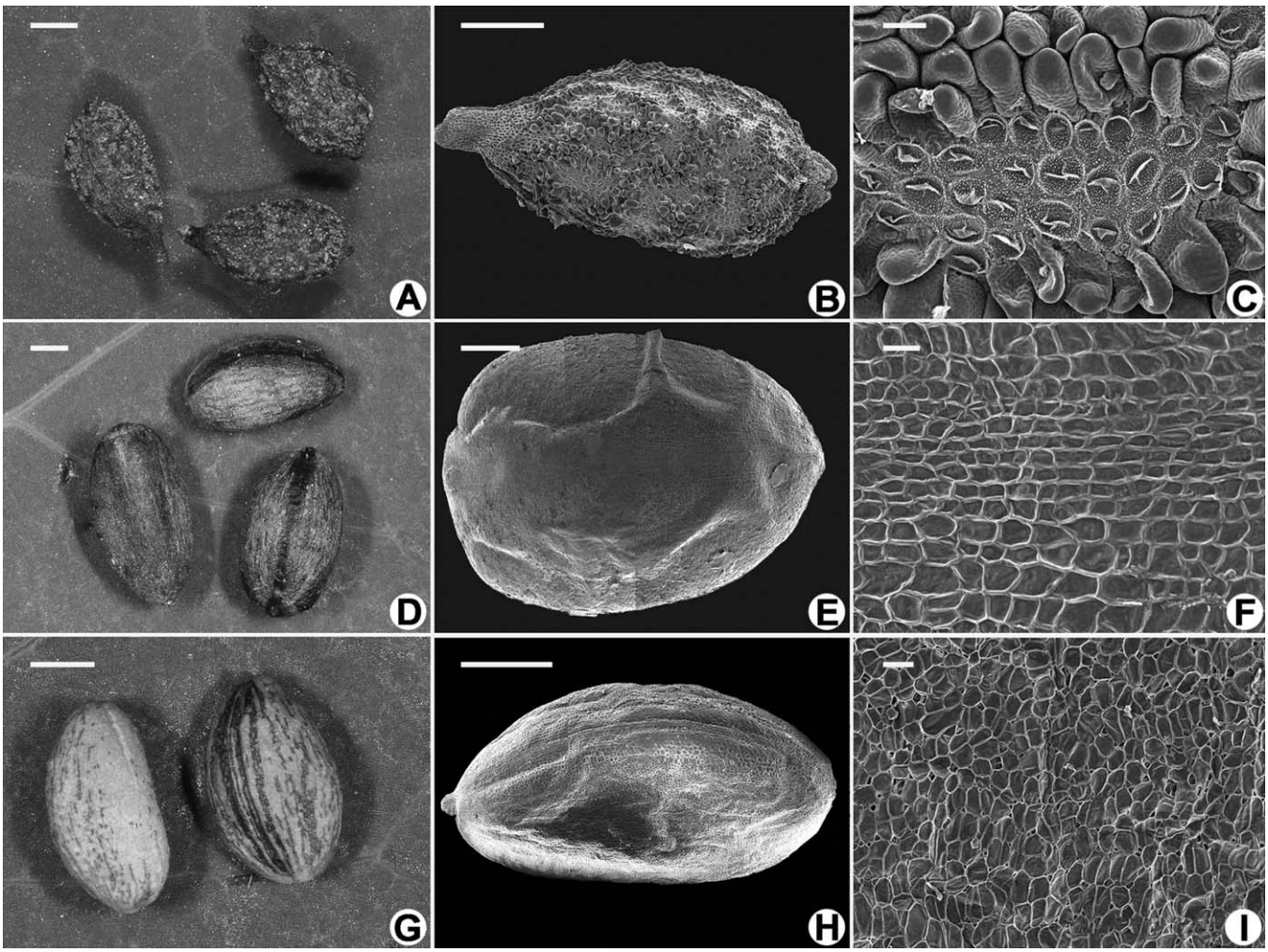

FIG. 5. Seeds of the three new species of *Impatiens*. A.-C.: *I. aconitoides* (*Y. M. Shui and W. H. Chen 32393*); D.-F.: *I. purpureifolia* (*Y. M. Shui et al. 82529*); G.-I.: *I. rugata* (*Y. M. Shui et al. 82528)*. B., C., E., F., H., and I. under SEM. Scale bars: 40 μm-C., F., I.; 1mm- A., B., D., E., G., H.

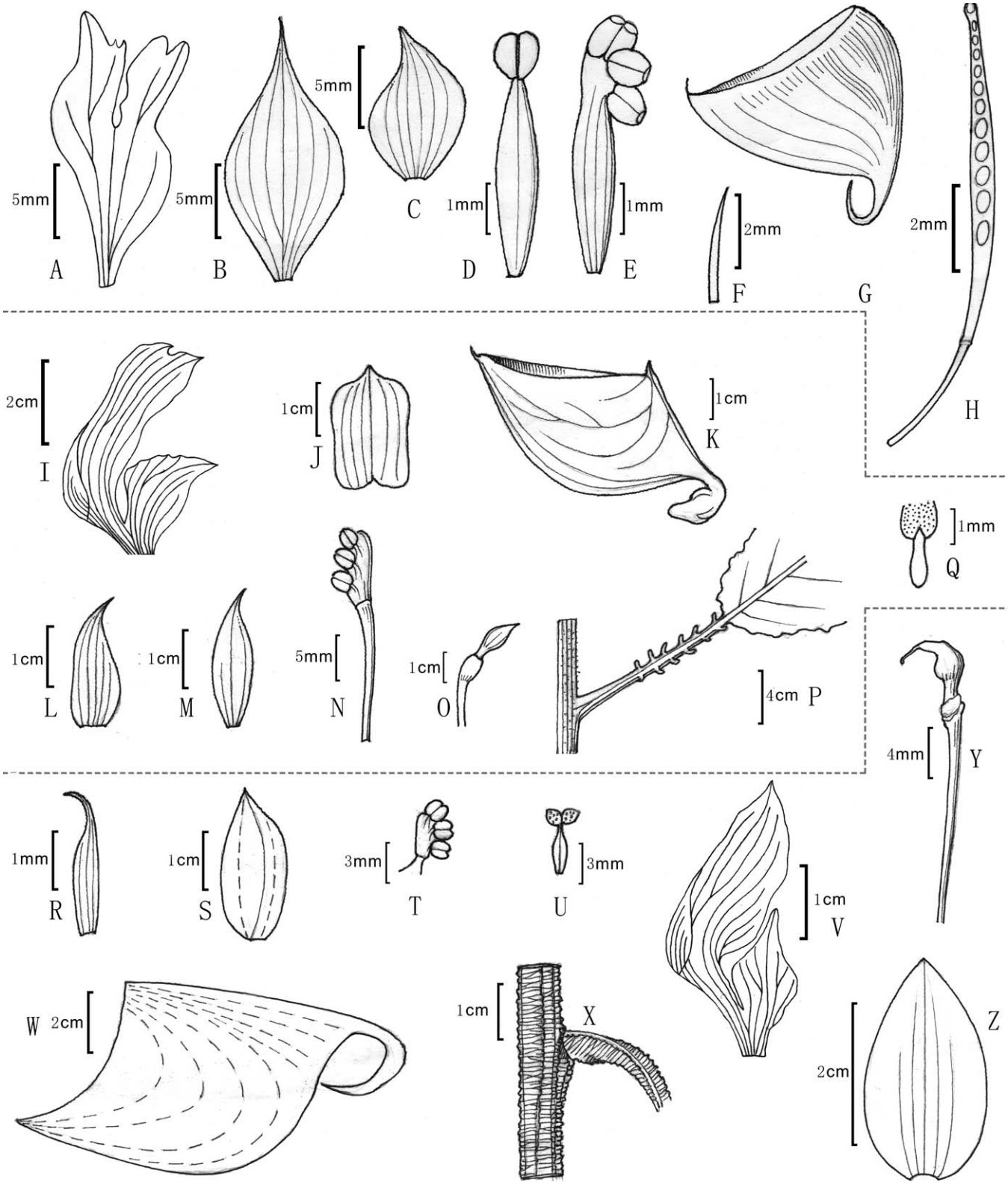

FIG. 6. Three new species of *Impatiens*. A.-H. *I. aconitoides* (*Y. M. Shui & W. H. Chen 32393*) A. Lateral united petal. B. Dorsal petal. C. Lateral sepal. D. Back face of stamens. E. Wing face of stamens. F. Bract. G. Lip. H. Fruit. I.-Q. *I. purpureifolia* (*Y. M. Shui, W. H. Chen, and J. S. Sheng 30583*) I. Lateral united petal. J. Dorsal petal. K. Lip. L. Outer sepal. M. Inner sepal. N. Wing face of stamens. O. Young fruit. P. Petiole showing glands. Q. Anther; R.-Z. *I. rugata* (*Y. M. Shui, W. H. Chen, & J. S. Sheng 30108*) R. Inner sepal. S. Outer sepal. T. Wing face of stamens. U. Anther. V. Lateral united petal. W. Lip. X. Rugate stem and petiole. Y. Fruit. Z. Dorsal petal. Drawn by X. L. Wu.

Phenology—Flowering from July to Sept., fruiting from Aug. to Nov.

Geographic Distribution and Ecology—The new species is only known from Malipo county of southeast Yunnan, China (Fig. 4). It grows on cliff surfaces with moist thin soil at the entrances of caves on limestone hills at alt. 1,800–2,000 m (Fig. 1A). There, the yearly and daily temperature changes are small, and the daily light duration is relatively constant. Outside the entrances of caves, however, the situation is completely different because of the bright and open environments. The main associated plants include *Saxifraga epiphylla* Gornall et H. Ohba (Saxifragaceae), *Boenninghausenia albiflora* (Hook.) Reich. ex Meisn (Rutaceae), *Pilea semisessilis* Hand.-Mazz. (Urticaceae), *Impatiens racemosa,* and *I. laojunshanensis* S. H. Huang, also *Primula* sp. (Primulaceae), *Petrocosmea* sp. (Gesneriaceae), and another possibly undescribed species of *Impatiens*. So, like an island, the karst cave habitat isolates some endemic plants from those outside the cave, and makes them narrowly endemic.

Notes—The new species is similar to *I. laojunshanensis* in having one pair of glands on the base of leaf limb, ovate dorsal petals, and 2–3-dentate tips of dorsal petals. In the new species, the stem is simple, leaf limb oblique at base, dorsal petals ovate ca. 1.3 cm long and adaxially villous on costa, the basal lobes of lateral united petals oblong, apex emarginate (Fig. 2C, 6A and B).

Impatiens purpureifolia S. H. Huang & Y. M. Shui, sp. nov.—TYPE: CHINA. Yunnan: Maguan Co., Gulinqing, Juziyuan, 103°55′39″ E, 22°44′29″ N, alt. 800 m, in evergreen broad-leaved forests of limestone hills, 13 Oct 2002, *Y. M. Shui, W. H. Chen and J. S. Sheng 30583* (holotype, KUN!; isotypes, NY!, MO!, PE!, US!).

Species haec habitu *Impatienti apalophyllae* Hook. f. similes, sed a qua foliis subtus saepe purpureis basi obliquis late cuneatis vel truncatis haud decurrentibus; petiolis superne glandulis 4–6 paribus clavatis instructis; vexillis subquadratis apice parce acutis; alis bilobatis, lobo basali alorum dolabrato, apice emarginatis ad superiori margine, extreme acutis, distali alorum apice emarginato antice leviter acuto; calcari labellis breviori 6–8 mm longo prope extremo inflatis facile differt.

Herb 30–50 cm tall. Stem erect, robust, slightly angulate, slightly puberulose toward the apex. Leaves alternate, thickly chartaceous, congregating at the upper parts of stems, obovate-elliptic or elliptic, 13–15.5 × 5.5–6.5 cm, apex caudate, base oblique and broadly cuneate or truncate, hardly decurrent, margin repand-crenate, glandular-mucronate between crenae, green on the adaxial surface, often purple on the abaxial surface, glabrous, lateral nerves 10–12-paired; petioles 2–3.5 cm long, angulate, above the middle with 4–6 pairs of clavate glands 1–1.2 mm long. Peduncles arising from the axils of leaves on the upper stem, 10–13 cm long, 10–12-flowered; pedicels 1.5–2.1 cm long; bracts caducous, ovate, 8–9 × 4.6–5 mm, apex caudate, margin entire, at the base of pedicel. Flowers yellowish, ca. 2 cm long when expanded; lateral sepals 4, yellowish green, slightly thick, outer 2 oblique and ovate, 11–12 × 4–5 mm, apex rostrate, rostra 3–4 mm long, base obtuse or truncate, inner 2 linear-lanceolate, 11–12 × 2.5–3.6 mm, apex acuminate, base obtuse; dorsal petal orbicular or subquadrate, 11–12 × 6–7 mm, apex slightly acute, base broadly cuneate or retuse, adaxially with slightly thick costae; lateral united petals bilobed, the basal dolabrate, 10–11 × 8–9 mm, shortly stipitate, apex with emarginate margin and an acute end, inside with purple dots, the distal recurved, oblong, 1.7–2 × 0.6–0.8 cm, emarginate at the superior part, end slightly acute, dorsal slightly auriculate, base long-istipitate, stipes 6–7 × 12–15 mm; lips saccate-infundibular, 2.5–2.8 cm long including spurs, limb oblique, ca. 2 × 1.2 cm, terminally mucronate; spurs of lips incurved, 6–8 mm long, inflated near tip, end slightly mucronate; stamens 6 mm long, filaments linear, anthers ovate, apex slightly acute; ovaries fusiform, 4-loculed, 6–7 mm long, apex rostrate, with 4 ovules on the top of each locule. Capsules clavate, 3.1–3.5 cm long. Seeds 4, brown, elliptic. Figures 1C, D, 2 G-I, 6L-Q.

Specimens Examined—CHINA. Yunnan: Hekou county, Nanxi comm., Machang to Liangzi, alt. 690 m, in dense forests along moist valley, 6 May 2004, *Y. M. Shui et al. 40896* (KUN). Hekou county, 103°53′ E, 22°40′ N, alt. 800 m, in secondary forests on rocky hills, in flower, 21 Nov. 2000, *Y. M. Shui et al. 14570* (KUN, PE). Maguan county, Gulinqing comm., Arboretum-examining Station, alt. 500 m, in monsoon rain forests of limestone hills, 23 July 2005, *Y. M. Shui et al. 44255* (KUN). The same locality and habitat, 24 Dec., 2008, in fl. and fr., *Y. M. Shui et al. 82529* (KUN). VIETNAM. Lao Cai: Bac Ha district, Ta Van Cho comm., Tenh Chu village, N22°36′28.05″, E104°15′56.73″, alt. 1,533 m, limestone secondary forest, 0.7–1.0 m tall, fl. yellow, 14 Nov., 2009, *CKF 157* (KUN); the same community, Su Manh Khang village, N22°35′20.72″, E104°14′56.62″, alt. 1,411 m, limestone secondary forest, 0.4 m tall, fl. yellow, 17 Nov., 2009, *CKF 219* (leaves purple beneath), *CKF 220* (leaves green beneath) (KUN); Lao Cai: Muong Hhuang district, Muong Khuong comm., Sa Ho village, N22°46′53.06″, E104°06′46.35″, limestone secondary forest, alt. 928 m, 0. 3 m tall, fl. yellow, 19 Nov., 2009, *CKF 231* (KUN); Muong Hhuang, Nam Chay comm., Moi village, N22°44′12.06″, E104°03′31.60″, alt. 902 m, in the forest, 20 Nov., 2009, 0.5 m tall, fl. yellow, *Y. M. Shui V-031* (KUN).

Etymology—The epithetic "purpureifolia" refers to the abaxial leaf surface being purple.

Phenology—Flowering from Oct. to Nov., fruiting from Nov. to Dec.

Geographic Distribution and Ecology—The new species is distributed in Hekou and Maguan counties of southeast Yunnan, China, Bac Ha, and Muong Hhuang districts of Lao Cai, north Vietnam (see Fig. 4). It only grows in the thick soil among limestone rocks of tropical limestone monsoon rain forests dominated by *Excentrodendron hsienmu* (Chun et How) H. T. Chang et R. H. Miau (Tiliaceae). The accompanying tall trees over ca. 30 m tall include *Dipterocarpus retusus* Bl., *Parashorea chinensis* Wang Hsie (Dipterocarpaceae) and *Antiaris toxicaria* Lesch. (Moraceae). Accompanying trees include *Rothmannia daweishanensis* Y. M. Shui et W. H. Chen (Rubiaceae), *Muricococcum sinense* Chun et How, and *Cleidon brevipetiolatum* Pax ex Hoffm. (Euphorbiaceae), *Campereia manillana* (Bl.) Merr. (Opiliaceae), and *Hydnocarpus annamensis* (Gagnep.) M. Lescot et Sleum. (Flacourtiaceae). The main shrubs include *Mananthes panduriformis* (R. Ben.) C. Y. Wu et C. C. Hu (Acanthaceae) and *Streblus macrophylla* Bl. (Moraceae). The main herbs are *Elatostema acuminatum* (Poir.) Brongn. (Urticaceae), *Aglaonema tenuipes* Engl. (Araceae), *Habenaria commelinifolia* (Roxb.) Lindl., *Liparis cordifolia* Hook. f. (Orchidaceae), *Impatiens rugata* S. H. Huang & Y. M. Shui, *I. balansae,* and *I. morsei* (Balsaminaceae). Most of these accompanying plants are rare and endemic. Therefore, the karst forest ecology with high endemism deserves further protection.

Notes—The new species is similar to *I. apalophylla* in having 4 lateral sepals, bilobed upper lateral united petals, recurved spurs, and ovate outer lateral sepals with acute apex, but is distinguished by its oblique cuneate or truncate leaf base, petiole with 4–6-paired clavate glands, yellowish flowers with few pink patches on the lateral united sepals, dorsal petal orbicular or subquadrate, with slightly acute apex, distal lobes of the

lateral united sepals emarginate on the superior margin, and lip spur with an inflated end. In *I. apalophylla*, the leaf base is decurrent, petiole with 2–3-paired clavate glands, flowers yellow with numerous red patchs on the lateral united petals and dorsal sepals, dorsal sepal is elliptic with a rounded and mucronate apex, and the lip spur is acuminate.

Impatiens rugata S. H. Huang & Y. M. Shui, sp.nov.—TYPE: CHINA. Yunnan: Maguan Co., Gulinqing, Arboretum-examining Station, 103°59′42″ E, 22°44′23″ N, alt. 560 m, in tropical seasonally rainy forests, 2 Oct. 2002, *Y. M. Shui, W. H. Chen & J. S. Sheng 30108* (holotype, KUN!; isotypes, IBSC!, PE!).

Species haec habitu *Impatienti clavigerae* Hook. f. similes, sed a qua superne caulis et ad nodos, dense rugatis; foliis crasse papyraceis, griseis, nervis lateralibus retinervisque densis et prominentibus; petioles dense rugatis; vexillis late ovatis apice mucrunatis haud carinatis, lobis basalibus alorum anguste obovatis apice retusis vel truncatis, lobis distalibus alorum oblique oblongis anteris apice acuminatis facile differt.

Herb ca. 1 m tall. Stem erect, robust, angulate, glabrous, base lignose, densely rugate. Leaves alternate, hard papyraceous, glabrous, grey, congregated at the upper stem; lamina obovate-elliptic or obovate-oblong, 19–22.5 × 7–8 cm, apex acuminate or caudate, base cuneate and attenuate, margin repand-crenate, glandular-mucronate between crenae, lateral nerves 11–13-paired and prominent on both surfaces, rectinerves dense and prominent; petioles angulate, densely rugate, glabrous, 0.8–1 cm long, ca. 5 mm diam. Peduncles arising from axils of the upper leaves, 4–6 cm long, 5–6-flowered; pedicels ca. 1.5 cm long, with bracts at the upper part; bracts lanceolate, ca. 4 × 1.5 mm, apex acute, at the base of pedicel. Flowers yellowish; lateral sepals 4, exterior 2, ovate-oblong, oblique, ca. 12 × 5 mm, apex acuminate, nerves 5, interior 2, linear-lanceolate, ca. 13 × 2 mm, apex long acuminate, recurved; dorsal petals broadly ovate, ca. 18 mm long, base ca. 13 mm wide, apex mucronate, base broadly cuneate or obtuse, with an obscure abaxial costa; lateral united petals 2-lobed, basal lobes anguste obovate, ca. 11 × 6 mm, apex acuminate, distal lobes stipitate, narrowly oblong, 2–3 cm × ca. 4 mm, apex acute, recurved, purpureo-punctate inside the inferior part, abaxial inconspicuously auriculate; lip saccate, 3.5–4 cm long including spur, limb oblique, 2.5–3 × ca. 1.5 cm, terminally rostrate, inside with a spur 1.2 cm long; stamens ca. 6 mm long, filaments filiform, widest at the upper part, anthers broadly ovate, apex slightly acute; ovaries clavate, ca. 3 mm long, apex rostrate, with 4 ovules on the top of each locule. Capsules clavate, 3.0–3.3 cm long. Seeds 4, brown, ellipsoid. Figures 1E, F, 2M-O, H-I, 6R-Z.

Specimens Examined—CHINA. Yunnan: Hekou Co., from Zhuchang to Anjiahe, near a village, 103°55′12″ E, 22°40′16″ N, alt. 450 m, broad-leaved forests, in heavily shaded forests on mountain slope, 10 July 1999, *C. I. Peng et al. 17528* (HAST, KUN). Hekou Co., Nanxi, Huayudong, alt. 300–500 m, in the secondary forest of the limestone hills, 22 July 2001, *Y. M. Shui 15311* (KUN, PE). Hekou Co., 104°58′ E, 22°41′ N, 15 Oct. 2009, in flower, *Z. G. Yang* 2009–01 (KUN). Maguan Co., Gulinqing, Arboretum-examining Station, 103°59′42″ E, 22°44′23″ N, alt. 560 m, in the tropical seasonal rain forests, 9 Oct 2002, *Y. M. Shui et al. 30959* (KUN). The same locality and habitat, 24 Dec., 2008, in fr., *Y. M. Shui et al. 82528* (KUN).

Etymology—The epithet "rugata" means that the stems and petioles are rugate.

Phenology—Flowering from July to Dec., fruiting from Sept. to Jan.

Geographic Distribution and Ecology—The new species is distributed in Hekou and Maguan counties of southeast Yunnan, China (see Fig. 4). It is similar to our new species *Impatiens purpureifolia* in both geographic distribution and ecology.

Notes—The new species is similar to *I. clavigera* in having lateral united petals which are bifid near the base and broadly ovate outer lateral sepals with caudate apices. Besides the above differences in flowers, the vegetative charcters are also different from the latter species. In the new species, the stem is rugate, angulate, leaf limb hard papyraceous, both surfaces grey, lateral nerves 11–13-paired, rectinerves dense, prominent on both surfaces, petiole rugate, angulate, glabrous, 0.8–1 cm long. In *I. clavigera* the stem is pilose to glabrescent, cylindrical without ridges, leaf limb membraceous, upper surface green, inferior surface greenish, lateral nerves 5–6-paired, rectinerves sparse, obscure on both surfaces, and the petiole is smooth, pilose, 1–2 cm long.

DISCUSSION

Importance of Flower Preparation in Impatiens—If the above steps of flower preparation had not been taken during field collections, it would have been difficult to separate the different floral organs, and we would not have been able to document the shape and precise position of the petals. Hooker and Thompson (1859) and Hooker (1874) realized the difficulties of identification of *Impatiens* collections, especially in a dried state. Hooker (1909) stated that the shape of the lateral united petals was only based on the sketch of a flower for a poorly preserved specimen in the description of some new species. Grey-Wilson (1980a) discussed how to collect and prepare *Impatiens* herbarium specimens in his Appendix 1. He suggested simple quick drawings to document the nature of various ridges and interlocking devices of flowers so that these characters would not be lost when the lateral united petals were pressed. Akiyama et al. (1995a, b, 1996) made excellent illustrations of flowers in the protologues of several new species, similar to the above procedures and mostly based on field observations. Chen (1978) also thought that the most useful information for *Impatiens* depends on careful field observation, detailed records, dissection of flowers, and timely illustrations and photos. Although he exhibited the dissections of 18 Chinese species of *Impatiens*, most of the dissections were based on dried specimens. Huang et al. (2003) followed a similar procedure to Chen (1978) with dried specimens and described 11 new taxa.

The characters of flowers were not accurately described in some protologues of *Impatiens*. According to field observations, Fig. 2D-F of *Impatiens laojunshanensis* shows that the lateral united petals have the basal lobes and dorsal lobes both with lobed tips. However, these characters were not in its protologue which was prepared from dried flowers (Huang et al. 2003). As to *Impatiens morsei* Hook. f., we were able to clarify its floral morphology through the careful comparisons with several accompanying species in the same locality (Hooker 1909; Chen et al. 2007b). Thus, some illustrations in the original protologue may be incorrect based on the poorly preserved specimens.

The flower characters deserve to be clarified especially in a region with an abundance of type localities. In China, Yunnan is the center of diversity for *Impatiens* with 105 species (Huang 2006). In Northeast Yunnan, about 10 new species were collected by E. Maire nearly 100 yr ago and poorly described by Léveillé (1916). According to Chen

(2001) and Huang (2006), the confusion arose largely from the unclear documentation of species with potentially incorrect descriptions including some new species described recently only based on dried specimens. Therefore, *Impatiens* species from Northeast Yunnan need to be surveyed and documented with the procedure we propose herein. The case may also be suitable in other regions with diverse type species of *Impatiens*.

Systematic Position of Three New Impatiens Species— Pollen and seeds can provide some important taxonomic characters for *Impatiens* (Lu 1991; Janssens et al. 2005). The 4-colpate pollen and protrusive-type seeds in *I. aconitoides* suggest that the new taxon is similar to *I. cyanantha* Hook. f. and *I. siculifer* Hook. f. (Song et al. 2005; Yu 2008). The 3-colpate pollen and ellipsoidal seeds in *I. purpureifolia* and *I. rugata* may link the two new species to *I. apalophylla*, *I. clavigera*, *I. balansae*, *I. guizhouensis*, and *I. angulata* (Lu and Chen 1991; Yu 2008). However, it is unclear if the group with 3-colpate pollen in *Impatiens* should include *Hydrocera*, a hydrophytic group with 3-colpate pollen in Balsaminaceae (Grey-Wilson 1980b; Janssens et al. 2005, 2006). Nevertheless, the correlation among pollen, seeds, chromosome numbers, and DNA sequences is relatively high (Akiyama et al. 1992; Song et al. 2003; Yuan et al. 2004; Janssens et al. 2007; Yu 2008). Recently, most new species descriptions usually have some pollen and seed characters (Cai et al. 2008; Cong et al. 2008a, b; Bi et al. 2009; Janssens et al. 2009b; Yu et al. 2009). We argue that pollen and seeds should be described whenever new taxa of *Impatiens* are described.

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