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Seedcoat micromorphology of *Impatiens* (Balsaminaceae) from China

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Seeds of 38 species of *Impatiens* mostly from south-western China were observed by scanning electron microscopy. High diversity was found in seedcoat micromorphology. Based on the structure and ornamentation of epidermal cells of the seedcoat, particularly the pattern of the arrangement of the cells and the degree of elevation of the anticlinal cell walls, four morphological types can be distinguished, viz, laevigate, granulate, reticulate and protrusive. The laevigate type and granulate type are unique, each occurring in only one species. The other two types are common and can be further divided into subdivisions according to the shape of the epidermal ornamentation of the seedcoat. Descriptions of seed size, shape and seedcoat types are summarized for the genus. Taxonomic and phylogenetic implications of the seedcoat micromorphology are also discussed, in comparison with the available gross morphological and molecular data.

ADDITIONAL KEYWORDS: evolution – SEM – seeds – taxonomy.

INTRODUCTION

The Balsaminaceae has two genera, *Hydrocera* with only one species that occurs in the Indo-Malaysian countries, and *Impatiens* with over 900 species occurring mostly in tropical and subtropical regions of the Old World (Grey-Wilson, 1980). Recent studies have been devoted to the morphology, cytology, taxonomy and molecular systematics of this family (Chen, 2001; Fischer & Rahelivololona, 2002, 2003; Song, Yuan & Küpfer, 2003; Yuan *et al*., 2004). Classification of *Impatiens* remains a strong challenge as the traditionally favourite characters, such as floral morphology and structure, are extremely variable (Grey-Wilson, 1980). The search for new taxonomic characters for *Impatiens* is therefore urgent.

The importance of seed morphology for classification has long been recognized (Hooker & Thomson, 1860). Recent examples of successful applications of seed morphology include studies on *Veronica* (Martinez-Ortega & Rico, 2001), Massonieae (Pfosser *et al*., 2003), *Aeschynanthus* (Mendum *et al*., 2001), *Phyllocladus* (Bobrov, Melikian & Yembaturova, 1999), Caryophyllaceae (Yildiz, 2002) and Brassicaceae (Abdel Khalik & Van der Maesen, 2002). However, seed morphology of Balsaminaceae has been observed for only 15 species (Shimizu, 1979; Lu & Chen, 1991). Two types of seedcoat micromorphology, laevigate and scabrous, have been described in *Impatiens*. The seed morphology of the genus was shown to provide useful taxonomic characters at species level, highly correlated with flower and pollen morphology (Shimizu, 1979; Lu & Chen, 1991). These studies, however, described only the main types of seedcoat, and it was not possible to elucidate the overall variation in seedcoat micromorphology and its implementation for taxonomy, due to insufficient sampling. Detailed observations on more species and a systematic overall evaluation of seedcoat micromorphology in *Impatiens* are still necessary. South-western China is one of the most important diversification centres of *Impatiens*. Scanning electron microscopy (SEM) observations on seeds of 38 species of *Impatiens*, mostly from south-

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western China, were therefore carried out to elucidate the systematic value of seedcoat micromorphological characters and, furthermore, to detect evolutionary trends among seedcoat microcharacters and their correlations with other traits.

MATERIAL AND METHODS

Seeds used for observations were collected in the field during the summers and autumns of 2000 and 2003. Two species of the western Himalayan origin were collected from a botanic garden. The origins of the samples and vouchers, together with information on seed size, shape, colour and length/width ratio, are shown in Table 1. Seed sizes were measured from five seeds randomly chosen. Voucher specimens are deposited in the Herbarium of the Botanical Institute of the University of Neuchâtel (NEU). For scanning electron microscopy, dried mature seeds were mounted on stubs, using double-sided adhesive tape, and were coated with about 2.3 nm of gold with a BALTEC SCD 005 sputter coater. Coated seeds were then examined and photographed with the Philips ESEM-FEG XL30 scanning electron microscope.

OBSERVATIONS

Morphological characteristics of the seeds such as size, shape, colour and structure of the seedcoat, as summarized in Table 1, were found to be highly variable within *Impatiens* and provided a set of useful taxonomic characters for most species analysed. Seeds range from 1.3–1.5 × 0.6–0.9 mm (length × width) (*I. xanthina* Comber) to 4.1–4.5 × 2.0–2.1 mm (*I. parviflora* DC.) in size, and are globose, ovate, ellipsoid, subellipsoid or long ellipsoid in outline. Their colour varies from yellow, beige, grey, to various shades of brown, to black. The 38 *Impatiens* species observed also show wide variation in seedcoat micromorphological patterns. Based on the structure and ornamentation of the epidermal cells of the seedcoat, four principal morphological types can be distinguished: laevigate, granulate, reticulate and protrusive; the latter two types can be further divided into several subdivisions.

Laevigate type (Fig. 1)

Found only in *I. chinensis* L., this type of seedcoat is characterized by an almost smooth surface without obvious sculpture (Fig. 1A). Seeds are 1.7–2.0 × 1.4–1.7 mm, globose, and shining black in colour. The cuticular pattern is fine and dense, rarely with fine hairs and granules on the cell surface that can be seen only at high magnification (Fig. 1B).

Granulate type (Fig. 2)

Seen only in *I. balsamina* L., this type of seedcoat is characterized by granulate protrusions evenly covering the entire surface (Fig. 2A). Seeds are 2.2–3.1 × 1.4–1.7 mm, subellipsoid and grey-brown in colour. In detail, each granule is a bud-like protrusion with radial pleats (Fig. 2B).

Reticulate type (Figs 3–20)

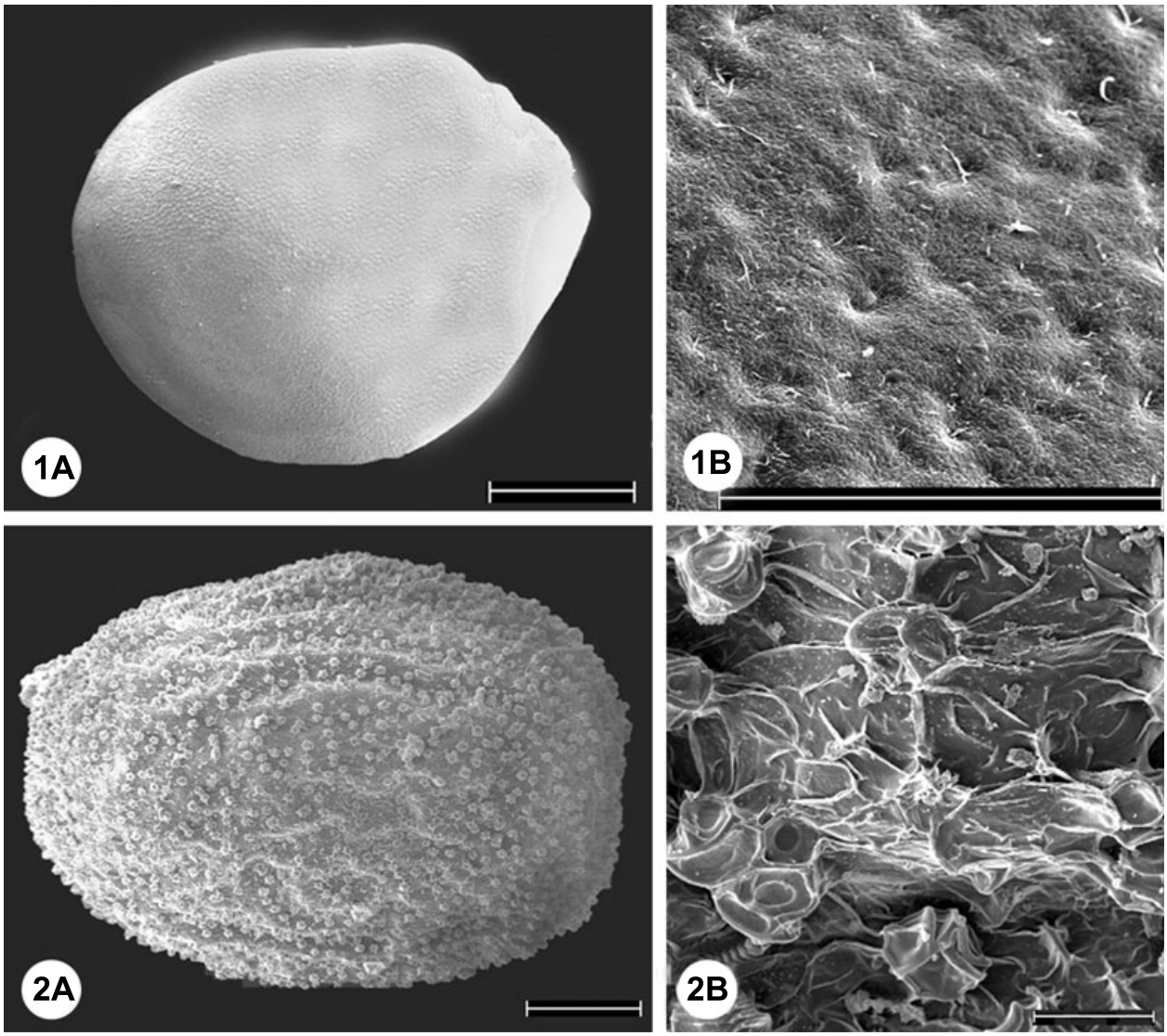
The outer periclinal wall of the epidermal cells of the seedcoat bulges, or becomes more or less bullate, forming a reticulate ornamentation with varied shapes and sizes of the meshes. This is the most common type observed. Based on the extent of the projection and the arrangement of the bullate epidermal cells, the following four subtypes can be distinguished.

Fine reticulate subtype (Figs 3–9)

The outer periclinal walls of all the epidermal cells of the seedcoat are evenly, but only slightly bulged. Eight of the species observed belong to this group, i.e. *I. dolichoceras* E. Pritz. ex Diels (Fig. 4), *I. gongshanensis* Y. L. Chen (Fig. 3), *I. holocentra* Hand.-Mazz., *I. forrestii* Hook. f. et W. W. Sm. (Fig. 5), *I. infirma* Hook. f. (Fig. 6), *I. noli-tangere* L. (Fig. 7), *I. rostellata* Franch. (Fig. 8) and *I. scabrida* DC. (Fig. 9). The seeds of this category vary in size and shape: 1.5–3.8 × 0.8–2.4 mm, round, ovate or ellipsoid, yellowish brown to dark brown in colour. *Impatiens gongshanensis* (Fig. 3A) and *I. holocentra* have comparatively small seeds (1.5–2.0 × 0.8–1.4 mm) with subcircular or ocellate testa cells (Fig. 3B). *Impatiens dolichoceras* has rhombic cells on the flat seedcoat surface with undulate pits inside (Fig. 4A, B). The seed of *I. forrestii* has a round shape (Fig. 5A) and the curvature of the periclinal wall of the seedcoat cells make up an undulated pattern (Fig. 5B). Cuticle granules can be seen under higher magnification (Fig. 5C). The seed of *I. infirma* has irregular elevations along the anticlinal walls of the epidermal cells (Fig. 6A, B). *Impatiens noli-tangere* has narrow and ellipsoid seeds with ornamentations similar to *I. infirma* (Fig. 7A, B). The seeds of *I. rostellata* are narrow and ellipsoid, with nearly round or polygonal testa cells. The anticlinal walls of the testa cells are curved or slightly undulate (Fig. 8A, B). *Impatiens scabrida* has ovate seeds with stellate-undulate pits on the seedcoat (Fig. 9A). Fine cuticle dusts can be seen under high magnification (Fig. 9B).

Colliculate subtype (Figs 10–15)

In comparison with the above fine reticulate subtype, the colliculate subtype was assigned to those seeds that have seedcoat cells with higher inflated or bulged outer periclinal walls. The epidermal cells with inflated or bulged outer periclinal walls are evenly



Figures 1–2. SEM micrographs of seedcoat of *Impatiens*. Fig. 1. Laevigate type. *I. chinensis*. Fig. 2. Granulate type. *I. balsamina*. A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.

distributed across the entire seedcoat and are densely reticulate. Seeds of the following species fall into this category: *I. aquatilis* Hook. f. (Fig. 10A, B), *I. chungtienensis* Y. L. Chen (Fig. 11A, B), *I. cyanantha* Hook. f., *I. fenghwaiana* Y. L. Chen (Fig. 12A, B), *I. pseudo-kingii* Hand.-Mazz., *I. purpurea* Hand.-Mazz. (Fig. 13A–C), *I. radiata* Hook. f., *I. rectangula* Hand.-Mazz., *I. apsotis* Hook. f. (Fig. 14A, B) and *I. soulieana* Hook. f. (Fig. 15A, B). Seeds are 1.5–3.4 × 0.8–2.3 mm, ovate, subellipsoid to subspheroid, yellowish brown to dark grey in colour. Dense pits are seen on the inflated outer periclinal walls of epidermal cells of the seedcoats of *I. aquatilis* and *I. fenghwaiana* (Figs 10B, 12B). The seeds of *I. radiata* and *I. rectangula* (not shown) are very similar to those of *I. purpurea* (Fig. 13A), being 1.8–2.4 × 1.3–1.7 mm, subspheroid, yellowish brown to dark brown in colour, and their seedcoats have undulate surfaces with round aculeate protrusions covering the entire testa (Fig. 13B). Fine cuticular granules can be seen all over the surface (Fig. 13C). *Impatiens apsotis* and *I. soulieana* have similar seedcoats with irregular polygonal or rounded cells. Fine cuticle dust covers the entire surface (Figs 14, 15).

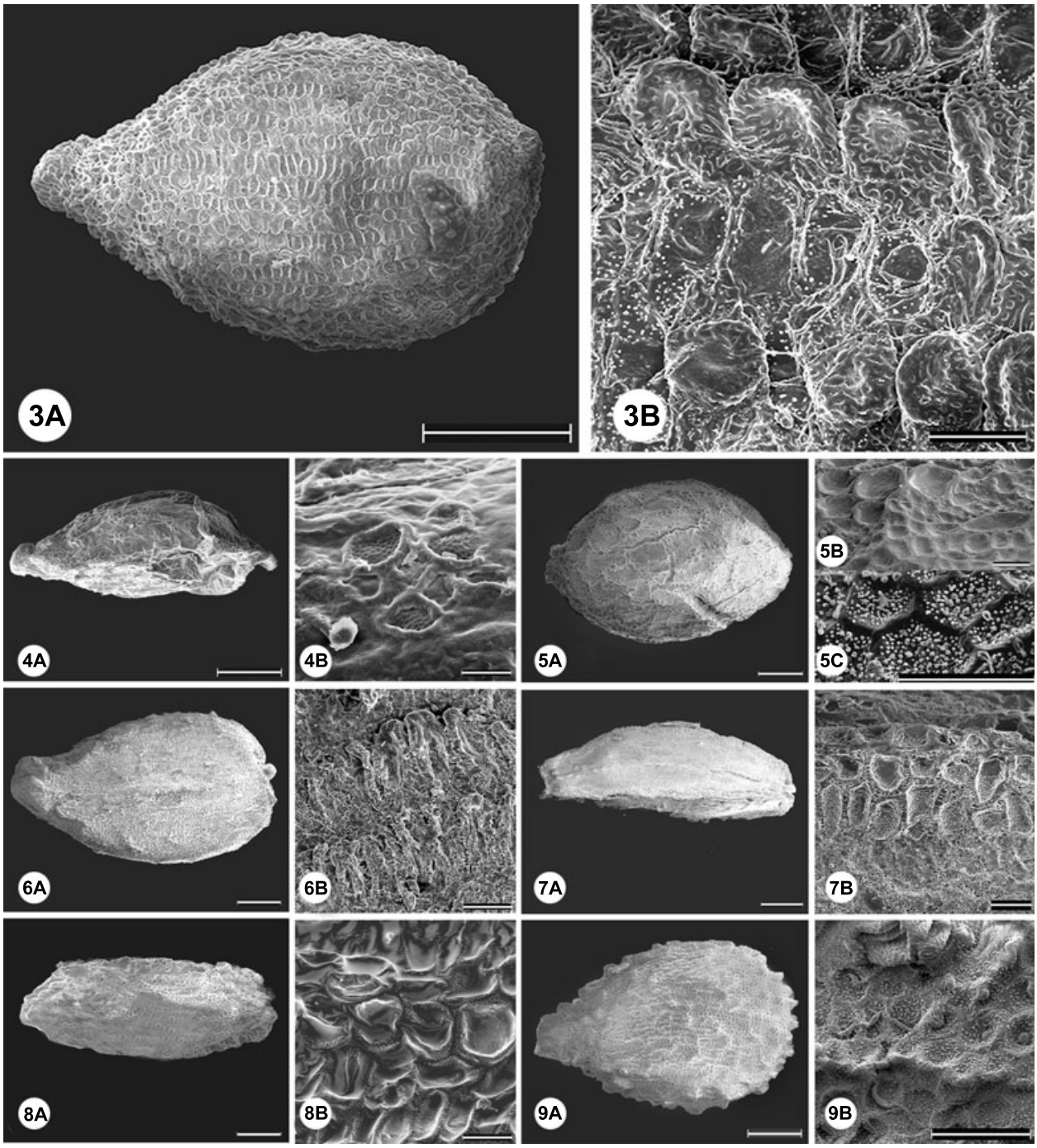
Carinate subtype (Figs 16,17)

The basic ornamentation of this subtype is very similar to the above. However, some of the seedcoat

Table 1. List of the *Impatiens* species observed, the origin and voucher of the samples, and the seed characteristics (L/W*, Length/Width; †Type refers to the types and subtypes described in the text)

			Seed characters					
Species	Origin	Voucher	Colour	Shape	Size (mm)	L/W* radio	Type†	Figure
I. arguta Hook. fl. et Thomson	Yunnan, China	CN2k-85	black brown	subspheroid	2.5–3.0 × 1.9–2.2	1.35	3.4	18
I. apsotis. Hook. f.	Sichuan, China	CN2k2–159	light brown-yellowish brown	ellipsoid	2.0–2.2 × 1.2–1.3	1.62	3.2	14
I. aquatilis Hook. f.	Yunnan, China	CN-Y017	brown	subovate	2.8–3.4x.1.8–2.2	1.55	3.2	10
I. aureliana Hook. f.	Yunnan, China	CN2k1–56	maroon-brown	subellipsoid	1.8–2.8 × 1.1–1.8	1.47	4.3	27
I. bahanensis Hand.-Mazz.	Yunnan, China	CN2k-30	grey-brown	subellipsoid	2.2–3.1 × 1.4–1.7	1.87	4.1	22
I. balfourii Hook. f.	Himalayan origin, Bot. Gard. Neuch., Switzerland	NE1	beige to grey	subellipsoid	2.1–3.0 × 1.6–2.2	1.40	3.4	19
I. balsamina L.	Yunnan, China	CN2k1-GSh	maroon–brown	subellipsoid	2.2–2.9 × 1.5–2.2	1.30	2	2
I. begoniifolia S. Akiyama et H. Ohba	Yunnan, China	CN2k1–51	brown	subellipsoid	1.7–2.2 × 1.1–1.6	1.46	4.3	25
I. chinensis L.	Yunnan, China	CN2k1–49	shiny black	globose	1.7–2.0 × 1.4–1.7	1.27	1	1
I. chungtienensis Y. L. Chen	Yunnan, China	CN2k2–179	dark-grey	subellipsoid	3.1–3.8 × 1.6–1.8	2.06	3.2	11
I. cyanantha Hook. f.	Yunnan, China	CN2k1–84	grey brown	subovate	2.9–3.1 × 1.8–2.3	1.50	3.2	
I. cyathiflora Hook. f.	Yunnan, China	CN2k-74	yellowish brown	subellipsoid	1.9–2.5 × 1.0–1.4	1.69	4.1	
I. davidi Franch.	Fujian, China	CN2k-09	yellow	ellipsoid	2.1–2.2 × 1.1–1.5	1.50	4.1	21
I. delavayi Franch.	Yunnan, China	CN2k-76	yellowish brown	ellipsoid	2.2–3.0 × 1.3–1.6	1.80	3.3	16
I. dolichoceras E. Pritz. ex Diels	Guangxi, China	CN2k1–80	yellowish brown	oblate ovate	2.0–2.8 × 1.1–1.6	1.92	3.1	4
I. drepanophora Hook. f.	Yunnan, China	CN2k-20	dark-brown	subellipsoid	2.2–2.9 × 1.3–1.7	1.67	4.1	
I. fenghwaiana Y. L. Chen	Guangxi, China	CN2k1–78	brown	ovate	2.3–2.5 × 1.3–1.7	1.50	3.2	12

I. forrestii Hook. f. et W. W. Sm.	Yunnan, China	CN2k-79	dark-brown	globose	2.2–2.9 × 2.0–2.4	1.18	3.1	5
I. gongshanensis Y. L. Chen	Yunnan, China	CN2k-23	dark-brown	ovate	1.6–2.0 × 0.8–1.4	1.58	3.1	3
I. holocentra Hand.-Mazz.	Yunnan, China	CN2k-54	brown	ovate	1.5–1.8 × 1.0–1.1	1.60	3.1	
I. infirma Hook. f.	Sichuan, China	CN2k2–162	black brown	long ovate	3.1–3.8 × 1.9–2.1	1.85	3.1	6
I. mengtszeana Hook. f.	Yunnan, China	CN2k1–60	green-brown	spheroid	1.4–1.9 × 1.0–1.5	1.31	4.3	26
I. noli-tangere L.	Jilin, China	CN2k-86	yellowish brown	long ellipsoid	2.9–3.2 × 1.2–1.5	2.21	3.1	7
I. parviflora DC.	Bern, Switzerland	Bern1	beige–dark grey	ellipsoid	4.1–4.5 × 2.0–2.1	2.10	3.4	20
I. poculifer Hook. f.	Yunnan, China	CN2k2–209	brown	subspheroid	2.9–3.1 × 1.6–2.0	1.50	3.3	17
I. pseudo-kingii Hand.-Mazz.	Yunnan, China	CN2k-31	yellow	ovate	1.5–2.0 × 0.8–1.0	2.13	3.2	
I. purpurea Hand.-Mazz.	Yunnan, China	CN-Y007	yellowish brown	subspheroid	2.1–2.2 × 1.4–1.7	1.31	3.2	13
I. radiata Hook. f.	Yunnan, China	CN2k-77	dark brown	subspheroid	2.1–2.4 × 1.3–1.6	1.47	3.2	
I. rectangula Hand.-Mazz	Yunnan, China	CN2k1–17	grey-brown	subspheroid	1.8–2.0 × 1.3–1.6	1.33	3.2	
I. rostellata Franch.	Sichuan, China	CN2k2–167	blackish brown	oblate ellipsoid	2.2–3.0 × 1.3–1.8	1.87	3.1	8
I. ruiliensis S. Akiyama et H. Ohba	Yunnan, China	CN2k1–50	dark yellow	subspheroid	1.6–2.0 × 1.2–1.6	1.38	4.2	24
I. scabrida DC.	Himalayan origin, Bot. Gard. Neuch., Switzerland	NE6	dark yellow	ovate	2.1–2.8 × 1.4–2.0	1.33	3.1	9
I. siculifer Hook. f.	Yunnan, China	CN2k-80	brown	ellopsoid	2.2–3.0 × 1.4–2.1	1.50	4.1	23
I. soulieana Hook. f.	Sichuan, China	CN2k2–163	yellowish brown	subspheroid	2.5–3.1 × 1.7–2.0	1.67	3.2	15
I. trichosepala Y. L. Chen	Yunnan, China	CN2k1–68	brown	subspheroid	1.3–1.6 × 0.9–1.2	1.36	4.3	
I. walleriana Hook. f.	African origin, Cult. Yunnan, China	CN-Y040	yellowish brown	subspheroid	1.7–2.0 × 1.2–1.4	1.46	4.4	28
I. xanthina Comber	Yunnan, China	CN2k-17	yellowish brown	ellipsoid	1.3–1.5 × 0.6–0.9	1.75	4.4	29
I. yingjiangensis S. Akiyama et H. Ohba	Yunnan, China	CN2k1–55	light brown	spheroid	1.4–1.7 × 1.1–1.4	1.25	4.3	

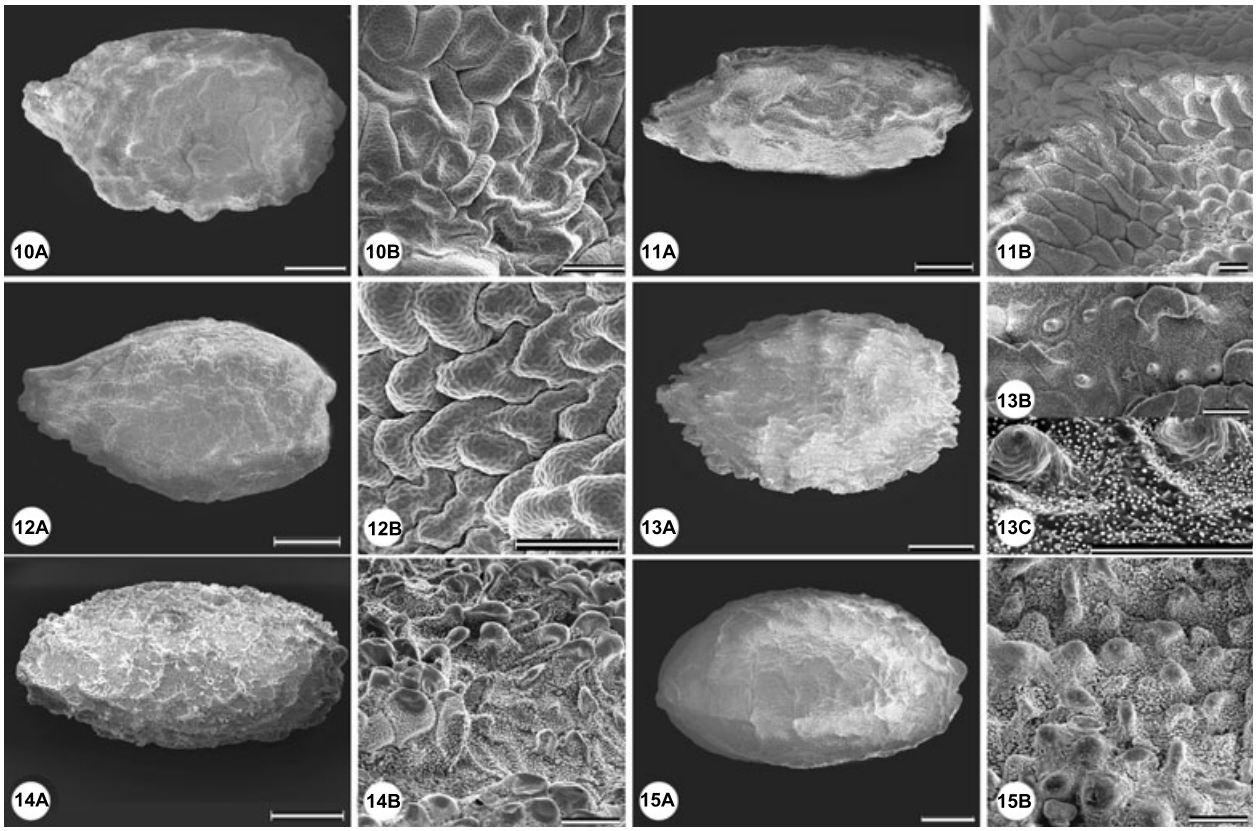


Figures 3–9. SEM micrographs of seedcoat of *Impatiens*: reticulate type – fine reticulate subtype. Fig. 3. *I. gongshanensis.* Fig. 4. *I. dolichoceras.* Fig. 5. *I. forrestii.* Fig. 6. *I. infirma.* Fig. 7. *I. noli-tangere.* Fig. 8. *I. rostellata.* Fig. 9. *I. scabrida.* A, entire seed. Scale bars = 0.5 mm. B, C, details of seedcoat. Scale bars = 0.05 mm.

epidermal cells are elevated significantly higher and arranged in ridges. This subtype is observed only in *I. delavayi* Franch. (Fig. 16A, B) and the closely related species, *I. poculifer* Hook. f. (Fig. 17A, B). Seeds are 2.2–3.1 × 1.3–2.0 mm, ellipsoid to subspheroid, yellowish brown to brown in colour. Both species also have a fine circle sculpture on the surface of the epidermal cells (Figs 16B, 17B).

Striate subtype (Figs 18–20)

The elevated epidermal cells are arranged in clear lines, as observed in *I. arguta* Hook. fl. et Thomson



Figures 10–15. SEM micrographs of seedcoat of *Impatiens*: reticulate type – colliculate subtype. Fig. 10. *I. aquatilis.* Fig. 11. *I. chungtienensis.* Fig. 12. *I. fenghwaiana.* Fig. 13. *I. purpurea.* Fig. 14. *I. apsotis.* Fig. 15. *I. soulieana.* A, entire seed. Scale bars = 0.5 mm. B, C, details of seedcoat. Scale bars = 0.05 mm.

(Fig. 18), *I. balfourii* Hook. f. (Fig. 19) and *I. parviflora* (Fig. 20). Seeds of these species are comparatively large, 2.1–4.5 × 1.6–2.2 mm, subspheroid to subellipsoid, black brown, beige to grey in colour. *Impatiens arguta* has rugose elevated cells on the seedcoat surface as pleats (Fig. 18A–C). *Impatiens balfourii* has colliculate or corrugate epidermal cells (Fig. 19A, B), while *I. parviflora* has elevated epidermal cells regularly arranged in lines isolated by nonelevated cells. Fine cuticular granules and pits are seen on the periclinal walls of the epidermal cells of the latter two species (Figs 19B, 20B).

Protrusive type (Figs 21–29)

Some of the epidermal cells of the seedcoat protrude significantly to form projections of varied shapes. According to the shapes of the projections, the following four subtypes can be recognized.

Digitiform subtype (Figs 21–23)

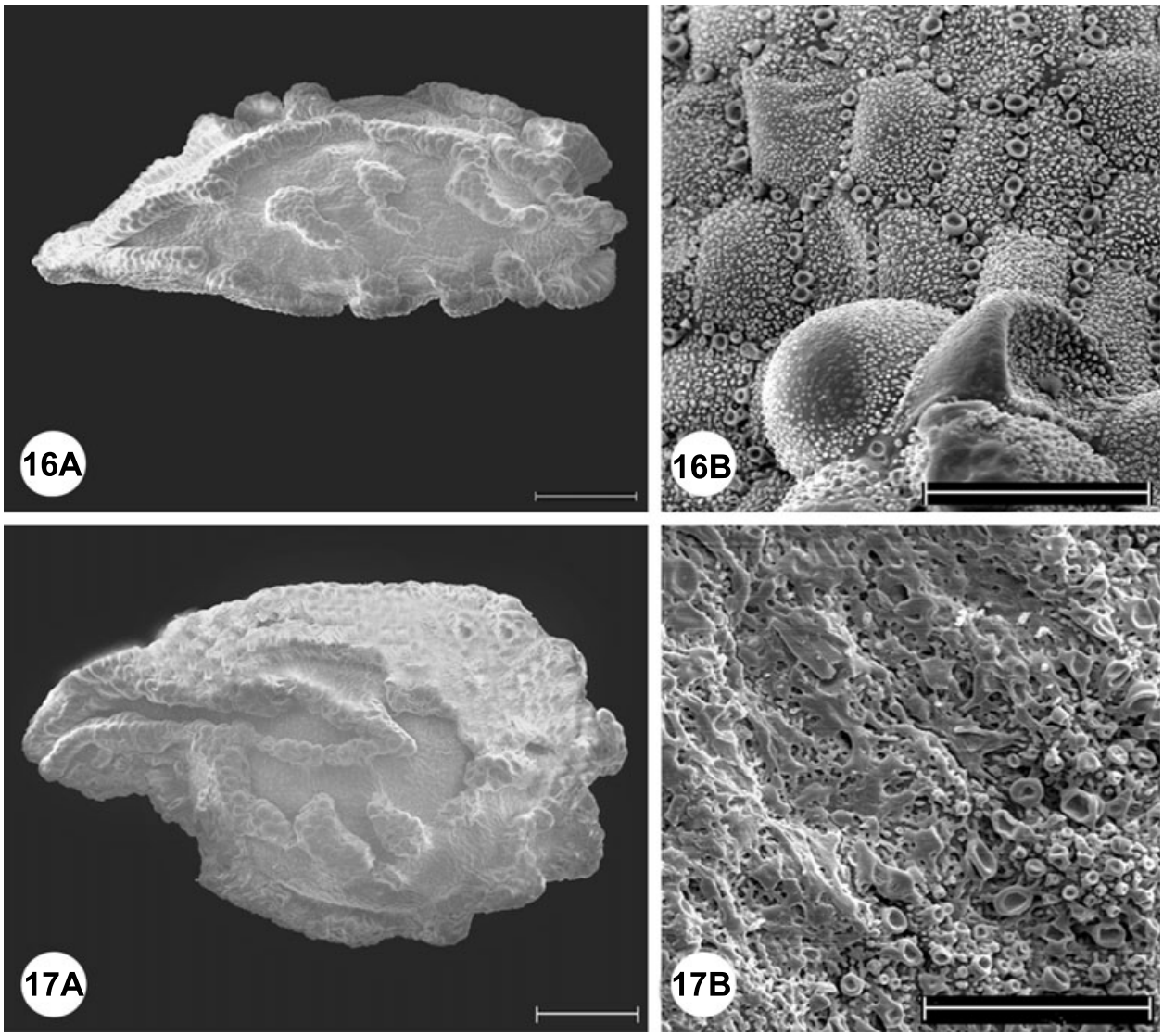
The protrusive epidermal cells of the seedcoat are finger-like, as shown by the species *I. davidi* Franch. (Fig. 21A), *I. bahanensis* Hand.-Mazz. (Fig. 22A), *I. drepanophora* Hook. f., *I. cyathiflora* Hook. f. and *I. siculifer* Hook. f. (Fig. 23A). Seeds are 1.9–3.1 × 1.0–2.1 mm, ellipsoid to subspheroid, yellow to brown in colour. In *I. davidi*, the protrusions have flat tops and numerous cuticular granules on their lateral surface (Fig. 21B), whereas in the other species of this group, the protrusions have numerous pits (Figs 22B, 23B).

Clustered subtype (Fig. 24)

As seen in *I. ruiliensis* S. Akiyama et H. Ohba, several spine-shaped projections cluster together (Fig. 24A). Seeds are 1.6–2.0 × 1.2–1.6 mm, subspheroid, and dark yellow in colour. Both the protrusive and flat epidermal cells have conspicuous pits (Fig. 24B).

Squamalate subtype (Figs 25–27)

The protrusions are scale-like. Squamalate protrusions sparsely but evenly cover the entire seedcoat, or they are denser near the hilum region. *Impatiens begoniifolia* S. Akiyama et H. Ohba (Fig. 25A), *I. yingjiangensis* S. Akiyama et H. Ohba, *I. mengtszeana* Hook. f. (Fig. 26A), *I. trichosepala* Y. L. Chen and *I. aureliana* Hook. f. (Fig. 27A) show



Figures 16–17. SEM micrographs of seedcoat of *Impatiens*: reticulate type – carinate subtype. Fig. 16. *I. delavayi.* Fig. 17. *I. poculifer.* A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.

this kind of seedcoat. Seeds are 1.3–2.8 × 0.9–1.8 mm, spheroid, ellipsoid and subellipsoid, light to dark brown in colour. Cuticular granules are found mostly on the nonprotrusive cells, while pits are conspicuous on the squamalate projections (Figs 25B, 26B, 27B).

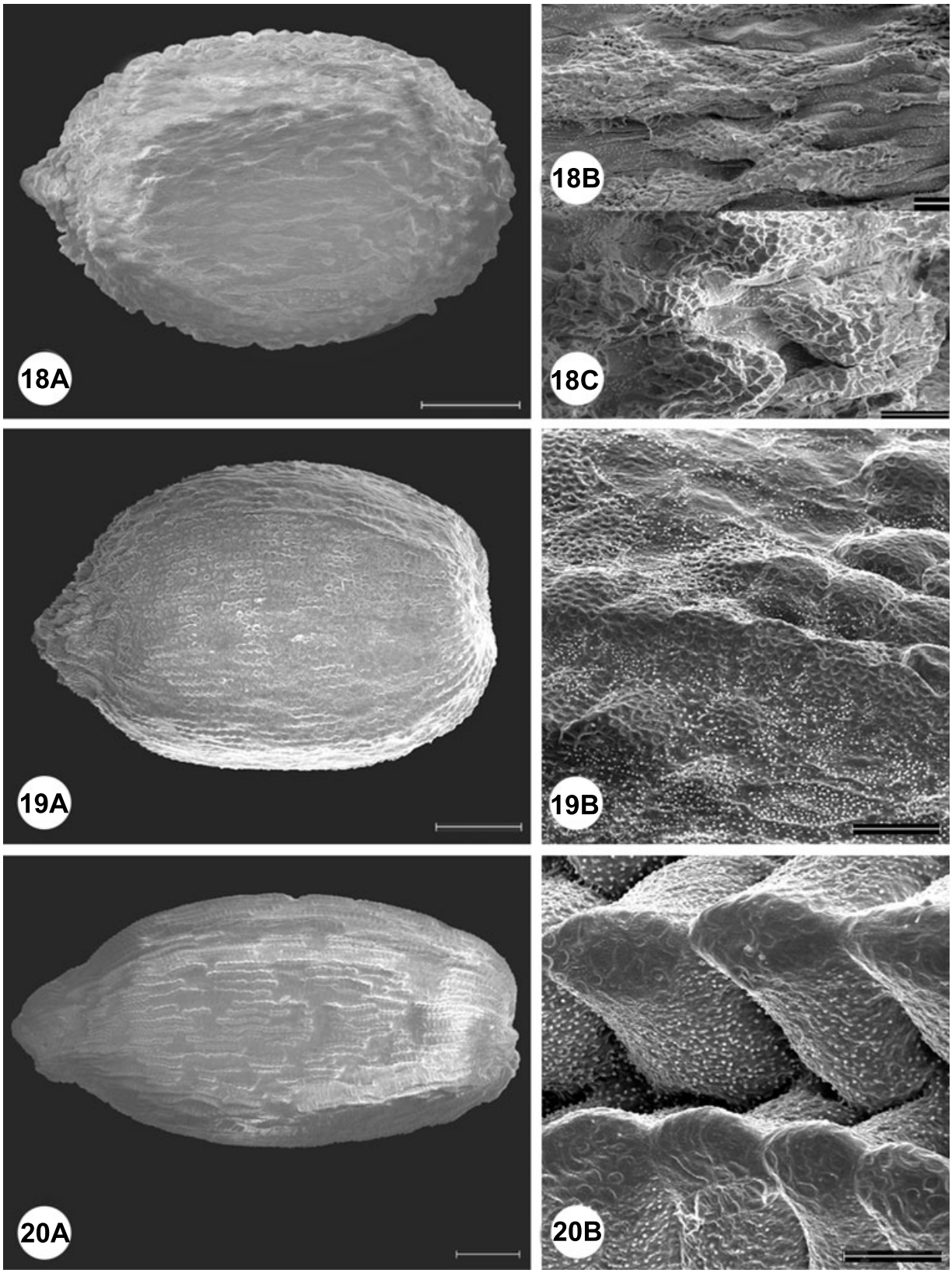
Cristate subtype (Figs 28, 29)

This subtype is similar to the above squamalate form, but the protrusions are more complex and are crest-like. Two species belong to this group: *I. walleriana* Hook. f. (Fig. 28A) and *I. xanthina* (Fig. 29A). Seeds are 1.3–2.0 × 0.6–1.4 mm, subspheroid to ellipsoid, and yellowish brown in colour. The seedcoat of *I. walleriana* is sparsely covered with cristate protrusions that have a reticulate sculpture, and fine cuticular granules densely cover the nonprotrusive epidermal cells (Fig. 28B). The protrusions of *I. xanthina* are spirally thickened, but no cuticular granules were seen (Fig. 29B).

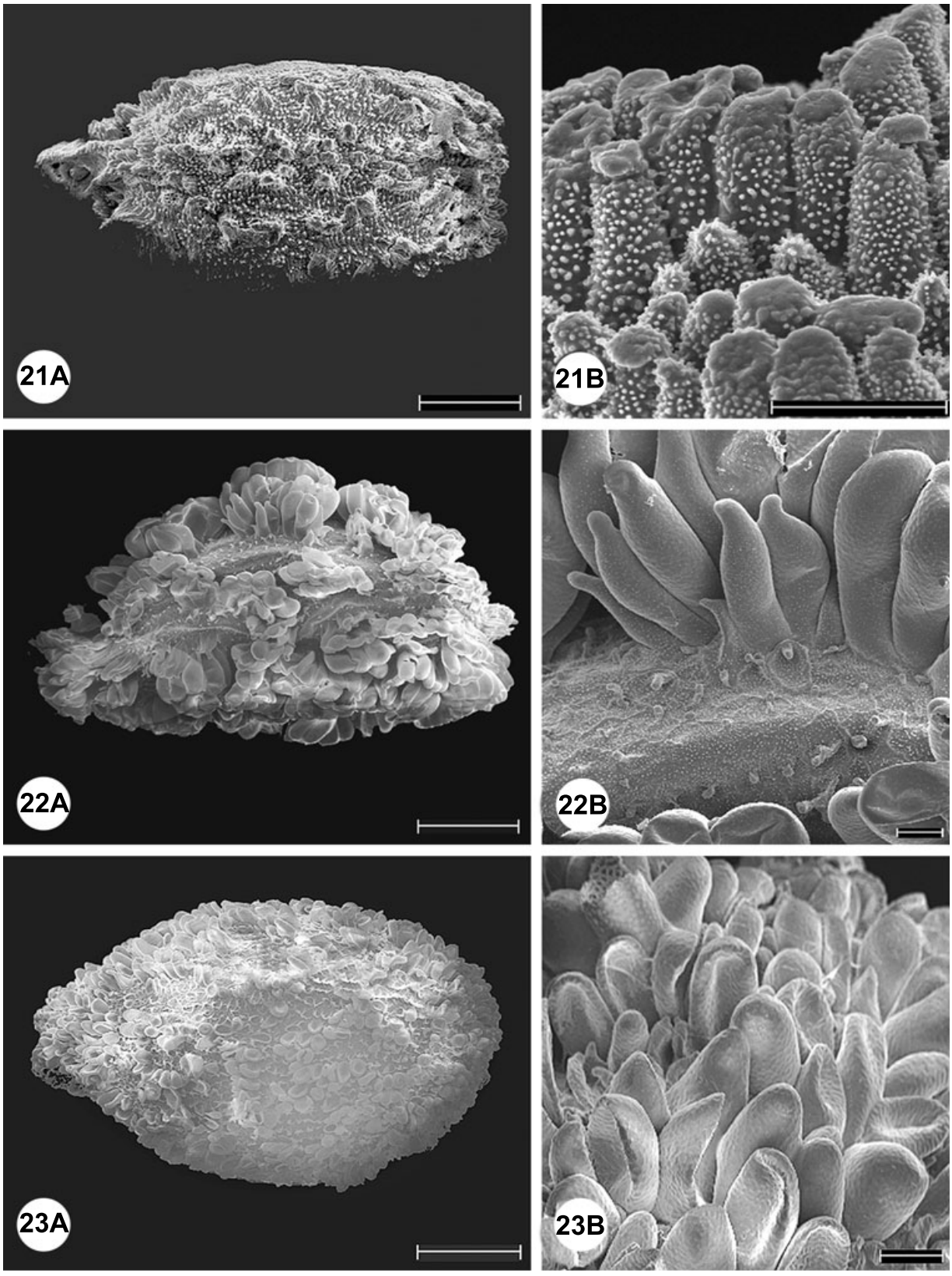
DISCUSSION

Diversity of seedcoat micromorphology in *Impatiens*

This examination of *Impatiens* species from China and other regions has revealed considerable diversity in seed size, shape, colour and seedcoat micromorphology. Four main types of seedcoat, laevigate, granulate, reticulate and protrusive, and detailed subdivisions of



Figures 18–20. SEM micrographs of seedcoat of *Impatiens*: reticulate type – striate subtype. Fig. 18. *I. arguta.* Fig. 19. *I. balfourii.* Fig. 20. *I. parviflora.* A, entire seed. Scale bars = 0.5 mm. B, C, details of seedcoat. Scale bars = 0.05 mm.



Figures 21–23. SEM micrographs of seedcoat of *Impatiens*: protrusive type – digiform subtype. Fig. 21. *I. davidi.* Fig. 22. *I. bahanensis.* Fig. 23. *I. siculifer.* A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.

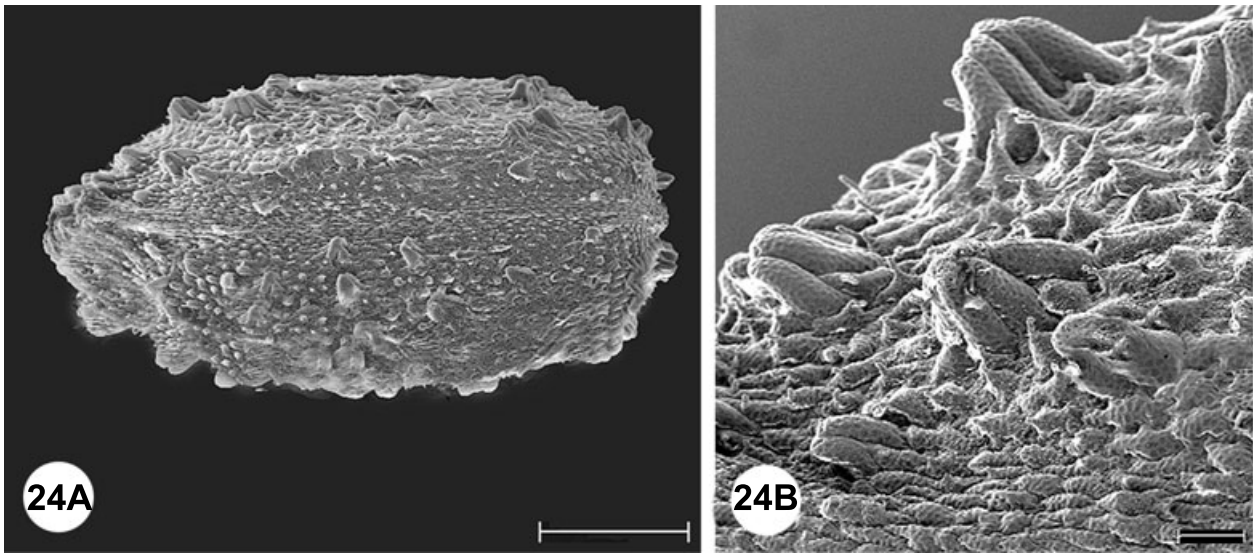
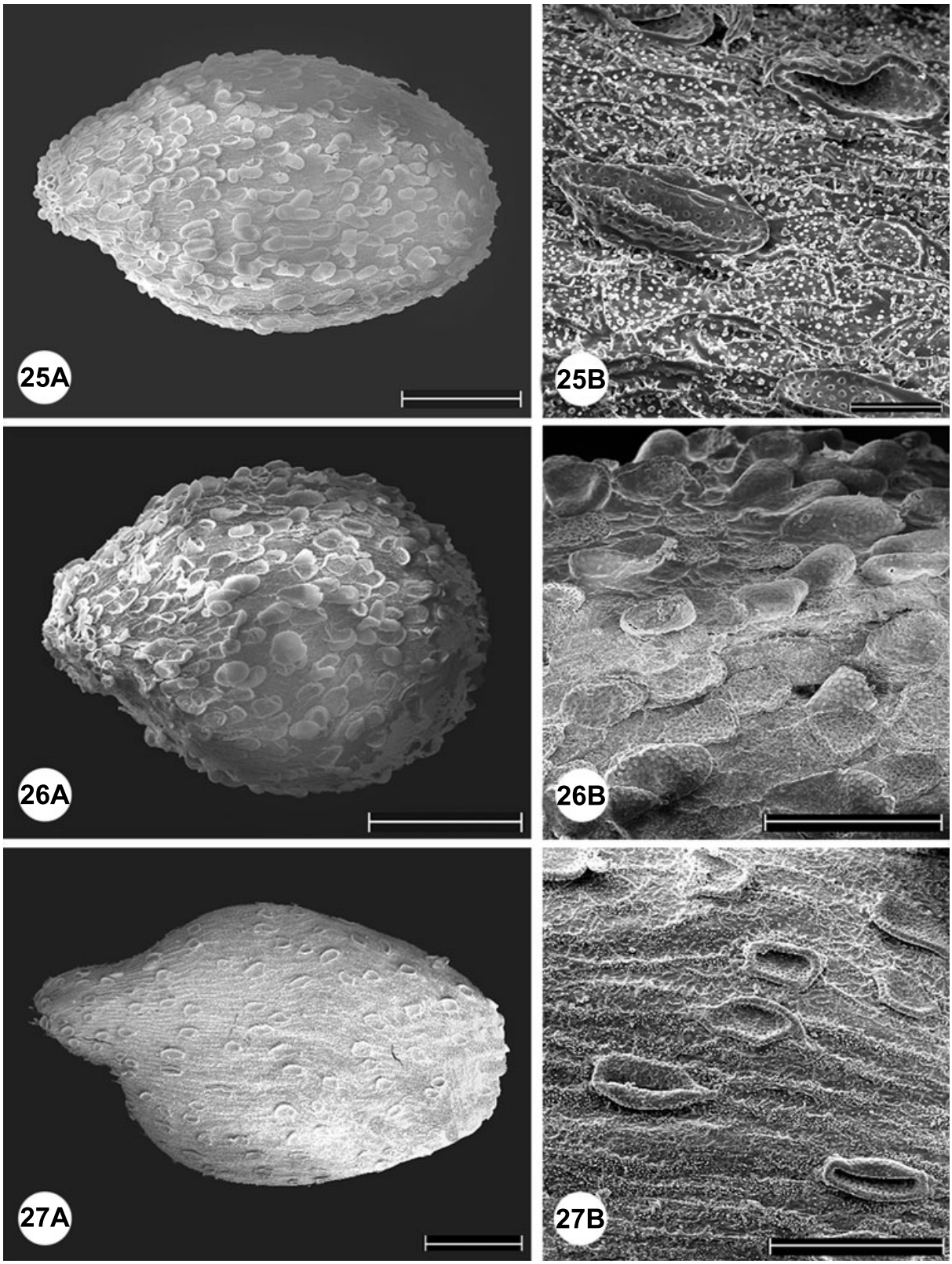


Figure 24. SEM micrographs of seedcoat of *Impatiens*: protrusive type – clustered subtype. Fig. 24. *I. ruiliensis.* A, entire seed. Scale bar = 0.5 mm. B, detail of seedcoat. Scale bar = 0.05 mm.

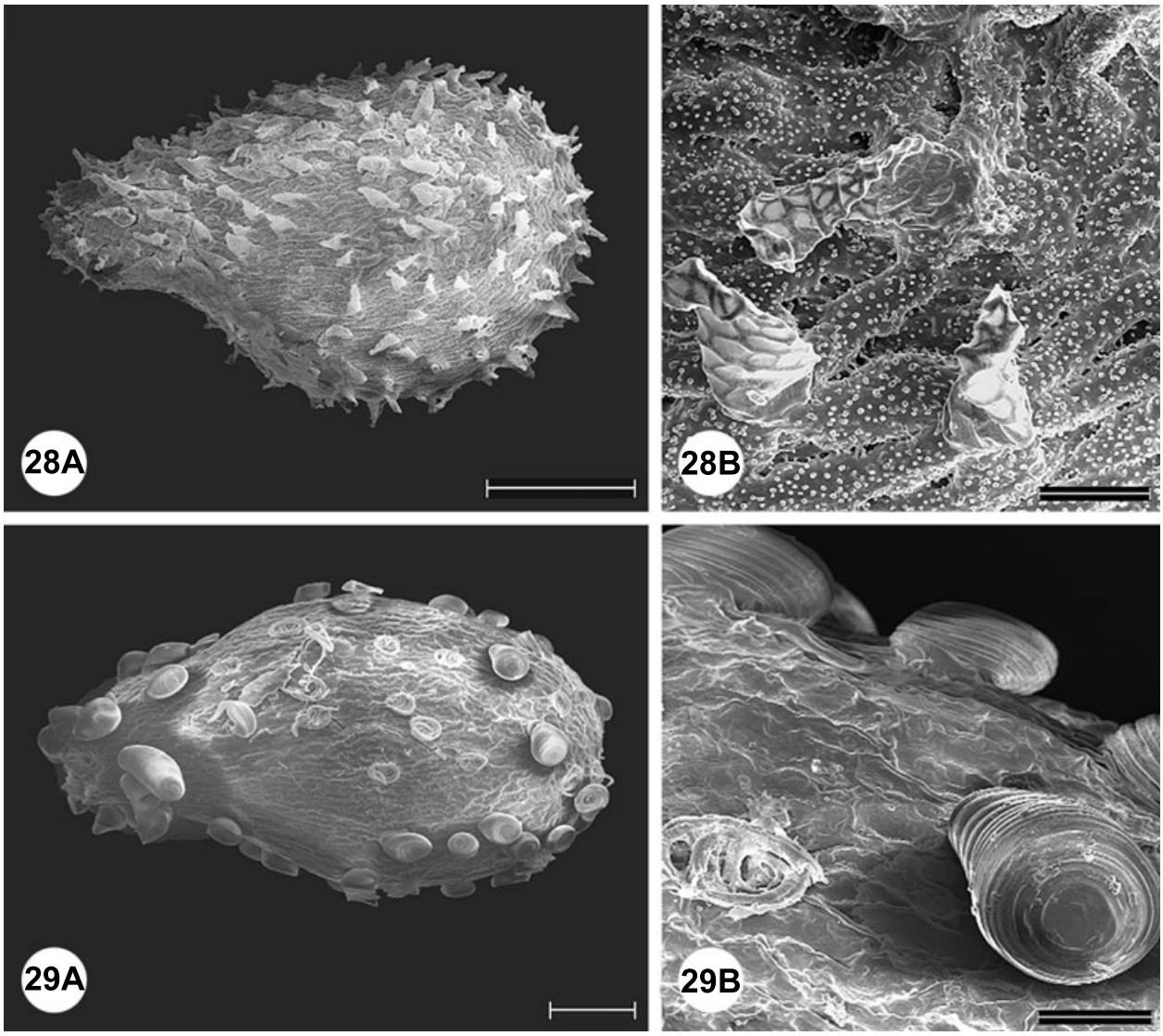
the reticulate and protrusive types, were distinguished according to the ornamentation of the seedcoat. The granulate type in *I. balsamina*, carinate subtype in *I. delavayi* and *I. poculifer*, striate subtype in *I. arguta*, *I. balfourii* and *I. parviflora*, digitiform subtype in *I. davidi*, *I. bahanensis*, *I. drepanophora*, *I. cyathiflora* and *I. siculifer*, and clustered subtype in *I. ruiliensis* were observed for the first time. Lu & Chen (1991) studied 12 species collected from Mt Omei, China. One species, *I. wilsonii* Hook. f., was described as having the laevigate seedcoat, which was found only in *I. chinensis* in this study and by Shimizu (1979). However, the micrographs of the species (Lu & Chen, 1991: plate 1, figs 1–6) appeared to represent the fine reticulate seedcoat we defined here. The remaining 11 species were described as having scabrous seedcoats, without further detailed classification of the types. The micrographs of these species show that they correspond to our reticulate and colliculate subtypes, except in *I. monticola* Hook. f. The seedcoat of *I. monicola* was described as having sieve-like discs that, in fact, correspond to our squamalate subtype of the protrusive category. Shimizu (1979) reported that the seed of the Thailand *I. violaeflora* Hook. f. has two types of projections, big and minute. The micrographs show that the morphology of the seedcoat of this species is very similar to the cristate subtype we observed in *I. walleriana* (Africa origin, cultivated in Yunnan, China): the big projections are the cristate sculpture while the minute projections seem to be the fine cuticular granules. In gross morphology, *I. violaeflora* is very similar to *I. aureliana* (distributed mainly in south-western China and Burma), which has a squamalate subtype of seedcoat. This suggests that the cristate and squamalate sculpture may have a similar origin. Our observations here on a limited number of species have revealed high diversity in seedcoat micromorphology. Nevertheless, we should note that the majority of the species of *Impatiens* remain to be studied. Further examination of more species, particularly of the African, Indian and Madagascan species, may reveal further diversity and seedcoat types. At that point, it will be possible to establish a more comprehensive scheme of terminology and classification of seedcoat micromorphology.

Phylogenetic and taxonomic implications of seedcoat micromorphology

Previous studies have suggested possible taxonomic implications of seedcoat micromorphological characters (Shimizu, 1979; Lu & Chen, 1991). Our present observations on more species confirmed the possibility that types or subtypes of seedcoat can be diagnostic or indicative of phylogenetic relationships. For example, the granulate seedcoat is unique to *I. balsamina* within the species observed so far. *Impatiens delavayi* and *I. poculifer*, both with a unique carinate seedcoat, are very similar to each other in gross morphology (Chen, 2001). The species with a squamalate subtype seedcoat, including *I. aureliana*, *I. begoniifolia*, *I. mengtszeana*, *I. trichosepala* and *I. yingjiangensis*, and the species with a cristate subtype seedcoat represented by *I. xanthina* and *I. walleriana*, all characterized by having small and globose seeds, are limited to a lineage of species that have globose or broadly



Figures 25–27. SEM micrographs of seedcoat of *Impatiens*: protrusive type – squamalate subtype. Fig. 25. *I. begoniifolia.* Fig. 26. *I. mengtszeana.* Fig. 27. *I. aureliana.* A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.



Figures 28–29. SEM micrographs of seedcoat of *Impatiens*: protrusive type – cristate subtype. Fig. 28. *I. walleriana.* Fig. 29. *I. xanthina.* A, entire seed. Scale bars = 0.5 mm. B, detail of seedcoat. Scale bars = 0.05 mm.

fusiform (length/diameter = 4) capsules (Grey-Wilson, 1980). Interestingly, species of this lineage were found to have lower basic chromosome numbers (x = 8, 7, 6) (Song *et al*., 2003), and a molecular phylogenetic study based on the sequences of the internal transcribed spacers of ribosomic DNA confirmed the monophyly of the lineage (Yuan *et al*., 2004). Thus, the squamalate and cristate sculpture of seedcoats seems to be associated with the fruit shape. In addition to capsule shape, these species are very similar to each other in other aspects of morphology, e.g. all species have relatively large flowers *c.* 3.5–4 cm long, obtuse stigmata, and are slightly pubescent on the upper surface of the leaves. The only exceptions amongst the observed species are the two species with a somewhat specialized seedcoat sculpture, *I. balsamina* with a granulate seedcoat and *I. chinensis* with a laevigate seedcoat. These two species have globose or broadly fusiform capsules, but do not have a squamalate or cristate sculpture on their seedcoats.

Of the various seedcoat types, the reticulate is the most common, as shown by 18 observed species. These species occupied relatively basal positions on the molecular phylogenetic tree (Yuan *et al*., 2004), suggesting that the reticulate seedcoat might represent a comparatively ancestral primitive type. For example, *I. arguta* is one of the most basal species in the phylogenetic tree and has a striate type of seedcoat. On the other hand, the species with complex sculptures, such as squamalate and cristate, on their seedcoats

occurred in the more apical branches of the phylogenetic tree. Thus, they may represent more advanced types.

Based on the samples mainly collected from one of the most important diversity centres, our observations have confirmed high diversity of seedcoat micromorphology in *Impatiens* and allowed development of a preliminary scheme to describe such diversity. Some important correlations among the micromorphological characters of the seedcoat and gross morphological characters have been identified. The sculpture on seedcoats thus offers a set of characters useful for the taxonomy of the genus. However, species from other diversity centres, such as southern India, tropical Africa and Madagascar, are needed for a similar study, that will allow widely applicable conclusions to be drawn. Meanwhile, studies on the developmental variation of seedcoat sculpture, which may provide insights into the better understanding of the evolutionary relationships among different types of sculpture, are also urgently needed.

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