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Authors' Contributions

RG collected material (*I. decipiens*,
I. drepanophora, *I. haridasanii*, *I.*
jurpia, *I. pulchra*, *I. spirifera*, and *I.*
sulcata) as well as initiated SEM
imaging of all species; SB
collected material (*I. anjawensis*
and *I. nyimana*); AR, WA, and RG
developed research designing;
WA performed literature survey;
AR, WA, RG, and SB wrote the
main manuscript text; AR, RG
prepared figures; RG prepared
Table 1; AR and WA prepared
Table 2

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ORIGINAL RESEARCH PAPER in RECENT DEVELOPMENTS IN TAXONOMY AND PHYLOGENY OF
PLANTS

New Data on Seed Coat Micromorphology of Several *Impatiens* spp. from Northeast India

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Abstract

This study aimed to analyze the seed coat structure of nine species from the genus *Impatiens* from Northeast India. A review of the available literature showed a scarcity of data on seed sizes and shapes, as well as a complete lack of information on the ultrastructure of seeds from five taxa of *Impatiens* determined with a scanning electron microscope (SEM). Photographs of the surface structure of seeds from the analyzed species were taken using an SEM and, from these, we measured the length and width of the seeds. The results showed differences in the seed ultrastructure and metric traits within the studied taxa. Based on the ornamentation of the epidermal cells, we distinguished three morphological types: protrusive, granulate, and reticulate. The seeds of the investigated species had ellipsoid, subellipsoid, or subspheroid shapes. Their lengths and widths ranged from 1.2 to 3.6 mm and 0.7 to 2.1 mm, respectively. The results of the study showed that the ultrastructures of *Impatiens* seeds are different among taxa and for some species can be used as a diagnostic character for their identification.

Keywords

Balsaminaceae; micromorphology; scanning electron microscope (SEM); seed surface; taxonomy

1. Introduction

The family Balsaminaceae contains two genera: monotypic *Hydrocera* and *Impatiens*, which has over 1,000 species (Fischer, 2004; Gogoi et al., 2018; Grey-Wilson, 1980; Ruchisansakun et al., 2018). The genus *Impatiens* is characterized by enormous species richness and extremely variable flowers, making it one of the most difficult groups to classify (Bhaskar, 2012; Gogoi et al., 2018; Song et al., 2005). New species of *Impatiens* continue to be described from different regions of the world (Gogoi et al., 2020; Z. C. Lu et al., 2020; Utami, 2020).

For years, plant species have been solely described based on macroscopic morphological characters (Hooker, 1913; Perrier de la Bathie, 1948; Tardieu-Blot, 1945–1946). Nowadays, an integrative taxonomic approach may help solve some uncertain or complicated cases of classification (Rahelivololona et al., 2018; Ruchisansakun et al., 2015; Shajitha et al., 2016; Yu et al., 2015). The progress in molecular techniques as well as in microscopic imaging using a scanning electron microscope (SEM) constitute additional approaches to determine the boundaries

between organisms (Brisson & Peterson, 1976; Godfray & Knapp, 2004). The SEM enables observation and study of small-sized structures that were impossible to examine using conventional light microscopy (Heywood, 1969; Stace, 1992).

Seed shape and seed coat micromorphology are useful for solving taxonomic problems in spermatophytes, including Balsaminaceae (see Cai et al., 2013; W. Chen, Liu, et al., 2007; Utami & Shimizu, 2005; Zhang et al., 2016). However, seed coat micromorphology using the SEM technique has been studied in fewer than one fifth of the *Impatiens* species (Maciejewska-Rutkowska & Janczak, 2016). Of about 150 balsams known from NE India, seeds of almost 20 were investigated this way, for which, however, material was collected from other distribution sites, and only in one case (*I. nagorum*; Moaakum et al., 2017) seeds came from NE India. Seed descriptions for *Impatiens* spp. are not common in literature and often include only macroscopic observations. Such studies are often incomplete and crucial information is missing, e.g., either measurements or only SEM images are published; these are rarely published together (Abid et al., 2011). To date, fewer than 200 species (<20%) have at least one study on seed ultrastructure published (Maciejewska-Rutkowska & Janczak, 2016), and only approximately 20 species (ca. 2%; our unpublished review) of balsams have more than one study available. However, studies have highlighted the importance of seed micromorphology as a useful feature in species identification (Y. Q. Lu & Chen, 1991; Shimizu, 1979) and that seed ultrastructure could be indicative of phylogenetic relationships (Song et al., 2003, 2005; Yuan et al., 2004).

The present study aimed to describe micromorphological characters (based on SEM and biometrical traits) of seeds from nine species of the genus *Impatiens* collected from Northeast India and provide data useful for solving its taxonomic problems.

2. Material and Methods

We analyzed seeds from nine species of the genus *Impatiens*. We collected two–three seeds from three individuals per species during the full fruiting period. The seeds were collected from Northeast India (Table 1). The seeds were sputter-coated with gold and micro-morphological data were studied using an SEM (FEI Quanta 200 SEM and Carl Zeiss EVO 18) at the Central National Herbarium, Botanical Survey of India, Howrah & Botanical Survey of India, ERC, Shillong, Meghalaya. The digital images obtained from the SEM were trimmed and arranged in plates using Corel Draw 2018. The measurements (approximately six–nine for each species) of the seeds (seed length – mm; seed width – mm) were performed based on the SEM pictures of the seeds using the biometric software Image J (Ferreira & Wayne, 2010). The types of seed surface and shape were adapted from Song et al. (2005) and Cai et al. (2013).

Among the nine presented taxa, two (*I. anjawensis* and *I. haridasanii*) are newly described (Gogoi et al., 2018; Hareesh & Sabu, 2017) and one (*I. nyimana*) is a recent addition to the flora of Northeast India (Gogoi et al., 2018; Lidén & Adhikari, 2019). Previously, *I. nyimana* was only known to occur in eastern Tibet (Y. L. Chen, Akiyama, & Ohba, 2007). *Impatiens nyimana* was included in molecular and morphological studies by Yu et al. (2015); however, its SEM images were not published. Recently, *I. mengtzeana*, which was previously known to be from China (Y. L. Chen, Akiyama, & Ohba, 2007), was synonymized under *I. pulchra*, a species known from Southeast Asia and Northeast India (Gogoi et al., 2018; Ruchisansakun et al., 2018). Five of the nine species analyzed in the present study have not been studied using the SEM technique before (Table 1).

Voucher specimens of studied taxa were deposited at Botanical Survey of India (CAL), Botanical Survey of India, Eastern Regional Center (ASSAM) & Botanical Survey of India, Arunachal Pradesh Regional Centre (ARUN) (Table 1).

3. Results

The ultrastructure of the seeds observed under the SEM showed three morphological types: reticulate (four taxa), protrusive (four taxa), and granulate (represented

Table 1 Studied *Impatiens* species.

Taxon	Studied material	Habitat of the species	Remarks
<i>Impatiens anjawensis</i> Borah, Kandwal, Chhetri & Gogoi	Arunachal Pradesh, Anjaw District, Walong, 4 km from Pimple top towards Walong, 96°58'32.27" E, 28°10'44.00" N, 3,690 m, 2016-09-07, S. Borah 34905 (CAL, ASSAM & ARUN)	Moist shady mountain slopes in subalpine forest	Newly described (Gogoi et al., 2018)
<i>Impatiens decipiens</i> Hook. f.*	Mangan Road, Pakchar, 2017-08-25, R. Gogoi & A. Kumar 73605 (CAL)	Moist hilly slopes and forest margins in subtropical forest	-
<i>Impatiens drepanophora</i> Hook. f.	North Sikkim, Kabi to Mangan, 1,100–1,700 m, 2017-08-25, R. Gogoi & A. Kumar 73604 (CAL)	Moist forest margins and road sides, also in open areas near streams in subtropical to lower temperate forest	-
<i>Impatiens haridasanii</i> Hareesh & M. Sabu*	Nagaland, Noklak, N. Odyuo & R. Domari 131936 (CAL)	Moist rocky open areas and in hilly slopes of subtropical forest	Newly described (Hareesh & Sabu, 2017)
<i>Impatiens jurpia</i> Buch.-Ham. ex Hook. f. & Thomson*	Arunachal Pradesh, Lower Subansiri District, Ziro, Monipuleng to Pine grove, 1,500 m, 2013-08-01, R. Gogoi 30340 (CAL & ARUN)	Moist hilly slopes and in road sides of subtropical primary forests	-
<i>Impatiens nyimana</i> C. Marquand & Airy Shaw	Arunachal Pradesh, Anjaw District, way to Pimple top from Helmet top, 96°59'34.52" E, 28°10'4.33" N, 3,170 m, 2016-09-07, S. Borah & M. K. Kandwal 34907 (ARUN)	Wet alpine meadows	Recently found in India (Gogoi et al., 2018; Lidén & Adhikari, 2019)
<i>Impatiens pulchra</i> Hook. f. & Thomson*	West Sikkim, Yuksum, Rimbi Waterfall, 2017-09-01, R. Gogoi & A. Kumar 73632 (CAL)	In highly moist areas near streams or waterfalls in subtropical forest	<i>Impatiens mengtszeana</i> was synonymized under <i>I. pulchra</i> (Ruchisansakun et al., 2018)
<i>Impatiens spirifera</i> Hook. f. & Thomson*	North Sikkim, in between Chungthang to Lachen, 2017-10-05, R. Gogoi 73701 (CAL)	Margins of primary forest and in moist road sides from subtropical to temperate forest	-
<i>Impatiens sulcata</i> Wall.	North Sikkim, way to Katao, 2017-10-08, R. Gogoi 73710 (CAL)	Open areas in the forest margins and road sides of subalpine forest	Species with wide Himalayan distribution (Y. L. Chen, Akiyama, & Ohba, 2007; Gogoi et al., 2018; Grey-Wilson, 1991; Nasir, 1980)

* Species tested for the first time using SEM.

by one taxon; Table 2). Their lengths and widths were different among the taxa and ranged from 1.2 to 3.6 mm and 0.7 to 2.1 mm, respectively. The seeds of the investigated species had ellipsoid, subellipsoid, or subspheroid shapes.

The reticulate type was characterized by epidermal cells that formed reticulate ornamentation with varied shapes and sizes. This group comprises *I. anjawensis* (Figure 1A,B), *I. decipiens* (Figure 1C,D), *I. nyimana* (Figure 2E,F), and *I. sulcata* (Figure 3E,F). These species belong to the fine reticulate subtype, with epidermal cells evenly distributed and slightly bulged. The seeds in this category had ellipsoid or subellipsoid shapes and the size was in the range of 1.8–3.6 (length) × 0.9–1.4 mm (width; Table 2). Only one species in this subtype, *I. decipiens*, had seeds with granules covering the epidermal cells.

The protrusive type, characterized by some epidermal cells of the seedcoat protruding significantly to form projections of varied shapes, included *I. drepanophora* (Figure 1E,F), *I. haridasanii* (Figure 2A,B), *I. pulchra* (Figure 3A,B), and *I. spirifera* (Figure 3C,D). In this type, three species had granules covering

Table 2 Comparison of the morphological characters of the seeds of the analyzed species from *Impatiens* genus with data from the literature.

Taxon	This study results Length × Width (mm)	Color	Shape	Ornamentation	Literature data	Source	Figure
<i>Impatiens ajiawensis</i> Borah, Kandwal, Chhetri & Gogoi	2.0 × 1.3	Light brown	Ellipsoid	Reticulate type Fine reticulate subtype	3.0 mm long, 2.5 mm wide; white; ovoid	Gogoi et al., 2018	Figure 1A,B
<i>Impatiens decipiens</i> Hook. f.	1.8 × 1.2	Light brown	Subellipsoid	Reticulate type Fine reticulate subtype Granules cover the epidermal cells	Not studied	-	Figure 1C,D
<i>Impatiens drepanophora</i> Hook. f.	1.4 × 1.1	Creamy or light brown	Subellipsoid	Protrusive type Digitiform subtype	2.2–2.9 mm long; 1.3–1.7 mm wide; dark-brown; subellipsoid; protrusive type, digitiform subtype	Song et al., 2005	Figure 1E,F
<i>Impatiens haridasanii</i> Hareesh & M. Sabu	1.4 × 0.7	Light brown	Ellipsoid	Protrusive type Squamalate subtype Granules cover the epidermal cells	Not studied	-	Figure 2A,B
<i>Impatiens jurpia</i> Buch.-Ham. ex Hook. f. & Thomson	2.4 × 2.1	Greenish or dark brown	Subspheroid	Granulate type	Not studied	-	Figure 2C,D
<i>Impatiens nyimana</i> C. Marquand & Airy Shaw	2.5 × 0.9	-	Subellipsoid	Reticulate type Fine reticulate subtype	Ovoid, corrugate	Yu et al., 2015	Figure 2E,F
<i>Impatiens pulchra</i> Hook. f. & Thomson	1.2 × 0.8	Greenish or dark brown	Subspheroid	Protrusive type Cristate subtype Granules cover the epidermal cells	Not studied	-	Figure 3A,B
<i>Impatiens spirifera</i> Hook. f. & Thomson	2.0 × 1.4	Grey	Subspheroid	Protrusive type Cristate subtype Granules cover the epidermal cells	Not studied	-	Figure 3C,D
<i>Impatiens sulcata</i> Wall.	3.6 × 1.4	Light brown or cream coloured	Subellipsoid	Reticulate type Fine reticulate subtype	3.2–4.0 mm long, 1.3–2.4 mm wide; brown; subellipsoid /ovate; areolate subtype/granulate; ovoid, corrugate	Abid et al., 2011; Cai et al., 2013; Yu et al., 2015	Figure 3E,F

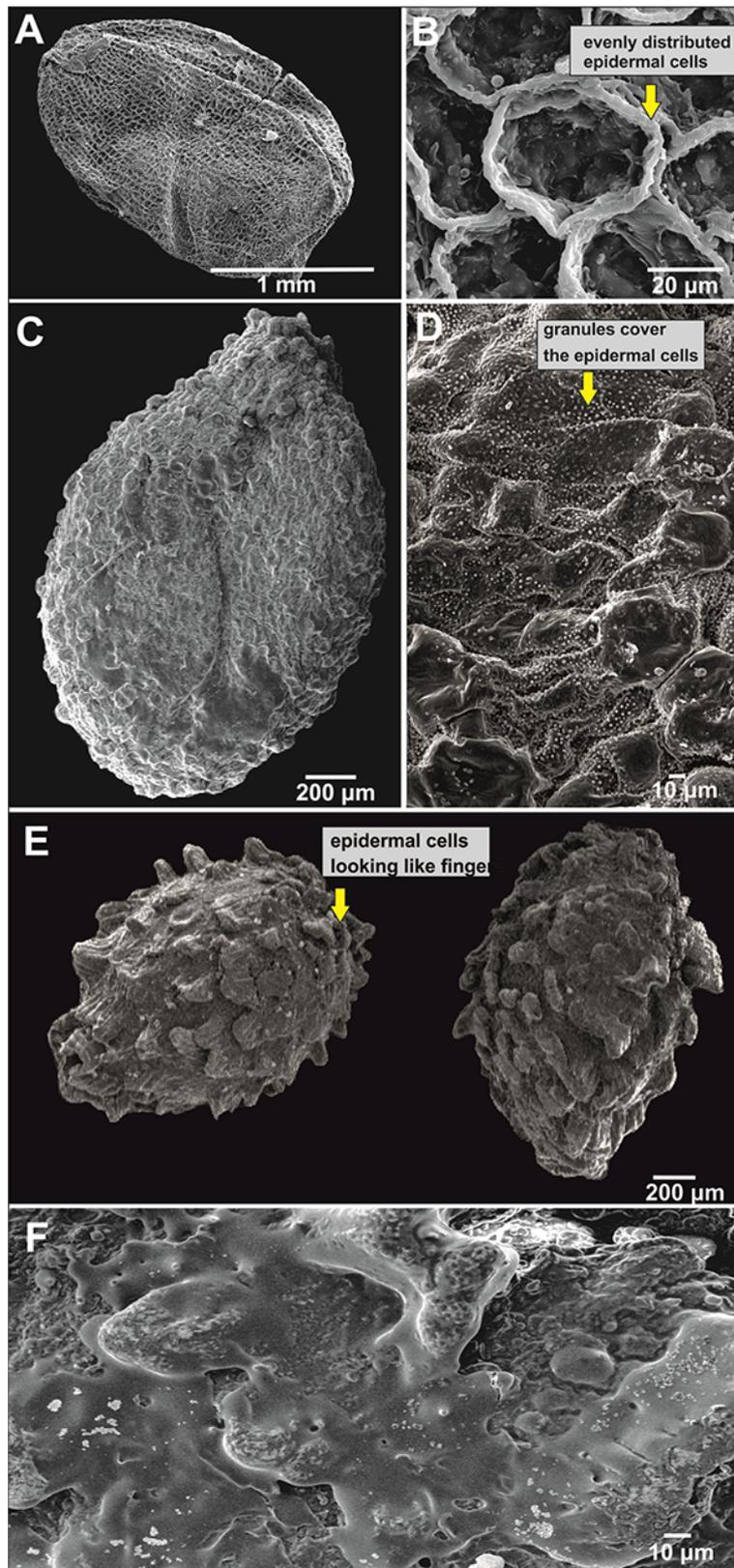


Figure 1 Seed general view and coat micromorphology of analyzed balsams from Northeast India. (A–D) Reticulate type: *Impatiens anjawensis* (A,B), *I. decipiens* (C,D); (E,F) protrusive type: *I. drepanophora*.

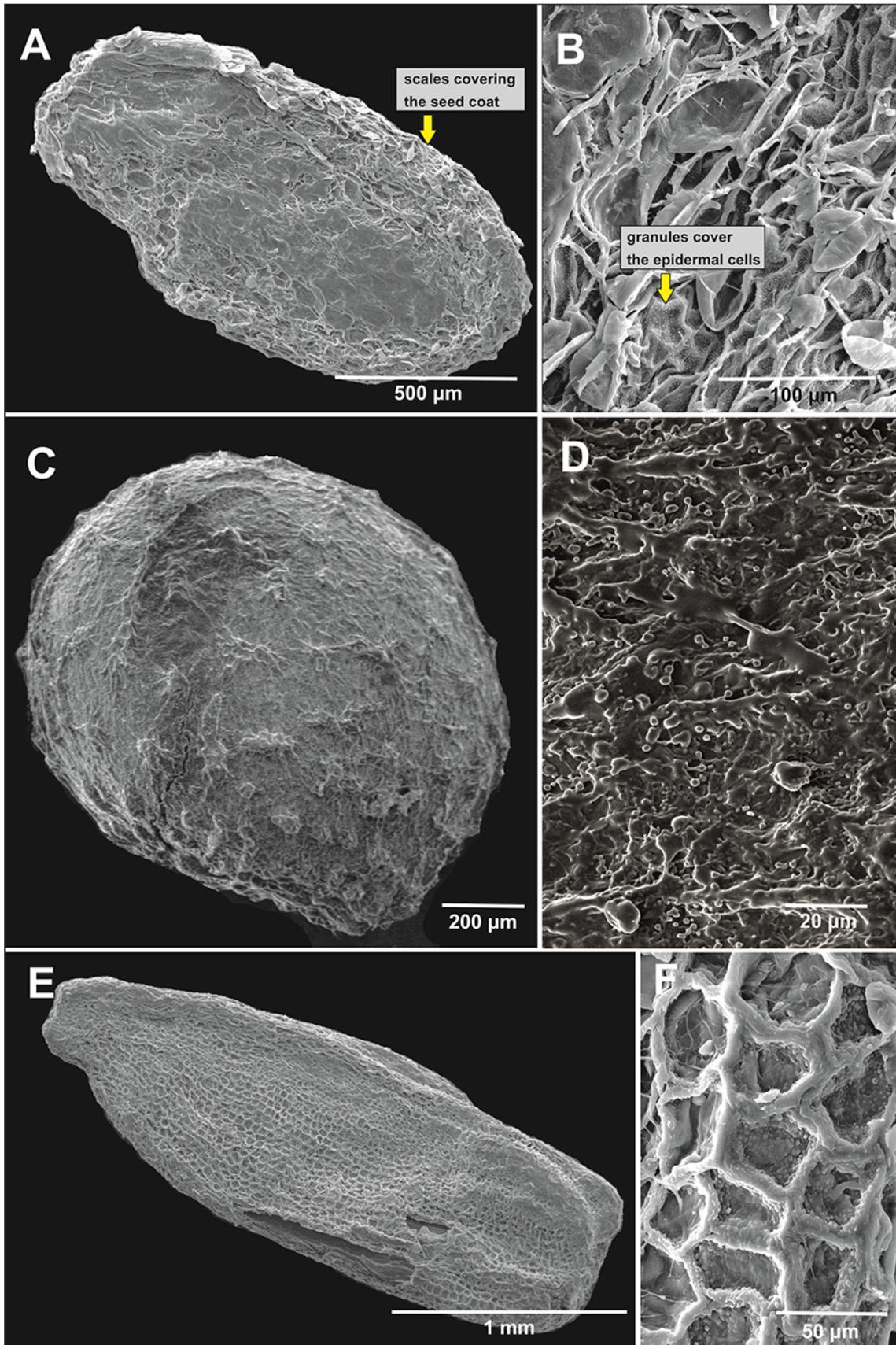


Figure 2 Seed general view and coat micromorphology of analyzed balsams from Northeast India. (A,B) Protrusive type: *Impatiens anjawensis*; (C,D) granulate type: *I. jurpia*; (E,F) reticulate type: *I. nyimana*.

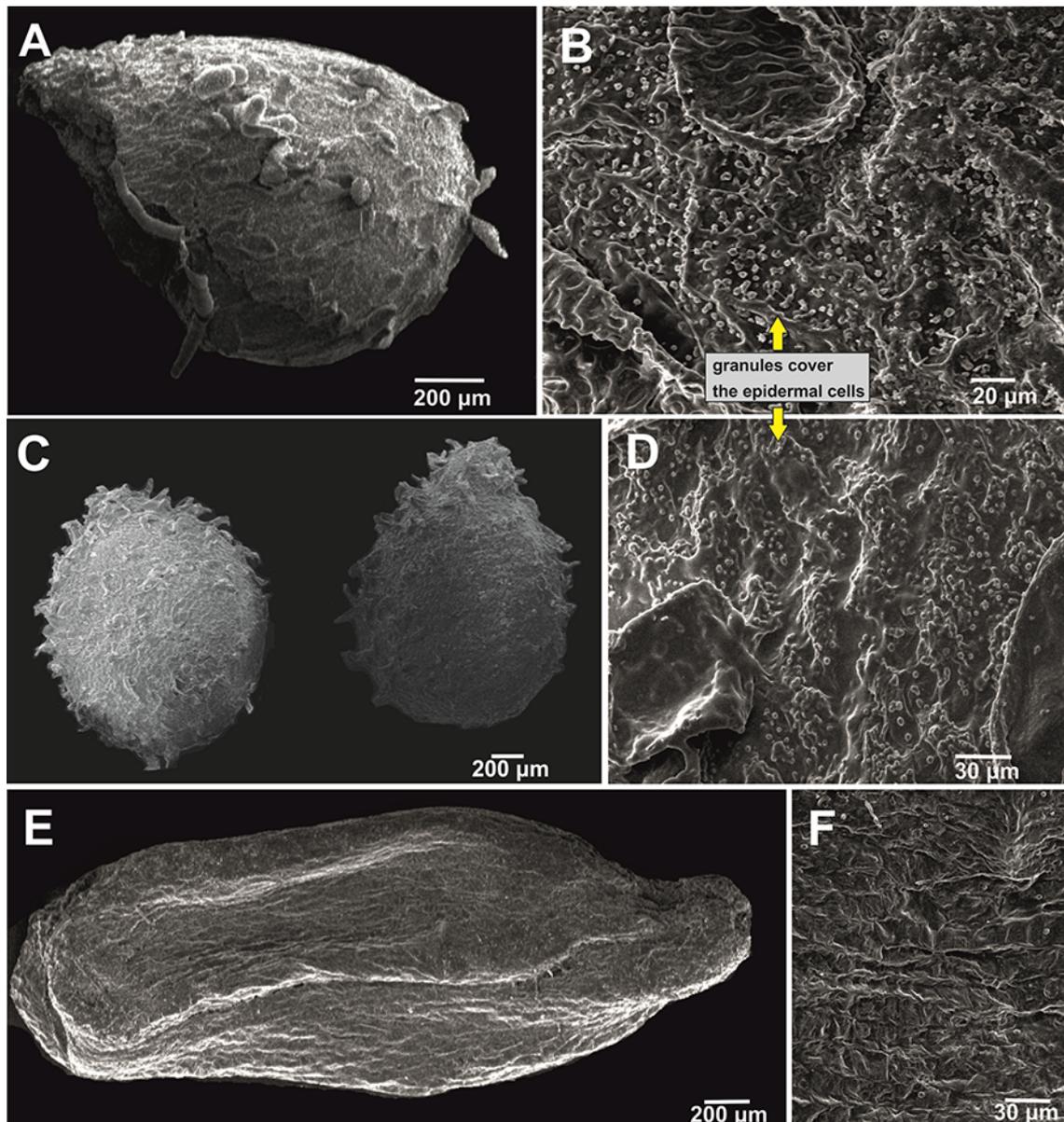


Figure 3 Seed general view and coat micromorphology of analyzed balsams from Northeast India. (A–D) Protrusive type: *Impatiens anjawensis* (A,B), *I. spirifera* (C,D); (E,F) reticulate type: *I. sulcata*.

their epidermal cells (Table 2). *Impatiens drepanophora* represented the digitiform subtype with epidermal cells looking like fingers. *Impatiens haridasanii* represented the squamulate subtype having scales covering the seed coat. The last two species in this type, *I. pulchra* and *I. spirifera*, belonged to the cristate subtype.

Only one species, *I. jurpia* (Figure 2C,D), represented the last type, granulate, which was characterized by granulate protrusions evenly covering the entire seed surface.

4. Discussion

Previous studies on seed sculpture have shown its usefulness in plant taxonomy, as seen for Asteraceae (Shabestari et al., 2013), Caryophyllaceae (Ullah, Papini, et al., 2019; Ullah, Zaman, et al., 2019), Poaceae (Martín-Gómez, Rewicz, Goriewa-Duba, et al., 2019), Ranunculaceae (Hadidchi et al., 2020; Martín-Gómez, Rewicz, & Cervantes, 2019; Rewicz et al., 2017), and Orchidaceae (Ortúñez et al., 2006; Rewicz et al., 2016). Our research is the first insight into the seed ultrastructure of five species from the genus *Impatiens* from Northeast India. Among the analyzed taxa, differences in seed ultrastructure (especially in *I. jurpia*, *I. pulchra*, and *I. spirifera*)

and shape distinguished them from each other. Studies have shown a large variation in the ultrastructure of the seed coat in *Impatiens* (Abid et al., 2011; Y. Q. Lu & Chen, 1991; Shimizu, 1979; Song et al., 2005) and our results confirmed this high diversity. In the four analyzed species, *I. decipiens*, *I. haridasanii*, *I. pulchra*, and *I. spirifera*, we found a prominent feature of the sculpture on seed coats, granules on the seed surface, which could be useful for the taxonomy of this genus. Abid et al. (2011) considered this feature as important for the recognition of two subspecies of *I. bicolor* (*I. bicolor* ssp. *bicolor* and *I. bicolor* ssp. *pseudo-bicolor*).

However, without testing seeds from a wide spatial and habitat range, we should be careful before considering seeds as taxonomically valid for identification. Further research may provide answers concerning the level of phenotypic plasticity of seeds in the analyzed species. It is necessary to determine whether the seeds of these species have a stable ultrastructure and shape to be useful as a tool in taxonomy. Species with a wide distribution, such as *I. sulcata* or *I. noli-tangere*, may be a model for such future studies.

Due to the scarcity of seed biometrics and seed coat ultrastructure data, we could only compare the obtained results to the data available for three species, *I. anjawensis*, *I. drepanophora*, and *I. sulcata* (Table 2). For two species, *I. anjawensis* and *I. drepanophora*, biometric analyses showed that our seed length and width values were lower than those described in the literature. Only the seed length of *I. sulcata* was higher than that in the literature data. Unfortunately, for *I. nyimana*, the literature data did not include seed measurements (Table 2).

Our results of seed shape and ultrastructure agree with the published data only in the case of *I. drepanophora* (Song et al., 2005). The seed shape and ornamentation information obtained in our study differs from that of the earlier studies in *I. sulcata* (Abid et al., 2011; Cai et al., 2013; Yu et al., 2015) (Table 2). For *I. drepanophora* and *I. sulcata*, this may indicate high phenotypic plasticity in these species. Therefore, seed characteristics may not be useful in identifying *I. drepanophora* and *I. sulcata*. Previous descriptions of the seeds of *I. nyimana* (Yu et al., 2015) differ from our results (ovoid, corrugate and subellipsoid, reticulate type, and the fine reticulate subtype). Results presented by Gogoi et al. (2018) do not include seed ornamentation information for *I. anjawensis*.

These differences could be an effect of deformation of seeds during air drying in storage or the collection of immature seeds. In the taxa with wide spatial distribution, this phenomenon could also be an effect of internal variability or even potential cryptic diversity. For example, the micrographs of *I. noli-tangere* seeds from China and Japan are different in their seed coat ultrastructure (W. Chen, Liu, et al., 2007; Jin et al., 2008; Song et al., 2005).

The most complicated case is *I. pulchra*, where there is no published data on seed ultrastructure. However, during the recent revision of Myanmarese balsams, Ruchisansakun et al. (2018) synonymized *I. mengtszeana*, known from China and Thailand, with *I. pulchra*. *Impatiens mengtszeana* has published data on its seed ultrastructure (Song et al., 2005; Utami & Shimizu, 2005). If seed shape and ultrastructure is considered as a stable taxonomic feature (Stace, 1992), our *I. pulchra* micrographs should be similar to the micrographs of *I. mengtszeana* (= *I. pulchra* sensu Ruchisansakun et al., 2018) from China (Song et al., 2005; Utami & Shimizu, 2005). However, our study showed different shape and ornamentation of seeds in *I. pulchra* (seeds of *I. pulchra* were subspheroid and had the protrusive type of ornamentation, while *I. mengtszeana* had spheroid seeds and the squamulate subtype of ultrastructure; Song et al., 2005) and the specimens we examined had granules covering their epidermal cells (Figure 3B). Thus, the taxonomic status of these two taxa requires further investigation.

Overall, our observations concerning the ultrastructure of seeds from *Impatiens* spp. provide knowledge of the diversity in this group.

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