# SEED MORPHOLOGY AND CLASSIFICATION OF IMPATIENS (BALSAMINACEAE)

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# SUMMARY

Seed coat morphology was observed through SEM for 65 species and 10 sections of *Impatiens*. Several types of seed coat are recognized in this study. On the basis of comparison with other characters such as chromosome numbers, floral characters and seedling morphology, systematic problems concerning *Impatiens* are briefly discussed.

Key words: Balsaminaceae, Impatiens, seed coat, classification.

# INTRODUCTION

The Balsaminaceae, a family consisting mostly of subsucculent annual or perennial herbs, is usually regarded as comprising two genera, the monotypic *Hydrocera* Blume ex Wight & Arn. and the prolific *Impatiens* L. with an estimated number of about 850 species (Grey-Wilson, 1980). The genus *Impatiens* is concentrated in mountainous regions of South-East Asia, South China, India and Africa. Like most genera of comparable or larger size *Impatiens* suffers from a rather confused taxonomy and the absence of recent comprehensive revisions.

Studies on seed coat morphology have been carried out by several researchers. Wunderlich (1967), Corner (1976) and Kumar & Singh (1990) pointed out the significance of seed coat morphology in solving problems of classification and in phylogenetic considerations. The seed coat ornamentation in the genus *Impatiens* has been given attention since Hooker & Thomson (1859), but without comprehensive surveys. In the following account the seeds of 65 species and 10 sections of *Impatiens* in the sense of Warburg & Reiche (1895) are described and the contribution that seed coat may make to the taxonomy of the family is considered.

# MATERIALS AND METHODS

Seed samples of 65 species of *Impatiens* representing two subgenera, 10 sections (2 sections of subg. *Acaulimpatiens* and 8 sections of subg. *Impatiens*) taken from the field or herbarium specimens in Kanazawa University (KANA), Japan and the Herbarium Bogoriense, Bogor Indonesia (BO) (See Table 1). The samples were air dried, mounted, coated with gold and observed with SEM (AKASHI ALPHA) 30A at 15 kV and magnification 30–300.

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| Subgenus                | Section              | Species   | Locality   | Voucher   |
|-------------------------|----------------------|---|--|---|
| Acaulimpatiens<br>Warb. | Scapimpatiens Warb.  | I. acaulis Arn.<br>I. scapiflora Heyne  | India: W Ghats<br>India: W Ghats   | KANA 190708<br>KANA 192267  |
|                         | Orchimpatiens Warb.  | I. modesta Wight.<br>I. stocksii<br>Hook.f. & Thomson   | India: W Ghats<br>India: W Ghats   | KANA 192266<br>KANA 192257  |
| Impatiens Warb.         | Enantiophyllon Warb. | I. chinensis L.<br>I. helferi Hook.f.<br>I. kleinii Wight. & Arn.<br>I. masoni Hook.f.<br>I. proctrata Hook.f.<br>I. gardneriana Wight.<br>I. griffithii<br>Hook.f. & Thomson<br>I. javensis Steud<br>I. platypetala L.   | Thailand: Loei<br>China<br>India: W Ghats<br>Thailand: Petchaburi<br>Indochina<br>India: W Ghats<br>Malaya: Pahang<br>Indonesia: W Java<br>Indonesia: W Java   | KANA 190740<br>BR (Perrotet 188)<br>KANA 194742<br>KANA 189638<br>P (type)<br>KANA 194740<br>KANA 194740<br>KANA 190762<br>KANA 189552<br>BO 114839 |
|                         |                      | I. relaxata Hook.f.   | Indochina  | P (isotype)   |
|                         | Macrocentron Warb.   | I. chiangdaoensis<br>T. Shimizu   | Thailand: Chiang Mai   | KANA 190274   |
|                         |                      | I. diffusa Hook.f.<br>I. longicalcarata Tardieu<br>I. monotrica Hook.f.<br>I. noei Craib<br>I. obscura Hook.f.<br>I. patula Craib<br>I. rara Tardieu<br>I. santisukii T. Shimizu<br>I. subaequalis Craib<br>I. thorelii Hook.f.<br>I. honslowiana Arn.<br>I. muscicola Craib<br>I. siamensis T. Shimizu   | Indochina<br>Indochina<br>Indochina<br>Thailand: Huiyang<br>Indochina<br>Thailand: Chiang Mai<br>Indochina<br>Thailand: Chiang Mai<br>Thailand: Prachuap<br>Khiri Khan<br>Indochina<br>Thailand: N. Tak<br>Indochina<br>Thailand<br>Thailand | P (type)  |
|                         | Microcentron Warb.   | I. balsamina L.<br>I. bunnackii T. Shimizu<br>I. charanii T. Shimizu<br>I. harmandii T. Shimizu<br>I. kanburiensis T. Shimizu<br>I. macrosepala Hook.f.<br>I. obesa Hook.f.<br>I. saraburiensis T. Shimizu<br>I. hongsonensis T. Shimizu<br>I. kerriae Craib<br>I. psittacina Hook.f.<br>I. verucifer Hook.f.<br>I. namkatensis T. Shimizu<br>I. capusii Hook.f.<br>I. glandulifera Royle |  | KANA 189623<br>KANA189805<br>P (Balansa 113)<br>P   |

Table 1. Impatiens species examined.

| Tabl | le 1 | (con | t.) |
|------|------|------|-----|
|      |      |      |     |

| Subgenus | Section               | Species                           | Locality                         | Voucher       |
|----------|-----------------------|-----------------------------------|----------------------------------|---------------|
|          | Megalocentron Warb.   | I. garrettii Craib                | Thailand: Chiang Mai KANA 1895   |               |
|          |                       | I. tapanuliensis<br>Grey-Wilson   | Indonesia: W Sumatra             | a KANA 189424 |
|          |                       | I. diepenhorstii<br>Grey-Wilson   | Indonesia: W Sumatra KANA189347  |               |
|          |                       | I. oncidioides Ridl.              | Malaya                           | KANA 187115   |
|          |                       | I. dewildeana Grey-Wilson         | Indonesia                        | BO 115674     |
|          |                       | I. mengtzeana Hook.f.             | South China, Thailand            | dKANA 189620  |
|          |                       | I. pseudoperezii<br>Grey-Wilson   | Indonesia: W Sumatra KANA 189367 |               |
|          |                       | I. sidikalangensis<br>Grey-Wilson | Indonesia: W Sumatra KANA 189396 |               |
|          |                       | I. vittelina Grey-Wilson          | Indonesia: W Sumatra KANA 189399 |               |
|          | Brachycentron Warb.   | I. hypophylla Makino              | Japan: Kyushu                    | KANA 153136   |
|          |                       | I. textori Miq.                   | Japan: Ishikawa                  | KANA 109208   |
|          |                       | I. noli-tangere L.                | Japan: Nagano                    | KANA 182684   |
|          |                       | I. alboflava Miq.                 | Indonesia: W Sumatra             | a KANA 189413 |
|          |                       | I. larsenii T. Shimizu            | Thailand: Surathani              | KANA 189594   |
|          | Longicornes Warb.     | I. cordata Wight                  | India: W Ghats                   | KANA 194866   |
|          | 0                     | I. walleriana Hook.f.             | Cultivated                       | NONE          |
|          | Salpingochillon Warb. | I. benthami Steenis               | India                            | KANA 196463   |
|          |                       | I. goughii Wight                  | India: W Ghats                   | KANA 194874   |
|          | Choniochillon Warb.   | I. omissa Hook.f.                 | India: W Ghats                   | KANA 196466   |
|          | I. viscosa Bedd.      | India: W Ghats                    | KANA 194922                      |               |

# RESULTS AND DISCUSSIONS

The Balsaminaceae have small- to medium-sized seeds, that are exarillate, exalbuminous and in most cases pachychalazal, the integument is not or scarcely multiplicative. The seed coat is formed by the expanded chalaza; the testa (outer or exotesta) is unspecialized or the cells have a papilla or a hair; the mesophyll is unspecialized, more or less crushed; the tegmen when present becomes crushed. The vascular bundle ends at the chalaza. The endosperm is cellular but absorbed. The embryo is straight (Corner, 1976). Parenchyma, tannin, crystal, mucilage, cork and sclerenchyma or collenchyma cells all contribute to the differentiation and build-up of the seed coat. The seed coat of Balsaminaceae usually contains crystals. However, the function of the crystals in the seed coat is unknown. They may serve as storage organs of waste products, defence against animal predators or, especially in the case of silicate crystals, assist in mechanical protection (Boesewinkel & Bouman, 1984).

In the last decade the SEM technique has opened an entire new field of micromorphological research on seed coats. Brisson & Peterson (1976) reviewed the use of SEM in the study of seed coats. They found that several features can be distinguished on the surface of the seed by SEM. Our study focuses on seed morphology of the genus *Impatiens*. The descriptions of the seed coats follow Grey-Wilson's (1980) and Barthlott's (1990) terminology. Scanning Electron Micrographs (SEM) of the representative seed coat types of *Impatiens* species examined are presented in Plate 1–4.

# A. Subgenus Acaulimpatiens Warb.

From Plate 1a & b (*I. acaulis* Arn., *I. stocksii* Hook.f. & Thomson) it can be seen that subg. *Acaulimpatiens* has two types of seed coat. The seeds of *I. acaulis* (Plate 1a) are covered with broad and ring patterned hairs of  $40-50 \mu m$  diam., while those of *I. stocksii* (Plate 1b) have long and slender hairs of  $30-37 \mu m$  diam. at both ends only. This indicates that the subgenus may be divided at least into two sections, supporting

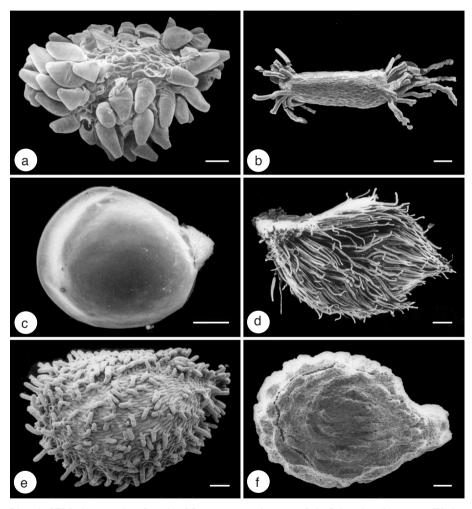


Plate 1. SEM-photographs of seeds of *Impatiens* species. — a & b: Subg. *Acaulimpatiens* Warb.: a. Sect. *Scapimpatiens* Warb.: *I. acaulis* Arn. with short and thick hairs on the surface; b. Sect. *Orchimpatiens* Warb.: *I. stocksii* Hook.f. & Thomson with long and slender hairs on the surface, only at both ends. — c–f: Subg. *Impatiens*: c & d. Sect. *Enantiophyllon* Warb.: c. *I. chinensis* L. with smooth seeds; d. *I. gardneriana* Wight with long hairs around the testa; e & f. Sect. *Macrocentron* Warb.: e. *I. patula* Craib: testa with tufts of helical 'hairs'; f. *I. henslowiana* Arn.: testa irregular with isodiametric cells with reticulate thickened outer walls. — Scale bars:  $a = 100 \mu m$ ;  $b = 175 \mu m$ ; c & e = $330 \mu m$ ;  $d = 250 \mu m$ ;  $f = 320 \mu m$ .

the classification of Warburg & Reiche (1895) who divided subg. *Acaulimpatiens* into two sections on the basis of floral shape: sect. *Scapimpatiens* Warb. with big, long-spurred flowers and sect. *Orchimpatiens* Warb. with small, short-spurred flowers. The two sections also show differences in chromosome numbers. In *I. scapiflora* F. Heyne of sect. *Scapimpatiens* 2n = 6, 8, 10, 16 and 20 have been counted, while in *I. stocksii* of sect. *Orchimpatiens* the chromosome number is n = 7 and 10 (Bhaskar & Razi, 1972). Shimizu et al. (1990) reported two anther types in this subgenus: tetrasporangiate (*I. acaulis* and *I. scapiflora*) and trisporangiate (*I. modesta* Wight and *I. stocksii*). The species *I. acaulis* and *I. scapiflora* have a seedling morphology of the 'third type' (Shimizu, 1982). Together seed coat morphology, seedlings, anther type and chromosome numbers support the classification given by Warburg & Reiche (1895).

## B. Subgenus Impatiens Warb.

The most interesting problems are to be found in subg. *Impatiens* in which the species show considerable diversity in their seed coat pattern. Eight sections were examined in this study. They are as follows:

#### 1. Section Enantiophyllon Warb.

This section is variable in seed coat patterns, which can be divided into two groups as shown in Plate 1c-f (I. chinensis L., I. gardneriana Wight, I. patula Craib, I. henslowiana Arn.). Group I is characterized by black globose seeds with a glabrous and shining surface as found in I. chinensis (Plate 1c), I. kleinii Wight & Arn., I. masoni Hook.f. and I. proctrata Hook.f. All species of this group share opposite or whorled leaves, but differ in seedling morphology. The seedling morphology of I. chinensis belongs to 'subgroup F', while I. masoni is assigned to 'subgroup H' (Shimizu, 1982). The chromosome number of *I. chinensis* and *I. kleinii* is 2n = 16 (Shimizu, unpubl.; Zinov'Eva-Stahevitch, 1981). Group II: Seeds densely covered with long hairs on the surface, whorled leaves and seedling morphology 'subgroup E' (Shimizu, 1982). The chromosome number of *I. gardneriana* (Plate 1d) and *I. platypetala* L. is 2n =16 (Bhaskar & Razi, 1974; Zinov'Eva-Stahevitch, 1981). Bhaskar & Razi (1978) divided sect. *Enantiophyllon* into two subsections on the basis of phyllotaxis, subsect. *Oppositifoliae* Bhaskar & Razi, including all of the members of Group I and subsect. Verticillatae Bhaskar & Razi which refers to Group II. As a result, his subdivision is well concordant with the present grouping, although it is not always supported by seedling type and chromosome number.

## 2. Section Macrocentron Warb.

This section is characterized by alternate leaves and only one or two flowers with long spurred lips per inflorescence. The seed coat morphology suggests division of the species examined into three groups. Group I has a testa with tufts of helical 'hairs' (Plate 1e, *I. patula*) and is further characterized by pink flowers, turgid and tomentose capsules and seedling type 'subgroup C' (Shimizu, 1982). Their distribution is strictly restricted to South-East Asia. This is a natural group to be treated as an independent taxon. *Impatiens henslowiana* of Group II (Plate 1f) is characterized by an irregular

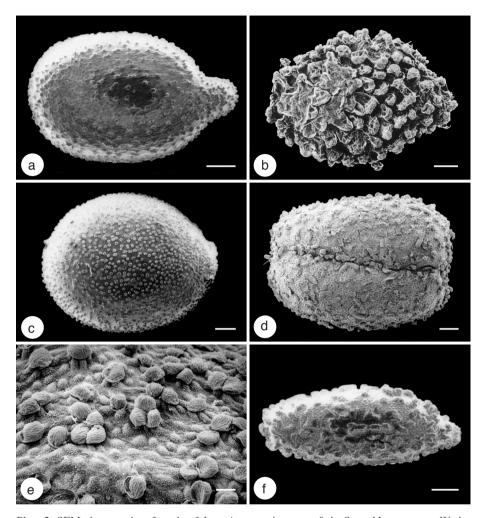


Plate 2. SEM-photographs of seeds of *Impatiens* species. — a & b: Sect. *Macrocentron* Warb.: a. *I. muscicola* Craib with short 'hair-like' protuberances; b. *I. siamensis* T. Shimizu with a 'warted' epidermis. — c-f: Sect. *Microcentron* Warb.: c. *I. balsamina* L.: testa covered with small peculiar wart-like outgrowths; d. *I. harmandii* Hook.f.: testa cells with reticulate thickened outer walls and long, helical 'hair-like' structures; e. *I. psittacina* Hook.f.: outer walls of the testa cells are granulate; f. *I. namkatensis* T. Shimizu: testa cells with bulging outer walls. — Scale bars: a, c & d = 330  $\mu$ m; b = 150  $\mu$ m; e = 33  $\mu$ m; f = 270  $\mu$ m.

testa with isodiametric cells with reticulate thickened outer walls (compare Plate 2b, *I. siamensis* T. Shimizu). The seedling belongs to 'subgroup E' (Shimizu, 1982). Group III, *I. siamensis* (Plate 2b), is characterized by a four-carpellate ovary and connate winged petals like Group II and III of section *Microcentron* Warb. The chromosome numbers of *I. violiflora* Hook.f. are 2n = 10 and 12 (Larsen, 1981) and that of *I. santisukii* T. Shimizu is 2n = 10 (Shimizu, unpubl.).

## 3. Section Microcentron Warb.

This section can be divided into six groups of species. Group I, I. balsamina L. is characterized by a vertucose seed coat (Plate 2c) and inflated tomentose capsules. These capsules are very characteristic and not seen in any of the other groups. The seedling type of this species belongs to 'subgroup C' (Shimizu, 1982). Its chromosome number is variable, 2n = 12, 14, 18, 20, 24 and 14 + 2 (Khoshoo, 1955, 1956, 1957; Chatterjee & Sharma, 1970). Group II is characterized by testa cells with reticulate thickened outer walls. This group includes I. arriensii (Zoll.) T. Shimizu, I. bunnackii T. Shimizu, I. charanii T. Shimizu, I. harmandii Hook.f. (Plate 2d), I. kanburiensis T. Shimizu, I. macrosepala Hook.f., I. obesa Hook.f. and I. saraburiensis T. Shimizu. Group III, including I. hongsonensis T. Shimizu, I. kerriae Craib, I. parishii Hook.f. and *I. psittacina* Hook.f. (Plate 2e), is characterized by a finely granulate seed coat. These species are very unique in having a glabrous, four-carpellate ovary. The species mentioned above, together with some others, should be treated as a third subgenus of Impatiens. This group is restricted to South Asia, from South China to South Indonesia. Group IV, represented by I. boni Hook.f., I. cardiophylla Hook.f. and I. namkatensis T. Shimizu (Plate 2f), is characterized by a 'warted' epidermis. These 'warts' appear to be glands derived from a single epidermal cell and are distributed over the entire surface of the testa. These species have four carpellate ovaries and connate winged petals. Like Group III, Group IV should be transferred to a new subgenus. Impatiens siamensis of section Macrocentron probably belongs to the same new subgenus. Group V is heterogeneous in floral characters: I. salaengensis T. Shimizu has a four-carpellate ovary, while I. tripetala Roxb. has a five-carpellate ovary. In this case, seed characters are considered as homoplastic. Group VI, I. muscicola Craib, should be regarded as a member of section Macrocentron on the basis of flower morphology and seedling type.

#### 4. Section Megalocentron Warb.

This section is characterized by alternate leaves, racemes with 2–5 flowers with long spurred lips. This section can be divided into two groups. Group I is characterized by a testa with papillae (Plate 3a, *I. garrettii* Craib). *Impatiens garrettii* has violet and red striped flowers with curved spurs. *Impatiens tapanuliensis* Grey-Wilson has yellow flowers with stripes and straight spurs. No features common to these species were found. Group II is represented by rheophytic plants with flabellate wing petals.

#### 5. Section Brachycentron Warb.

This section is divided into two groups. Group I is characterized by a reticulate seed coat as seen in *I. hypophylla* Makino, *I. textori* Miq. (Plate 3b) and *I. noli-tangere* L. (Plate 3c). The seedlings of *I. noli-tangere* and *I. textori* belong to 'subgroup C' (Shimizu, 1982). Group II, *I. alboflava* Miq. and *I. larsenii* T. Shimizu, is characterized by a seed coat with two kinds of granules: large and small (Plate 3d, e). These two species are peculiar in having non-pendulous flowers and asymmetric wing petals (Utami & Shimizu, 1998). Warburg & Reiche (1895) classified *I. alboflava* as a member of sect. *Brachycentron* together with *I. noli-tangere* and *I. textori*. However, the seed coat features indicate that *I. alboflava* and *I. larsenii* may better be placed in sect. *Microcentron*.

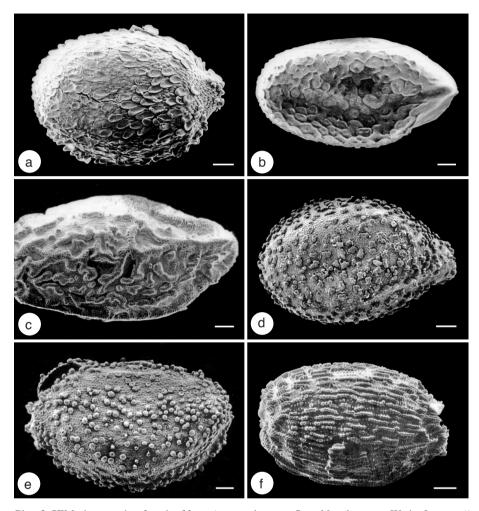


Plate 3. SEM-photographs of seeds of *Impatiens* species. — a. Sect. *Megalocentron* Warb.: *I. garrettii* Craib: testa with papillae. — b–e: Sect. *Brachycentron* Warb.: b. *I. textori* Miq.: testa with a reticulate pattern; c. *I. noli-tangere* L.: testa cells with reticulate thickened outer walls; d. *I. alboflava* Miq.: testa cells with granulate thickened outer walls; e. *I. larsenii* T. Shimizu: testa cells with granulate thickened outer walls; e. *Sect. Longicornes* Warb.: *I. cordata* Wight with longitudinally arranged testa cells. — Scale bars:  $a-c = 330 \mu m$ ;  $d = 260 \mu m$ ;  $e = 210 \mu m$ ;  $f = 310 \mu m$ .

## 6. Section Longicornes Warb.

This section is characterized by alternate leaves, many-flowered umbels and flowers with a long spur. *Impatiens cordata* Wight of India and *I. walleriana* Hook.f. ex Oliv. of Africa belong to this section. These two species differ greatly in their seed coat structure: *I. cordata* (Plate 3f) is peculiar in having ciliate seeds, while *I. walleriana* (Plate 4a) has a pattern similar to that of sect. *Macrocentron*, Group I. However, the chromosome number of *I. walleriana* is 2n = 16 (Arisumi, 1980), while Group I of sect. *Macrocentron* has 2n = 10 or 12.

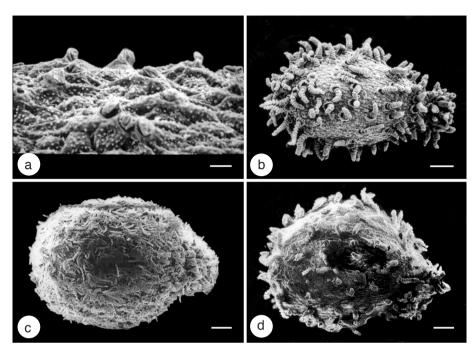


Plate 4. SEM-photographs of seeds of *Impatiens* species. — a. Sect. *Longicornes* Warb.: *I. walleriana* Hook.f. ex Oliv.: outer walls of the testa cells nipple-shaped. — b & c: Sect. *Salpingochillon* Warb.: b. *I. goughii* Wight: testa cells with reticulate thickened outer walls; c. *I. benthami* Steenis with 'long hairs' around the testa. — d. Sect. *Choniochillon* Warb.: *I. omissa* Hook.f.: testa cells with reticulate thickened outer walls. — Scale bars:  $a = 34 \mu m$ ; b &  $d = 170 \mu m$ ;  $c = 250 \mu m$ .

#### 7. Section Salpingochillon Warb.

Impatients goughii Wight (Plate 4b) and I. benthami Steenis (Plate 4c) of India are characterized by opposite leaves and inflorescences with few flowers with a short spur. These two species have a seed coat similar to that of several species of sections *Enantiophyllon* (Group II), *Megalocentron* (Group III) and *Choniochillon* Warb.

## 8. Section Choniochillon Warb.

This section is characterized by opposite leaves and inflorescences with many flowers with a long spur. The two species of this section *I. omissa* Hook.f. (Plate 4d) and *I. viscosa* Bedd. of India, have the same type of seed coat as section *Enantiophyllon* (Group II), *Megalocentron* (Group III) and *Salpingochillon*. The chromosome numbers of these two species are 2n = 28 for *I. omissa* and 2n = 16 for *I. viscosa* (Rao, 1972).

#### CONCLUSION

The genus *Impatiens* has a most diverse and elaborately sculptured seed coat. The seed coat patterns can be used for distinguishing groups or aggregates. However, seed coat morphology alone does not provide universally applicable key characters for identification, but it can be as helpful as many other characters used in taxonomy. These

seed coat types were sometimes well concordant with the classification of the genus but sometimes not. Especially in the South-East Asian species, the seed coat characters agrees well with other morphological characters to form natural groups.

As a result of the morphological and seed coat morphological examinations carried out, it is concluded that a number of good species groups should be recognized in *Impatiens* as more natural. Several species in sect. *Microcentron* which are characterized by four carpellate ovaries, connate winged petals and seed coat pilose with long hairs or granulate and finely granular form a new subgenus in *Impatiens* that will be published in a separate paper.

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