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Comparative floral and pollen morphology of some invasive and native *impatiens* species

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ABSTRACT

To evaluate the hypothesis of competitive superiority of invasive species, we compared the invasive *Impatiens parviflora* DC. and *I. Glandulifera* royle, the naturalized *I. Nevskii* pobed. and the native *I. Noli-tangere* L. in the flowers' morphometric characters at different phases of anthesis. The characters in which alien species have a competitive superiority over closely related *I. Noli-tangere* are revealed. Morphological variability was studied by morphometric observations of the following characters: bud: length and diameter; spurred sepal: length and width; spur: length and diameter; lateral sepal: length and width; largest petal: length and death; large lobe of lateral petal: length and width; small lobe of lateral petal: length and width; anther: length; stamen's filament: length; calyptra: length and width; ovary: length and diameter; length of a style, length of a stigma. There is a tendency for alien *Impatiens* species of the earlier development of androecium and gynoecium: caliptra is formed at the stage of uncolored bud, the pistil is differentiated in ovary, short style and stigma is formed at the stage of colored bud. No other flowers' morphometric characters, representing competitive advantage of the invasive *I. Glandulifera* and *I. Parviflora* over the native *I. Noli-tangere* and naturalized *I. Nevskii* were identified.

KEYWORDS: *Impatiens*, alien species, invasion, floral biology.

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Comparación de morfología floral y polen de algunas especies de *Impatiens* invasoras y nativas

RESUMEN

Para evaluar la hipótesis de la superioridad competitiva de las especies invasoras, comparamos la invasiva *Impatiens parviflora* DC. e *I. Glandulifera* royle, la *I. Nevskii* pobed naturalizada y el nativo *I. Noli-tangere* L. en los caracteres morfométricos de las flores en diferentes fases de la antesis. Se revelan los caracteres en los que las especies exóticas tienen una superioridad competitiva sobre *I. Noli-tangere* estrechamente relacionada. La variabilidad morfológica se estudió mediante observaciones morfométricas de los siguientes caracteres: brote: longitud y diámetro; sépalo estimulado: largo y ancho; espuela: longitud y diámetro; sépalo lateral: largo y ancho; pétalo más grande: longitud y muerte; lóbulo grande de pétalo lateral: largo y ancho; lóbulo pequeño de pétalo lateral: largo y ancho; antera: longitud; filamento del estambre: longitud; caliptra: largo y ancho; ovario: longitud y diámetro; longitud de un estilo, longitud de un estigma. Hay una tendencia para las especies exóticas de *Impatiens* del desarrollo anterior de androceo y gineceo: el caliptra se forma en la etapa de yema incolora, el pistilo se diferencia en ovario, el estilo corto y el estigma se forma en la etapa de yema coloreada. No se identificaron los caracteres morfométricos de otras flores, que representan una ventaja competitiva de la *I. Glandulifera* e *I. Parviflora* invasoras sobre la *I. Noli-tangere* nativa y la *I. Nevskii* naturalizada.

PALABRAS CLAVE: *Impatiens*, especies exóticas, invasión, biología floral.

Introduction

Potentially invasive species can be identified before they start to spread by comparing their traits with those of successful invaders. A powerful tool for delimiting the traits associated with invasiveness are analyses of a number of species of the same genus. Since the influence of traits on invasion success may differ with respect to the stage of the plant's life cycle, comparative studies should address the whole life cycle (Cuda et al., 2016).

This study is a continuation of a series of works on the micromorphological study of flower organs in invasive taxa belonging to *Lupinus* L. (Vinogradova et al., 2012), *Robinia* L. (Vinogradova et al., 2013) and *Caragana* Fabr. (Kuklina et al., 2015; Vinogradova, 2016). It was found that the signs by which invasions can be predicted, apparently, are not common to all taxa. Even among worst invasive species, there is not any plant with all the characteristics of the "ideal weed" (Vinogradova et al., 2010). In this article, we compared flower biology of alien *Impatiens* species with the native *Impatiens noli-tangere* L.

Impatiens noli-tangere, - native in Europe from Scandinavia to the Apennines and temperate Asia to the Pacific (Pobedimova, 1949). Pollinated by bees.

I. glandulifera Royle (= *I. roylei* Walp.) is an Asian ornamental plant introduced to Europe in the XIXth century from the Himalayas, becoming established as an escape from cultivation in Europe and North America (Clements et al., 2008; DAISIE, 2019; Maslo and Šaric, 2019). The species is included in the Black Book of the Flora of Central Russia (Vinogradova et al., 2010) and the Black Book of the Flora of Siberia (Ebel et al., 2016). As a result of the expansion of *I. glandulifera*, the native populations of *I. noli-tangere* are declining (Daumann, 1967). *I. glandulifera* outcompetes native plant communities for pollinators (Bartomeus et al., 2010).

I. parviflora DC., is one of the most widespread invasive plant species in Central Europe (DAISIE, 2019; Maslo and Šaric, 2019). This species disturbs the natural vegetation composition in many European forests where it is frequently mixed with the native *I. noli-tangere*. Extinctions have sometimes been reported for *I. noli-tangere*, and knowledge is lacking about the possible contribution of *I. parviflora* to this phenomenon (Godefroid and Koedam, 2010). Nevertheless, both mechanisms and consequences of its invasion are still poorly understood (Reczynska et al., 2015; Jarcuska et al., 2016; Florianova and Munzbergova, 2017; Skálová et al., 2019).

I. nevskii Pobed., naturally grows in Central Asia (Pobedimova, 1949). Currently, the taxonomic status of *I. nevskii* is not defined: “not establish this name either as an accepted name or as a synonym with original publication details: Fl. URSS 14: 746 1949 (The Plant List). Our early research showed that *I. parviflora* and *I. nevskii* have overlapping natural distribution ranges, a similar ecology, similar phenology, morphology of flowers and seeds both in nature and in the garden experiment, which causes doubt of the species autonomy of *I. nevskii* (Maitulina, 1988; Vinogradova et al., 2010).

Flowers of all studied species are hermaphrodite, strongly zygomorphic, combined in racemes. Sepals 3, petaloid; the lowest (the posterior sepal is at the bottom of the flower because of the twisting of the pedicel) large and spurred; the 2 lateral small. Petals 5, the upper one is large, the 4 lower usually united in pairs on each side of the flower. Stamens 5, filaments broad, short, connate above; anthers connate round the ovary forming a deciduous calyptra. Ovary with a short style, and a single stigma or 5 stigmas (Gleason and Cronquist, 1993; Clapham et al., 1962).

The purpose of this study is an attempt to identify the characteristics contributing to the wide resettlement of alien *I. parviflora* and *I. glandulifera*. The objective of this study is a comparative analysis of the micromorphology of the flower during different stages of development in taxa of the genus *Impatiens*: native *I. noli-tangere*, invasive *I. parviflora*, invasive *I. glandulifera* and alien *I. nevskii* showing low capability of naturalization.

1. Materials and methods

We studied four species of *Impatiens* spontaneously growing in the Main Botanical Garden Russian Academy of Sciences (Moscow, Russia). Some samples were collected in other localities (Table).

Table. Localities of studied *Impatiens* samples

Taxon	Locality	Latitude	Longitude
<i>I. noli-tangere</i>	Russia, Moscow, Ostankino, protected oak forest in the Main Botanical Garden named after N.V. Tsitsin Russian Academy of Sciences	55°49'N	37°35'E
<i>I. glandulifera</i>	Russia, Moscow, Zelenograd district, bank of the Skhodnya river	55°98'N	37°19'E
<i>I. glandulifera</i>	Russia, Moscow, Ochakovo, General Dorokhov street, bank of the Setun- river	55°69'N	37°47'E
<i>I. parviflora</i>	Russia, Moscow, Ostankino, protected oak forest in the Main Botanical Garden named after N.V. Tsitsin Russian Academy of Sciences	55°49'N	37°35'E
<i>I. parviflora</i>	Russia, Moscow, Mytishchi, the national reserve «Losinyy Ostrov»	55°89'N	37°76'E
<i>I. nevskii</i>	Russia, Moscow, Main Botanical Garden named after N.V. Tsitsin Russian Academy of Sciences	55°98'N	37°19'E

For the analysis, the flowers were collected from 5-10 individuals per the species at the following stages of development: (I) the phase of beginning of budding (buds are green), (II) the phase of budding (buds are colored), (III) the phase of beginning of flowering, (IV) the phase of full flowering, (V) – ending of flowering and beginning of fruiting. The morphological and biometric traits of different organs of the flower were determined using a Keyence VHX 1000 digital microscope. The sampling was 10 fresh

flowers at each of the first two phases of development and 30–40 flowers at the subsequent phases. The morphological variability was defined by morphometric investigations of the following characteristics: bud length (BL), bud diameter (BD), spurred sepal, length (SSL), spurred sepal, width (SSW), length of the spur (SL), diameter of the spur (SD), lateral sepal, length (LSL), lateral sepal, width (LSW), largest petal, length (LPL), largest petal, width (LPW), large lobe of lateral petal, length (LLPL), large lobe of lateral petal, width (LLPW), small lobe of lateral petal, length (SLPL), small lobe of lateral petal, width (SLPW), anther, length (AL), stamen's filament, length (FL), calyptra, length (CL), calyptra, width (CW), ovary, length (OL), ovary, diameter (OD), length of style (STL), length of stigma (OSL). The size of the freshly collected pollen without water on a glass slide was calculated; the pollen fertility was determined by staining the pollen grains with acetocarmine under slight heating. The total number of pollen grains for each species to determine their size is 30-50. The average volume of the pollen was determined according to the formula for ellipsoid: $4/3\pi(l/2)(d/2)^2$. The scanning electron microscope LEO 1430 VP (Zeiss, Oberkochen, Germany) was used for examining the pollen and its exine surface of different taxa of *Impatiens*. Specimens were observed in a high vacuum, at magnification from x400 (overview) to x5000, accelerating voltage of 20 kV, and operating distance of 9 mm. Air-dried specimens were mounted directly on copper plates, underwent cathode sputtering with gold in argon, and then were viewed in a high vacuum. Special fixation of the material was not applied because of specimens' air-drying, which resulted in maximal preservation of the biological objects' native structure. Basic statistical analyses were performed using Microsoft Excel and PAST 2.17. Data was analysed with ANOVA test and differences between means compared through the Tukey-Kramer test ($\alpha = 0.05$). The statistical significance of differences of morphometric traits was tested by the Student's t -test.

2. Results

Impatiens noli-tangere (Fig.1)

Phase I (Beginning of budding): The inflorescences started to form in early June. The buds are green, 3.3×1.9 mm in length, the corolla is completely hidden in the calyx. Lateral sepal is 3–4 mm long and 0.3–0.5 mm wide. Spurred sepal is 3-3.5 mm long and 0.8

mm depth, but the spur isn't visible yet. The largest uncoloured petal is 2.9-3.2 mm long and 1.3-1.5 mm wide.

Phase II (Budding): The buds are greenish-yellow, 5.2×3.1 mm. Lateral sepal is 3.8-5.2 mm long and 2.8-3.6 mm wide. Spurred sepal is 4-5.1 mm long and 2.8-5 mm depth with a spur 4-4.5 mm long and 0.4 mm diameter. The corolla projects over the calyx. The largest petal is 3.7×2.9 mm. Each of the five stamens have filament 0.4 mm long and white free anther 2.5-3.1 mm long. The undifferentiated ovary length is 1.8 mm.

Phase III (Beginning of flowering): The open buds are yellow, 8.1×4.5 mm. Yellow lateral sepal is 3.9 mm long and 4.0 mm wide. Spurred sepal is 7 mm long and 5.3 mm depth with a curved spur 8.2 mm long and 0.8 mm diameter. The largest petal is 7.0×4.8 mm, bright yellow with small brown spots.

The large lobe of lateral petal is 8.5×4.4 mm, and the small one is 4.1×2.0 mm. Staminal filament is 0.9 mm, the length of anthers is 1.8-2.9 mm. The pistil (3.1 mm long) is slightly differentiated into the ovary (2.5×0.7 mm) and stigma (0.3 mm long). The style isn't visible.

Phase IV (Full flowering): The size of the lateral sepal increases (6.5-6.9×3.9-4.0 mm). Spurred sepal is 7.2 mm long and 9.5 mm depth with a curved spur 10.7 mm long and 3.2 mm diameter. The largest petal is 7.6×10.9 mm. The large lobe of lateral petal is 15.3×13.6 mm, and the small one is 6.9-7.6×3.3-3.5 mm. The anthers connate round the ovary forming a calyptra 3.6×1.5 mm. They release pollen and decrease in size. The pistil total length reaches 3.2-4.6 mm (the stigma is 0.3 mm and the ovary is 2.5-3.8 mm long and 0.8-0.9 mm in diameter). The style isn't visible.

Ending of flowering and beginning of fruiting (Phase V): The corolla turns brown and falls off. The staminal filaments drive off at the base, and the calyptra falls off completely together with them. The fruit is formed only in two or three flowers per raceme and contains 2-3 seeds.

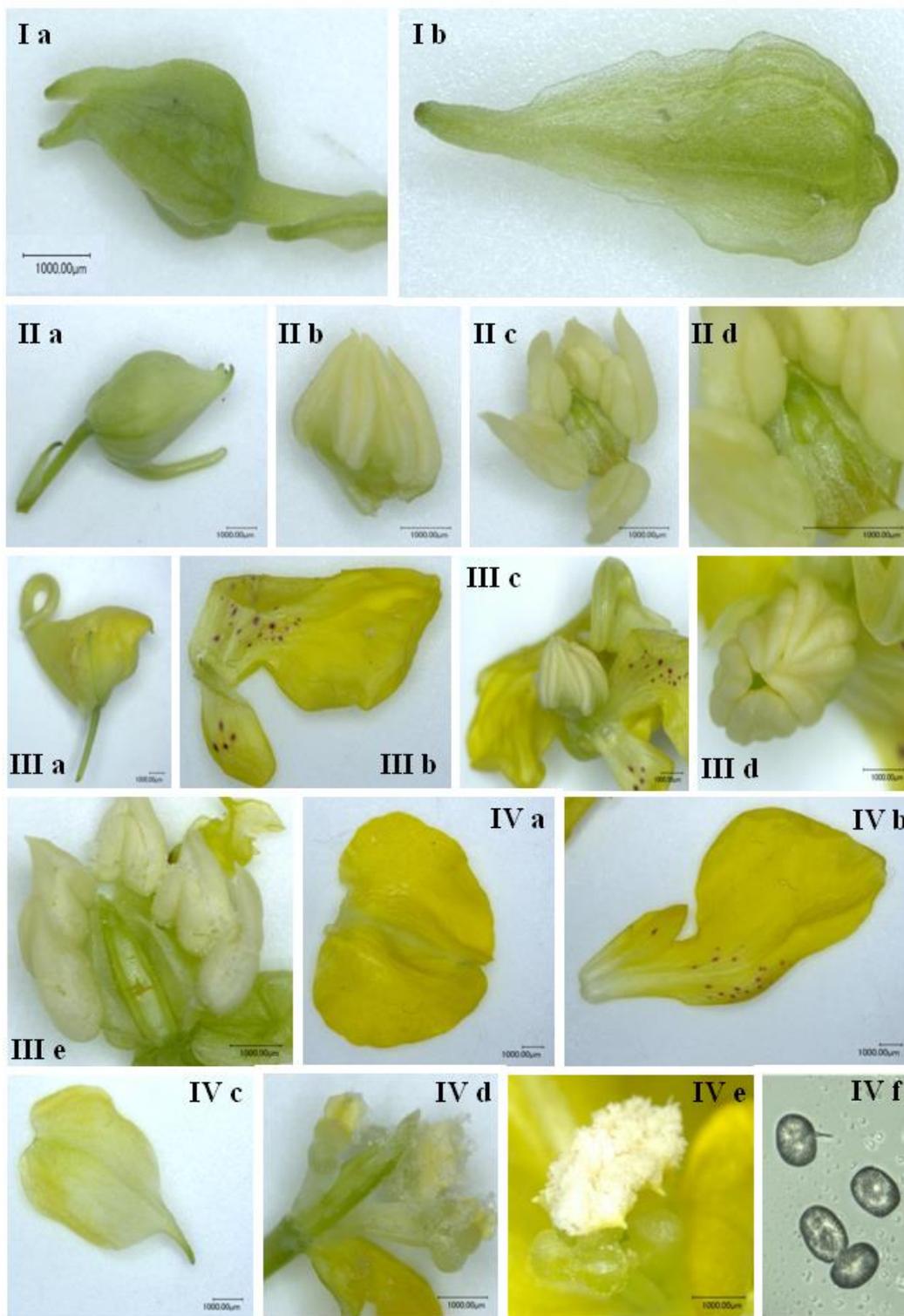


Figure 1. *Impatiens noli-tangere*

I – Beginning of budding: a – bud; b – large petal; II – Budding: a – bud; b – androecium; c – stamens and ovary; f – ovary; III – Beginning of flowering: a – bud; b – lateral petal; c – androecium and corolla; d – androecium; e – ovary; IV – Full flowering: a – large petal; b – lateral petal; c – lateral sepal; d – ovary and corolla; f – pollen.

Impatiens glandulifera (Fig.2)

In contrast to *I. noli-tangere*, *I. glandulifera* begins to bloom a half month earlier and continues to bloom almost to frost. The raceme has an average up to -100 flowers during the growing season. Flowers are purplish, pink, and rarely white.

Phase I (Beginning of budding): The buds are green 4.7-5.8×4.0-5.2 mm, the corolla is completely hidden in the calyx. Lateral sepal is 6.5-7.6 mm long and 3.9-4.8 mm wide. Spurred sepal is 7.2-9.1 mm long and 2.6-5.6 mm depth, with a spur 1.8-2.6 mm long and 0.5-0.8 mm diameter. The largest uncolored petal is 5.7-6.1 mm long and 5.6-6.1 mm wide. The large lobe of lateral petal is 6.6-7.4×2.8-3.2 mm, and the small one is 3.1-4.1×2.6-3.6 mm. Stamens have filament 1.5-1.9 mm long and white free anther 4.0-4.6 mm long. The pistil is differentiated in ovary (1.4-1.6 mm length) and stigma (0.5 mm length).

Phase II (Budding): The buds are colored, 9.6-9.7×5.5-8.6 mm. Lateral sepals are 7.4-10.3 mm long and 3.7-5.5 mm wide. Spurred sepal is 11.3-13.2 mm long and 4.4-7.5 mm depth with a spur 3.8-6.5 mm long and 0.9-1.2 mm diameter. The corolla projects over the calyx. The largest petal is 7.3-10.1×9.6-9.7 mm. The large lobe of lateral petal is 7.9-12.1×4.7-6.9 mm, and the small one is 4.2-6.1×6.0-9.4 mm. The stamens have filament 2.2-3.9 mm long and white free anther 4.4-5.8 mm long. The pistil is differentiated in ovary 2.4-3.6×1.4 mm, style 0.1-0.7×0.3 mm and stigma 0.5-0.7×0.1-0.3 mm.

Phase III (Beginning of flowering): The open buds are pink or purple. Lateral sepal is 9.4 mm long and 4.2 mm wide. Spurred sepal is 13.0-14.0 mm long and 7.3 mm depth with a spur 5.4-6.6 mm long and 1.2 mm diameter. Staminal filament is 3.7-4.9 mm, the length of anthers is 4.8-5.2 mm. The anthers connate round the ovary forming a calyptra.

Phase IV (Full flowering): The size of the lateral sepal increases 7.1-9.4×4.1-5.3. Spurred sepal is -17 mm long and 19 mm depth with a short erect spur 5-6 mm long and 1-2 mm diameter. The largest petal is 12-14×19-22 mm. The large lobe of lateral petal is 28.3-39.6×13.7 mm, and the small one is 11.0-11.7×8.9-10.0 mm. The anthers connate round the ovary forming a calyptra 4.7×4.2 mm. They release pollen and decrease in size. The pistil total length reaches 4.5-5.1 mm (the stigma is 0.6-1.0 mm, the style is 0.4 mm, and the ovary is 3.5-3.8 mm long and 1.6 mm in diameter).

Ending of flowering and beginning of fruiting (Phase V): As well as *Impatiens noli-tangere*, the corolla turns brown and falls off. The staminal filaments drive off at the base, and the calyptra falls off completely together with them. Almost every flower forms a fruit.

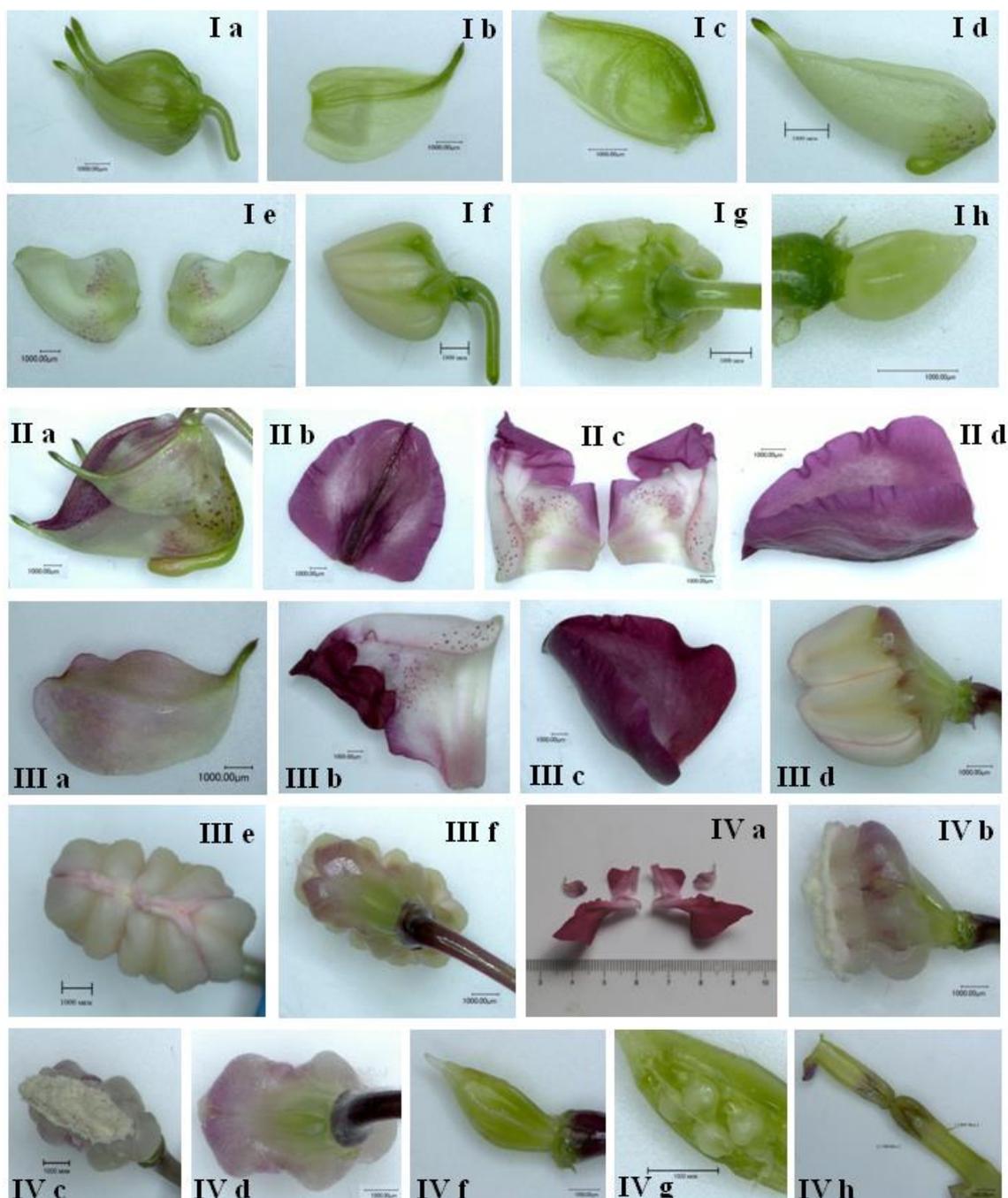


Figure 2. *Impatiens glandulifera*

I – Beginning of budding: a – bud; b – lateral sepal; c – large petal; d – spurred sepal; e – two lateral petals; f – androecium; g – stamen’s filament; h – ovary; II – Budding: a – bud; b – large petal; c – two lateral petals; d – largest petal; III – Beginning of flowering: a – lateral sepal; b – lateral petal; c – largest petal; d – androecium; e – calyptra, top view; f – calyptra and stamen’s filament, bottom view; IV – Full flowering: a – two lateral petals; b – calyptra, side view; c – calyptra, top view; d – calyptra and stamen’s filament, bottom view; e – ovary; f – ovules.

Impatiens parviflora (Fig.3)

In contrast to *I. noli-tangere*, *I. parviflora* begins to bloom a half month earlier and continues to bloom almost to frost (similar with *I. glandulifera*). The raceme has an average up to 50 flowers during growing season.

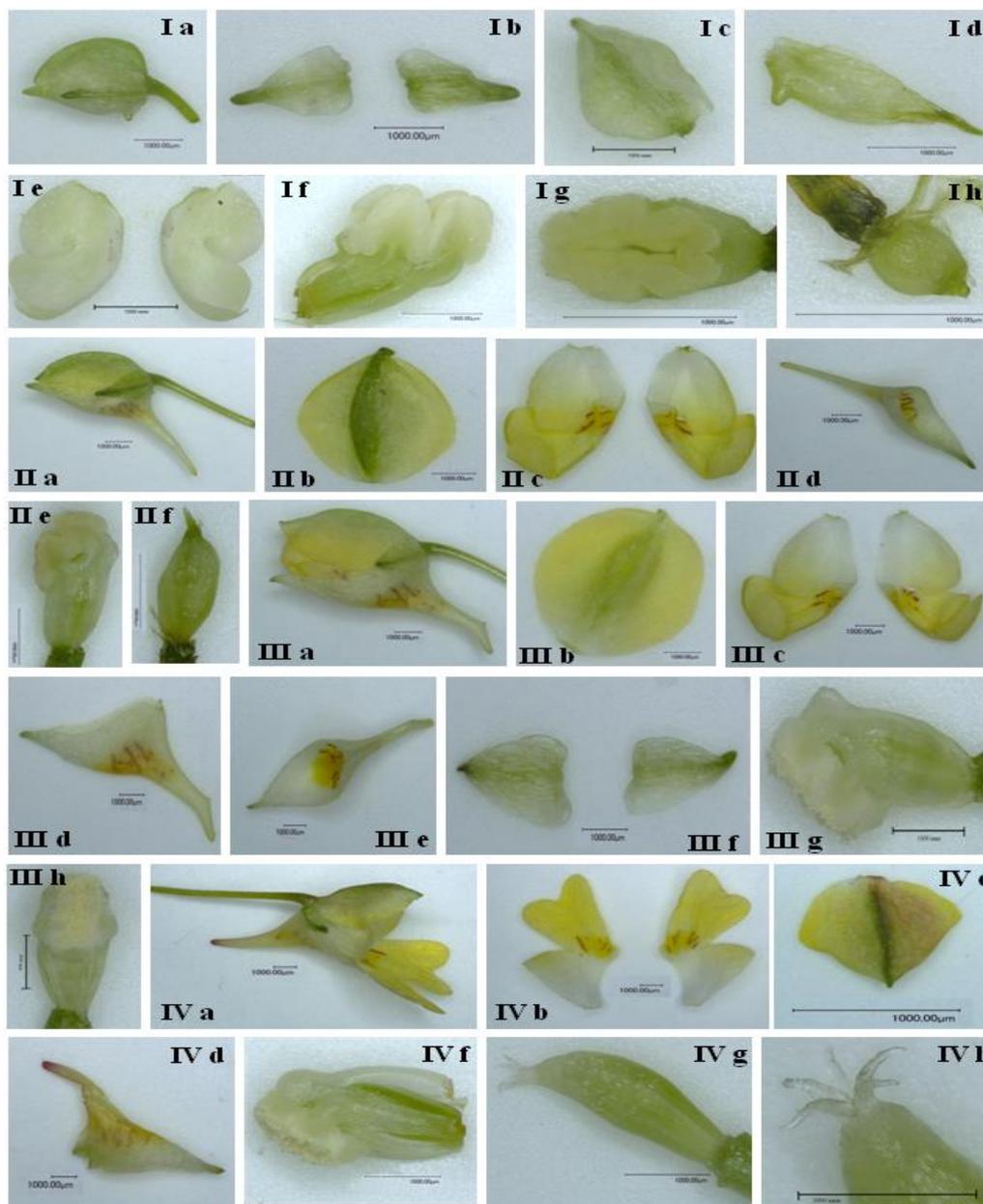


Figure 3. *Impatiens parviflora*

I – Beginning of budding: a – bud; b – lateral sepal; c – large petal; d – spurred sepal; e – two lateral petals; f – androecium; g – calyptra, side view; h – ovary; II – Budding: a – bud; b – large petal; c – two lateral petals; d – spurred sepal; e – androecium; f – ovary; III – Beginning of flowering: a – bud; b – large petal; c – two lateral petals; d – spurred sepal, side view; e – spurred sepal, top view; f – lateral sepal; g – androecium; h – ovary; IV – Full flowering: a – flower, side view; b – two lateral petals; c – large petal; d – spurred sepal, side view; f – androecium, side view; g – ovary; h – five stigmas.

Phase I (Beginning of budding): The buds are green, smaller than both above mention species, 2.6-3.1×2.0-2.1 mm. Lateral sepal is 1.7–1.9 mm long and 1.0–1.2 mm wide. Spurred sepal is 2.8-3.3 mm long and 0.7-0.8 mm depth, with a spur 0.1-1.0 mm long and 0.1-0.3 mm diameter. The largest uncolored petal is 2.6-3.9 mm long and 2.0-3.7 mm wide. The large lobe of lateral petal is 2.2-3.2×1.0-1.1 mm, and the small one is 1.1-1.6×1.0-1.3 mm. Stamens have filament 1.0-1.3 mm long. White anthers 4.0-4.6 mm long connate round the ovary forming a calyptra 1.0-1.3×0.8-1.0 mm. The pistil is differentiated in the ovary (0.6-0.8 mm length), the style (0.1 mm length) and stigma (0.1-0.2 mm length).

Phase II (Budding): The buds became colored, 3.6-5.7×2.7-3.7 mm. Lateral sepals are ~2.7 mm long and 1.2–1.6 mm wide. Spurred sepal is 3.9-5.8 mm long and 0.9-2.1 mm depth with a spur 0.9-4.9 mm long and 0.3-0.9 mm diameter. The corolla projects over the calyx. The largest petal is 2.9-3.9×3.0-3.8 mm. The large lobe of lateral petal is 3.3-5.2×1.6-2.5 mm, and the small one is 1.6-2.7×1.9-2.5 mm. The stamens have filament 1.6-2.4 mm long. White anthers 0.7-1.0 mm long form a calyptra 1.5×1.1 mm. The pistil is differentiated in ovary 1.3-1.9 mm length, style 0.1 mm length and stigma 0.3 mm length.

Phase III (Beginning of flowering): The open buds are pale-yellow. Lateral sepal doesn't increase as compare with phase II. Spurred sepal is 6 mm long and 2-3 mm depth. The largest petal is 5.0×4.6 mm. Staminal filament is 2.4-2.5 mm, calyptra is 1.7×1.1 mm.

Phase IV (Full flowering): Spurred sepal is 5.7-6.2 mm long and 4.2-5.6 mm depth. The largest petal is 5.3×5.2 mm. The large lobe of lateral petal is 9.0-11.0×3.5-3.9 mm, and the small one is 4.4×2.8-4.6 mm. Staminal filament is 2.4-2.5 mm, calyptra is 1.7×1.1 mm. The pistil is differentiated in ovary 2.0 mm length, style 0.3 mm length and stigma 0.3 mm length.

Ending of flowering and beginning of fruiting (Phase V): Almost every flower forms a fruit.

Impatiens nevskii (Fig.4)

I. nevskii differs from *I. parviflora* only in the lilac (vs. yellow) color of the corollae. No differences in all other studied parameters were noted. Multivariate analysis and pairwise Hotelling's tests of morphological variability of quantitative traits of *I. nevskii* and *I. parviflora* showed the fail of statistically significant differences (Wilks' lambda 0.9; $P <$

0.0001) between the species. This gave us reason to "close" this species and consider it to be the lilac-flowered form of *I. parviflora* (Maitulina, 1988).

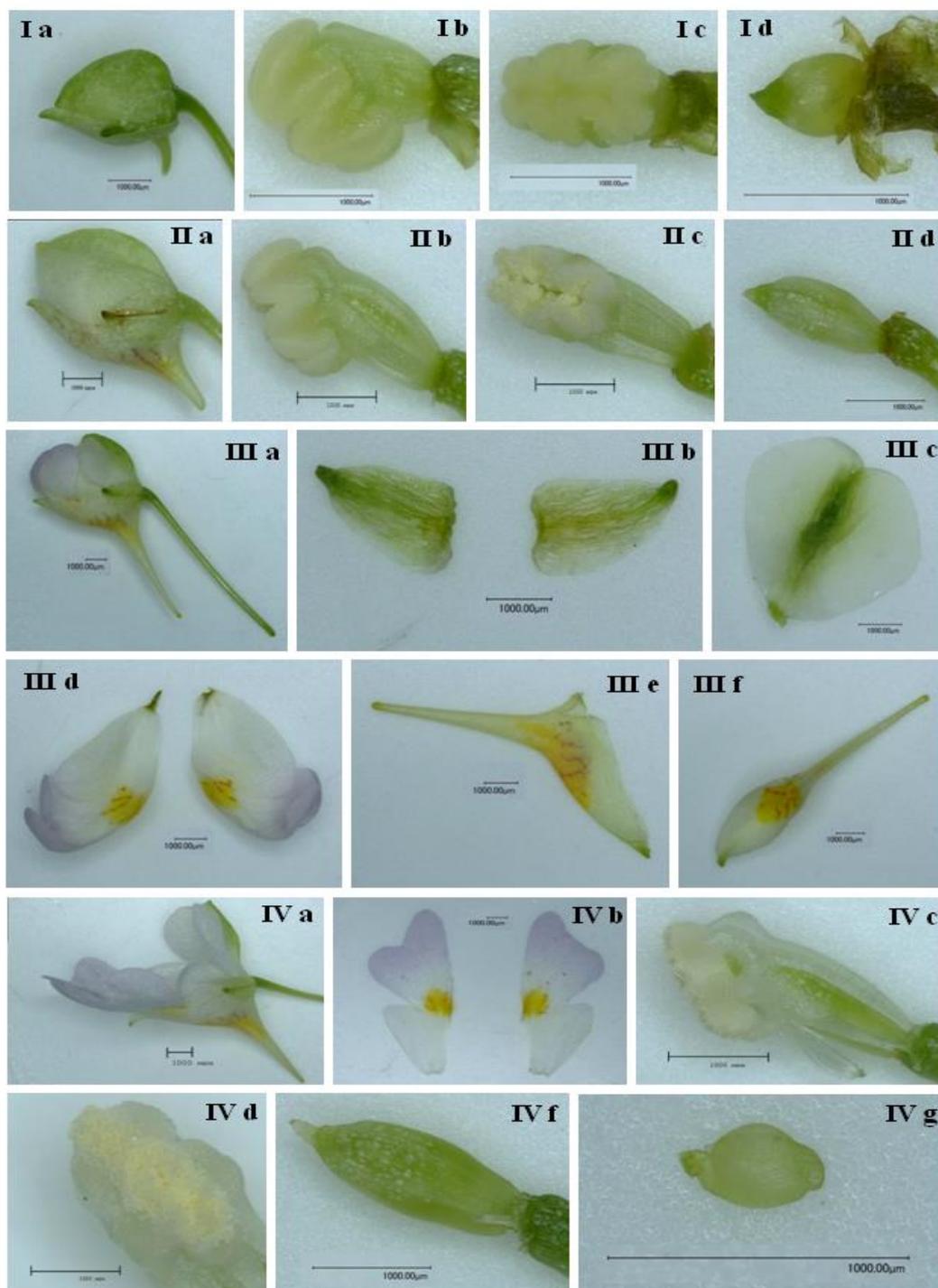


Figure 4. *Impatiens nevskii*

I – Beginning of budding: a – bud; b – androecium, top view; c – calyptra, top view; d – ovary; II – Budding: a – bud; b – androecium, top view; c – calyptra, top view; d – ovary; III – Beginning of flowering: a – bud; b – two lateral sepals; c – large petal; d – two lateral petals; e – spurred sepal, side view; f – spurred sepal, top view; IV – Full flowering: a – flower, side view; b – two lateral petals; c – androecium, side view; d – calyptra, top view; f – ovary; g – ovules.

The discriminant analysis between the four species made it possible to completely separate them. The plot (Fig. 5) shows that the *I. noli-tangere* and *I. glandulifera* data clouds diverge greatly. And only samples *I. nevskii* and *I. parviflora* tend to cluster close to each other.

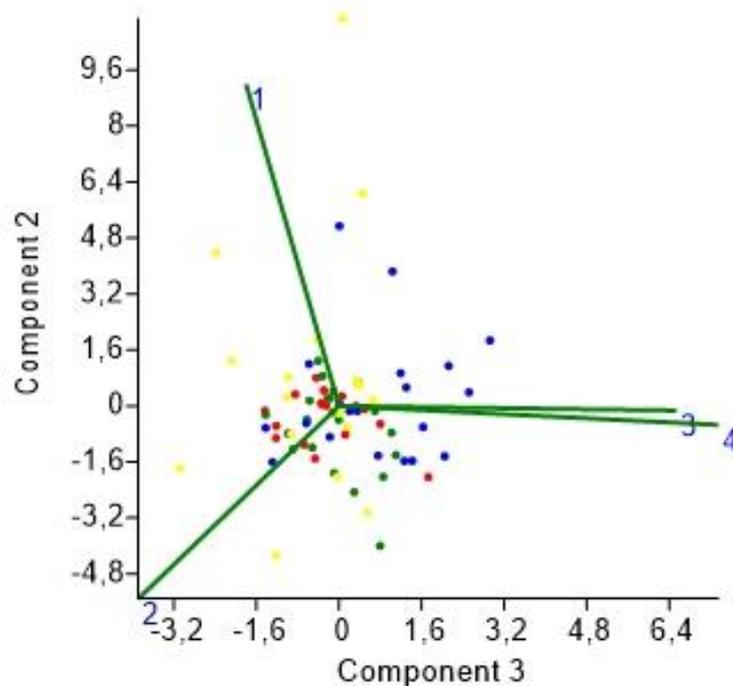


Figure 5. Morphometric parameters of flower in *Impatiens* species at different phases of development

1 - *I. noli-tangere*, 2 - *I. glandulifera*, 3 - *I. parviflora*, 4 - *I. nevskii*.

We studied the variability of sizes and shapes of the pollen in the four taxa (Figs. 6 and 7).

All studied taxa have medium-sized colpate pollen (Vinogradova and Kuklina, 2016; The Plant List). In native *I. noli-tangere*, the pollen grains are small: the mean length of polar axis (l) is $24.91 \pm 0.29 \mu\text{m}$, and their mean equatorial diameter (d) is $17.71 \pm 0.26 \mu\text{m}$; shape index (the ratio of length to diameter) is 1.4. The pollen fertility is quite low, 39%.

In invasive *I. glandulifera*, the pollen sizes are small, too: the mean length of polar axis (l) is $27.16 \pm 0.21 \mu\text{m}$, and their mean equatorial diameter (d) is $16.36 \pm 0.18 \mu\text{m}$; shape index is 1.7. The pollen fertility is high, up to 98%.

In invasive *I. parviflora*, the pollen is finer: the mean length of polar axis (l) is $32.39 \pm 0.25 \mu\text{m}$, and their mean equatorial diameter (d) is $22.00 \pm 0.27 \mu\text{m}$; shape index is 1.5. The pollen fertility is high, up to 98%.

In naturalized *I. nevskii*, the pollen is the largest: the mean length of polar axis (l) is $34.94 \pm 0.29 \mu\text{m}$, and their mean equatorial diameter (d) is $20.49 \pm 0.21 \mu\text{m}$; shape index is 1.7. The pollen fertility is high, up to 95%.

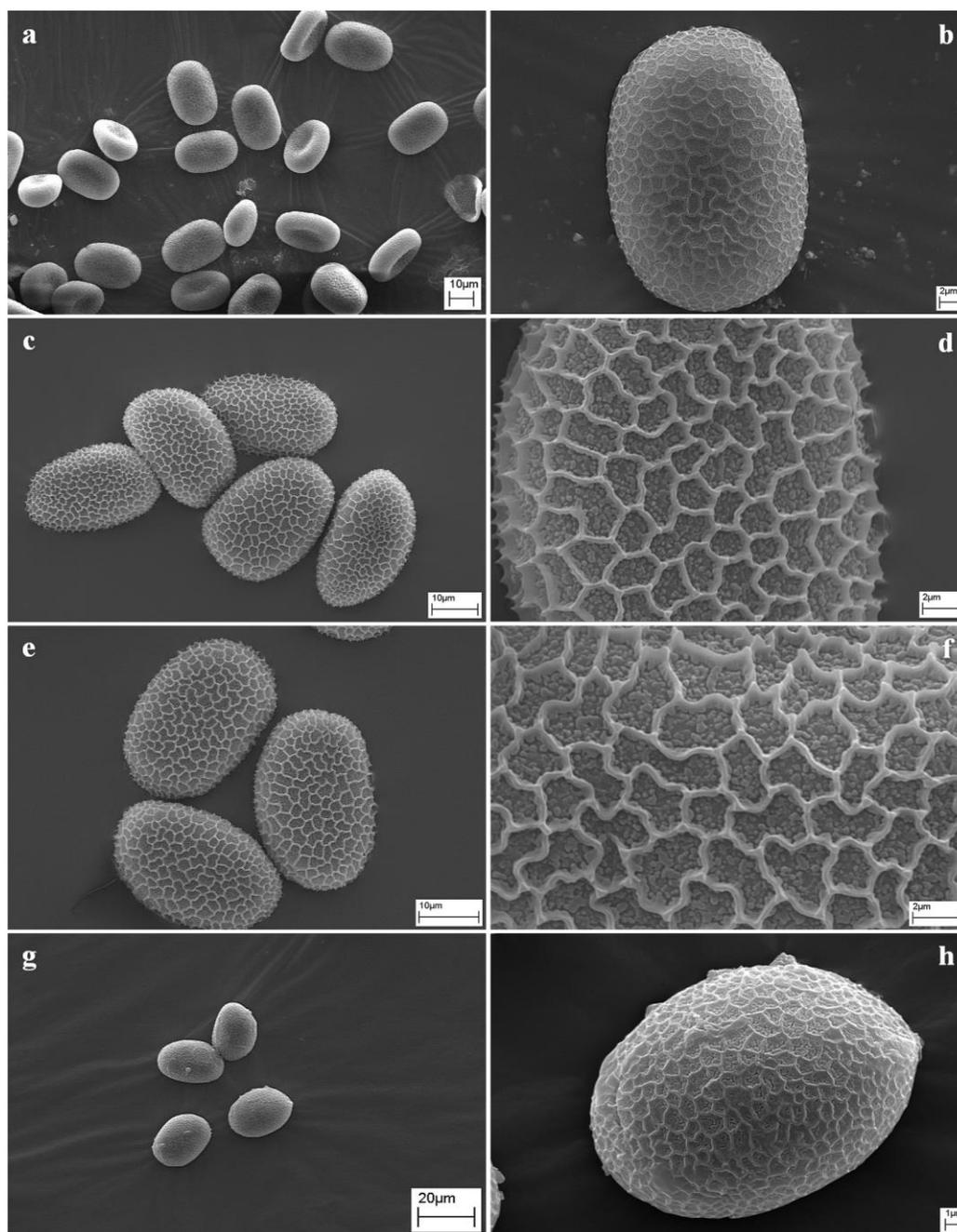


Figure 6. Pollen and its exine surface of different taxa of *Impatiens*

a, b – *I. glandulifera*; c, d – *I. parviflora*; e, f – *I. nevskii*; g, h – *I. noli-tangere*.

Thus, the size of the pollen grain decreases in the order *I. parviflora* → *I. nevskii* → *I. noli-tangere* → *I. glandulifera*. The largest pollen grains are observed for *I. parviflora* and *I. nevskii*. In *I. parviflora*, the volume fluctuates from 4987 to 11433 (mean $8291 \pm 235 \mu\text{m}^3$). In *I. nevskii*, the volume fluctuates from 5645 to 9517 (mean $7710 \pm 175 \mu\text{m}^3$). These two species aren't statistically different each from other on this parameter. The pollen grains of *I. glandulifera* are twice smaller: the volume fluctuates from 2013 to 6166 (mean $3873 \pm 104 \mu\text{m}^3$). Native *I. noli-tangere* isn't different from invasive *I. glandulifera* on the average volume of pollen: it fluctuates from 2020 to 5719 (mean $4131 \pm 142 \mu\text{m}^3$).

Four species differ also in a shape of pollen. Pollen grains are elongate ($l/d > 1.5$) for *I. glandulifera* and *I. nevskii*. Pollen grains of *I. parviflora* are oval ($l/d = 1.5$), and pollen grains of *I. noli-tangere* are rounded ($l/d < 1.5$).

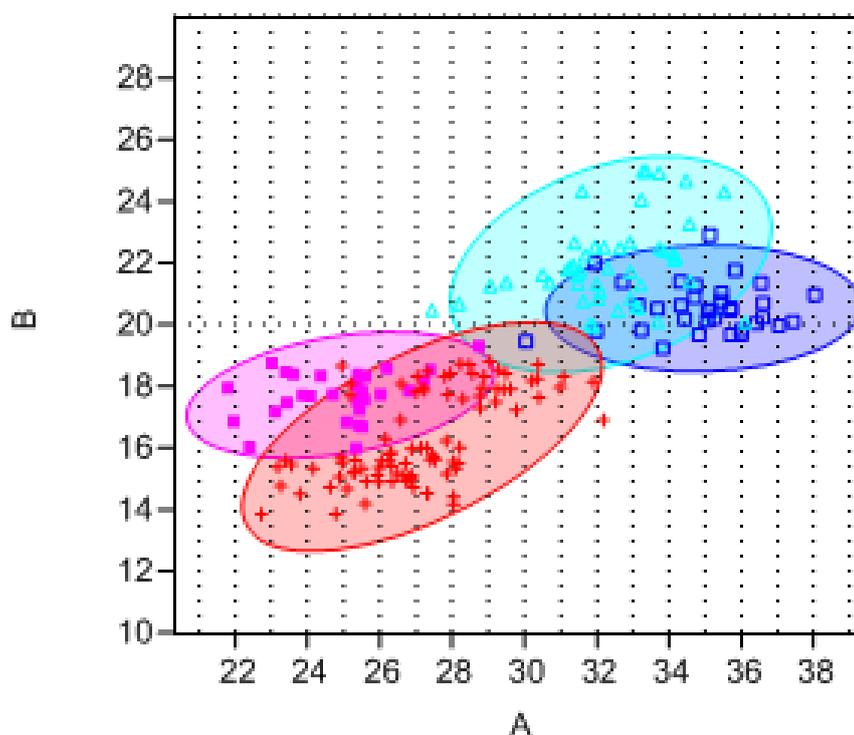


Figure 7. Morphometric parameters of pollen in different taxa of *Impatiens*

I. nevskii (dark blue), *I. parviflora* (light blue), *I. noli-tangere* (violet), *I. glandulifera* (red).

In alien *I. glandulifera* and *I. parviflora*, almost every flower forms a fruit; in *I. nevskii*, fruiting is less abundant, while in native *I. noli-tangere*, the fruits are formed only in two or three flowers per raceme (Fig. 8). Earlier we determined, that floral biology differed among species, and *I. glandulifera* is a cross-pollination species, while *I. parviflora* is a self-pollination one (Maitulina, 1988; Vinogradova, 2008). Other botanists obtained the same results. So, *I. glandulifera* and *I. noli-tangere* presented large quantities of sucrose-dominant nectar, contrary to *I. parviflora* (Vervoort et al., 2011).

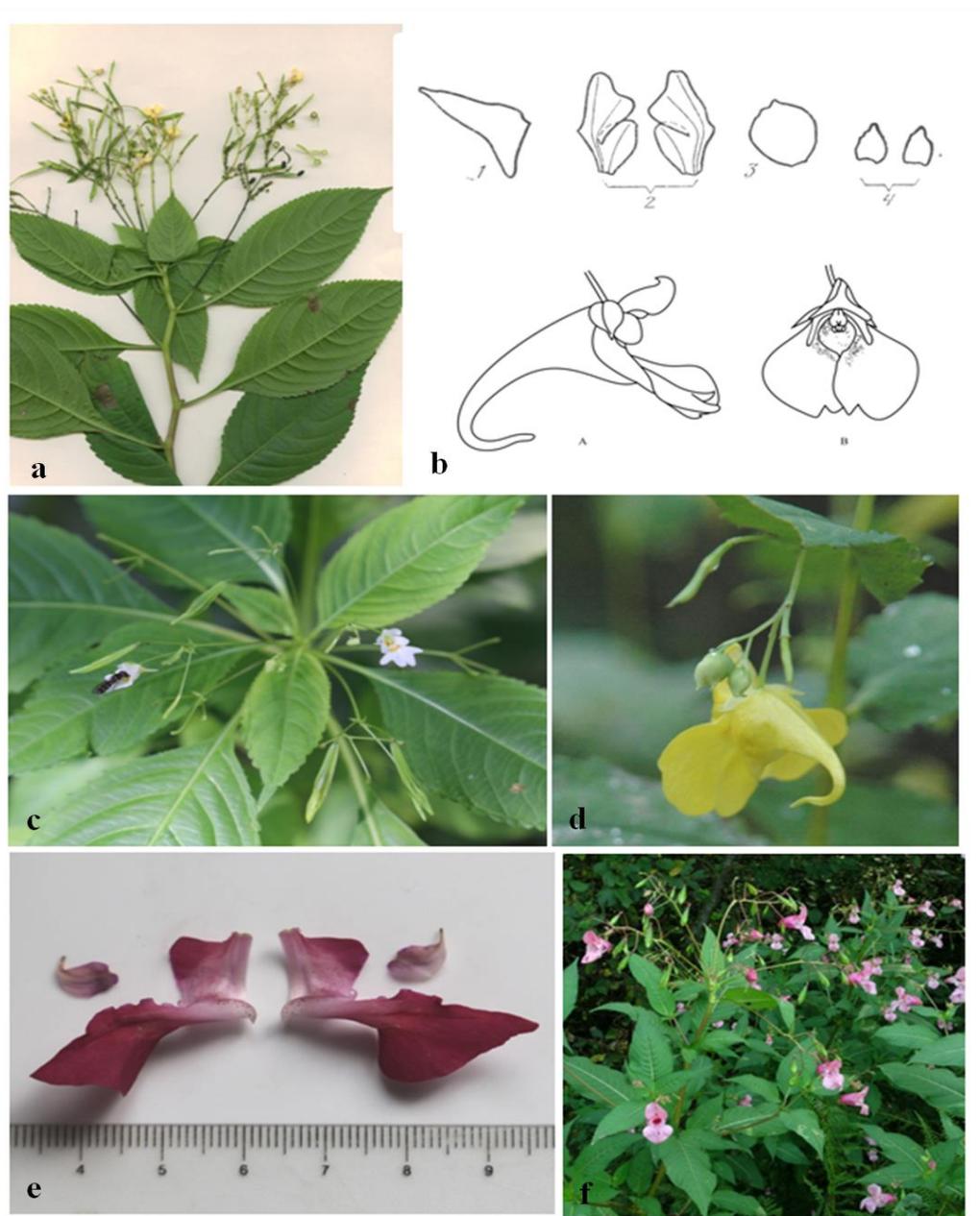


Figure 8. Morphology of inflorescences in different taxa of *Impatiens*

a - herbarium specimen of *I. parviflora*; b - scheme of flower's structures of *Impatiens* spp.; c - *I. nevskii*; d - *I. noli-tangere*; e - two lateral petals of *I. glandulifera*; f - *I. glandulifera*.

In Ireland, *Bombus pascuorum* is a highly effective pollinator of *I. glandulifera* due to its high visitation frequency, the morphological fit with flowers and individuals removing large pollen quantities (Nienhuis and Stout, 2009). *I. parviflora* had high autonomous selfing ability (81.4% fruit set) linked to complete self-compatibility, whereas *I. glandulifera*, showed lower autonomous selfing (9.3% fruit set), with high self-compatibility (Vervoort et al., 2011).

It is known that the reproductive traits and the breeding system could indicate some trends that might favour population spread and invasion (Jacquemart et al., 2015; Florianova and Munzbergova, 2018). However, the flower development of the four *Impatiens* species proceeds quite similarly. With aging of the flower, the corolla sizes increase, the pistil is differentiated, and the sepals and corolla became colored. At all the stages of the flower development, the pistil is shorter than the stamens. At all the stages of development, *I. parviflora* and *I. nevskii* have the smallest sizes of the flower organs.

The study of the features of the flower development in the four taxa of the genus *Impatiens* allowed distinguishing in them five phases of development, which differ not only in terms of quantitative but also in terms of qualitative criteria. The phase I finishes when the corolla acquires a form peculiar to the species and becomes longer than the calyx. At the same time, sepals and petals become colored. The phase II finishes, when the corolla disperses from the calyx. The phase III finishes when anthers burst and release pollen. The phase IV finishes when the brownish corolla shanks off, and virtually all the pollen pours out from the anthers.

The factors responsible for invasion success of *I. glandulifera* and *I. parviflora* are poorly known, though they may be related to frost tolerance and its popularity as an ornamental plant (Najberek et al., 2017). There is information, that light availability in dense forest canopy could influence on abundance the local population of *I. parviflora* (Barabasz-Krasny et al., 2018). Influences natural enemies and arbuscular mycorrhizal fungi have on plant performance, should take into consideration, too (Tanner et al., 2014). According to our observations, the native *I. noli-tangere* is more susceptible by diseases than *I. parviflora*. Polymorphism in the structure of DNA in plants from natural populations of *I. noli-tangere* is lower (7.3%) than in invasive populations of *I. glandulifera* (45.6%) and *I. parviflora* (49.7%) (Kupcinskiene et al., 2015).

There is a tendency for alien *Impatiens* species to earlier development of androecium and gynaecium: calyptra is formed at the stage of uncolored bud, the pistil is differentiated in ovary, short style and stigma at the stage of colored bud. Not any other flower morphometric traits which would offer more competitiveness of invasive *I. glandulifera* and *I. parviflora* as compared to the native *I. noli-tangere* and naturalized *I. nevskii* were identified.

Conclusions

There aren't any taxonomic traits for differentiate *I. parviflora* from *I. nevskii* in the morphology of the floral sphere, excepting the color of sepals and petals.

The invasive *I. glandulifera* has competitive advantage over other studied species in terms of large sizes of organs of the floral sphere. The invasive *I. parviflora* has no competitive advantage over *I. nevskii* and *I. noli-tangere* in the floral sphere. On the contrary, it is inferior to native *I. noli-tangere* and it is similar with slightly naturalized *I. nevskii*. The invasive *I. parviflora* has the largest pollen grains, but in the same time invasive *I. glandulifera* has the smallest pollen grains. Thus, the hypothesis concerning the competitive advantage in the secondary distribution range of "the more powerful" plants is not supported by our results in full.

Compared to the native *I. noli-tangere*, flowers of alien *Impatiens* species, develop faster. For example, the calyptra is observed in the earliest phase of development, into uncolored buds; the pistil is differentiated in ovary, short style and stigma at the stage of colored bud.

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