POLLEN MORPHOLOGY AND POLLEN PRODUCTION OF INVASIVE AND NATIVE *IMPATIENS* SPECIES GROWING IN BULGARIA

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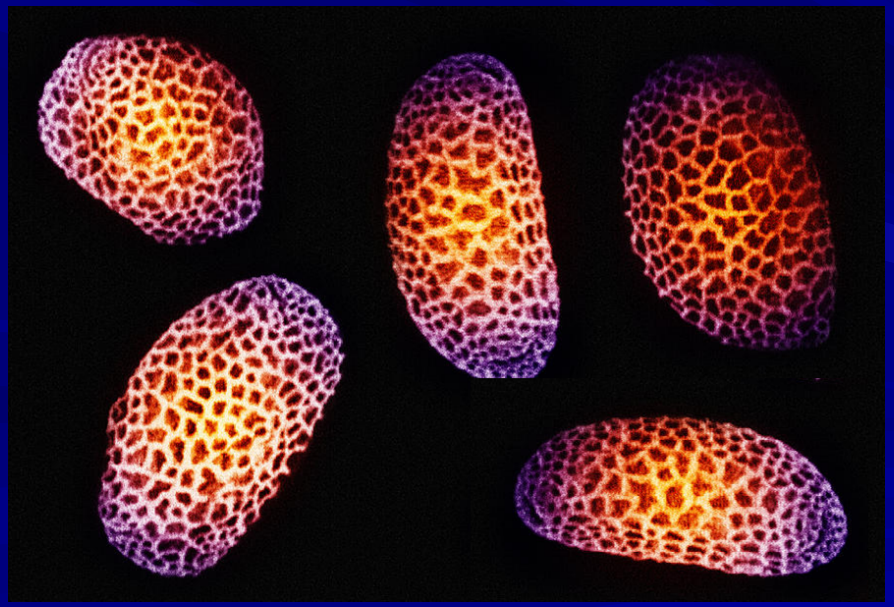


Purpose of the study

The purpose of this study was to establish baseline data concerning certain aspects of pollen biology of the invasive alien species *Impatiens balfourii* and *I. glandulifera,* and the native *I. noli-tangere* to the flora of Middle part of Iskar river gorge (Western Bulgaria)

Impatiens glandulifera

Impatiens balfourii

Impatiens noli-tangere

The aims of the study

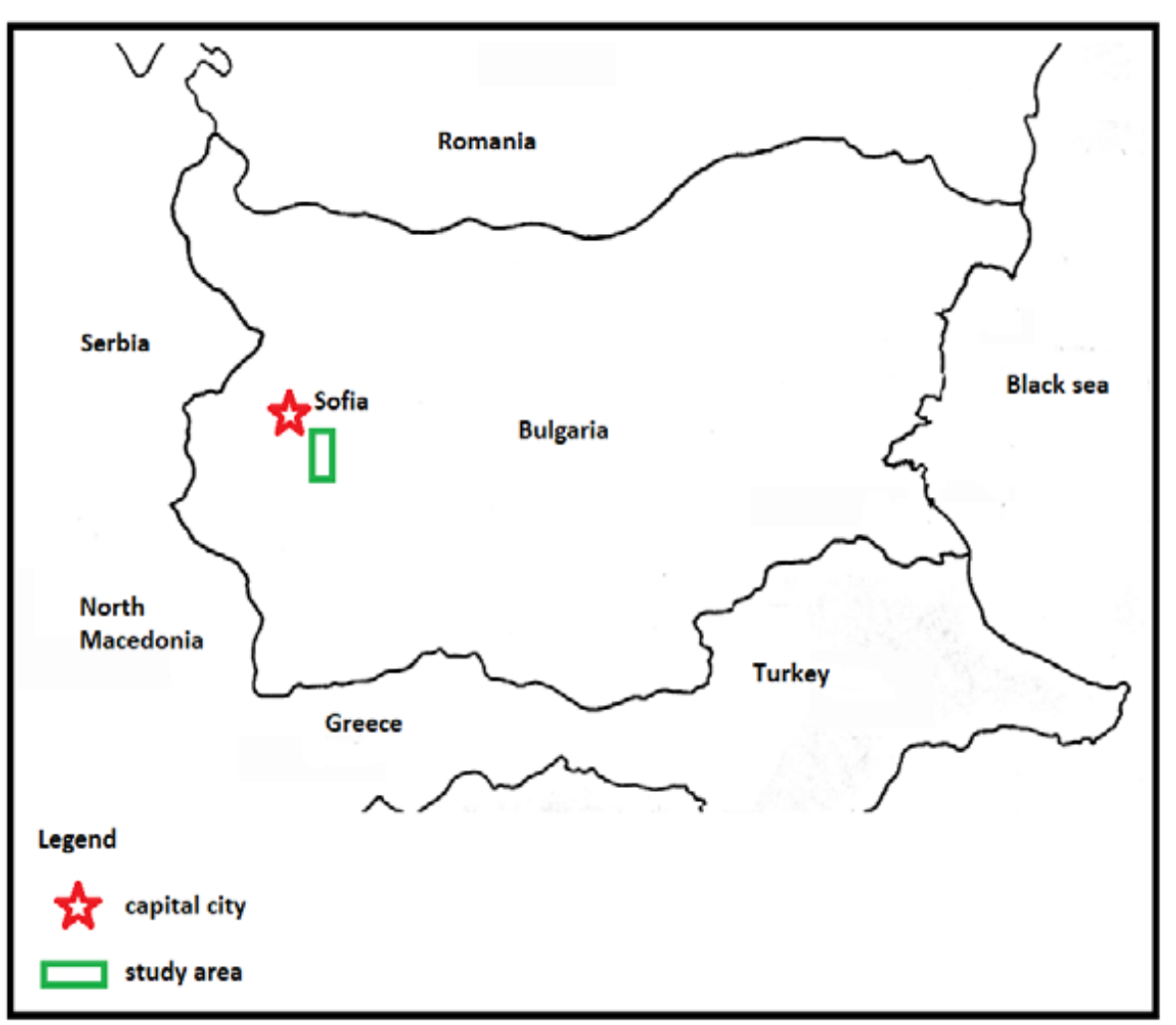
1. To evaluate variation in pollen morphology, pollen production and fertility/sterility in the populations of *I. glandulifera* in order to obtain data for its invasive success and compare with the other two species;

1. To analyze differences in pollen biology in two flower color forms (pink and violet).

Pink flowers

Violet flowers

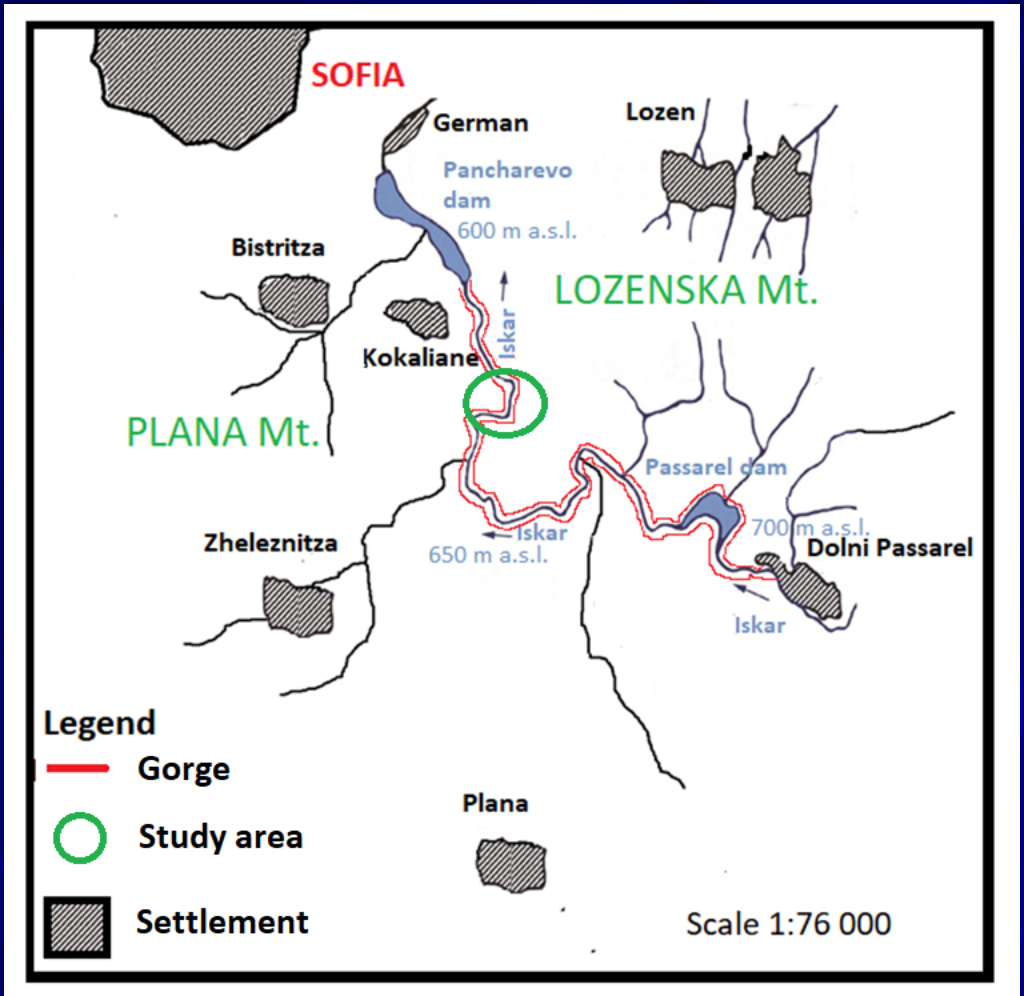
Materials and methods



The study area is located 10 km from the capital of Bulgaria- Sofia.

The investigation was conducted in the period June-September, 2020.

Materials and methods



Parameters of the area:
Iskar River Gorge (Western Bulgaria)
650 m. a.s.l.; 3 sq. km,

Average monthly air humidity is the lowest in July (63%) and the highest in December and January (85%).

Monthly temperature is the highest in July, 21.5 °C and the lowest in January, - 2.5 °C.

The average annual precipitation is 645 mm.

Materials and Methods

List of the localities of the studied populations of *Impatiens* species.

Population №	Species	Locality	Site code	Geographical coordinates	Altitude [m]
1	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., near the "Devil's Bridge"; soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Lz 1	42°34'58.674''N 23°25'39.212''E	650
2	*I. glandulifera*	Vitosha Mt. floristic Region, Plana Mt., near village Dolni Okol, soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Pl 2	42°30'27.873''N 23°30'29.057''E.	880
3	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., near the "Fallen tree bridge" on the left bank of the Iskar River, soil type: Fluvisols (WBR, 2014), part of hygrophyte grass community.	Lz 3	42°34'5.006''N 23°25'42.95''E	610
4	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., along the road Sofia -Samokov, dry open terrain, soil type: Leptosols (WBR 2014), part of ruderal grass community.	Lz 4	42°34'06.8"N 23°25'50.1"E	680
5	*I. glandulifera*	Sredna gora floristic Region, Lozenska Mt., screes on the right bank of the Iskar River; initial stage of soil formation, community of *Fagus sylvatica* L. close to the screes.	Lz 5	42°34'2.076''N 23°25'48.594''E.	630
6	*I. balfourii*	Sredna gora floristic Region, Lozenska Mt., Passarel village soil type: Leptosols (WBR 2014), with partial shade under the *Acer pseudoplatanus* trees.	Lz 6	42°32'29.0"N 23°29'52.9"E	700
7	*I. noli-tangere*	Sredna gora floristic Region, Lozenska Mt., soil type: Fluvisols (WBR, 2014), part of community *Alnus glutinosa and Alnus incana* community on the right bank of the Iskar River.	Lz 7	42°34'02.1"N 23°25'48.6"E	615

Materials and Methods

Collecting samples

Flowers (20-0) from 10 individuals with same color were collected before opening, placed in plastic bags, transported to the laboratory and separated for analyses

Pollen morphology

Pollen grains (30) were measured for six features: polar diameter (P), equatorial diameter (E), apocolpium (A), distance between colpi edges in polar view (M), long diameter in polar view (Ld), and short diameter in polar view (Sd). The P/E ratio and Ld/Sd ratio were calculated to determine the pollen shape.

Materials and Methods

Pollen production and fertility/ sterility

Eighteen anthers from unopened flowers from 6 different individuals (three flowers per individual) per population were placed in separate vials and treated with 1 mL of 0.1% aqueous solution of a detergent and Alexander stain (Alexander, 1969) in the ratio 9 : 1. The Alexander stain is used for differential staining of aborted and non-aborted pollen (grains stained in red are considered fertile).

Statistical analysis

Univariate and multivariate statistical procedures were applied to examine variation among the *Impatiens* populations. Data for pollen production and fertility/sterility were analyzed using analysis of variance (ANOVA) and the means were statistically grouped by Tukey's (HSD, honestly significant difference) test ($P < 0.05$).

Mean pollen production, fertile and sterile pollen ± standard deviation of three replicates for each flower form of the *Impatiens glandulifera* populations, *I. balfourii,* and *I. noli-tangere*. Significant differences (based on one-way ANOVA and Tukey HDS-tests) are indicated with different letters, $P < 0.05$. Site codes correspond to data in Table 1. Abb. pink flower (p); violet flower (v)

Site Code	Pollen production flower	Pollen production stamen	Fertile pollen (%)	Sterile pollen (%)
Impatiens glandulifera				
Lz 1p	1022951 ± 82405abc	204590 ± 16481abc	89.3 ± 4.53a	10.7 ± 4.53a
Lz 1v	997413 ± 245688abc	199483 ± 49138abc	85.82± 10.39a	14.18± 10.39a
Pl 2p	1358602 ± 469551a	271720 ± 93910a	87.95± 9.57a	12.05 ± 9.57a
Lz 3p	1350000 ± 126997a	270000 ± 25399a	88.27 ± 5.53a	11.73 ± 5.53a
Lz 3v	1239392 ± 227536ac	247878 ± 45507ac	82.46 ± 3.87a	17.54 ± 3.87a
Lz 4p	1368637 ± 378471a	273727 ± 75694a	91.09± 1.58a	8.91± 1.58a
Lz 5p	885677 ± 79850bc	177175 ± 15970bc	88.6 ± 4.1a	11.4 ± 4.1a
Lz 5v	772613 ± 181566b	154523± 36313b	82.49 ± 6.89a	17.47 ± 6.89a
Impatiens balfourii				
Lz 6	197639 ± 53351d	39528 ± 10670d	87.59 ± 5.85a	12.41 ± 5.85a
Impatiens noli-tangere				
Lz 7	194444 ± 62765d	38889 ± 12553d	88.45 ± 7.23a	11.55 ± 7.23a

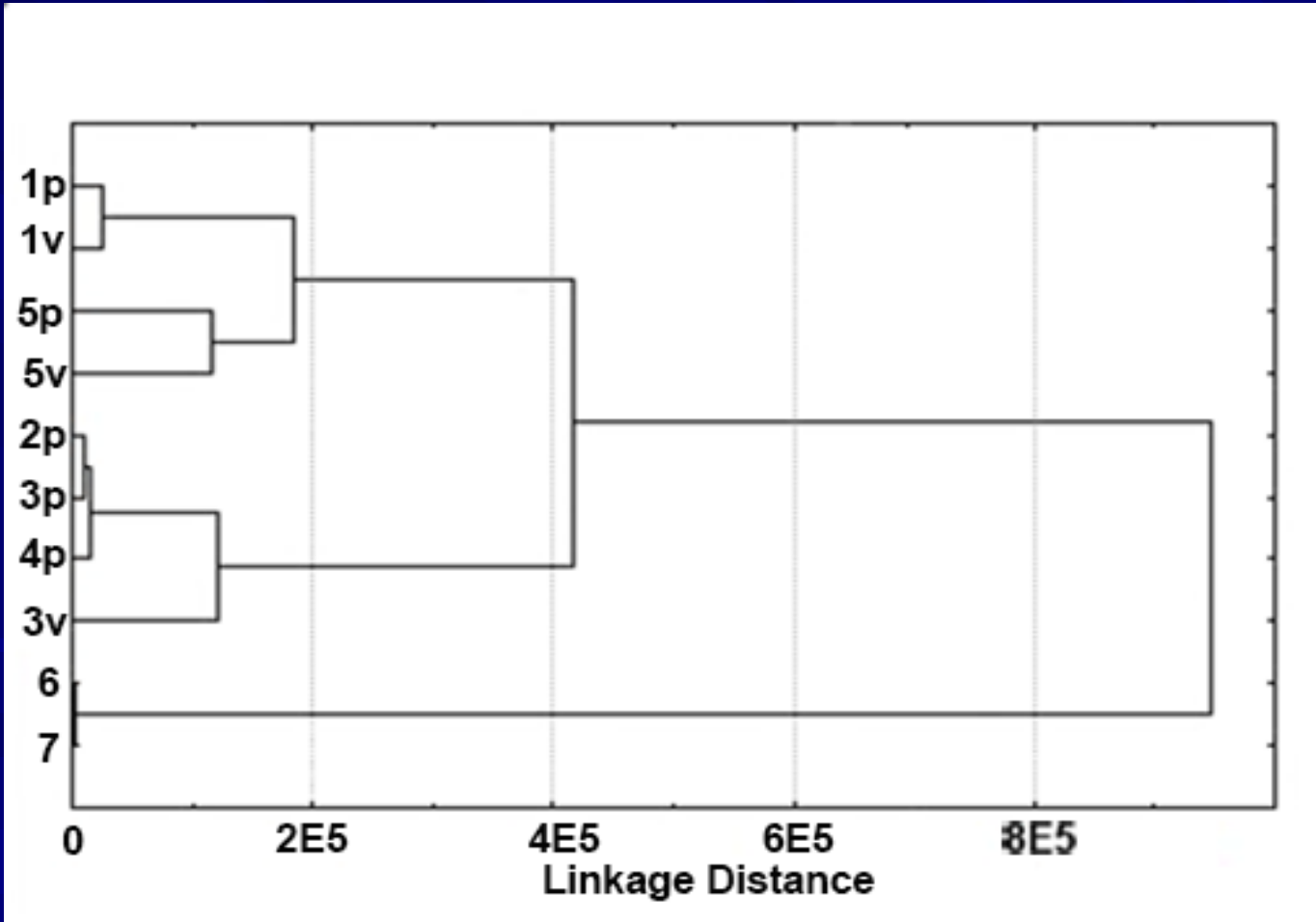
Pollen morphological data of the examined populations of *Impatiens* species with measurements (µm) for each flower form with mean (in brackets) ± standard deviation and ranges of the pollen characters: polar (P) and equatorial (E) diameter, apocolpium (A), long distance between colpi in polar view (M), long diameter in polar view(Ld), short diameter in polar view (Sd), index P/E, and index Ld/Sd. pink flower (p); violet flower (v).

Species	Site code	P	E	A	M	P/E	Ld	Sd	Ld/Sd
I. glandulifera	Lz 1p	16.5-19.5 (18.59±0.7)	33-39.8 (37.2±1.36)	22.5-30 (26.46±1.6)	18-28 (22.71±1.49)	0.5-0.6 (0.5±0.03)	33-40.5 (37.1±1.36)	25.5-31.5 (28.77±1.23)	1.7-2.3 (1.94±0.09)
	Lz 1v	16.5-22.5 (18.54±0.59)	34.5-42.0 (38.2±1.17)	24-37.5 (37.5±1.96)	18-28.5 (24.54±1.66)	0.4-0.6 (0.6±0.04)	36-42.0 (38.7±1.03)	24-30 (28.61±1.09)	1.8-2.4 (2.03±0.09)
I. glandulifera	Pl 2p	13.5-19.5 (16.5±0.82)	31.5-37.5 (34.88±1.24)	19.5-28.5 (25.55±1.29)	15-24 (20.57±1.41)	0.39-0.59 (0.47±0.05)	30-39 (35.46±1.52)	24-42 (27.7±2,3)	1.28-2.2 (1.94±0.15)
I. glandulifera	Lz 3p	15-22.5 (17.36±1.17)	31.5-43.5 (39.38±1.46)	22.5-31.5 (27.96±1.39)	19.5-28.5 (24.27±1.7)	0.34-0.62 (0.44±0.03)	34.5-43 (39.21±1.41)	24-30 (27.16±1.17)	1.8-2.6 (2.18±0.14)
	Lz 3v	15-19.5 (17.41±0.88)	34.5-45 (38.2±1.5)	15-30 (26.46±1.91)	12-27 (21.75±1.88)	0.4-0.52 (0.46±0.03)	30-39 (37.07±1.33)	25.5-33 (28.93±1.08)	1.67-2.16 (1.93±0.09)
I. glandulifera	Lz 4p	15-19.5 (16.77±0.94)	30-40.5 (35.41±1.75)	22.5-30 (25.88±1.29)	16.5-25.5 (20.89±1.33)	0.4-0.59 (0.48±0.04)	30-40.5 (35.14±1.64)	22.5-28,5 (26.04±1,06)	1,66-2,4 (2.03-0.13)
I. glandulifera	Lz 5p	16.5-19.5 (17.7±0.59)	36-45.5 (38.9±0.86)	22.5-33 (28.8±1.41)	21-30 (24.4±1.26)	0.41-0.52 (0.45±0.02)	36-48 (41.2±1.73)	25.5-36 (30.4±2.12)	1.75-2.45 (2±0.1)
	Lz 5v	15-18 (16.7±0.7)	34.5-40.5 (37.39±0.94)	22.5-31.5 (26.89±1.36)	18-28.5 (22.88±1.6)	0.37-0.52 (0.45±0.03)	30-40.5 (37.23±1.36)	22.5-30 (26.03±1.06)	1.81-2.5 (2.15±0.11)
I. balfourii	Lz 6	15-22.5 (19.23±0.92)	40.5-49.5 (45.99±1.31	30-37.5 (34.13±1.53)	34.5-42 (38.04±1.47)	0.34-0.48 (0.42±0.03)	39-52.5 (45.71±2.01)	19.5-34.5 (28.29±1.95)	1.96-3.1 (2.44±0.17)
I. noli-tangere	Lz 7	15-25.5 (20.19±1.38)	37.5-46.5 (41.36±1.48)	18-36 (28.55±2.06)	18-30 (24.43±1.66)	0.39-0.63 (0.49±0.05)	34.5-49.5 (42.08±2.08)	21-36 (29.87±2.3)	1.68-3 (2.13±0.14)

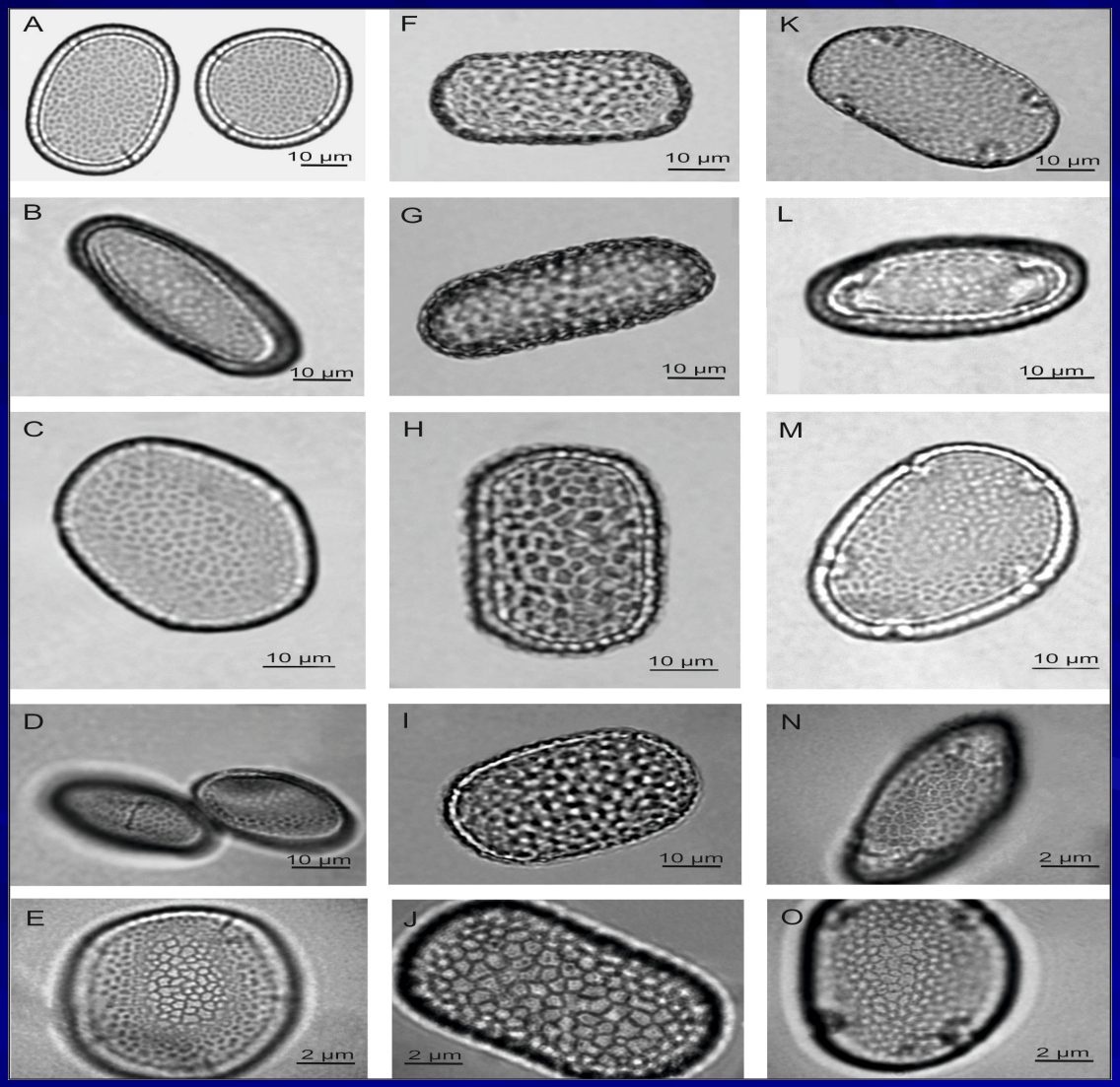
Pollen morphological data of the examined populations of *Impatiens* species with measurements (µm) for each flower form with mean (in brackets) ± standard deviation and ranges of the pollen characters: polar (P) and equatorial (E) diameter, apocolpium (A), long distance between colpi in polar view (M), long diameter in polar view(Ld), short diameter in polar view (Sd), index P/E, and index Ld/Sd. pink flower (p); violet flower (v).

Variable	FPP	P	E	A	M	Ld	Sd
FPP	ns	-0,79**	-0,81**	ns	-0,72*	-0,83**	ns
P	-0.79**	ns	0,72*	ns	ns	0,74*	0,63*
E	-0,81**	0,72*	ns	0,69**	0,94**	0,96**	ns
A	ns	ns	0,69*	ns	0,80**	0,68*	ns
M	-0,72*	ns	0,94**	0,80**	ns	0,91**	ns
Ld	-0,83**	0,74*	0,96**	0,68*	0,91**	ns	ns
Sd	ns	0,63*	ns	ns	ns	ns	ns

Similarity dendrogram obtained by cluster analysis (Euclidean distances) applied to data of the measured pollen characters and calculated flower pollen production and fertility.



LM micrographs of *Impatiens glandulidera* (A-E), *I. balfourii* (F-J), and *I. noli-tangere* (K-O) pollen grains: (A, F, K) rectangular-obtuse and subcircular pollen in polar view; (B, G, L) peroblate pollen in equatorial view; (C, M) 5-colpate and (H) 4-colpate pollen; (D, N) colpus; (D, J, O) reticulate ornamentation.



Conclusions

The pollen morphology was investigated with light microscope and the pollen grains are 4(5)-zonocolpate oblate or peroblate in equatorial view, and rectangular-obtuse or suborbicular in most 4-zonocolpate grains in polar view, with a reticulate ornamentation.

The mean pollen production per flower and stamen was very high in all three species comparable with anemophilous plants.

The sterile pollen was between 8.9% and 17.54%.

The variations in pollen biology can be related to different evolutionary adaptations of the flowers to be more attractive for pollinators and successful in reproduction and distribution.

The results are of importance for taking measures to reduce the invasiveness of these alien species.