Relating Invasibility and Invasiveness: Case Study of *Impatiens parviflora*

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Data on alien species show that plant invasions are caused by a complex combination of characteristics of invasive species (invasiveness) and characteristics of invaded environment (invasibility). *Impatiens parviflora* is one of Europe's top invasive species. The present study aimed to evaluate molecular diversity of populations of highly invasive in Lithuania *I. parviflora* by applying several DNA markers and relating genetic parameters to abiotic and biotic environment. For sampling, urban forests, riparian forests, and agrarian shrublands were selected. Three different DNA-based techniques, Simple Sequence Repeats (SSR), Inter Simple Sequence Repeat (ISSR) markers, and Random Amplified Polymorphic DNA (RAPD), were used for detecting genetic variation between 21 populations. All population individuals were monomorphic and homozygotic for four loci and heterozygotic for one locus by SSR analysis. Hierarchical analyses of molecular variance at ISSR and RAPD loci revealed significant differentiation of populations depending on geographic zones of the country. Bayesian Structure analyses of molecular data demonstrated existence of many genetic clusters and this finding is in support to multiple introduction of the species. The polymorphism extent at ISSR loci was positively correlated with the total coverage of herbaceous plant species. The coverage by *I. parviflora* was negatively correlated with the total number of herbaceous species and light in the sites. Our results indicate that Lithuanian sites with *I. parviflora* might be distinguished by high soil nutrient levels. According to the principal component analysis, the coverage by *I. parviflora* was a more important variable of populations compared to molecular data or parameters of abiotic environment. In conclusion, complexity of invasibility and invasiveness factors determine the variability of *I. parviflora* sites, including genetic traits, coverage of invasive species and conditions of environment that were significant and interrelated.

Keywords: small balsam, genetic diversity, ISSR, Balsaminaceae, alien plants, herbaceous plants, phytosociology

INTRODUCTION

The world is facing multiple environmental challenges simultaneously, including chemical and biological pollution, climate change, degradation, and loss of biological diversity. Tackling these challenges has become increasingly complicated due to the raise of temporal and spatial human-environment interactions (Wang, 2021). Phenomenon of species invasion is considered among the most harmful processes, creating threats to agriculture and forestry, health and the global economy (Schmidt et al., 2012). To date, an unusually large amount of time and money has been spent controlling invaders (Haubrock et al., 2021).

Forests provide valuable habitat for various living beings, supplying them with food and shelter, cleaning and enriching air, mitigating the influence of cold and droughts, ameliorating climate change and chemical pollution. However, forests are trampled, burned, felled, ignorantly replanted and otherwise modified by human, making them highly susceptible to plant invasion (Wagner et al., 2017, 2021). Forest alien species of herbaceous plants might be unfavorable for recreation, diminishing the number of local species, causing soil erosion and other changes of habitats. The impact of specific invaders on forest ecosystem services is not clear enough and requires wider insights. Exotic species interfering with tree communities are extremely difficult to eradicate.

European woodlands, covering a third of Europe's terrestrial area, are prone to alien plant invasions because many sites show typical symptoms of hemeroby, such as disturbance, fragmentation, high concentration of soil nutrients and pressure of foreign propagules. The regulation of invasive alien species has become a top priority for conservation policy at the national and European levels (European Parliament, 2014; EU Regulation 1143/2014).

The first target of invasive alien species regulation is the most aggressive invaders. Small balsam (*Impatiens parviflora* from Balsaminaceae) could be classified in this category as alien species that is extremely penetrative into forest ecosystems (Coombe, 1956; Trepl, 1984; Eliáš, 1999; Tanner, 2008; Chmura, 2014). As summarized by Coombe (1956) in 1831 *I. parviflora* was cultivated in the Botanic Garden in Geneva and within the next few decades was recorded as an escape in many places in Europe, often near botanic gardens: Geneva, Vienna, Prague, Tartu, Königsberg, and Berlin. There is a lot of convincing data about the type of further random invasion of *I. parviflora* (Trepl, 1984). In Poland and Latvia naturalization of this species was recorded in the second half of 19th century (Tokarska-Guzik, 2005; Priede, 2009b) and the first records for this species occurrence in the current territory of Lithuania were published later (Wisnewski, 1934). From the second half of the former century *I. parviflora* has been listed as adventive species of Lithuania (Natkevicaite-Ivanauskiene, 1951) and nowadays it belongs to the most widely spread aliens in the country (The Ministry of Environment of the Republic of Lithuania, 2015). Different European countries have reported continued spread of this species within recent decades (Priede, 2009a; Reczyńska et al., 2015; Jarčuška et al., 2016; Wagner et al., 2017). Invasive strength

of this plant is proven by both the size of occupied territory and the variety of habitats (Klimko and Piskorz, 2003; Chmura and Sierka, 2006). *I. parviflora* is an exceptionally successful invader of forest vegetation and is presently the most common alien plant in European woodlands (Wagner et al., 2017, 2021). This plant has been very frequently discussed when evaluating impact on forest regeneration (Langmaier and Lapin, 2020). Many studies have been carried out focusing on physiology and ecology of *I. parviflora* (Trepl, 1984; Chmura, 2014; Reczyńska et al., 2015; Jarčuška et al., 2016; Florianová and Münzbergová, 2017). Information about steps and vitality of populations of alien herbs in North-East parts of Europe still remains very fragmental.

To counteract expansion of harmful species to the new areas, comprehensive knowledge in both traditional and modern biology is required (Tokarska-Guzik, 2005; Cross et al., 2011). Neutral molecular genetic markers can be used as the tools to study ecological processes such as colonization, dispersal, or community interactions (Nybom et al., 2014). Data in molecular genetics may provide unique insights into the sources, routes and mechanisms of alien species spread. There is a growing understanding that integrating genetics and ecology is critical in the context of biological invasions, since the two are explicitly linked (Handley et al., 2011). Information on the genetic make-up of an invasive emerging population is important for answering one of the most fundamental questions in invasion biology: what determines the success of the spread of alien species? In addition, understanding these processes is seen as essential for implementing proper management policies. Molecular markers differ in terms of the number of fragments per primer, the extent of polymorphic fragments per primer. SSR loci are known as simple di- or tri-nucleotide repeats with higher rate of mutation compared to the average for the genome, ISSR cover genomic areas flanked with the inverted repeats of SSR loci, and RAPDs are related to anonymous sequences scattered all over the genome (Grover and Sharma, 2016; Bechtold and Field, 2018; Garrido-Cardenas et al., 2018; Younis et al., 2020). Studying closely related genotypes, the analysis of variability is more informative in case more than one DNA-based technique is applied (Costa et al., 2016; Yasui, 2020). Molecular genetics of populations has provided valuable information about *Lupinus polyphyllus* as alien forest species of Finland (Li et al., 2016), and Lithuania (Vyšniauskienė et al., 2011). When compared with other molecular techniques, ISSR markers are easy to use, specific enough, and are applicable for all plant groups. Overall, these markers showed great promise as a method of elucidating relationships among individuals within populations and between populations and is still much used nowadays (Nybom et al., 2014; Lazzaro et al., 2018). ISSRs have been employed for evaluation of genetic diversity of *Alliaria petiolata* which scope of invasion in North America is similar to *Impatiens parviflora* expansion in Europe (Meekins et al., 2001).

Information about steps and vitality of populations of alien herbs in North-East parts of Europe still remains very fragmental. Till now, *I. parviflora* remains among the least genetically examined alien plant species world-wide. Extensive investigations of *I. parviflora* has started long before the dawn of molecular methods for population studies (Coombe, 1956;

Trepl, 1984). To the best of our knowledge, till the present study, genetic diversity of two Polish populations was evaluated by amplified fragment length polymorphism (AFLP) markers (Komosińska et al., 2006) and cpDNA from one United Kingdom plant was recently analyzed (Kurose et al., 2020). However, the relations between genetic data and assemblages of herbaceous species in sites with *I. parviflora* have never been traced for the species. This aspect might be important while searching for unique invasive plant abilities to extend into secondary areas. The present study is aimed at evaluation of genetic diversity of Lithuanian populations of *I. parviflora* using a set of molecular markers and relating genetic parameters to species abiotic and biotic environment.

MATERIALS AND METHODS

Study Area and Species

Twenty-one populations of small balsam (*Impatiens parviflora* DC.) were selected in the way to cover all the territory of Lithuania (**Figure 1**). Sites with *I. parviflora* were named by three letters, reflecting their geographical location. Geographical coordinates of the examination are ranged from 53°59′ to 56°21′ latitude (N), from 20°59′ to 26°09′ longitude (E), with annual rainfall of 722–936 mm, and mean annual temperature of 6.0–6.8°C (Kupcinskiene et al., 2013).

Impatiens parviflora sites were described by a number of properties (**Table 1**). According to the biotope they were divided into urban forests, riparian forests and agrarian shrublands. Sites were divided into three groups based on the traffic intensity along the road close to the site: a road without blacktop in the forest, a

blacktop road with low intensity traffic, and a blacktop road with intensive traffic. Herbaceous plant species growing along with populations of *I. parviflora* were recorded in 100 m^2 plots at each site. The coverage of each species was calculated using Braun-Blanquet methodology (Braun-Blanquet, 1964) and transformed into mean-percentage values for each degree (0.1, 0.5, 3, 15, 38, 63, and 88%). For evaluation of environmental preferences of *I. parviflora* in each site, Ellenberg indicator values (EIV; Ellenberg et al., 1992), i.e. – light, temperature, continentality, soil moisture, soil reaction and soil nutrients, were quantified in proportion to the corresponding species coverage, employing weighted average (W-EIV) method, as it was described previously (Marozas, 2014; Marozas et al., 2015).

For molecular analyses 15 plants (315 individuals in total), were sampled from each site in the way described above (Kupcinskiene et al., 2013).

Molecular Analyses

Plant leaf DNA was extracted using DNA purification kit #K0512 (Thermo Fisher Scientific, Vilnius, Lithuania), following the instructions of the producer with some modifications (Zybartaite et al., 2011).

For SSR analyses the primer pairs IGNSSR101-EF025990, IGNSSR104-EF025992, IGNSSR106-EF025993, IGNSSR203-EF025994, IGNSSR210-EF025995, and IGNSSR240-EF025997 (Metabion International AG, Steinkirchen, Germany) were used following Provan et al. (2007). The polymerase chain reaction (PCR) was carried out using 6.25 µl PCR Master mix, 5 pmol of each primer in the pair and 25 ng of genomic DNA. Temperature conditions for PCR were as follows: initial denaturation at 94°C for 3 min, 35 cycles including denaturation at 94°C for 30 s,
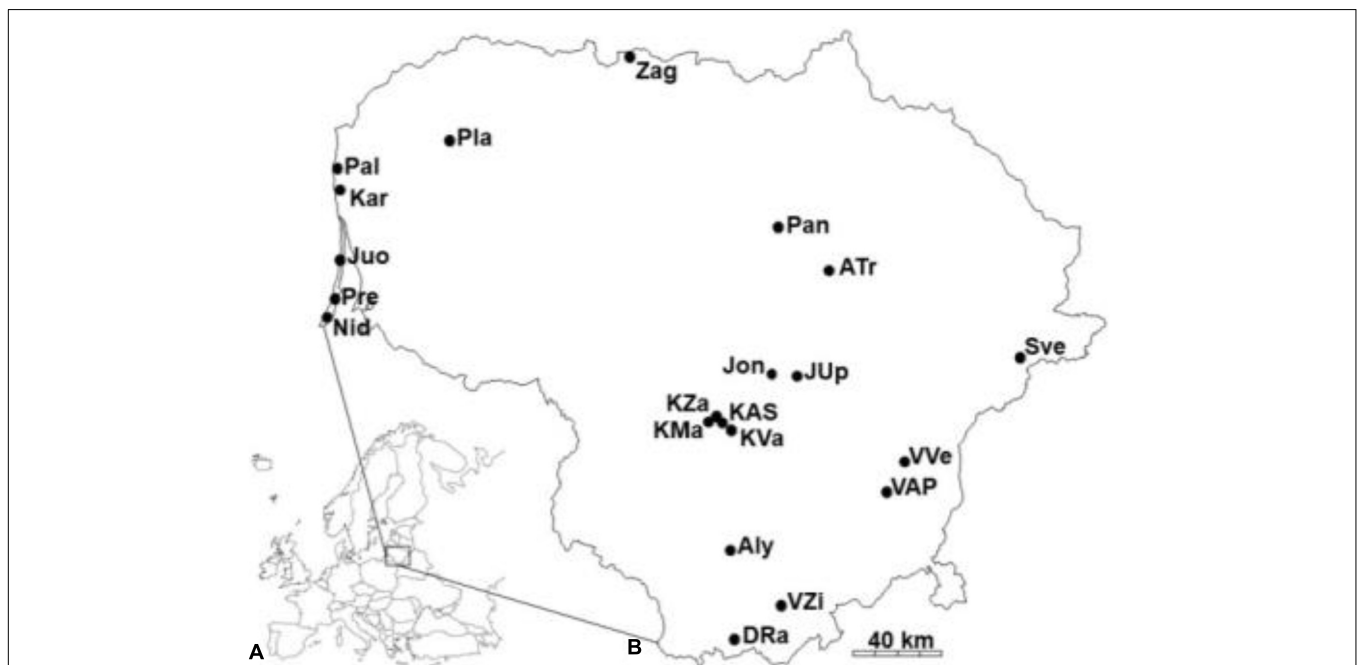

FIGURE 1 | Location of Lithuania **(A)**, and sites with *Impatiens parviflora* in the country **(B)**. Populations are named by three letters.

TABLE 1 | Characteristics of sites with *Impatiens parviflora*.

Population title	Biotopes[A]	Geographic zones[B]	Roads[C]
VVe	urban forest	SE	low traffic road
VAP	urban forest	SE	low traffic road
Sve	agrarian shrubland	SE	unpaved road
VZi	riparian forest	SE	unpaved road
DRa	riparian forest	SE	unpaved road
Aly	agrarian shrubland	SE	high traffic road
KVa	urban forest	C	high traffic road
KAS	urban forest	C	high traffic road
KZa	urban forest	C	high traffic road
KMa	urban forest	C	unpaved road
Juo	urban forest	NW	low traffic road
Pre	urban forest	NW	low traffic road
Nid	urban forest	NW	low traffic road
Kar	riparian forest	NW	low traffic road
Pal	urban forest	NW	high traffic road
Pla	urban forest	NW	unpaved road
Zag	agrarian shrubland	NW	low traffic road
Pan	agrarian shrubland	C	low traffic road
ATr	agrarian shrubland	C	low traffic road
Jon	agrarian shrubland	C	low traffic road
JUp	riparian forest	C	low traffic road

*(A) Biotopes: , urban forest; , riparian forest; , agrarian shrubland. (B) Geographic zones: NW, North-West part of Lithuania; C, central part of Lithuania; SE, South-East part of Lithuania. (C) Roads: , unpaved road; , low traffic road; , road of high traffic intensity. Populations are titled by three letters (for details of locations, see **Figure 1**).*

annealing at 58°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 5 min.

Inter simple sequence repeat-polymerase chain reaction was carried out using primers former employed to develop microsatellites for *Impatiens glandulifera* (Provan et al., 2007): (1) GGCC(AG)$_8$, (2) GGCC(AC)$_8$, (3) CCGG(AG)$_8$, (4) CCGG(AC)$_8$, (5) GCGC(AG)$_8$, and (6) GCGC(AC)$_8$ (Metabion International AG, Steinkirchen, Germany). Of the six tested primers, four (2–3, 5–6) generated clear and reproducible fragments of DNA and were used for population studies. Eight RAPD markers (OPA-20, OPD-20, 222, 250, 269, 340, 474, and 516) applied for populations of *I. glandulifera* (Zybartaite et al., 2011) later were employed for *Impatiens parviflora* (Kupcinskiene et al., 2013).

For genetic analyses of 315 individuals of *I. parviflora* ISSR-PCR amplification was carried out in a final volume of 20 μL containing 100 ng of template DNA, 20 pmol primer, 1 × *Taq* buffer, 200 μM dNTPs, 2.5 mM MgCl$_2$ and 1 U of *Taq* polymerase, following Hatcher et al. (2004) and Provan et al. (2007). DNA amplification was carried out in Eppendorf Mastercycler® ("Eppendorf," Germany) under such a program: initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C, annealing at 60°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 5 min. The products of PCR were fractionated by electrophoresis on 1.5%

TBE-agarose gels stained with ethidium bromide and imaged using UV transilluminator ("Herolab", Germany).

Statistical Analysis

Both ISSR and RAPD data were evaluated by Mantel test, hierarchical analyses of molecular variance (AMOVA), principal coordinate analyses of individuals, and Bayesian clustering. In addition, genetic parameters of *I. parviflora* were related to biotic environment (herbaceous plant species data).

Standard parameters of genetic diversity were obtained using GENALEX version 6.5 (Peakall and Smouse, 2012). An unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on pairwise Nei's unbiased genetic distances (Nei, 1978) was used to assess genetic relationships among populations. Mantel test (9999 permutations; Mantel, 1967) and principal coordinate analyses (PCoA) were done in GENALEX program, version 6.5. Hierarchical analyses AMOVA were done using GENALEX, which allowed partition the total genetic variation into three hierarchical levels: among regions, among sites within regions, and within sites. To explore patterns in genetic variation of *I. parviflora*, and to relate this parameter to the traits of abiotic and biotic environment, a hierarchical analysis of molecular variance (AMOVA at ISSR and RAPD loci) was conducted among populations depending on biotope (agrarian shrubland, urban forest, riparian forest), geographic zone (North-West, Central, and South-East of Lithuania), and road type.

For SSR data (21 population, 15 individuals in each) observed heterozygosity (H_o), expected heterozygosity (H_e) and inbreeding coefficient (F_{IS}) were calculated using ARLEQUIN version 3.1 (Excoffier et al., 2005).

For identification of gene clusters of *I. parviflora* populations, Bayesian analysis was performed using STRUCTURE, version 2.3.1 (Pritchard et al., 2000; Falush et al., 2003, 2007). A priori estimation of clusters was set to $K = 1$–21, the highest probable number of the clusters corresponded to the number of populations. To test for significant patterns of clustering, the procedure described by Evanno et al. (2005) was implemented. Twenty runs were carried out for each K and the rate of change in the log probability of the data between successive likelihood values (ΔK) was estimated using a 10^4 steps burn-in period followed by 10^5 iterations of Markov chain Monte Carlo.

To assess the similarity of assemblages of herbaceous plant species in the sites, a cluster analysis was performed, applying program PC-ORD, version 6.0 (McCune and Mefford, 2011). For comparison of features of the sites, median values of W-EIV were used and differences among sites were calculated using STATISTICA, version 7.0 (StatSoft Inc, 2004).

To relate genetic data of *I. parviflora* and environment data, Spearman correlations were calculated, drawing correlogram by R package "corrplot," version 0.90 (Wei and Simko, 2021). Principal component analysis was carried out using two genetic variables [percentage of polymorphic loci (PLP) at ISSR loci and RAPD loci], two biotic variables (coverage by *I. parviflora*, and the number of herbaceous species), six abiotic variables [W-EIV for light (L), temperature (T), continentality (K), soil moisture (F), soil reaction (R), and soil fertility (nutrients, hereinafter N)

RESULTS

Profiles of Molecular Markers of *Impatiens parviflora* Populations

No alleles were obtained by IGNSSR104-EF025992, one allele was produced by each out of four primer pairs: IGNSSR101-EF025990, IGNSSR106-EF025993, IGNSSR203-EF025994, and IGNSSR240-EF025997, and two alleles were synthesized by IGNSSR210-EF025995. The size of DNA fragments ranged within 98–148 bp. The test of conformity of locus IGNSSR210-EF025995 heterozygosity to the Hardy-Weinberg equilibrium revealed, that for all populations inbreeding coefficient, (F_{IS}) was −1, observed heterozygosity (H_O) was 1 and expected heterozygosity (H_E) was 0.517 ($p < 0.001$).

Among 21 populations examined, 108 alleles were generated at four ISSR loci and 218 alleles at eight RAPD loci. All ISSR and RAPD loci were polymorphic at the species level. In case of ISSR markers the range of polymorphic loci percentage (PLP–ISSR) per population was 10.2–25.9, the mean being 16.5 (**Figure 2**). Among populations Nei's gene diversity (h–ISSR) range was 0.030–0.078 and Shannon's index (I–ISSR) range was 0.046–0.121, the respective mean values per population were 0.047 and 0.073. For RAPD loci the range of PLP per population was 7.3–39.0, the mean being 21.0 (PLP–RAPD). Among populations Nei's gene diversity (h–RAPD) range was 0.052–0.141 and

Shannon's index (I–RAPD) range was 0.079–0.225, the respective mean values per population were 0.095 and 0.146. There were 13 unique alleles at ISSR loci and seven alleles at RAPD loci. Of 20 unique (either for ISSR or RAPD loci) alleles, two alleles were identified in the *I. parviflora* populations in the South-East Lithuania, 10 alleles in the Central and eight alleles in the North-West Lithuania.

Estimated by ISSR markers, Nei's (1978) pairwise genetic distances (GD) between the populations were in the range of 0.083–0.405. Mantel test for those markers did not show correlation between the genetic and geographic distances of populations (**Figure 3**). Interval of pairwise genetic distances at RAPD loci was 0.135–0.426. Mantel test showed significant correlation between the genetic and geographic distances of the populations. Dendrograms split populations into clades of 9th (at ISSR loci) or 12th order (at RAPD loci) (**Figure 4**). In both dendrograms of genetic relationships at ISSR and RAPD loci, one of the three most distinct populations were DRa and in opposite situation was VVe.

Hierarchical analyses of molecular variance (AMOVA) showed that *I. parviflora* populations, grouped according to the biotope (urban forest, riparian forest, and agrarian shrublands), differed significantly at RAPD loci accounting for 2% of the variation (**Table 2**).

Hierarchical AMOVA for distinct geographic zone (North-West part, Central part, and South-East part of Lithuania) populations reflected significant genetic structure of the populations, accounting for 6% of the variation at ISSR loci and 2% of the variation at RAPD loci. Hierarchical AMOVA

FIGURE 2 | ISSR and RAPD loci based parameters of genetic diversity of 21 *Impatiens parviflora* populations: PLP, polymorphic loci percentage; indices: *h*, Nei's gene diversity; *I*, Shannon's information index. The central line of each box indicates the median value; the boxes, the lower (25%) and upper (75%) quartiles, and the whiskers, are from 10 to 90 percentiles (typical range), the points are outliers.

FIGURE 3 | Mantel test of isolation by distance at ISSR loci **(A)** and RAPD loci **(B)** for populations of *Impatiens parviflora*. Each point represents Nei's genetic distance (*h*) plotted against geographic distance.
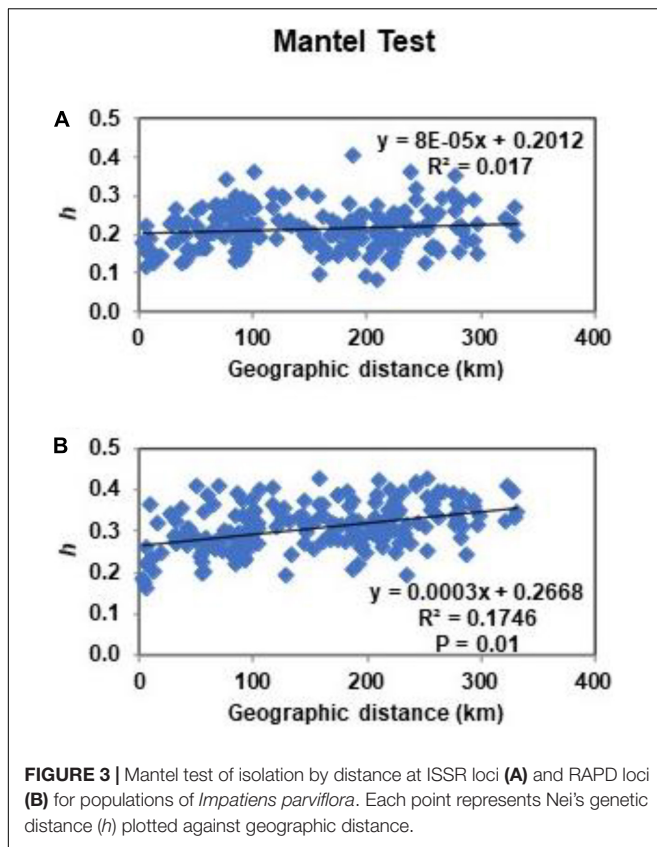
markers, the populations within groups (A–C cases of grouping) were significantly different (accounting for 71–79% of the genetic variation), while the within-population proportion of variance was smaller and very similar for all cases of grouping (21–24%, respectively).

According to the analysis PCoA of ISSR data, the first three coordinates accounted for about 28.2% of the genetic variation of individuals of *I. parviflora* populations (**Figure 5**), the first, the second, and the third coordinate explained, respectively, 10.6, 9.7, and 7.9% of the total variation. In all ISSR based PCoA plots (axis 1 vs. 2, 1 vs. 3, and 2 vs. 3) the most distinct individuals belonged to DRa, ATr, and Jon populations. Individuals of the North-West populations were mostly overlapping with individuals of the Central zone populations.

Based on RAPD data PCoA for the first three coordinates accounted for about 21.4% of the genetic variation of individuals of *I. parviflora* populations. The first, the second, and the third coordinate explained, respectively, 7.7, 7.1, and 6.7% of the total variation. Defined by PCoA at RAPD loci (axis 1 vs. 3, axis 2 vs. 3), the most distinct were individuals of the population DRa. For both systems of molecular markers (ISSR and RAPD), PCoA analyses did not differentiate populations into three distinct subgroups corresponding to the different geographic zones.

The Bayesian analysis of ISSR data of 315 individuals of *I. parviflora* revealed that a simulation equilibrium (likelihood of K's) provided by the genomic structure was reached at $K = 11$ (**Figure 6**). The next likelihood of K was three genetic clusters ($K = 3$). The Bayesian analysis of RAPD data of the same populations suggested the most likely *K* value being 13.

For ISSR STRUCTURE barplot at $K = 3$, the 1st gene pool was predominant among five populations (24%), each of the remaining two gene pools was characteristic for groups of eight populations (**Figure 7**). Populations with the same prevalent gene pools were scattered along all the territory under investigation. For ISSR STRUCTURE barplot at $K = 11$, there were eight pairs of

analysis of ISSR data showed significance of population grouping according to the neighboring road type: 2% of molecular variance was related to differences between the groups of populations. At RAPD loci, differentiation of population groups was not significant. Based on two types of molecular

FIGURE 4 | Dendrograms based on UPGMA cluster analysis of Nei's genetic distances at ISSR loci **(A)** and RAPD loci **(B)** of genetic relationships between the populations of *Impatiens parviflora*. Three letters denote the populations (for details of their location, see **Figure 1**. Explanations of the pictogram are provided in the **Table 1**).

TABLE 2 | ISSR and RAPD loci based hierarchical analyses of molecular variance (1) among groups of populations differing in biotope (A), geographic zone (B), type of neighboring road (C); (2) among populations within groups, and (3) within populations of *Impatiens parviflora*.

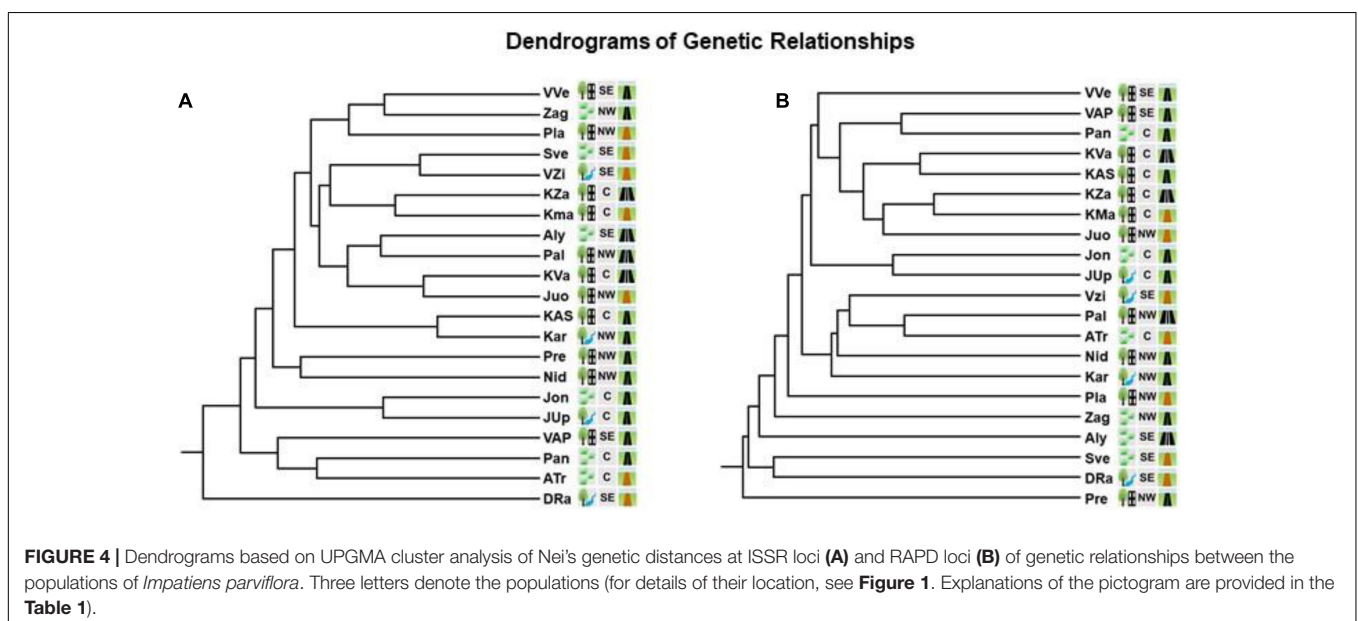| Level of variation	df	SS	MS	Est. Var.	%	Φ
ISSR loci						
A. Among groups of populations in sites of different biotopes	2	281	140.7	0.000	0	$\Phi_{CT} = -0.011^{NS}$
Among populations within groups	18	2791	155.0	10.127	76	$\Phi_{SC} = 0.764^{***}$
Among individuals within populations	294	919	3.1	3.127	24	$\Phi_{ST} = 0.761^{***}$
Total	314	3991		13.253	100	
B. Among groups of populations in different geographic zones	2	448	224.1	0.751	6	$\Phi_{CT} = 0.056^{***}$
Among populations within groups	18	2624	145.8	9.509	71	$\Phi_{SC} = 0.753^{***}$
Among individuals within populations	294	919	3.1	3.127	23	$\Phi_{ST} = 0.766^{***}$
Total	314	3991		13.386	100	
C. Among groups of populations besides different road type	2	358	179.1	0.283	2	$\Phi_{CT} = 0.021^{***}$
Among populations within groups	18	2714	150.8	9.842	74	$\Phi_{SC} = 0.759^{***}$
Among individuals within populations	294	919	3.1	3.127	24	$\Phi_{ST} = 0.764^{***}$
Total	314	3991		13.252	100	
RAPD loci						
A. Among groups of populations in sites of different biotopes	2	1056	528.1	0.700	2	$\Phi_{CT} = 0.018^{***}$
Among populations within groups	18	8285	460.3	30.25	77	$\Phi_{SC} = 0.788^{***}$
Among individuals within populations	293	2390	8.2	8.16	21	$\Phi_{ST} = 0.791^{***}$
Total	313	11732		39.10	100	
B. Among groups of populations in different geographic zones	2	1068	534.1	0.715	2	$\Phi_{CT} = 0.018^{***}$
Among populations within groups	18	8274	459.6	30.195	77	$\Phi_{SC} = 0.778^{***}$
Among individuals within populations	293	2390	8.2	8.158	21	$\Phi_{ST} = 0.791^{***}$
Total	313	11732		39.068	100	
C. Among groups of populations besides different road type	2	867	433.6	0.000	0	$\Phi_{CT} = -0.010^{NS}$
Among populations within groups	18	8474	470.8	30.945	79	$\Phi_{SC} = 0.791^{***}$
Among individuals within populations	293	2390	8.2	8.158	21	$\Phi_{ST} = 0.789^{***}$
Total	313	11732		38.980	100	

df, degree of freedom; SS, sum of squares; MS, mean squares; Est. Var., estimated variability; %, percentage of variation; Φ, genetic differentiation. A. Biotopes: 1, urban forest; 2, riparian forest; 3, agrarian shrubland. B. Geographic zones: 1, North-West part of Lithuania; 2, central part; 3, South-East part. C. Roads: 1, unpaved road; 2, low traffic road; 3, intensive traffic road. ***P ≤ 0.001, NS, P > 0.05.

populations (VVe-VAP, SVe-VZi, KZa-KMa, Pre-Nid, Pla-Zag, Juo-Pal, Pan-ATr, and JUp-Jon) with the same prevailing gene cluster. For RAPD STRUCTURE barplot at $K = 13$, there were two the same pairs (like in case of ISSR, KZa-KMa and JUp-Jon) out of seven pairs of populations with the prevalence of identical gene cluster. The extent of admixture of gene pools was negligible for most of the populations, with the following exceptions: ATr at ISSR loci and Pal at RAPD loci.

Analyses of Biotic Variables of Sites

In urban forests, the characteristic tree species were *Pinus sylvestris*, *Acer pseudoplatanum*, *Tilia cordata*, *Quercus robur*, *Fraxinus excelsior*, and *Ulmus glabra*. In riparian forests *Salix alba*, *S. fragilis*, and in some sites *Alnus glutinosa* were growing. Agrarian shrublands consisted of shrubs such as *Corylus avellana*, *Ribes nigrum*, *Euonymus europaeus*, *E. verrucosus*, and *Lonicera xylosteum* and/or single trees such as *Fraxinus excelsior*, *Acer platanoides*, *Tilia cordata*.

Evaluating the sites with *I. parviflora* according to the number of herbaceous plant species, 138 (62) species were recorded. The most frequent were species belonging to following families: Asteraceae (16), Fabaceae (14), Poaceae (12),

Rosaceae (11), Lamiaceae (10), Apiaceae (8), Caryophyllaceae (7), Plantaginaceae (5), Polygonaceae (5), Ranunculaceae (5), Onagraceae (4), and Brassicaceae (4). The number of herbaceous plant species in the sites ranged from 13 to 32 (**Figure 8**). The most commonly species found near the *I. parviflora* individuals were hemicryptophytes, such as *Urtica dioica* (frequency of occurrence in the sites was 100%), *Anthriscus sylvestris* (76%), *Chelidonium majus* (67%), *Geum urbanum* (62%), *Aegopodium podagraria* (57%), and *Alliaria petiolata* (48%).

The most diverse in species were the following sites: Zag with 32 species (23.2%), ATr – 29 (21.0%), Nid, Pla – 26 (18.8%). In the sites, the coverage by *I. parviflora* ranged from 5% (population Zag) to 70% (populations KAS and Juo), the mean for all populations was 35%. In the sites (KAS and Juo) with the most abundant *I. parviflora* (mean coverage 42.5%) the number of species was not large (13 species per site). Coverage by herbaceous plants other than *I. parviflora* ranged from 12.2 to 81.9%, the mean coverage being 42.5%. Total coverage by herbaceous plants (covT) ranged from 31.6 to 99.9% (populations Nid and Aly, respectively), the mean coverage being 76%.

In the cladogram based on the similarities of the assemblages of herbaceous plant species, the sites split into clusters of 14

FIGURE 5 | Principal coordinate analyses of *Impatiens parviflora* individuals of 21 populations according to: **(A–C)** ISSR loci, **(A)** – axis 1 vs. 2, **(B)** – axis 1 vs. 3, **(C)** axis 2 vs. 3; **(D–F)** RAPD loci, **(D)** – axis 1 vs. 2, **(E)** – axis 1 vs. 3, **(F)** – axis 2 vs. 3. Color of the labels denotes location of populations: blue – North-West, gray – Central, and red – South-East part of the country.
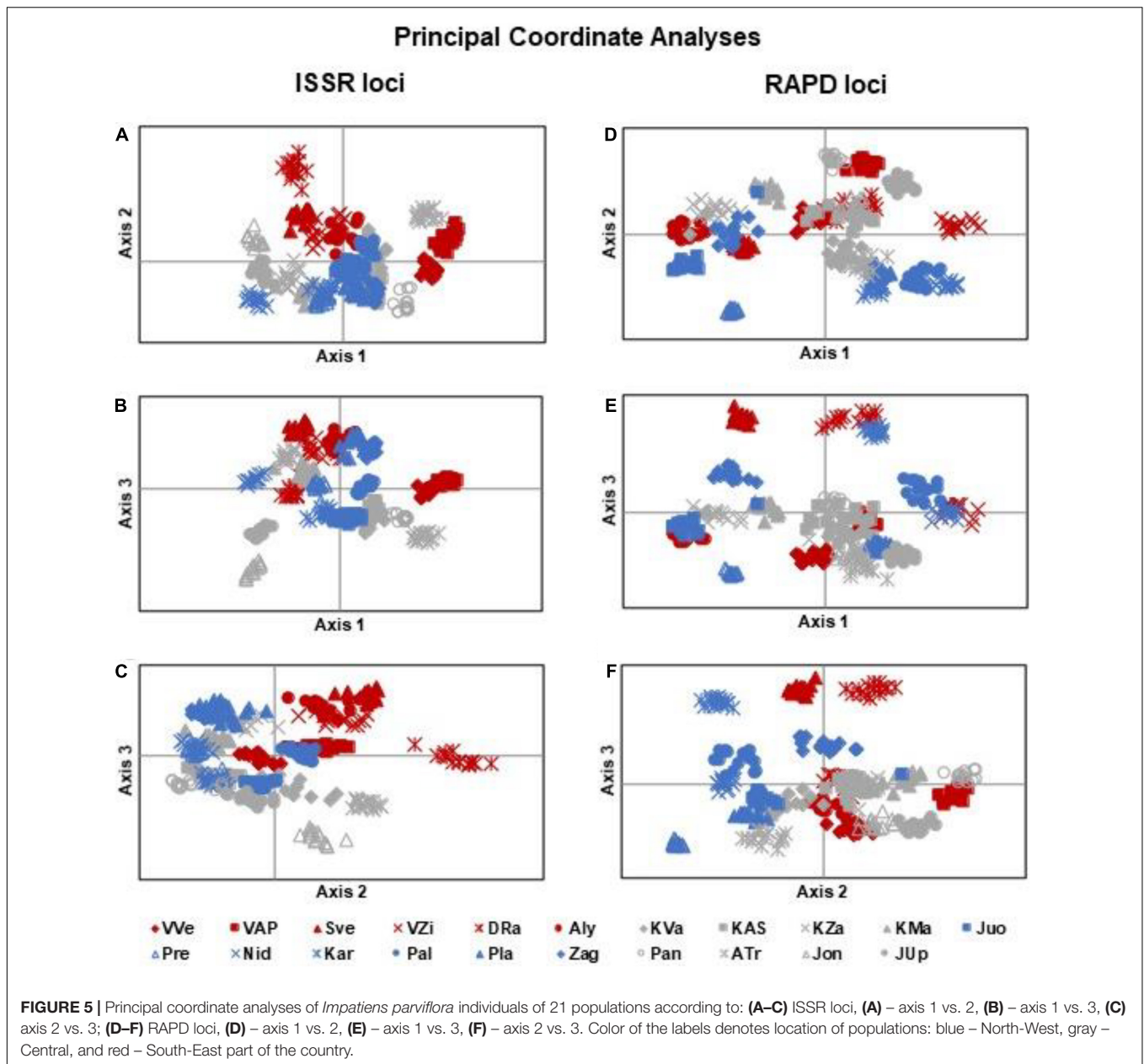
orders (**Figure 9**). Clade of the 1st order involved Zag site only (it had the lowest coverage by *I. parviflora* – 5% and the highest number of herbaceous species – 32).

Clades of the 2nd–6th order (Aly, Sve, ATr, Nid, KMa, KVa, Kar, Jon, and DRa) encompassed the sites which had low *I. parviflora* coverage (mean 20%, range 8–30%), and the mean number of species in these sites was 23 (range 19–29). Clades of 7th–14th order included 10 sites of the most similar assemblages (Pal, Juo, Pla, JUp, Pre, Pan, KAS, VAP, KZa, and VVe) which had high *I. parviflora* coverage (mean 50%, range 30–70%) and the mean number of species was 17 (range 13–26). Neighboring clades encompassed the sites scattered geographically and located near the roads with different traffic intensity. Sites of the most distinct four clades (Zag, Aly,

Sve, and ATr) belonged to the agrarian shrubland biotope, eight out of 10 sites of the 7th–14th order clades belonged to the urban forest biotope.

Assessing the abiotic environment of the sites with Ellenberg indicator values (W-EIV), the range of light (L) between the sites was 4.87–6.37 (Juo and Zag, respectively), temperature (T) – 5.20–6.00 (KZa and Juo, respectively), continentality (K) – 3.63–4.67 (Sve and JUp, respectively), soil moisture (F) – 4.81–6.30 (Nid and Kar, respectively), soil reaction (R) – 4.99–6.91 (KZa and VZi, respectively), and soil nutrients (N) – 5.78–7.22 (Pla and Aly, respectively) (**Figure 10**).

Interrelating parameters of the sites, PLP, coverage, and W-EIV data, Spearman correlations were calculated (**Figure 11**). The PLP-ISSR was positively correlated with the total coverage

FIGURE 6 | Evanno et al. (2005) Δ*K* statistics according to ISSR **(A)** and RAPD **(B)** markers for 21 populations of *Impatiens parviflora*.
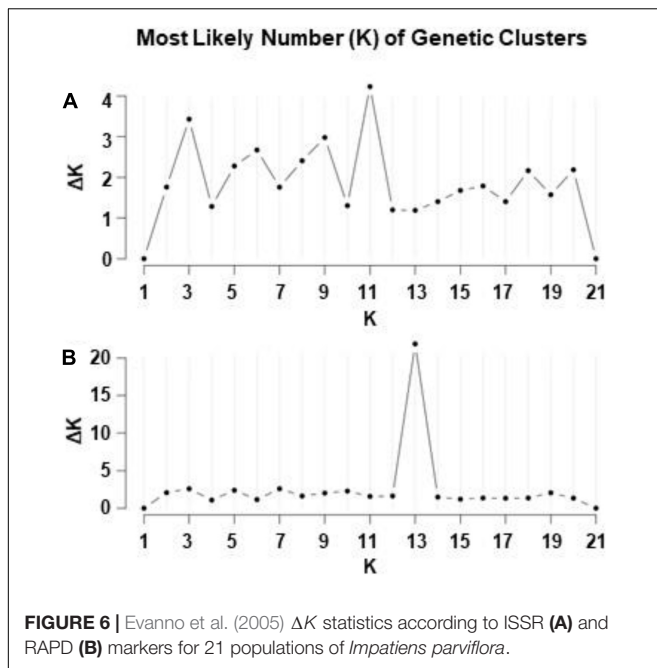
(covT) of herbaceous plant species (Rs = 0.44, *P* < 0.047). The covIP was positively correlated with covT (Rs = 0.45, *P* < 0.039), continentality (K) of the site (Rs = 0.58, *P* < 0.006), and was negatively correlated with the total number of herbaceous species (Rs = −0.23, *P* < 0.044), and light (L) in the site (Rs = −0.70, *P* < 0.001). Total number of herbaceous plant species showed positive correlation with light (Rs = 0.45, *P* < 0.042).

To explain the most important parameters for the variability of *I. parviflora* populations, principal component (PC) analysis was performed (**Figure 12**) on two genetic variables (PLP at ISSR loci and PLP at RAPD loci) and *I. parviflora* coverage, two biotic variables of *I. parviflora* environment (total coverage by herbaceous plant species, and total number of herbaceous species), and six abiotic variables of *I. parviflora* environment (W-EIV traits – light, temperature, continentality, moisture, acidity of the soil, and soil nutrients). The first four PCs were quite informative accounting for approximately 73.64% of the overall variance for the entire variables. For separate principal components PC1, PC2, PC3, and PC4 the variance (with eigenvalues) was: 30.08% (3.01), 18.96% (2.32), 14.35% (1.70), and 10.26% (1.06), respectively. PC1 variability was mainly caused by the number of herbaceous plant species (NSp), light (L), continentality (K), soil moisture (F) and *I. parviflora* coverage (covIP). The contribution of NSp, L, and F was positive while the contribution of K, and covIP was negative. For variability of PC2 PLP-RAPD, PLP-ISSR, the total coverage by herbaceous plant species (covT), soil moisture (F), temperature (T), and soil reaction (R) were the most important. The contribution of PLP-RAPD, PLP-ISSR, covT, and F was positive and the contribution of T, and R was negative. According to the variability of the parameters, displayed in the two principal component biplot by the vectors of different length, the importance of the variables (in descending order) in

PC1 was as follows: covIP > L > K > NSp > F > T > N > PLP-ISSR > R > covT > PLP-RAPD. The order of importance of the variables in PC2 was slightly different: PC2: covT > T > F > R > PLP-RAPD > covIP > N > PLP-ISSR > L > NSp > K. Compared to the remaining sites, (1) the variables L, F, NSp, and covT had higher values and the variables T, R, K, and covIP had lower values for the sites Zag, ATr, DRa, Kar, Aly, and KVa; (2), the variables K, covT, and covIP had higher values, while the variables L, F, T, R, and NSp had lower values for the sites KZa, VAP, Pla, KAS, and Pal; (3) the variables T, R, K, and covIP had higher values and the variables L, F, NSp, and covT had lower values for the sites JUp, Juo, VVe, Pan, and Pre; (4) the variables L, F, T, R, and NSp had higher values, while the variables K, covT, and covIP had lower values for the sites Sve and Nid. The most extreme locations in the PC biplot were characteristic for the sites Kar, Pre, Nid, and Zag.

DISCUSSION

Molecular Features of *Impatiens parviflora* Populations

Invasiveness of alien *Impatiens parviflora* was documented in various parts of Europe with 21% frequency of occurrence among alien plants (Wagner et al., 2017). The most comprehensive and numerous studies devoted exclusively for this species were performed in the countries of Central and Western Europe – Great Britain (Coombe, 1956), Germany (Trepl, 1984), Slovakia (Eliáš, 1999; Jarčuška et al., 2016), Poland (Obidziński and Symonides, 2000; Klimko and Piskorz, 2003; Chmura and Sierka, 2006; Komosińska et al., 2006; Chmura et al., 2007; Chmura, 2014; Reczyńska et al., 2015; Barabasz-Krasny et al., 2018; Grabowska et al., 2020), Belgium (Vervoort and Jacquemart, 2012), Hungary (Csiszar and Bartha, 2008; Csontos et al., 2012), Czechia (Hejda, 2012; Florianová and Münzbergová, 2017; Skálová et al., 2019), and summarized data for many aforementioned regions of the continent (Reczyńska et al., 2015). The first attempts to characterize populations of *I. parviflora* of the Baltic States were rather limited in terms of scope and quantification (Priede, 2009a; Dobravolskaitė, 2012). Congeneric for *I. parviflora* species, *Impatiens glandulifera* is also an annual plant existing in Europe already for the second century, known as top of widespread and worrying alien of the continent. Surprisingly, to date, molecular analyses of *I. parviflora* populations lag far behind *I. glandulifera* analyses performed in various countries by various types and sets of DNA markers (Walker, 2001; Provan et al., 2007; Walker et al., 2009; Zybartaite et al., 2011; Hagenblad et al., 2015; Jocienė, 2015; Nagy and Korpelainen, 2015; Cafa et al., 2020; Kurose et al., 2020; Korpelainen and Elshibli, 2021).

Based on successful application of SSR markers (Provan et al., 2007) for Lithuanian populations of *I. glandulifera* (Jocienė, 2015), analyses we employed the same simple sequence repeat markers for *I. parviflora* analyses. The decision was supported by the fact that SSR markers developed for the *Impatiens lateristachys* appeared to be applicable for the other four congeneric species *I. oxyanthera*, *I. faberi*, *I. imbecilla*, and
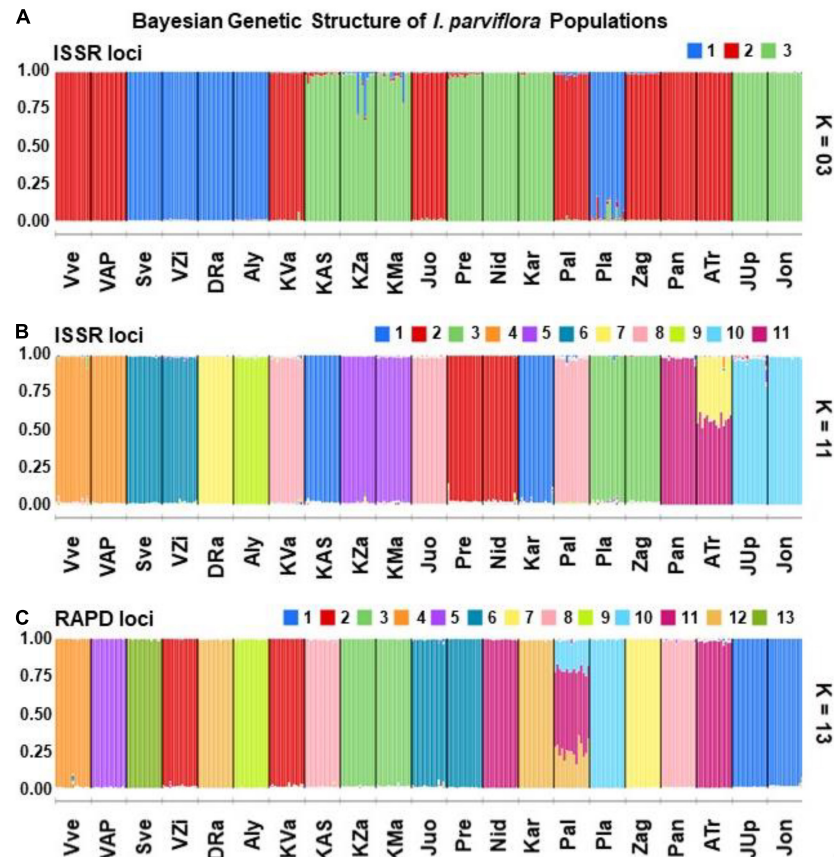
FIGURE 7 | Barplots of the membership of *Impatiens parviflora* individuals/populations from the 21 sites in each of K genetic clusters identified from the ISSR or RAPD datasets using the Bayesian clustering in the STRUCTURE: **(A)** ISSR (K = 3, as next to the highest K); **(B)** ISSR (K = 11, the highest K); **(C)** RAPD (K = 13, the highest K). White lines separate bars of different individuals, black lines separate bars of different populations. Bars are partitioned into K colored segments that represent the individual's/population's estimated membership fractions (the amount of the genetic diversity) in K clusters. Three letters denote the populations (Pop) (for details of their locations, see **Figure 1**).

I. rostellata (Li et al., 2008). In addition, such strategy was also based on the close position of *Impatiens parviflora* and *Impatiens glandulifera* in the *Impatiens* phylogenetic trees built on the basis of nDNA and cpDNA markers (Yuan et al., 2004; Janssens et al., 2006, 2007). In our study of *I. parviflora* no alleles were detected in case of one out of six SSR markers, four out of five remaining markers were monoallelic and one marker was diallelic (Kupcinskiene et al., 2013). According to the length of SSR locus (IGNSSR203-EF025994) *I. parviflora* populations in Lithuania were very similar by size of the amplified DNA fragments to those of *I. glandulifera* populations in Great Britain (Provan et al., 2007). The remaining loci were either shorter in several nucleotides (IGNSSR240-EF025997; IGNSSR101-EF025990) or longer (IGNSSR240-EF025997). Comparison of *Impatiens parviflora* and *Impatiens glandulifera* data of the same SSR loci revealed bigger numbers of alleles for populations of *I. glandulifera* examined in a similar scope study (Jocienė, 2015). Species specific structure of evaluated loci could explain the observed differences.

To compensate shortage of SSR markers for *I. parviflora*, inter simple sequence repeats (ISSRs) were chosen as SSR-related

markers, located between microsatellite sites in the genome (Zietkiewicz et al., 1994). Possibly similar reasons for the selection of ISSR were in the numerous assessments of other *Impatiens* species (Hatcher et al., 2004; Gao et al., 2012; Li et al., 2013; Zhong et al., 2014), with the latest ISSR use approvement for analyses of genetic diversity on *Impatiens* (Tamboli et al., 2018).

Comparison of our *I. parviflora* molecular data (21 populations, 315 individuals) with *I. glandulifera* results obtained applying the same eight RAPD markers, examining similar numbers of individuals and populations of close geographical location (20 populations, 400 individuals) showed, that the percentage of polymorphisms at RAPD loci in *I. parviflora* populations (mean PLP 21.0; **Figure 2**) was twice lower than the *I. glandulifera* populations (mean PLP 46.1) (Zybartaite et al., 2011). The genetic diversity at ISSR loci in our populations of *I. parviflora* was even lower than that of RAPD loci (mean PLP – 16.5). Unique alleles were observed in *I. parviflora* populations located very close to Western (Pre, Nid, Kar, Pal), Northern (Zag), and Eastern borders (Sve) of the country. *I. parviflora* populations (KVa, KAS, KZa, and KMa) near the second largest city of Lithuania (Kaunas) were also
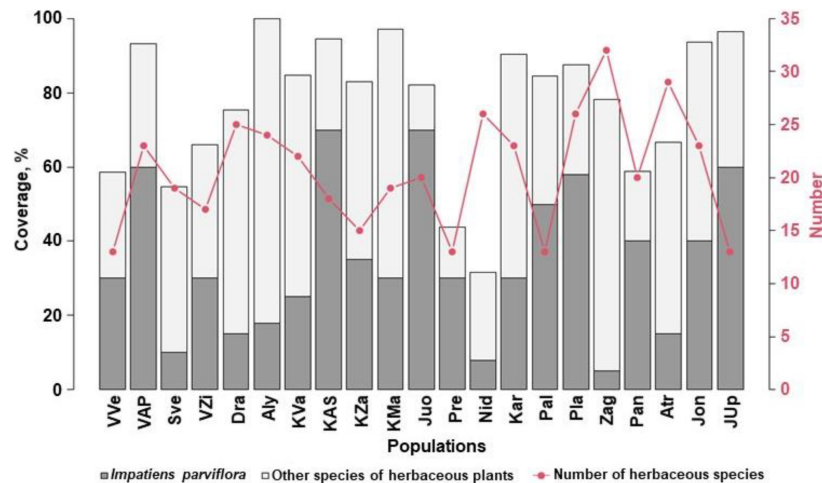
FIGURE 8 | The number of herbaceous species and coverage by *Impatiens parviflora* and herbaceous plant species in the sites. Three letters denote populations (for details of their locations, see **Figure 1**).

characterized by unique alleles, whereas no unique alleles were found in the populations near the largest city (Vilnius) and the Southern border of the country. There are many factors that may determine the diversity level of populations. Absence of data about the native populations of *I. parviflora* does not allow to relate low genetic polymorphism at RAPD or ISSR loci either to species level features or population level fitness. Often, new populations are formed by a few individuals, which potentially lead to a decreased population-level genetic diversity as a result of genetic bottle necks and founder effects (Nei, 1978).

Although information on the diversity of *I. parviflora* populations at six SSR loci (Provan et al., 2007) was not obtained, application of other distinct marker systems (ISSR and RAPD) allowed us to reach the same conclusion about the possible multiple introductions of *I. parviflora* within the area of the investigation.

Data about *I. parviflora* performance in temperate climate forests of Europe (Chmura, 2014; Jarčuška et al., 2016) were complemented by our study on species behavior in hemiboreal forest zone (**Figure 1**). Levels of invasion of aliens have been found to increase with increasing proportion of urban land and proximity to roads (Wagner et al., 2021). In Latvia, *I. parviflora* was most commonly registered in the vicinity of roads in the semi-natural slightly disturbed forest (Priede, 2009b). Our study encompassed environmental characteristics of *I. parviflora* populations including several biotopes (urban forest, agricultural shrubland, and riparian forests, the main habitats in which this species usually grows in Lithuania) and traffic intensity differences along the road close to the site (**Table 1**). Species occurrence in Vilnius, 1934, was not supported by the botanical literature of the 1940s about species in the areas located west from Vilnius (toward the Baltic Sea). During the subsequent period of supposed intensive spread of *I. parviflora*, various socio-economic communications for nearly a half of century were limited to the Soviet Union. Due to this reason, geographical zonation within the country was also taken into account in our

studies of invasion pathways. We determined genetic variation of *I. parviflora* in 21 sites across a longitudinal gradient (East-West) that might reflect the invasion history of the species within the country.

Presence of significant isolation of populations by distance (Mantel test) was documented only in case of RAPD loci (**Figure 3**). Based on ISSR and RAPD data, UPGMA clustering of populations did not reveal relations of populations neither to the geographical location nor to the traffic intensity
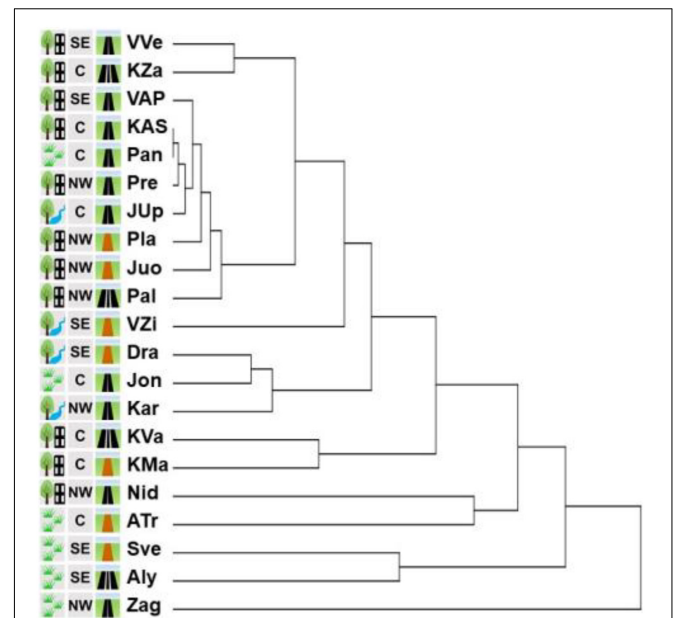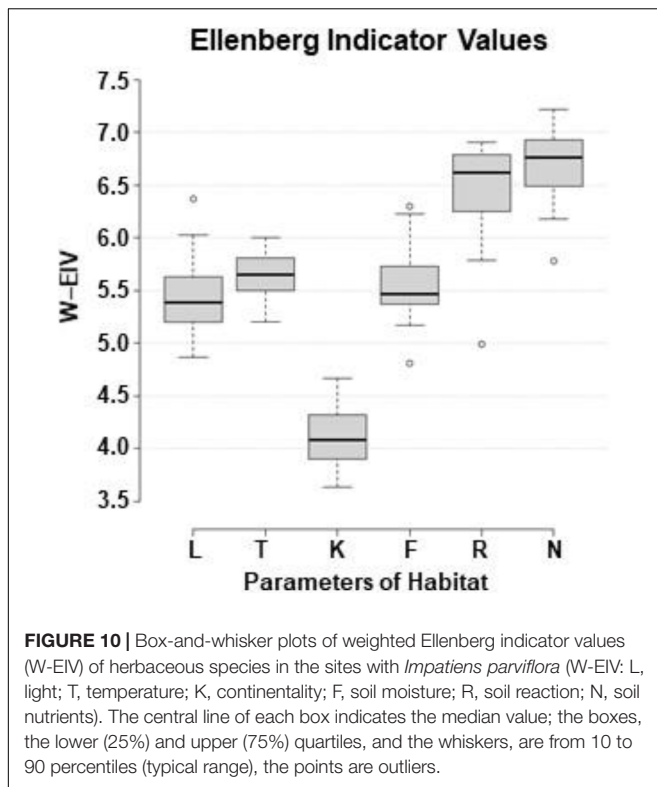

FIGURE 9 | Cluster analysis of the similarity of the assemblages of herbaceous plant species in 21 sites with *Impatiens parviflora*. Three letters denote populations (location is provided in the **Figure 1**. Pictogram explanations are provided in the **Table 1**).

FIGURE 10 | Box-and-whisker plots of weighted Ellenberg indicator values (W-EIV) of herbaceous species in the sites with *Impatiens parviflora* (W-EIV: L, light; T, temperature; K, continentality; F, soil moisture; R, soil reaction; N, soil nutrients). The central line of each box indicates the median value; the boxes, the lower (25%) and upper (75%) quartiles, and the whiskers, are from 10 to 90 percentiles (typical range), the points are outliers.

FIGURE 11 | The correlogram represents the Spearman correlation coefficients (Rs) from −1 to 1 for all pairs of variables in the sites with *Impatiens parviflora*. Blue color represents positive and red color represents negative correlations. The intensity of the color indicates the strength of the correlation, so the stronger the correlation, the darker the figures. Correlations numbers inside colored figures indicate the level of significance, thin ellipsoid figures show significant (*P* < 0.05), figures toward spherical shape – insignificant correlations. PLP-RAPD, percentage of polymorphic loci at randomly amplified polymorphic DNA; PLP-ISSR, percentage of polymorphic loci at inter simple sequence repeats; covIP, coverage by *I. parviflora*; covT, total coverage by herbaceous plant species; NSp, number of herbaceous plant species; L, light; T, temperature; K, continentality; F, soil moisture; R, soil reaction; N, nutrients as weighted Ellenberg indicator values (W-EIV).

along the road (**Figure 4**). In the case of RAPD loci based dendrogram, urban forest biotope was very rare among the most dissimilar populations and was prevailing among the most similar populations (three of 13 cases and seven of eight cases, respectively). With the exception of the Juo population, the populations (VVe, VAP) most similar at RAPD loci were sampled from the sites close to the biggest cities of Lithuania (Vilnius, Kaunas, and Panevėžys). The dendrograms of genetic relationships at ISSR and RAPD loci were similar according to the most distant populations: one of the three most distinct populations was located near the southern border of the country (DRa) while opposite results were detected for populations located near Vilnius (VVe).

An important factor influencing genetic diversity within the introduced range of invasive species is the number of introduction events (Bossdorf et al., 2005). The differences found among the population dendrograms built on RAPD and ISSR data could be partially explained by the distinct nature of RAPD and ISSR loci.

Unweighted pair group method with arithmetic mean cluster analyses at both ISSR and RAPD loci and the lack of correlation between genetic and geographic distances at ISSR loci (**Figures 3**, **4**) suggest that the populations might have received genotypes from several donors. Hereby, our study supports the assumption that multiple introductions of invasive plants appear to be dominant pattern of species introduction (Hagenblad et al., 2015).

We used hierarchical AMOVA to group populations according to various environmental criteria to determine which
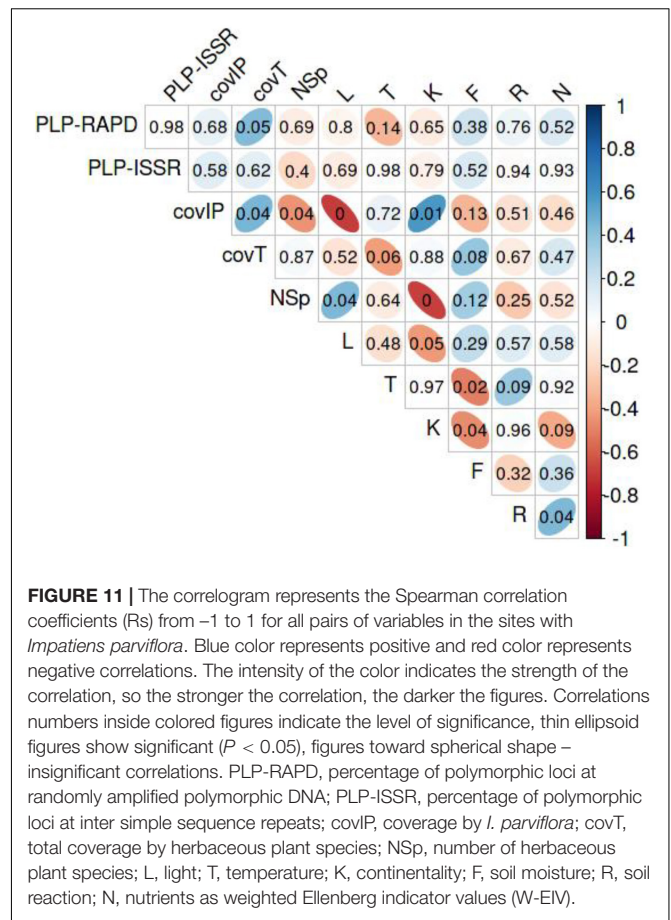
of these factors would divide the populations into significant differentiation groups. Hierarchical analyses of molecular variance at both type markers' loci revealed significant small extent differentiation (causing 6% of the total variation at ISSR loci and 2% of the total variation at RAPD loci) among population groups of distinct geographic zones (**Figure 1** and **Table 2**). The least polymorphic at both ISSR and RAPD loci were populations located by the western border near the Baltic Sea (PLP 14.55 and 16.45, respectively). The geographical subdivision of our study populations into North-West, Central and South-East corresponded to the climatic zones based on several temperature parameters (average annual temperature, warmest and coldest months, absolute max and absolute min, annual precipitation, period with snow cover, and duration of sunshine) (Lithuanian Hydrometeorological Service under the Ministry of Environment, 2021). Hereby, significant differentiation among the populations of North-West, Central and South-East parts of the country might be related to climate effect. This observation was also supported by positive correlation of the covIP with continentality (K) of the site (Rs = 0.58, *P* < 0.006) (**Figure 9**).

Significant differentiation of population groups at one of the two markers loci was related to the road type (causing 2% of the total variation at ISSR loci), and biotope (causing 2% of the total variation at RAPD loci). Among population groups with
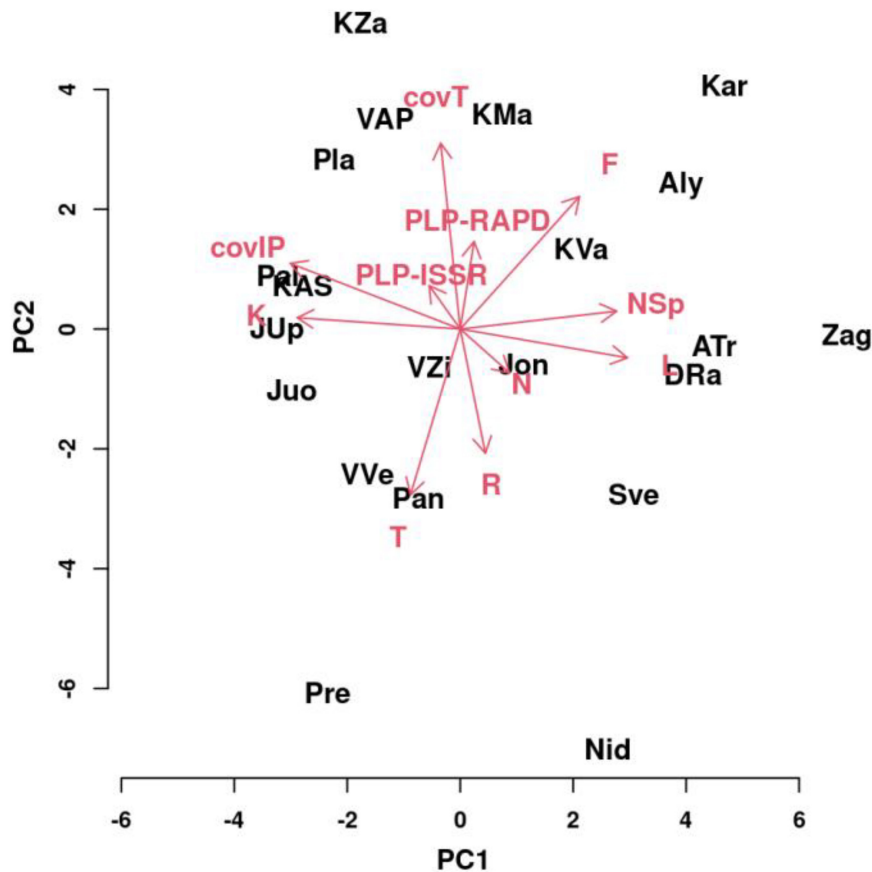
FIGURE 12 | Biplot of the principal component analysis for the first two principal components of a model testing variation of populations depending on molecular markers (PLP at ISSR and RAPD loci), coverage of *Impatiens parviflora*, total coverage of herbaceous plant species, the number of herbaceous plant species, and abiotic environment features such as W-EIVs' (L, light; T, temperature; K, continentality; F, moisture; R, pH; N, nutrients). Three black letters denote populations and red arrows with letters denote variables.

different traffic intensity roads, the least polymorphic at ISSR loci (PLP 13.89) were *I. parviflora* populations located near the low intensity traffic road with a blacktop. The least polymorphic at RAPD loci (PLP 17.09) were *I. parviflora* population groups of riparian forest, when compared to population groups of urban forest or agrarian shrubland. The small among-region variance component implies that regions are only slightly differentiated from one another. Low differentiation among groups of populations belonging to distinct geographic zones was evident from PCoA plots (**Figure 5**). In agreement with AMOVA results were PCoA data, indicating the biggest differentiation among populations within groups, and much lower extent of differentiation among individuals within populations. For invasive species, low intrapopulation genetic differentiation may be due to a combination of genetic drift, founder effects, a short life span, autogamy or limited pollination or dispersal of seeds, small number of individuals in a population, and environmental variation (Barrett and Husband, 1990).

Based on the large number of genetic clusters identified by two different molecular markers (11 according to ISSR loci and 13 according to RAPD loci) (**Figures 6**, **7**), Lithuanian

I. parviflora populations highly differed from the number of gene clusters of the congeneric species *Impatiens glandulifera* populations sampled from three continents employing other molecular markers (three gene clusters at simple sequence repeats loci) (Hagenblad et al., 2015; Nagy and Korpelainen, 2015). Multiple introductions for European *I. glandulifera* were suggested based on numerous assessments (Hagenblad et al., 2015). We did not find relations between Bayesian genetic clusters and geography of the sites with *I. parviflora*, suggesting unrestricted geographically gene flow. Our Mantel test, PCoA and Bayesian clustering data (**Figures 4–7**) are in support for multiple introductions of *I. parviflora*. Since the naturalization of *I. parviflora* in all neighboring countries were registered earlier species might entered Lithuania from several places. We must point out three as the second most probable number of the gene clusters at ISSR loci (**Figure 6**) and this could be related to the probable external donors. In most cases the extreme locations in the biplot (**Figure 12**) of principal component analyses were characteristic for the populations close to the borders of the country, either western (Kar, Pre, Nid) or northern (Zag). Quite distant location in the biplot was also

characteristic for the populations close to the eastern border (Sve) or the southern border (DRa). Therefore, our study of genetic diversity of *I. parviflora* populations indicates multiple introductions of this species in Lithuania. In addition, invaded areas might become re-colonized from the other sites due to high production of seeds (tens, hundreds up to 10,000 seeds might be produced annually by an individual) (Coombe, 1956; Trepl, 1984). Presence of two pairs of geographically close populations with the same prevailing gene cluster at two different loci (Jon and JUp, also KZa and KMa) (**Figure 7**) might be the evidence for occurrence of short distance unidirectional transport of seeds.

Indications Interrelating Invasiveness and Invasibility

Negative correlation (**Figures 9**, **11**) between coverage of *I. parviflora* and herbaceous plant diversity of invaded forests supports the facts documented by other European forest studies (Obidziński and Symonides, 2000; Chmura and Sierka, 2006; Jarčuška et al., 2016). Coverage of *I. parviflora* was used as a measure of invader success (Jarčuška et al., 2016).

In the dendrogram of similarity of assemblages of herbaceous plant species in the sites with *I. parviflora* (**Figure 9**), with increasing similarity of the assemblages, *I. parviflora* coverage was also increased, whereas the number of herbaceous plant species in the sites (**Figure 8**) was decreased. Eight out of 10 the most similar sites according to herbaceous plant species belonged to urban forest. The dendrogram of similarity of assemblages of species was very similar to the genetic dendrogram (built for RAPD data) in terms of the most genetically similar populations (**Figure 4B**), among which seven of eight also belonged to urban forest. Based on our results, it might be concluded, that both the genetic diversity and herbaceous species diversity could be diminished in urban forest.

Biotope-related differences in association with environment conditions were noted among our study sites. Agricultural shrubs as the open areas with single trees and shrubs comprised numerous herbaceous plant species, especially those with the higher demand for light (Zag) and moisture. Riverside forests had higher numbers of species which require the soil of high humidity and fewer species with the higher demand for light than agrarian shrubs, but more than urban forests. Abundance of *I. parviflora* was highest in urban forests with the lowest number of herbaceous plant species with higher demand for light. In urban forests with *I. parviflora* signs of eutrophication were obvious, with the tree species such as *Acer pseudoplatanum*, *Tilia cordata*, *Quercus robur*, *Fraxinus excelsior*, and *Ulmus glabra*, which were typical for fairly fertile, non-acidic soils.

There are many studies which aimed to evaluate environment characteristics of European *Impatiens parviflora* based on EIV of neighboring herbaceous plant species (Chmura, 2014; Reczyńska et al., 2015; Jarčuška et al., 2016; Barabasz-Krasny et al., 2018). Despite difference in latitudes, defined by EIV (**Figure 10**) temperature conditions at our sites were similar to those in Poland (Reczyńska et al., 2015) or Slovakia (Jarčuška et al.,

2016). Values of our *I. parviflora* sites for continentality, moisture and soil reaction were slightly higher compared to the aforementioned studies.

Impatiens parviflora is well-known as a species growing in semi-shaded, shaded habitats (Coombe, 1956; Trepl, 1984). Studies of *I. parviflora* with defined EIV were conducted in different biotopes with a wide variety of dominant tree species such as alders, poplars, willows, elms, oaks, beeches, fir, and fir-spruce forests (Chmura, 2014; Reczyńska et al., 2015; Jarčuška et al., 2016) which had different light conditions for understory herbaceous species. Our results coincide well with most these studies, although light demand was higher compared to those performed with underground cover of a tree layer ≥50% (Jarčuška et al., 2016). In support to many other studies, our data show that covIP was negatively correlated with light (L) in the site (Rs = −0.70, $P < 0.001$) (**Figure 10**).

Analyses of European woodlands disclosed the fact, that high soil nutrient levels are favorable factors for alien plant invasions (Wagner et al., 2017). Our results indicate that Lithuanian sites with *I. parviflora* might be distinguished by high levels of soil nutrients (**Figure 8**): the mean of Ellenberg indicator values (EIV) was very high (8 among possible top score 9) for the soil nutrients among 6 the most common herbaceous plant species growing near the *I. parviflora* (Ellenberg et al., 1992), while mean range for our populations was lower (6.49–6.93; **Figure 10**). Our results are in agreement with the positive association between nutrient (EIV) availability and *I. parviflora* coverage observed in understories in Western Carpathians (Jarčuška et al., 2016). In oak forests of Central Europe, *I. parviflora* was common in soils of medium or higher fertility and was absent or infrequent in less fertile areas (Reczyńska et al., 2015).

A wide range of information is available on genetic diversity and variability of invasive species at a geographic level (Meekins et al., 2001; Hagenblad et al., 2015). However, the interaction between genetic characteristics and neighboring species in sites has been poorly documented until now. Relationships between genetic differentiation and various environmental variables such as temperature, humidity, and nitrogen were documented for *P. arundinacea* within invasive distribution range (Kettenring et al., 2019). For a better interrelation of the genetic and community data, we conducted correlation and a principal component analyses (**Figures 11**, **12**). In our study, the only case of significant relations was observed: the PLP-ISSR was positively correlated with total coverage (covT) of herbaceous plant species (**Figure 11**). We did not find significant relationships between *I. parviflora* molecular polymorphisms and site characteristics based on EIV of herbaceous plant species. Coverage by *I. parviflora* (covIP) was the most important variable of populations for the 1st principal component and total coverage by herbaceous plants (covT) was the most important variable for the 2nd principal component (PC2; **Figure 12**). Importance of molecular data for PC1 was less significant compared to weighed Ellenberg indicator values of herbaceous plant species for light, temperature, continentality, soil moisture, and nutrients. Importance of molecular data for PC2 was less compared to temperature, soil moisture, and soil reaction. According to the principal component analysis, the coverage by *I. parviflora* was

more important variable of populations compared to molecular data or parameters of abiotic environment.

CONCLUSION

Use of non-indigenous species to study genetic variation is not only valuable in an evolutionary context, but might also be important for the management of invasion control and eradication. Complexities in human-nature interactions exist across multiple invasiveness and invasibility scales. The present study showed that the pervasion of *I. parviflora* in Lithuania was likely due to human-mediated activities, such as nature tourism, especially fishery, road building that are transporting seeds along road borders, which may also explain the lack of a relationship between genetic variation and neighboring species. Other molecular tools, including amplified fragment length polymorphism markers and chloroplast DNA, could also be used in conjunction with ISSR and RAPD data to present a more complete picture. Further studies of molecular, ecological, and geographic relationships of *I. parviflora* populations should examine sites across a broader range from Western Europe to Eastern part of Eurasia. This would help to evaluate the impact of multiple East-South Asian introductions of *I. parviflora*

populations to Europe and provide a more extensive overview of *I. parviflora* genetic diversity in Europe.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

EKu conceived and designed the study. EKu, LJ, and RJ collected the material in the field. RJ, GR, and LJ obtained the laboratory data. TR, VM, EKr, and RJ did the statistical analyses and data visualization. RJ, LJ, EKu, EKr, VM, TR, and AP wrote the manuscript. All authors have read and agreed to the final version of the manuscript.

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