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Genetic diversity of populations of *Bidens* genera invasive and native species in Lithuania

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Abstract

River riparian ecosystems deteriorate because of anthropogenic factors that are related to industry, intensive agriculture and tourism. Two native species, *Bidens tripartita* and *B. cernua*, are weeds widespread in Lithuania on river banks and other habitats. An alien species, *B. frondosa*, is also widespread in the same habitats. We investigated the genetic diversity of *B. frondosa*, compared its population structure to that of related species and assessed the possibility of genetic interaction between *B. frondosa* and native congeners. Genetic analysis of populations was based on inter-simple sequence repeats (ISSR) polymorphism. Our results revealed high genetic differentiation in the *B. frondosa* populations (48.2%) and high genotypic diversity ($i = 0.99$). Bayesian cluster analysis using the program *Structure* revealed signs of gene flow between *B. frondosa* and the native species; however, principal coordinate analysis did not support this result. Our results do not indicate the role of interspecific hybridization in the invasiveness of *B. frondosa*.

Key words: *Bidens*, hybridization, invasive species, population genetic structure.

Introduction

Human-mediated changes in the river environment create favourable conditions for non-native species introduction, long-term settlement and spread (Richardson et al., 2007). The spread of alien plant species in the new area can endanger or even destroy the integrity of natural communities because of the negative effects of invaders on native species and ecosystem stability (Pauchard, Alaback, 2004). It may initiate evolution of weed communities and appearance of new weeds in agroecosystems (Danuso et al., 2012). Rivers are important corridors for alien plant dissemination because propagules can be carried along the river by water, wind, humans and animals (Johansson et al., 1996; Richardson et al., 2007). High levels of disturbance that are typical of river banks in anthropogenically affected territories are also beneficial for the establishment of newcomers. Two annual species of the genus *Bidens* (*B. cernua* L. and *B. tripartita* L.) are native to Lithuania and common in riverside plant communities. The non-native congener *B. frondosa* L. was first observed in 1983 in the middle

reaches of the Nemunas River and recently has become widespread along this river and its tributaries and on the shores of the Curonian Lagoon (Gudžinskas, 1997; 2003). Quite often, this species occurs in the wet forests or in human-modified habitats (Gudžinskas, 1997).

B. frondosa is considered one of the most invasive species in Europe (Lambdon et al., 2008), and recently, this plant species has been included in the list of invasive plants of Lithuania. In some European countries this species is known as agricultural and environmental weed (Groves, 1999). *B. frondosa* spreads in arable fields of North Italy and is frequently found in summer crops (sugarbeet, maize, etc.). In Lithuania, *B. frondosa* and its close relative *B. tripartita* are weeds that are usually found in uncultivated areas (Čiuberkis, Vilkonis, 2013).

In Europe, *B. frondosa* was introduced from North America, where its native range occupies a large territory extending from Alaska into the central part of the continent. Supposedly *B. frondosa*, as with some other invasive plants, escaped from European botanical

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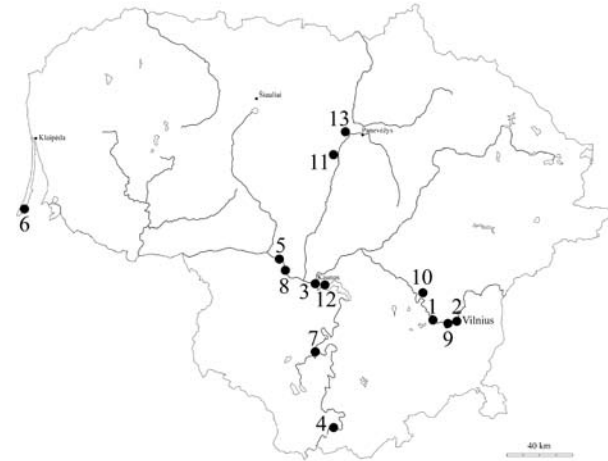
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gardens and spread into natural ecosystems (Galkina et al., 2015). The intensive expansion of this weed began after World War II, and the species became naturalized in many European countries (Chytrý et al., 2008), as well as in Lithuania (Gudžinskas, 1997; 2003). *B. frondosa* produces many seeds, which adhere to the surface of animal fur or human clothing and can be easily dispersed over long distances. The river water flow is also an important factor in the seed dispersal of *B. frondosa* (Kostrakiewicz-Gieralt, Zajac, 2014). *B. frondosa* has a highly flexible vegetative phase and can grow taller and produce more seeds than its congener, *B. tripartita* (Danuso et al., 2012). In parts of Europe, *B. frondosa* has displaced the native *B. tripartita* and *B. cernua* in some habitats (Vinogradova et al., 2014). According to the literature, the impact of alien species on native plant communities and native congeners could be dual: ecological – out-competed native species, and genetic – interspecific hybridization between the invader and native species (Bleeker et al., 2007). Interspecific hybridization is known to sometimes produce genotypes with increased weedy properties and invasiveness (Ellstrand, Schierenbeck, 2000). The genetic impact of *B. frondosa* on the native species is controversial because plants of intermediate morphology have been found in natural ecosystems; however, no proof of hybridization has been obtained for the studied genotypes (Crowe, Parker, 1981; Vinogradova et al., 2014; Galkina et al., 2015). The analysis of the genetic structure of invasive populations of *B. frondosa* has not received sufficient attention until now and is limited to a genetic diversity analysis of a small number of genotypes (Galkina et al., 2015).

The aim of our study was (1) to explore *Bidens frondosa* population structure and genetic diversity measures and compare these parameters to those of two native species *B. tripartita* and *B. cernua* and (2) to determine if a genetic interaction exists between *B. frondosa* and the native species.

Materials and methods

Plant materials. Populations were studied in river ecosystems that were considered possible corridors for the spread of *Bidens frondosa* in Lithuania. The study was performed in 2015–2016. On the river banks, *B. frondosa* grows as big stands near water. *B. frondosa*, *B. tripartita* and *B. cernua* plants were collected on the Nemunas, Neris, Vokė, Nevėžis and Jiesia rivers, and lagoon shores (Fig. 1).



Note. The sites indicated by the numbers as in Table 1.

Figure 1. Geographic location of the sample collection sites of *Bidens* species in Lithuania

A total of 286 plants were collected from 23 populations in 13 different sites (Table 1).

Samples of *B. frondosa* were gathered randomly with a minimal distance between samples of at least 8–10 metres. The number of *B. frondosa* plants collected per population ranged between 6 and 20. The native

Table 1. Collection sites and habitats of *Bidens frondosa* and its congeneric native species, *B. tripartita* and *B. cernua*

Site No.	Site name	<i>Bidens</i> species	Latitude, N	Longitude, E	Habitats	River
1.	Grigiškės	<i>B. frondosa</i>	54°40'21"	25°05'08"	Riverside, near gardens	Vokė
		<i>B. tripartita</i>	54°39'55"	25°06'12"		
		<i>B. cernua</i>	54°39'55"	25°06'12"		
2.	Vilnius	<i>B. frondosa</i>	54°41'41"	25°18'05"	Riverside, city centre	Neris
		<i>B. tripartita</i>	54°41'20"	25°17'29"		
3.	Kaunas	<i>B. frondosa</i>	54°53'36"	23°53'33"	Riverside, city centre	Nemunas
		<i>B. tripartita</i>	54°53'34"	23°53'47"		
		<i>B. cernua</i>	54°53'34"	23°53'47"		
4.	Druskininkai	<i>B. frondosa</i>	54°01'24"	23°58'35"	Riverside, city centre	Nemunas
		<i>B. tripartita</i>	54°01'24"	23°58'35"		
		<i>B. cernua</i>	54°01'24"	23°58'35"		
5.	Vilkija	<i>B. frondosa</i>	55°02'32"	23°33'53"	Riverside, near path	Nemunas
		<i>B. tripartita</i>	55°02'38"	23°33'17"		
6.	Nida	<i>B. frondosa</i>	55°19'02"	21°01'14"	Curonian lagoon	
		<i>B. cernua</i>	55°19'02"	21°01'14"		
7.	Merkinė	<i>B. frondosa</i>	54°09'29"	24°09'29"	Riverside near bridge	Nemunas
8.	Kulautuva	<i>B. frondosa</i>	54°56'27"	23°37'46"	Riverside, landing-place	Nemunas
9.	Žvėrynas	<i>B. frondosa</i>	54°41'36"	25°14'19"	Riverside near buildings	Neris
10.	Šilėnai	<i>B. frondosa</i>	54°43'21"	25°02'49"	Riverside near angler path	Neris
11.	Krekenava	<i>B. frondosa</i>	55°32'11"	24°05'29"	Riverside near grassland and the bush	Nevėžis
		<i>B. cernua</i>	54°51'29"	23°56'02"		
12.	Jiesia	<i>B. tripartita</i>	54°51'26"	23°55'52"	Riverside near path	Jiesia
		<i>B. cernua</i>	54°51'29"	23°56'02"		
13.	Varpučiai	<i>B. frondosa</i>	55°43'10"	24°13'34"	Riverside, livestock watering-place	Nevėžis

populations of *B. tripartita* and *B. cernua* are also spread in the riverside habitats. *B. tripartita* and *B. cernua* population samples were collected from areas where the plants were intermixed with *B. frondosa* (both native species in Druskininkai) and from areas where they grow in the vicinity (*B. tripartita* in Grigiškės or *B. cernua* in Vilkija) or grow separately (*B. tripartita* and *B. cernua* in Jiesia) from *B. frondosa*. In some collection sites, only the invasive *B. frondosa* was found (Table 1).

DNA extraction and inter-simple sequence repeats-polymerase chain reaction (ISSR-PCR). Total genomic plant DNA was extracted from the dried leaves. DNA extraction and purification was performed using the Genomic DNA Purification Kit, K0152 (Thermo Fisher Scientific Baltics, Lithuania), according to the manufacturer's instructions. The concentration and quality of the DNA samples were assessed spectrophotometrically and by electrophoresis on agarose gels.

ISSR polymorphism was assessed using four primers previously used by Tanahara and Maki (2010) in *Bidens* studies. ISSR-PCR with these primers produced clear and reproducible patterns of DNA bands in agarose gels. The ISSR-PCR reaction mixtures had a total volume of 10 μ l. The reaction mixtures contained 0.5 U of recombinant *Taq* DNA polymerase (Thermo Fisher Scientific Baltics), 0.1 μ M primer (Biomers, Germany), 0.2 mM of each dNTP, 1 \times *Taq* buffer with (NH₄)₂SO₄ (Thermo Fisher Scientific Baltics), 2.5 mM MgCl₂ and 10 ng of template DNA. Amplification was performed in a thermocycler (Biometra GmbH, Germany) programmed as follows: 4 min at 95°C for the initial denaturation, 45 cycles of 30 s at 95°C (denaturation), 45 s at 50°C (annealing) and 2 min at 72°C (extension). The final reaction extension step was carried out at 72°C for 5 min (Tanahara, Maki, 2010). The reproducibility of the DNA band patterns of the studied genotypes was tested by repeating experiments at least twice. ISSR-PCR products were separated on 1.5% agarose gels in 0.5 \times TAE buffer under conditions of constant voltage of 4.0 V cm⁻¹ for 3.5 h. The size of the scored DNA fragments was estimated with the DNA band size standard GeneRuler 1 kb DNA Ladder (Thermo Fisher Scientific Baltics) using software *BioDocAnalyze* (Biometra).

Statistical analysis. ISSR polymorphisms data generated from 286 individual plants were scored into a binary matrix according to the absence (0) or presence (1) of ISSR fragments. An analysis of molecular variance (*AMOVA*) was carried out using software *GenAlEx* v.6.5 (Peakall, Smouse, 2012). Based on the DNA band presence / absence binary matrix, the expected heterozygosity (*H_j*) was calculated using program *AFLP-*

SURV v.1.0 (Vekemans et al., 2002). *AFLP-SURV* v.1.0 was also used to evaluate the genetic differentiation (*F_{st}* – fixation index) among populations. A significance test for *F_{st}* was based on 1000 permutations. The percentage of polymorphic bands (PLP), the polymorphic band proportion at the 5% level (PLP 5%) and band richness (Br) were calculated in program *AFLPdiv* v.1.1 (Coart et al., 2005) for five individuals per population used for rarefaction. Shannon's diversity index (*I*) was calculated using software *POPGENE* v.1.32 (Yeh et al., 1997). Genotypic diversity was assessed by calculating the proportion of distinguishable genotypes: $i = G/N$, where *G* is the number of genotypes, and *N* – the total number of studied individuals. The hierarchical *AMOVA* was performed in software *GenAlEx* v.6.5 (Peakall, Smouse, 2012) to evaluate partitioning of diversity within (Φ_{PT}) and among (Φ_{RT}) species. The significance test for variance components was based on 999 permutations. In addition, we used principal coordinates analysis (PCoA) implemented in software *GenAlEx* v.6.5 to evaluate the relations among the populations. A software package *TREECON* for *Windows* was used to evaluate the genetic distances among genotypes and identify the identical genotypes (Van De Peer, De Wachter, 1994). To assess genetic structures of *Bidens* species population and allocate genotypes to clusters, we performed Bayesian cluster analysis using the program *Structure* v.2.3.4. The "admixture" model was chosen for analysis. After a period of testing (500 000 cycles), 500 000 MCMC repetitions were done for a number of clusters (*K*) ranging from one to 23.

We used the Mann-Whitney *U* test for a standardized sample size (five individuals from each population) and population number (six populations per species) to investigate whether statistically significant differences existed among the population diversity measures of *B. frondosa*, *B. tripartita* and *B. cernua*. The statistical analysis was performed using software *IBM® SPSS® Statistics* v.23 for *Windows*.

Results

Molecular diversity at the species level. We scored 85 reproducible polymorphic DNA bands that were generated with four primers. A similar number of polymorphic DNA bands were detected in ISSR profiles of studied species: *B. frondosa* – 71, *B. tripartita* – 72 and *B. cernua* – 71. On average, 18 bands were identified with one primer for each species. The sizes of the DNA fragments used for analysis were 250–2000 bp (Table 2).

Table 2. ISSR primers used for analysis of *Bidens* species and the number of identified DNA bands per primer

Sequence (5'-3')	DNA bands (polymorphic bands) in ISSR profiles			
	<i>B. frondosa</i>	<i>B. tripartita</i>	<i>B. cernua</i>	Size range (bp)
(AG) ₈ TC	17 (16)	18 (18)	21 (21)	450–2000
(GACA) ₄	18 (18)	18 (18)	17 (16)	250–1300
(CTC) ₄ CRC	19 (19)	20 (20)	22 (22)	250–2000
SSWN(GACA) ₃	18 (18)	17 (16)	14 (12)	250–2000
Total	72 (71)	73 (72)	74 (71)	
Mean \pm SE	18 \pm 0.8	18.25 \pm 0.25	18.50 \pm 3.62	

SE – standard error

However, the number of studied individuals of *B. frondosa* was more than three times larger (185) than that of *B. tripartita* (54) and *B. cernua* (47). Only two genotypes among 185 *B. frondosa* samples were detected in duplicate. So, the genotypic diversity of *B. frondosa* was high ($i = 0.99$). Among 47 *B. cernua* individuals, the number of identical genotypes was higher: one genotype was detected in three individuals, and two genotypes were

detected in two individuals. All 54 studied *B. tripartita* plants were genetically different.

ISSR polymorphism within the *B. frondosa* populations. The average percentage of polymorphic bands (PLP) in *B. frondosa* populations was 34.4 ± 3.0 , the average expected heterozygosity (H_j) – 0.124 ± 0.01 and the average value of Shannon's index (I) – 0.161 ± 0.015 (Table 3).

Table 3. Summary of *Bidens frondosa*, *B. tripartita* and *B. cernua* populations genetic diversity estimates

No.	Population	IDpop	N	loc P	PLP	H _j	I	Br (5)	PLP 5% (5)
<i>Bidens frondosa</i>									
1.	GrigiškėsF	1F	17	36	42.4	0.159	0.226	1.320	0.424
2.	VilniusF	2F	15	28	32.9	0.122	0.168	1.245	0.329
3.	KaunasF	3F	17	17	20	0.070	0.094	1.142	0.200
4.	DruskininkaiF	4F	20	33	38.8	0.128	0.167	1.263	0.388
5.	VilkijaF	5F	15	31	36.5	0.141	0.164	1.281	0.365
6.	NidaF	6F	18	49	57.6	0.197	0.265	1.404	0.576
7.	MerkinėF	7F	16	26	30.6	0.098	0.121	1.205	0.306
8.	KulautuvaF	8F	17	23	27.1	0.101	0.124	1.203	0.271
9.	ŽvėrynasF	9F	12	27	31.8	0.114	0.141	1.235	0.318
10.	ŠilėnaiF	10F	15	33	38.8	0.154	0.202	1.307	0.388
11.	KrekenavaF	11F	17	32	37.6	0.124	0.174	1.256	0.376
12.	VarpučiaiF	12F	6	16	18.8	0.079	0.089	1.171	0.188
	Mean		15.4	29.3	34.4	0.124	0.161	1.253	0.344
	SE		1.0	2.5	3.0	0.010	0.015	0.020	0.030
<i>Bidens tripartita</i>									
13.	GrigiškėsT	1T	9	41	48.2	0.200	0.261	1.404	0.482
14.	VilniusT	2T	5	15	17.6	0.081	0.078	1.176	0.176
15.	KaunasT	3T	19	58	68.2	0.256	0.345	1.511	0.682
16.	DruskininkaiT	4T	6	37	43.5	0.204	0.244	1.414	0.435
17.	VilkijaT	5T	9	24	28.2	0.104	0.121	1.217	0.282
18.	JiesiaT	6T	6	20	23.5	0.101	0.131	1.216	0.235
	Mean		9.0	32.5	38.2	0.158	0.197	1.323	0.382
	SE		2.1	6.5	7.7	0.029	0.042	0.056	0.077
<i>Bidens cernua</i>									
19.	GrigiškėsC	1C	21	27	31.8	0.123	0.179	1.249	0.365
20.	KaunasC	2C	5	39	45.9	0.239	0.269	1.459	0.459
21.	DruskininkaiC	3C	10	31	36.5	0.174	0.202	1.329	0.365
22.	NidaC	4C	6	24	28.2	0.130	0.255	1.265	0.282
23.	JiesiaC	5C	5	21	24.7	0.102	0.126	1.247	0.247
	Mean		9.4	28.4	33.4	0.153	0.206	1.310	0.344
	SE		3.0	3.1	3.7	0.025	0.026	0.040	0.037

IDpop – short population name, N – the number of analysed plants per population, loc P – the number of polymorphic bands, PLP – the percentage of polymorphic bands, H_j – expected heterozygosity, I – Shannon's index of genetic diversity, Br (5) – band richness with sample size rarefied to five individuals, PLP 5% (5) – polymorphic band proportion with sample size rarefied to five individuals at the 5% level; SE – standard error

The average number of identified polymorphic bands in *B. frondosa* populations was 29.3 ± 2.5 . The lowest genetic diversity measures were found in the 12F population (PLP = 18.8, H_j = 0.079, I = 0.089); the highest were found in the Nida population (PLP = 57.6, H_j = 0.197, I = 0.265). The genetic diversity measures (H_j and PLP) in the *B. frondosa* population did not correlate with the number of analysed individuals ($p > 0.05$). When the number of individuals per population was rarefied to five individuals per population, the same tendency was noted for the genetic diversity distribution in the populations. The highest proportion of polymorphic bands was detected in the 6F population (PLP 5% = 0.576, Br = 1.404), and the lowest, in the 12F (PLP 5% = 0.188), except for the Br measure, which was least in the 3F population (Br = 1.142). Although the 3F population of *B. frondosa* formed a large stand, with 17 individuals being collected for analysis, only 20 polymorphic bands were identified in the studied genotypes. The highest measures of genetic

diversity among the studied species were revealed in *B. tripartita*: loc P = 32.5, PLP = 38.2, H_j = 0.158, I = 0.197, Br (5) = 1.323 and PLP 5% (5) = 3.82 (Table 3).

When the numbers of populations and individuals were approximated for all species (5–6 populations per species, 47 individuals per species), the measures of genetic diversity of *B. frondosa* decreased to 24 polymorphic bands per population (PLP = 28.2, H_j = 0.11, data not shown). Nevertheless, the Mann-Whitney test did not reveal significant differences between species in these measures.

Comparison of population genetic structures.

The molecular genetic diversity analysis (AMOVA) of the 23 populations revealed high differentiation among the populations ($\Phi_{iPT} = 0.636$, $p < 0.001$). Analysis showed that 36% of the genetic diversity was located within populations (Table 4).

Similar results were generated with the program AFLP-SURV ($F_{st} = 0.59 \pm 0.04$). An assessment of the

Table 4. Molecular variance among and within Lithuanian populations of three *Bidens* species according to ISSR markers

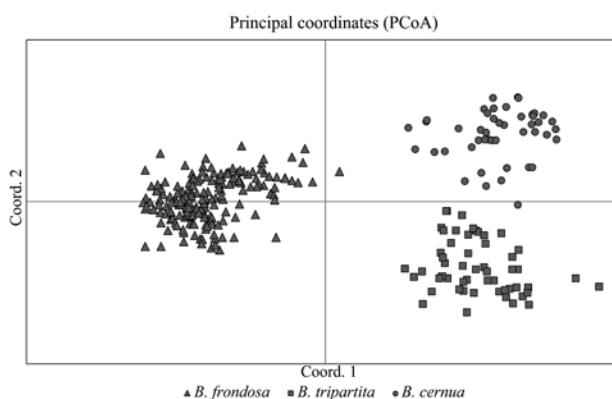
Source of <i>AMOVA</i> of all populations	df	SS	MS	Total variance %	Φ_{PT}	<i>p</i>
Among species	2	958.985	479.492	33		
Among pops	20	1343.380	67.169	30		
Within pops	263	1581.338	6.013	36		
Total	285	3883.703		100		
					0.636	<0.001
<i>B. frondosa</i>						
Among pops	11	881.742	80.158	48		
Within pops	173	906.441	5.240	52		
Total	184	1788.184		100		
					0.482	<0.001
<i>B. tripartita</i>						
Among pops	5	208.773	41.755	34		
Within pops	48	373.227	7.776	66		
Total	53	582.000		100		
					0.339	<0.001
<i>B. cernua</i>						
Among pops	4	204.875	51.219	47		
Within pops	42	257.976	6.142	53		
Total	46	462.851		100		
					0.466	<0.001

df – degree of freedom, SS – sum of squares, MS – mean squares, Φ_{PT} – genetic differentiation among populations, *p* – significance level

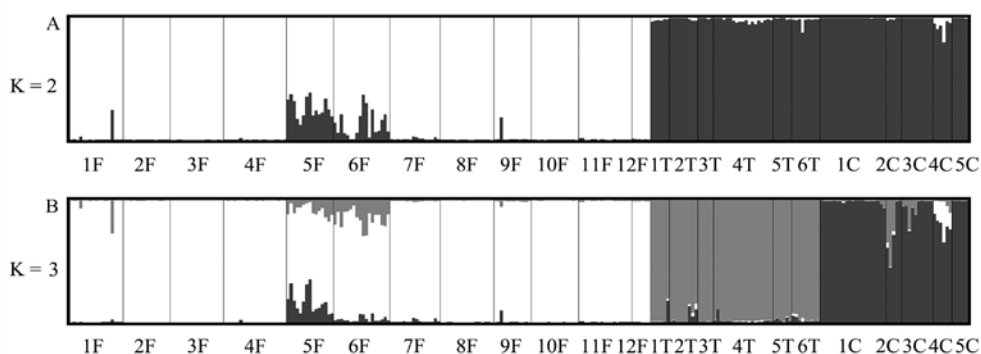
partitioning of the genetic variability within species revealed genetic differentiation that was somewhat greater among populations of *B. frondosa* ($\Phi_{PT} = 0.482$, $p < 0.001$) compared with *B. cernua* ($\Phi_{PT} = 0.466$, $p < 0.001$) and considerably higher than the genetic differentiation existing among populations of *B. tripartita* ($\Phi_{PT} = 0.339$, $p < 0.001$). Hierarchical *AMOVA* was performed to establish the variance component among species. Populational diversity accounted for 33% of the differences between species.

The results of principal coordinates analysis (PCoA), disclosing the relations between *B. frondosa* and the two native species, are shown as a two-dimensional plot (Fig. 2). The first two coordinates in the PCoA explained 46.97% of the variance. In this analysis, individuals of the different species were organized into distinct groups, and no overlapping groups were seen as potential hybrids.

We used the Bayesian procedure provided by the program *Structure* to elucidate the population structures in three species. The analysis grouped all populations into the two most likely clusters ($K = 2$) (Fig. 3A).

**Figure 2.** Two-dimensional plot envisioning relationships between *Bidens* species

Cluster 1 (white) included all *B. frondosa* populations. Cluster 2 (black) contained populations of the two native species. The smaller peak was detected at $K = 3$. In this case, the clustering corresponded to *B. frondosa*, *B. tripartita* and *B. cernua*. The assignment of populations in three clusters largely corroborated



Note. The clusters are denoted by white, grey and black; individuals are represented by vertical white, grey or black bars; populations are separated by vertical black lines; populations: 1F–12F – *B. frondosa*, 1T–6T – *B. tripartita*, 1C–5C – *B. cernua*.

Figure 3. Likelihood of the groupings of *Bidens frondosa*, *B. tripartita* and *B. cernua* plants to the genetic clusters ($K = 2$ and $K = 3$) identified by the Bayesian clustering method

the results of the PCoA. However, the Bayesian analysis revealed two admixed populations (5F and 6F) of *B. frondosa* (Fig. 3B). Certain signs of admixture were seen in some populations of the native species. However, in general, most populations were genetically homogeneous.

Discussion

An analysis of the genetic diversity of invasive species populations is a relevant topic for invasion biology as it provides a basis for a more reliable assessment of the species evolutionary potential in the new range, and a better understanding of invasion mechanisms and history and can contribute to more effective species control measures (Wang et al., 2012; Shou-Li et al., 2016). *Bidens frondosa* intensively spreads in Lithuania, especially in riparian habitats. According to theory, the introduction of an alien species is usually associated with founding events (Novak, Mack, 2005; Dlugosch, Parker, 2008) that later can be counteracted by multiple introductions, genetic recombination and other mechanisms (Bossdorf et al., 2005; Kelager et al., 2013). Unfortunately, we cannot assess how much genetic diversity was lost or acquired during the invasion process because we did not have information about the genetic diversity of the *B. frondosa* native populations. Nevertheless, we attempted to compare the level of diversity with that of two related native species. A comparison of diversity parameters among related species with different breeding system (Sun, Wong, 2001), native and invasive (Beckmann et al., 2009), common and endangered (Tanahara, Maki, 2010; Chung et al., 2012), endemic and widespread (Bednarska, Brazauskas, 2017) is a widely accepted approach in population ecology. We identified high genotypic diversity among the studied *B. frondosa* plants. Only a few genotypes were found to be duplicated, which, considering the sampling strategy, indicates the absence of large clones in the *B. frondosa* populations. A similar level of genetic diversity was established in populations of the native *B. cernua* and *B. tripartita* (Tanahara, Maki, 2010). No statistically significant differences were detected in the genetic diversity measures among the three *Bidens* species. The percentage of polymorphic bands for *B. cernua* and *B. tripartita* in our study was similar to that in the study of Tanahara and Maki (2010), except that we found slightly higher ISSR polymorphism in *B. tripartita*, whereas in the previous study, this polymorphism was higher in *B. cernua*.

In summary, our results indicate high genotypic and genetic diversity in the studied populations of *B. frondosa* in spite of rather short invasion history. The genetic differences among the *B. frondosa* populations ($Bf\Phi_{PT} = 0.482$) were only slightly higher than in native congener ($Bc\Phi_{PT} = 0.466$, $Bt\Phi_{PT} = 0.339$). Similar values of genetic differentiation observed among populations of *B. cernua* ($\Phi_{PT} = 0.438$) and *B. tripartita* ($\Phi_{PT} = 0.413$) in Japan are described in a previous study (Tanahara, Maki, 2010). Rather high genetic variability of *B. frondosa* populations could indicate that introductions from different source populations contributed to the current pattern of genetic structure in the invasive species (Durka et al., 2005; Kupcinskiene et al., 2013; Patamsytė et al., 2013).

A threat of invasive species is the genetic corruption of the native species by gene flow and hybridization (Ellstrand, Schierenbeck, 2000; Bleeker et al., 2007), and many studies have reported genetic assimilation and loss of integrity in native species

(Bleeker et al., 2007; Schierenbeck, Ellstrand, 2009; Vyšniauskienė et al., 2015). The structure analysis of the studied *Bidens* populations showed signs of admixture in some populations of *B. frondosa* and in some populations of the native species (Fig. 3).

Interestingly, our results indicate a possible larger impact of the native species on the *B. frondosa* than *vice versa*. The genetic structures of the 5F and 6F populations of *B. frondosa* show signs of introgression from *B. cernua* and *B. tripartita*. Overlapping of ranges from biological invasion makes hybridization of related species possible. *B. frondosa* produces pollen of high viability; however, at least in part of genotypes the usual fertilization is rare and gene flow between *B. frondosa* and related species is problematic because of the different ploidies of these species (Crowe, Parker, 1981). Crowe and Parker (1981) considered *B. connata* a possible hybrid between *B. frondosa* and *B. cernua*; however, they did not find reliable evidence for this hypothesis. Few works about potential *B. frondosa* hybrids with other related species are reviewed in Vinogradova et al. (2014). The most recent information on this topic is provided from studies in the upper basin of Volga on potential hybrids by V. G. Papchenkov and other Russian botanists, which were critically revised by Vinogradova et al. (2014) and Galkina et al. (2015). Based on morphological and molecular (ISSR) data analysis, these authors concluded that low hybridogenic activity existed with *B. frondosa*. Knope et al. (2013) also studied the hybridization between alien and native *Bidens* species. They hybridized *B. alba* and *B. pilosa* with nine native Hawaiian species and did not obtain viable hybrids. In our study, principal coordinate analysis of the three *Bidens* species populations, based on the molecular diversity patterns, did not show an overlap between species in the two coordinate systems (Fig. 2) and do not indicate intermediate genotypes; this outcome agrees with the results of Galkina et al. (2015).

The morphological characteristics of the plants involved in our study from populations that, according to Bayesian cluster analysis, are characterized as admixed and possess genetic material from gene pools of different species, did not attract our attention as having discrepancies from the typical morphology of *B. frondosa*. In addition, *AMOVA* also showed significant differentiation between *Bidens* species ($\Phi_{RT} = 0.33$). These results, taken together, also support the absence of genetic interactions between *B. frondosa* and native species.

Intensive studies of plant invasion phenomena revealed plant characteristics that are favourable for invasiveness (Rejmánek, 1996; Bossdorf et al., 2005). High seed production and ecological plasticity are characteristic of *B. frondosa* (Danuso et al., 2012). Ellstrand and Schierenbeck (2000) proved that interspecific hybridization is also an important mechanism for invasiveness as it increases genetic variation and produces genotypes with new characteristics. High genetic diversity also is favourable for the adaptation and spread of alien plants (Lavergne, Molofsky, 2007).

Conclusion

In the current study, we detected comparatively high genotypic and genetic diversity of invasive *Bidens frondosa* that is comparable with similar parameters of native species *B. cernua* and *B. tripartita*. The analysis of the population genetic structure of *B. frondosa* indicates that hybridization with native species, if this process occurs at all, is not an important factor for invasiveness.

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***Bidens* genties invazinių ir vietinių Lietuvos rūšių populiacijų genetinė įvairovė**

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Santrauka

Įvairūs antropogeniniai veiksniai (intensyvi ūkinė veikla, turizmas, klimato kaita ir kt.) pažeidžia upių ekosistemas, sudarydami sąlygas plisti invaziniam augalams. Lietuvoje upių, ypač Nemuno, ir kitų vandens telkinių pakrantėse sparčiai plinta invazinis ilgakotis lakišius (*Bidens frondosa* L.). Ši rūšis sąveikauja su vietinėmis rūšimis ir dažniausiai jas išstumia. Upių pakrantėse ir kitose natūraliose bei antropogenuose augavietėse paplitusios dvi vietinės lakišiaus rūšys – triskiautis (*B. tripartita*) ir pelkinis (*B. cernua*). Triskiautis lakišius yra žinomas ir kaip laukų bei daržų piktžolė. *B. frondosa* kai kuriose Europos šalyse irgi yra laikoma dirbamųjų laukų piktžole. Siekiant nustatyti, kokie genetiniai veiksniai gali būti palankūs plisti ilgakočiui lakišiu, tirta ir palyginta šių trijų rūšių populiacijų genetinė sandara bei įvairovė. DNR polimorfizmas buvo nustatytas paprastųjų pasikartojančių sekų tarpų (angl. *inter-simple sequence repeats* – ISSR) metodu.

Tyrimo rezultatai parodė didelę *B. frondosa* populiacijų genetinę diferenciaciją ir genotipų įvairovę. Nors Bayeso klasterių analizė, atlikta programa *Structure*, rodė tam tikrus genų srauto tarp rūšių požymius, tačiau pagrindinių koordinacinių analizė šio rezultato nepatvirtino. Tyrimo rezultatai parodė, kad tarprūšinė hibridizacija nėra *B. frondosa* invazyumo priežastis. Šiai svetimkraštei rūšiai plisti palankus veiksnys gali būti didelė populiacijų genetinė ir genotipinė įvairovė.

Reikšminiai žodžiai: *Bidens*, hibridizacija, invazinės rūšys, populiacijų genetinė sandara.