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# Evaluation and comparison of the genetic structure of *Bunias orientalis* populations in their native range and two non-native ranges

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**Abstract** We studied the invasive warty cabbage *Bunias orientalis* (Brassicaceae) in three geographically distinct areas. Using inter-simple sequence repeat fingerprinting, we analyzed warty cabbages, including non-native populations, from the eastern Baltic and western Siberian regions and native populations from southwestern Russia. The eastern Baltic region and western Siberia represent the two opposite directions of *B. orientalis* spread in climatically different zones. The genetic structures of the native and non-native *B. orientalis* populations were assessed through analysis of molecular variance (AMOVA) and the Bayesian clustering method and by determining the main measures of genetic diversity. AMOVA revealed considerable population differentiation in both the native and invasive ranges. Our results did not indicate a decrease in genetic diversity in the non-native populations of *B. orientalis*. Similar measures of genetic diversity and genetic structure were determined in the invasive populations in two geographically and ecologically distinct, non-native regions located in Europe and Asia. In both of these regions, higher genetic diversity was detected in the non-native populations than in the native region populations, which may be due to multiple introductions. However, Bayesian clustering analysis revealed slightly different sources of invasive populations in the two non-native regions. Genetic diversity patterns revealed the lack of isolation by distance between populations and confirmed the influence of anthropogenic factors on the spread of *B. orientalis*. The significance of native

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populations as germplasm resources for breeding is discussed.

**Keywords** Genetic diversity · *Bunias orientalis* · Biological invasions · Multiple introductions · ISSR · Warty cabbage

## Introduction

Biological invasions by alien species are recognized as a significant component of global environmental changes caused by human activity on our planet (Vitousek et al. 1997; Diekmann et al. 2016). During the establishment of a population in a new environment, genomic and genetic changes may arise and are determined by genetic drift, natural selection in novel environmental conditions, hybridization, and other factors (Nei et al. 1975; Bossdorf et al. 2005). The evolutionary perspective of the introduced species may also depend on the genetic structure of the native population, the reproductive system of the species, and its introduction history (Novak and Mack 1993; Shirk et al. 2014). Dlugosch and Parker (2008) performed a meta-analysis of 80 alien species and revealed that the loss of genetic diversity due to genetic drift is a frequent (but not universal) outcome of introductions. If the native populations are highly structured and represent only a limited part of the total genetic diversity of a species, founder effects are more likely and severe in the new range, at least in the initial period. In contrast, if the native populations exhibit low genetic structure, the high genetic diversity available in the source populations decreases the founder effect and the loss of alleles during an introduction (Shirk et al. 2014). Conversely, high genetic diversity is advantageous but not obligatory for biological invasions (Dlugosch and Parker 2008). Populations of some invasive species restore or even increase genetic diversity through multiple introductions and genetic recombination in the new range (Bossdorf et al. 2005; Pairon et al. 2010; Kelager et al. 2013; Barriball et al. 2015), while others can spread as nearly monomorphic populations or as populations consisting of a low number of advantageous genotypes (Li et al. 2006; Le Roux et al. 2007; Voss et al. 2012). Therefore, it is not easy to predict which pattern of genetic diversity will be most likely in the case of a particular species. Gaskin et al. (2014) reported that three closely related invasive knotweed species exhibited great variation in genetic structure in new areas. By considering genetic diversity across native and non-native ranges, an increase in the genetic diversity of invasive populations has been revealed for species such as *Bromus tectorum* (Novak and Mack 1993) and *Phalaris arundinacea* (Lavergne and Molofsky 2007). In some cases, neutralization of the founder effect is governed by repeated introductions that translate indigenous between-populational variation into higher within-populational variation in the non-native range (Kolbe et al. 2004). Therefore, it is desirable to empirically establish the genetic diversity patterns of invading populations and compare them with those of the native populations to elucidate suitable management approaches for invasive species.

Here, we studied warty cabbage (*Bunias orientalis*), which is widely spread across Europe (except the southern part), western Asia, Siberia, Russian Far East, and North America. *B. orientalis* belongs to the Brassicaceae family, which is rich in invasive species (Müller 2009). In Europe, except in the steppe zone, *B. orientalis* is a non-native perennial plant species that is well adapted to spread in anthropogenized habitats (Dietz et al. 1999b; Kiełtyk and Mirek 2015). In many parts of central and northern Europe, this species is considered an aggressive invader, penetrating semi-natural grassland communities, meadows, and protected natural areas (Müller 2009; Kiełtyk and Mirek 2015). The invasiveness of this species may depend on several factors. *B. orientalis* has been demonstrated to benefit from excessive disturbance relative to dominant native grass species, to cope very well with mowing due to rapid rosette regrowth and increased seedling recruitment (Dietz and Ullmann 1997; Woitke and Dietz 2002) and to form tall, dense stands that successfully compete with native grasses for sun, nutrients, and pollinators (Schürkens and Chittka 2001; Birnbaum 2006). *B. orientalis* also probably experiences reduced insect herbivore attacks due to the production of toxic chemicals (Müller 2009; Harvey et al. 2010), produces large numbers of seeds that form a dense seed bank, and can effectively germinate from seeds and regenerate from root fragments (Steinlein et al. 1996; Dietz et al. 1999b). The penetration of this species into semi-natural habitats is also promoted by disturbances caused by animals (Kiełtyk and Mirek 2015). Warty cabbage in

some countries is valued as an unconventional crop of multipurpose use that can be grown as a fodder, medicinal and edible plant (Birnbaum 2006; Mihovich et al. 2009; Avetisyan and Avetisyan 2017).

It is thought that *B. orientalis* is native to the steppe zone of eastern and southeastern Europe, including southwestern Russia, the Caucasus, and western Asia (Steinlein et al. 1996; Woitke and Dietz 2002; Birnbaum 2006). Its spread into Europe started in the eighteenth century, mainly due to anthropogenic factors (Laiviņš et al. 2006). At the end of the eighteenth century, *B. orientalis* spread to contemporary Estonia (1796), and a few decades later (1820), it was found near St. Petersburg (Kuusk et al. 1993; Laiviņš et al. 2006). The history of the spreading of warty cabbage in Latvia starts in 1803 and is rather well documented (Laiviņš et al. 2006). *B. orientalis* was first detected in Germany in 1810 and in Poland in 1858 (Laiviņš et al. 2006; Kiełtyk 2014). In Lithuania, the first record of this species comes from 1885 on the Baltic coast (Gudžinskas 1997). The spreading of warty cabbage has also extended to the east and northeast, towards eastern Siberia and the Far East. In Siberia, the status of *B. orientalis* is uncertain; it is usually considered a non-native species with a complicated introduction history (Ebel 2011). Botanical records indicate that introduction in eastern Siberia occurred at the beginning of the twentieth century, with the first records coming from Tobolsk (1907) and Krasnoyarsk (1909) (Bush 1931) and then near Barnaul, in 1912 (Krylov 1931). Currently, *B. orientalis* is common in most of the northwestern part of the Altai-Sayan floristic province (Ebel 2012). *B. orientalis* grows mainly in forest and steppe zones through river valleys and then penetrates into semi-deserts. However, taking into account new archeological data (Dashkovskiy et al. 2014), *B. orientalis* can be attributed to the archaeophytes in Siberia, as fruits of this species were found in a Chineta II burial mound (IV–III century BC) in northwestern Altai. Unfortunately, it is practically impossible to resolve the question of whether this species has been preserved in this territory since the initial introduction, or was introduced later, or if there have been multiple introductions (Ebel 2011). To the best of our knowledge, no attempts have yet been made to compare the genetic diversity between native and non-native populations of *B. orientalis*. The genetic structures of warty cabbage populations have been studied only on a small scale in the non-native range (Dietz et al. 1999a; Patamsytė et al. 2011, 2013). In this context, the use of dominant molecular markers allows the assessment of the population genetic structure in this species in which intensive genome studies are lacking.

In this study, we investigated the population genetic structure underlying the spread of *B. orientalis* in the eastern Baltic region of Europe and in Siberia using inter-simple sequence repeat (ISSR) markers. We asked the following questions: (1) What is the genetic structure of *B. orientalis* in the eastern Baltic region of Europe? (2) How do the obtained results correspond to the multiple-introduction hypothesis of *B. orientalis* in the eastern Baltic region? (3) Does the distribution of intra- and interpopulation genetic variation differ between native and non-native populations? (4) Is there a genetic difference between the non-native populations of Europe and Siberia?

## Materials and methods

### Population sampling

The investigation of *B. orientalis* was conducted in three geographically remote areas and included non-native populations from the eastern Baltic and western Siberian regions as well as native populations from southwestern Russia (Online Appendix Table 1; Fig. 1). The two non-native areas represent different climate zones; i.e., the maritime climate is characteristic of the eastern Baltic region, while western Siberia is in the strongly continental climate zone. We investigated 52 Baltic populations (Poland, 8; Lithuania, 17; Latvia, 14; Estonia, 11; and Russia, 2), including 832 individuals in total. The set of populations used to compare the genetic diversity in the native and non-native ranges included 23 populations. The mean geographic distance between the sampling sites of the 8 randomly chosen Baltic region populations (PL3, PL6, LT3, LT4, LV5, LV8, EE5, and RUS1) was 524 km, while that between the 8 populations from the native region in southwestern Russia was 519 km, and that between the 7 Siberian populations was 138 km. In this part of study, 360 individuals were analyzed in total. Leaves were collected in 2013–2014 and transported to the laboratory dried in silica gel. The samples were collected randomly from plants that were located at least

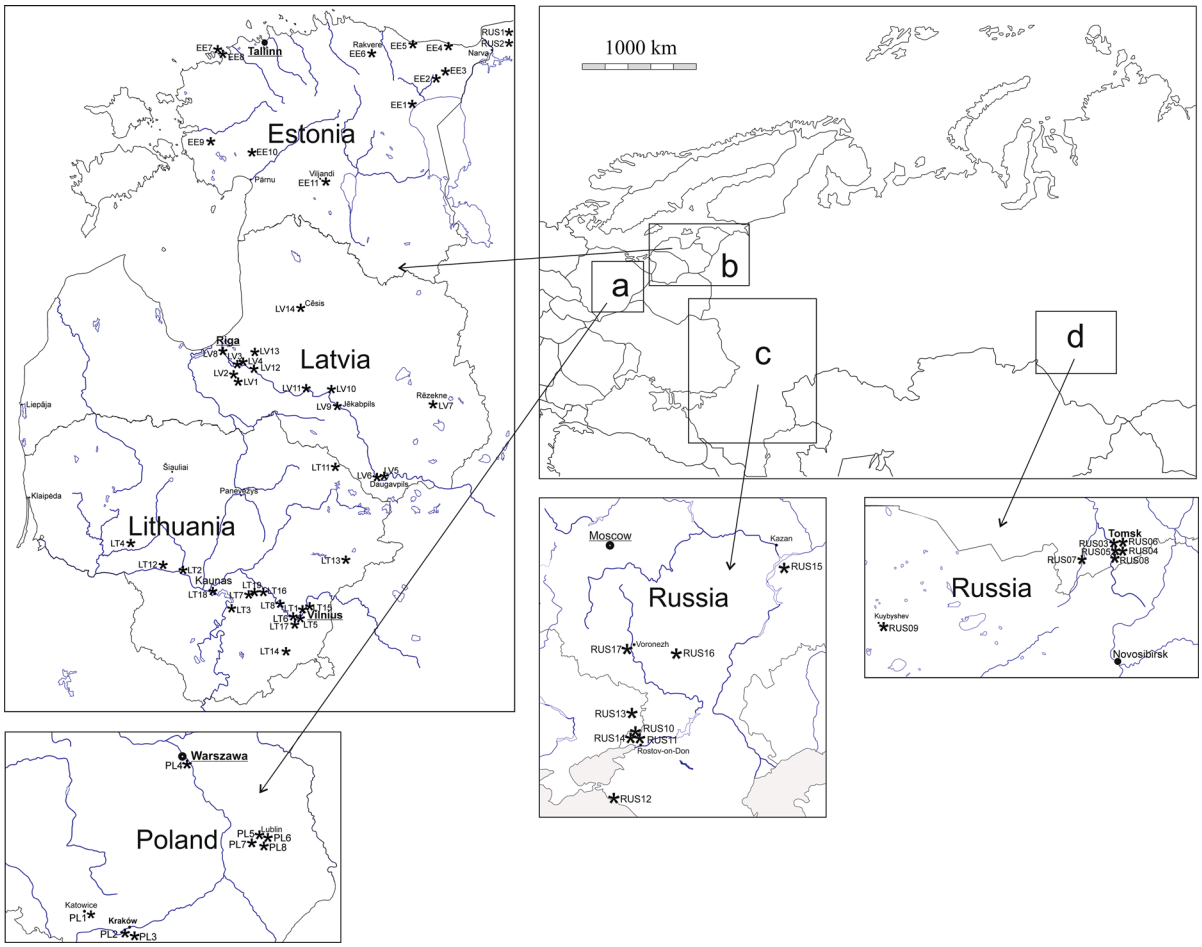


**Fig. 1** Map of the geographic distribution of the studied non-native and native *Bunias orientalis* populations in the Eastern Baltic region (**a**, **b**), southwestern Russia (**c**), and western Siberia (**d**). For population abbreviations and more detailed information, see Online Appendix Table 1

10–20 m apart. The number of plants sampled per population varied from 5 to 24.

## DNA sample preparation and ISSR analysis

DNA was isolated from dry leaves using a modified CTAB method (Doyle and Doyle 1990) adapted for very small volumes. The quantity and quality of DNA were assessed spectrophotometrically and via electrophoresis in agarose gel.

ISSR-PCR was performed in a volume of 10 µL containing 10 ng of genomic DNA, 1× PCR buffer, 200 µM dNTPs, 3 mM $MgCl_2$, 5 µM primers, and 0.4 U *Taq* DNA polymerase (Thermo Fisher Scientific/Baltics, Vilnius, Lithuania). Six preselected oligodeoxynucleotide primers (Patamsytė et al. 2011) were used for sample genotyping. ISSR-PCR was performed under the following conditions: one cycle at 94 °C for 7 min; 32 cycles of 30 s at 94 °C, 45 s at the primer-specific annealing temperature (Online Appendix Table 2) and 2 min at 72 °C; and a final step of 7 min at 72 °C.

The ISSR-PCR products were fractionated in 1.2% agarose gels using 0.5× TBE buffer and stained with ethidium bromide. Gel images were recorded using a BioDocAnalyze gel documentation system (Biometra, Göttingen, Germany).

GeneRuler$^{TM}$ DNA Ladder Mix, 100–10,000 bp (Thermo Fisher Scientific/Baltics, Vilnius, Lithuania) was used for DNA band size assessment. The DNA samples were examined at least twice. Band size adjustment among samples from different populations

and genotyping error rate calculation were performed as previously described (Patamsytė et al. 2013). The genotyping error rate in our study was 4.7%.

### nrITS DNA analysis

To determine whether we were dealing with polymorphic populations of one species or probably morphologically indistinguishable but separate (cryptic) species, we sequenced the nuclear ribosomal internal transcribed spacer (nrITS), which is one of the most widely employed plant molecular markers in phylogenetics (Hollingsworth et al. 2011; Cheng et al. 2016) and usually separates species with substantial divergence. Universal primers (ITS1 and ITS4) were used for amplification of this region (White et al. 1990). The PCR products were subsequently cloned into the pTZ57R/T vector using the InsTAclone PCR Cloning Kit (Thermo Fisher Scientific/Baltics, Vilnius, Lithuania). Two to three clones carrying the nrITS region insert from each plant were sequenced at BaseClear B.V. (Leiden, the Netherlands). The sequences were aligned and checked by eye with the program MEGA 7.0.25 (Kumar et al. 2016). The size of the *B. orientalis* nrITS region was 1266 bp. No polymorphisms of the nrITS sequence were detected in individuals from the native (RUS10, 11, 15, 16, 17) or non-native (RUS9, LT1) populations, and this marker was not included in the further analyses. Sequences representing different populations were deposited in GenBank (accession nos. MF356944-MF356950).

### Data analysis and population structure assessment

Reproducible DNA bands generated via ISSR-PCR were used to construct a binary 0/1 data matrix that was used for further analyses. The genetic diversity within populations was determined as Nei's gene diversity (Hj) following Lynch and Milligan (1994) and using AFLP-SURV (Vekemans et al. 2002) with the allele frequencies expressed as the DNA band frequencies. Additionally, the proportion of polymorphic loci (PLP, 5% level) and band richness (Br) were established by setting the number of individuals per population for rarefaction as $N = 10$ (for 52 Baltic region populations) or $N = 5$ for assessing the genetic diversity among non-native and native populations (23 populations). Rarefaction was performed with AFLP-DIV v1.1 (Coart et al. 2005).

The genetic differentiation of populations located in different regions and between regions was determined according to Fst values using AFLP-SURV v.1.0 based on 10,000 random permutations of the data. Analysis of molecular variance (AMOVA) in GenAlEx v.6.5 (Peakall and Smouse 2012) was used to calculate the subdivision of genetic diversity within and between populations and between countries from eastern Baltic non-native regions. Principal coordinate analysis (PCoA) was also performed using GenAlEx v.6.5 to observe the genetic relationships among 23 populations from native and non-native regions.

To determine and compare population genetic structures between ranges and allocate individuals into clusters (K), STRUCTURE software version 2.3.4 was employed (Pritchard et al. 2000; Falush et al. 2007). Using the admixture model, the probabilities of $L(K)$ and $\Delta K$ for each value of $K$ from $K = 1$–20 were calculated, and the best K was identified. Ten independent runs ($K = 1$–20) were conducted with 500,000 burn-in iterations followed by 500,000 Markov chain Monte Carlo repetitions. The results were processed using the STRUCTURE HARVESTER (Earl and vonHoldt 2012), and the output was visualized using DISTRUCT v1.1 (Rosenberg 2004).

A Mantel test was conducted using the GenAlEx v.6.5 program to estimate the correlations between the geographic and Nei's genetic distance matrices between 23 populations from native and non-native regions and between 52 populations from the Baltic region. Significance was determined by matching the outcomes with 9999 permutations.

The population diversity parameters (i.e., Hj, PLP, Br) between regions were compared using the Mann–Whitney *U*-test in IBM® SPSS® Statistics v.23 for Windows.

## Results

### Genetic diversity and differentiation of populations from the eastern Baltic region

Genetic diversity was assessed in 52 populations (832 individuals) of *B. orientalis* from the Baltic region using 6 ISSR primers. In total, 69 polymorphic ISSR bands were identified (Online Appendix Table 2). The number of polymorphic loci per population ranged

from 9 (LT19) to 32 (PL6, LV1, LV5, and EE8), with an average of 23.6 ± 0.70 (mean ± SE) (Table 1). The proportion of polymorphic loci at the 5% level, when the number of individuals per population was rarefied to $N = 10$, ranged from 0.132 (LT19) to 0.471 (EE8) (mean 0.352 ± 0.011), and Br ranged from 1.127 (LT19) to 1.457 (LV1) (mean 1.326 ± 0.01). The average Hj within populations was 0.130 ± 0.004. The population differentiation (Fst) in the eastern Baltic region was high (0.480 ± 0.026). A hierarchical AMOVA revealed 12% genetic variation between countries and 38% between populations within countries. An equal proportion of the genetic variation occurred within populations (50%). AMOVA revealed the highest percentage of between-population variation in Lithuania (47%) and the lowest in Latvia (36%). For the Polish and Estonian populations, these values were 42 and 43%, respectively. The Mantel test revealed a very weak correlation ($R^2 = 0.0416$, $P = 0.01$) between the Nei's genetic and geographic distances of the 52 populations. No isolation by distance (IBD) between populations was detected within each of the tested countries, and these results together indicate that there is no correlation between genetic and geographical distances at the scale of this study.

### Genetic diversity and differentiation of populations from native and non-native regions

A total of 66 polymorphic ISSR bands were identified across the 23 surveyed populations. The genetic diversity parameters detected in the non-native populations from the eastern Baltic and Siberian regions were similar (Br = 1.308, PLP (5%) = 0.386, Hj = 0.155 and Br = 1.303, PLP (5%) = 0.413, Hj = 0.151, respectively, Table 2). The Mann–Whitney test revealed that the population genetic diversity parameters of the two non-native ranges were not significantly different (Br: $U = 24$, $P = 0.69$; PLP (5%): $U = 21, P = 0.46$; Hj: $U = 24, P = 0.69$). The native populations from southwestern Russia exhibited lower values of corresponding genetic diversity parameters (Br = 1.183, PLP (5%) = 0.233, Hj = 0.091), and these values were significantly different from those of the non-native populations of both the eastern Baltic (Br: $U = 3$, $P = 0.001$; PLP (5%): $U = 4.5$, $P = 0.002$; Hj = 3, $P = 0.001$) and Siberia (Br: $U = 1$, $P = 0.001$; PLP (5%): $U = 2.5$, $P = 0.001$; Hj = 1, $P = 0.001$). The genetic differentiation between the 23 populations was high (Fst = 0.495 ± 0.052). The greatest genetic difference was found between the non-native Siberian and native southwestern Russian population groups (Fst = 0.216 ± 0.062). The genetic difference between the eastern Baltic and native population groups was somewhat lower (Fst = 0.168 ± 0.109). The smallest genetic difference was found between the two groups of non-native populations from the eastern Baltic and Siberian regions (Fst = 0.064 ± 0.051). The genetic differentiation within regions also differed. The highest genetic differentiation between populations was detected in the native range (Fst = 0.583 ± 0.067). The genetic differentiation between the populations of the Baltic region was Fst = 0.432 ± 0.060. The Siberian non-native populations exhibited the lowest population differentiation (Fst = 0.380 ± 0.079); however, the mean geographic distance between these populations was considerably smaller. Hierarchical AMOVA also revealed that the genetic differentiation between the regions was 10% ($P = 0.001$). The intra-population component constituted 51% of the total genetic diversity.

### Cluster analysis and genetic relationships between populations

PCoA (Fig. 2) divided the 23 populations into two groups that generally corresponded to the native and non-native ranges of *B. orientalis*. The larger group mostly included non-native populations and one native (RUS15) population, while the smaller, native population group showed relatedness only between native populations. The EE5 and PL2 populations were clear outliers of both groups. The populations from non-native regions (eastern Baltic and Siberia) largely overlapped in the two-coordinate system.

In the STRUCTURE analysis, $\Delta K$ exhibited a large peak at $K = 5$ ($\Delta K = 439.7$) with a smaller peak at $K = 2$ ($\Delta K = 274.9$) (Online Appendix Fig. 1). The strongest support was obtained for five clusters; however, this grouping did not correlate with the geographic locations or range statuses (native or non-native) of the analyzed populations. Three of the eight native populations (RUS10, 11, 16) were grouped into the gray cluster (Fig. 3), while two (RUS12 and 13) were assigned to the white cluster, and two (RUS14 and RUS15) were demonstrated to be highly admixed

**Table 1** Genetic diversity parameters by country for ISSR data from non-native *Bunias orientalis* populations from the eastern Baltic region

| No. | Population | *N* | LocP | Br (10) | PLP (10) 5% | Hj |
|---|---|---|---|---|---|---|
| Poland | | | | | | |
| 1 | PL1 | 24 | 19 | 1.286 | 0.324 | 0.122 |
| 2 | PL2 | 24 | 25 | 1.352 | 0.412 | 0.141 |
| 3 | PL3 | 12 | 27 | 1.389 | 0.397 | 0.168 |
| 4 | PL4 | 24 | 26 | 1.359 | 0.412 | 0.143 |
| 5 | PL5 | 12 | 19 | 1.274 | 0.279 | 0.120 |
| 6 | PL6 | 12 | 32 | 1.453 | 0.471 | 0.188 |
| 7 | PL7 | 12 | 23 | 1.314 | 0.338 | 0.116 |
| 8 | PL8 | 12 | 18 | 1.252 | 0.265 | 0.099 |
| Mean | | 16.5 | 23.6 | 1.335 | 0.362 | 0.137 |
| SE | | 2.20 | 1.71 | 0.024 | 0.026 | 0.010 |
| Lithuania | | | | | | |
| 1 | LT1 | 20 | 29 | 1.376 | 0.426 | 0.140 |
| 2 | LT2 | 20 | 24 | 1.318 | 0.353 | 0.121 |
| 3 | LT3 | 14 | 28 | 1.382 | 0.412 | 0.150 |
| 4 | LT4 | 14 | 20 | 1.278 | 0.294 | 0.119 |
| 5 | LT5 | 14 | 25 | 1.333 | 0.368 | 0.124 |
| 6 | LT6 | 12 | 16 | 1.227 | 0.235 | 0.098 |
| 7 | LT7 | 11 | 23 | 1.330 | 0.338 | 0.136 |
| 8 | LT8 | 18 | 20 | 1.273 | 0.294 | 0.117 |
| 9 | LT11 | 20 | 19 | 1.251 | 0.279 | 0.106 |
| 10 | LT12 | 15 | 30 | 1.396 | 0.441 | 0.150 |
| 11 | LT13 | 20 | 17 | 1.222 | 0.250 | 0.091 |
| 12 | LT14 | 20 | 22 | 1.254 | 0.324 | 0.089 |
| 13 | LT15 | 20 | 20 | 1.275 | 0.294 | 0.107 |
| 14 | LT16 | 22 | 20 | 1.293 | 0.324 | 0.118 |
| 15 | LT17 | 20 | 23 | 1.274 | 0.338 | 0.098 |
| 16 | LT18 | 20 | 27 | 1.330 | 0.397 | 0.130 |
| 17 | LT19 | 12 | 9 | 1.127 | 0.132 | 0.050 |
| Mean | | 17.2 | 21.9 | 1.291 | 0.323 | 0.114 |
| SE | | 0.89 | 1.28 | 0.016 | 0.019 | 0.006 |
| Latvia | | | | | | |
| 1 | LV1 | 13 | 32 | 1.457 | 0.471 | 0.186 |
| 2 | LV2 | 11 | 30 | 1.432 | 0.441 | 0.173 |
| 3 | LV3 | 24 | 28 | 1.407 | 0.441 | 0.169 |
| 4 | LV4 | 12 | 27 | 1.377 | 0.397 | 0.150 |
| 5 | LV5 | 15 | 32 | 1.435 | 0.471 | 0.172 |
| 6 | LV6 | 12 | 30 | 1.421 | 0.441 | 0.164 |
| 7 | LV7 | 12 | 28 | 1.396 | 0.412 | 0.160 |
| 8 | LV8 | 12 | 22 | 1.318 | 0.324 | 0.138 |
| 9 | LV9 | 16 | 20 | 1.277 | 0.294 | 0.114 |
| 10 | LV10 | 12 | 25 | 1.350 | 0.368 | 0.136 |
| 11 | LV11 | 10 | 24 | 1.353 | 0.353 | 0.141 |
| 12 | LV12 | 12 | 23 | 1.330 | 0.338 | 0.139 |

**Table 1** continued

| No. | Population | *N* | LocP | Br (10) | PLP (10) 5% | Hj |
|---|---|---|---|---|---|---|
| 13 | LV13 | 10 | 19 | 1.279 | 0.279 | 0.118 |
| 14 | LV14 | 22 | 28 | 1.399 | 0.471 | 0.148 |
| Mean | | 13.8 | 26.3 | 1.374 | 0.393 | 0.151 |
| SE | | 1.13 | 1.13 | 0.015 | 0.018 | 0.006 |
| Estonia | | | | | | |
| 1 | EE1 | 20 | 24 | 1.316 | 0.353 | 0.120 |
| 2 | EE2 | 18 | 23 | 1.304 | 0.338 | 0.126 |
| 3 | EE3 | 12 | 22 | 1.303 | 0.324 | 0.104 |
| 4 | EE4 | 15 | 22 | 1.296 | 0.324 | 0.111 |
| 5 | EE5 | 12 | 16 | 1.232 | 0.235 | 0.100 |
| 6 | EE6 | 27 | 28 | 1.374 | 0.412 | 0.149 |
| 7 | EE7 | 10 | 22 | 1.324 | 0.324 | 0.140 |
| 8 | EE8 | 20 | 32 | 1.416 | 0.471 | 0.166 |
| 9 | EE9 | 15 | 20 | 1.277 | 0.294 | 0.108 |
| 10 | EE10 | 12 | 15 | 1.213 | 0.221 | 0.081 |
| 11 | EE11 | 14 | 19 | 1.266 | 0.279 | 0.108 |
| Mean | | 15.9 | 22.1 | 1.302 | 0.325 | 0.119 |
| SE | | 1.50 | 1.47 | 0.017 | 0.022 | 0.007 |
| Russian populations from eastern Baltic region | | | | | | |
| 1 | RUS1 | 18 | 28 | 1.395 | 0.412 | 0.171 |
| 2 | RUS2 | 22 | 28 | 1.390 | 0.441 | 0.147 |
| Mean | | 20 | 28 | 1.393 | 0.427 | 0.159 |
| SE | | 2 | 0 | 0.003 | 0.015 | 0.012 |
| Total mean | | 16 | 23.6 | 1.326 | 0.352 | 0.130 |
| Total SE | | 0.64 | 0.70 | 0.010 | 0.011 | 0.004 |

Number of individuals studied per population (N); number of polymorphic loci per population (LocP); band richness based on 10 individuals (Br); percentage of polymorphic loci based on 10 individuals (PLP); Nei gene diversity (Hj)

(especially RUS14). Finally, one native population (RUS17) was attributed to a small dark gray cluster together with an invasive population from Estonia (EE5). The black cluster included the non-native and geographically related Siberian populations from the Tomsk region (RUS3, 4, 5). These populations showed relatedness to populations from Latvia (LV8) and Lithuania (LT3). The populations constituting the light gray cluster were also geographically scattered. This cluster included one native (RUS15) population and several non-native populations from the eastern Baltic region (LT4 and partially LT3) and the Siberian range (RUS6, 7, 9). The white cluster included two native populations (RUS12 and RUS13), four eastern Baltic populations (PL3, PL6, LV5, and RUS1), and one invasive Siberian population (RUS8). The grouping of the populations into two clusters ($K = 2$) correlated well with the population grouping into the native and non-native categories, which supported the PCoA results (Fig. 3). All of the non-native populations were grouped in the light gray cluster, regardless of their geographic territory. This cluster also included one native population (RUS15). Two native populations (RUS12 and RUS13) exhibited a mix between the light gray and white clusters. Five native populations (RUS10, 11, 14, 16, 17) were assigned to the white cluster.

## Discussion

### Genetic diversity and structure of populations from the eastern Baltic region

The available historical records indicate that the eastern Baltic region was among the first areas where

**Table 2** Genetic diversity statistics by region for ISSR data from non-native and native *Bunias orientalis* populations

| No. | Population | *N* | Loc P | Br (5) | PLP (5) 5% | Hj |
|---|---|---|---|---|---|---|
| Non-native populations from eastern Baltic region | | | | | | |
| 1 | PL3 | 12 | 27 | 1.338 | 0.409 | 0.170 |
| 2 | PL6 | 12 | 32 | 1.386 | 0.485 | 0.194 |
| 3 | LT3 | 14 | 27 | 1.307 | 0.409 | 0.152 |
| 4 | LT4 | 14 | 20 | 1.241 | 0.303 | 0.123 |
| 5 | LV5 | 15 | 32 | 1.358 | 0.485 | 0.177 |
| 6 | LV8 | 12 | 22 | 1.28 | 0.333 | 0.142 |
| 7 | EE5 | 12 | 16 | 1.204 | 0.242 | 0.103 |
| 8 | RUS1 | 18 | 28 | 1.347 | 0.424 | 0.176 |
| Mean | | 13.6 | 25.5 | 1.308 | 0.386 | 0.155 |
| SE | | 0.75 | 2.02 | 0.022 | 0.031 | 0.011 |
| Non-native populations from western Siberia region | | | | | | |
| 9 | RUS3 | 23 | 25 | 1.302 | 0.439 | 0.148 |
| 10 | RUS4 | 21 | 27 | 1.327 | 0.47 | 0.163 |
| 11 | RUS5 | 22 | 26 | 1.318 | 0.424 | 0.160 |
| 12 | RUS6 | 21 | 25 | 1.303 | 0.424 | 0.150 |
| 13 | RUS7 | 21 | 29 | 1.370 | 0.500 | 0.187 |
| 14 | RUS8 | 20 | 18 | 1.229 | 0.273 | 0.117 |
| 15 | RUS9 | 21 | 22 | 1.271 | 0.364 | 0.135 |
| Mean | | 21.3 | 24.6 | 1.303 | 0.413 | 0.151 |
| SE | | 0.36 | 1.36 | 0.017 | 0.028 | 0.008 |
| Native population from southwest Russia region | | | | | | |
| 16 | RUS10 | 15 | 15 | 1.145 | 0.227 | 0.068 |
| 17 | RUS11 | 14 | 11 | 1.11 | 0.167 | 0.054 |
| 18 | RUS12 | 5 | 11 | 1.167 | 0.167 | 0.081 |
| 19 | RUS13 | 10 | 15 | 1.19 | 0.227 | 0.095 |
| 20 | RUS14 | 5 | 12 | 1.182 | 0.182 | 0.093 |
| 21 | RUS15 | 12 | 19 | 1.198 | 0.288 | 0.096 |
| 22 | RUS16 | 21 | 16 | 1.210 | 0.273 | 0.106 |
| 23 | RUS17 | 20 | 22 | 1.263 | 0.333 | 0.134 |
| Mean | | 12.8 | 15.1 | 1.183 | 0.233 | 0.091 |
| SE | | 2.14 | 1.38 | 0.016 | 0.022 | 0.009 |
| Total mean | | 15.7 | 21.6 | 1.263 | 0.341 | 0.131 |
| Total SE | | 1.10 | 1.36 | 0.016 | 0.023 | 0.008 |

Number of individuals studied per population (N); number of polymorphic loci per population (LocP); band richness based on 5 individuals (Br); percentage of polymorphic loci based on 5 individuals (PLP); Nei gene diversity (Hj)

*B. orientalis* was introduced in Europe (Laiviņš et al. 2006). Genetic structure analysis of the Baltic populations indicated that the populations are differentiated, and multiple species introductions may have occurred in this area. The genetic differentiation (12%) between the populations from the various countries of this region supports the hypothesis of independent introductions of germplasms of this species to different locations in this region (Zhao et al. 2013; Kupcinskiene et al. 2015). The higher population differentiation observed within countries (38%) than between countries also implies that multiple introductions occurred (Durka et al. 2005; Okada et al. 2007). The economic and political situation in the eighteenth to twentieth centuries meant that the flow of transportation between the eastern Baltic region and the rest of the Russian Empire (later, the USSR) favored the arrival of new colonizing propagules and genetic mixing between distant populations of *B. orientalis*. Seeds of *B.*

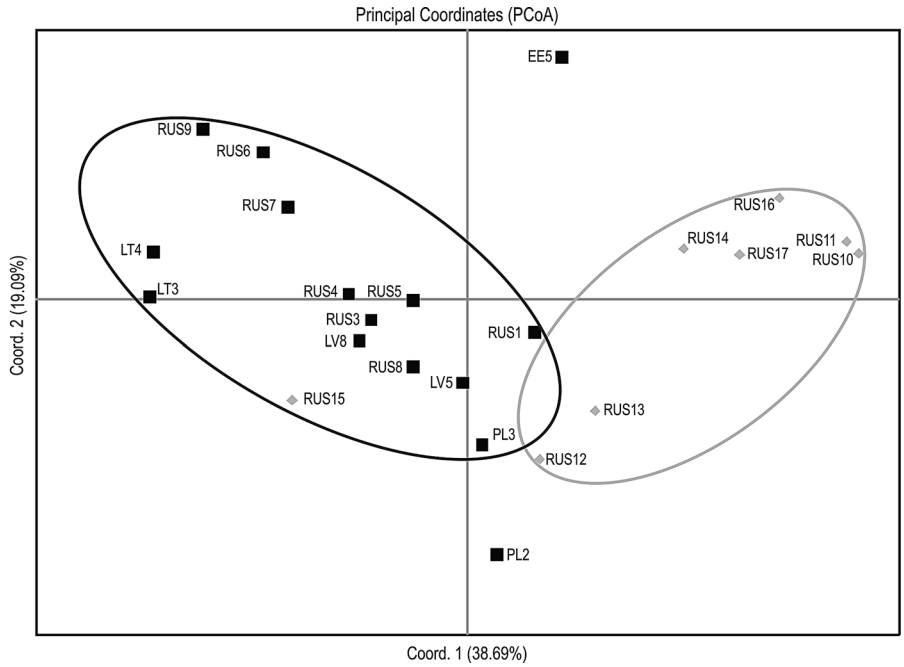


**Fig. 2** Distribution of the 23 *Bunias orientalis* populations from the non-native (black) and native (gray) ranges, based on principal coordinate analysis (PCoA) of the genetic distance matrix generated from ISSR data. Coordinate 1 explained 38.69% of the variance, and coordinate 2 explained 19.09% of the variance

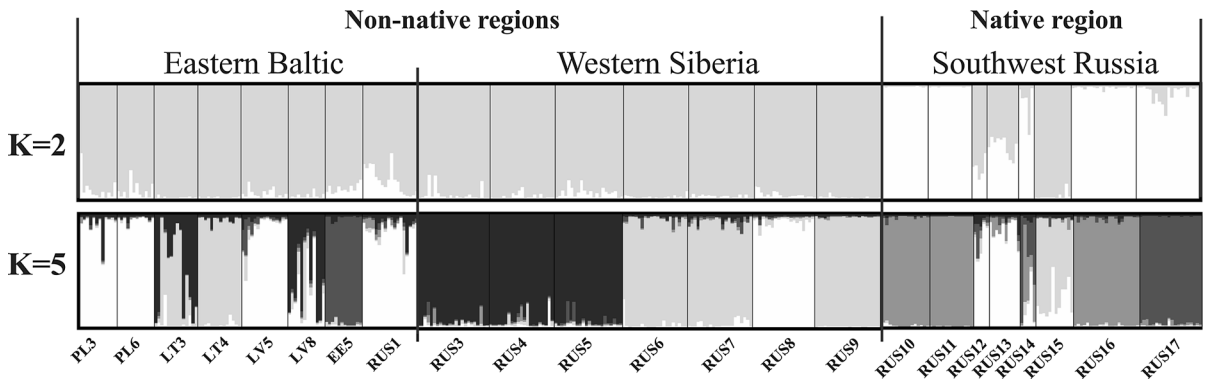


**Fig. 3** Population genetic structure of *Bunias orientalis* populations from non-native (eastern Baltic and western Siberia) and native (southwestern Russia) regions, determined using the Bayesian clustering program STRUCTURE with two ($K = 2$) and five ($K = 5$) clusters as the most plausible grouping. Clusters are indicated in white, light gray, gray, dark gray, and black

*orientalis* have been repeatedly imported as contaminants of grains or forage from Russia mainly via railway lines (Jehlík and Hejný 1974; Birnbaum 2006). Thus, a considerable number of the non-native populations are located along railways/roadsides (Steinlein et al. 1996; Laiviņš et al. 2006; Patamsytė et al. 2013) (Online Appendix Table 1). The high percentage of between-population variability detected in the populations from the Baltic region may have been influenced not only by the introduction history (introduction of propagules from genetically distant populations, limited gene flow, founder effects) but also by species biological properties (e.g., predominating cross-fertilization versus tolerance of autogamy) or adaptive differentiation (Kloss et al. 2011; Durka et al. 2017). Based on the Mantel test, a statistically significant but very weak correlation between geographic and genetic distance was detected at the regional scale, and no IBD was found within the countries in this region. The absence of IBD in the studied warty cabbage populations indicates that genetic drift in these populations is much greater than

the gene flow between them (Joyce and Pullin 2003; Durka et al. 2005). These molecular data confirm the influence of anthropogenic activity on the genetic structure of populations (Marrs et al. 2008; Kelager et al. 2013).

In general, population genetic diversity in different countries of the eastern Baltic region was found to be similar, albeit lower in Lithuania, where the populations also exhibited the highest genetic structure. For some of the Lithuanian populations (LT19), the proportion of polymorphic bands is very low, likely indicating a stronger influence of the founder effect in certain populations. According to the available historical records (Gudžinskas 1997), the spread of this species in Lithuania started approximately 100 years later than that in neighboring territories. Hence, founder effects and limited gene flow could have influenced the greater isolation of *B. orientalis* populations in this part of the Baltic region. Nevertheless, the genetic diversity of some other populations (LT1, LT3, and LT12) were demonstrated to be rather high, which could be the result of genetic mixing between populations from genetically distinct origins (Table 1).

## Genetic differences between native and non-native populations

Comparison of genetic diversity parameters (Br, PLP, Hj) between populations from the native region and two non-native regions revealed significant trends in the genetic diversity patterns of these regions (Table 2). Differences between the populations from the native and non-native groups were found in both cases, despite the locations of the non-native populations. In contrast, no statistically significant difference in these diversity parameters was detected between the groups of populations from the two different non-native regions. PCoA also supported this pattern of the distribution of genetic diversity and revealed separate groupings of most native and non-native populations (Fig. 2). Our results did not indicate that the invasion of *B. orientalis* was associated with a loss of genetic diversity. We found higher genetic diversity in the non-native populations than in the native populations, and the highest values of genetic diversity parameters were obtained in the non-native populations of the eastern Baltic range. Genetic differentiation was also 2–3 times higher between populations from the native and non-native groups than between populations from non-native regions. Thus, our results confirm the findings of some previous studies in which no decrease in genetic diversity was observed between non-native populations and native populations. According to these findings, biological invasions are not necessarily associated with genetic bottlenecks, and the initial decrease in genetic diversity may be counteracted by multiple introductions and by genetic recombination between genetically divergent individuals (Novak and Mack 1993; Kelager et al. 2013). For example, Bossdorf et al. (2005) compared 13 independent studies of within-population genetic diversity and noted that the diversity of non-native populations was decreased in only four species, whereas it was increased in two species. Higher average genetic diversity in introduced populations than in native populations can be explained by various factors, including (1) multiple introductions from genetically divergent sources, which can alleviate the loss of genetic diversity in the non-native range (Novak and Mack 1993); (2) introductions from areas not included in the part of the native range under investigation (Kelager et al. 2013; Shirk et al. 2014); (3) evolution of the species in a non-native area, which could generate new patterns of genetic diversity adapted to spreading under the diverse conditions of the new range (Lavergne and Molofsky 2007); or (4) changes in native populations toward reduced genetic diversity, possibly due to major environmental fluctuations. All of these factors may play a role in the formation of diversity patterns. The results of STRUCTURE analysis indicated high genetic heterogeneity of the native populations, corroborating the AMOVA results, which showed high between-population diversity. The genetic structure analysis of the studied populations and the available information about the spreading history of this species allowed us to consider the impact of multiple introductions on the current genetic diversity patterns of the non-native populations of *B. orientalis* responsible for the lack of a decrease in genetic diversity. The observed genetic variation was almost equally distributed between and within populations, and both the non-native and native populations were highly structured, with genetic differentiation being higher between native populations. This partitioning of genetic diversity is not common for outcrossing perennial species, which usually show low or moderate population differentiation (Nybom

and Bartish 2000). The rather high genetic differentiation of the *B. orientalis* populations may be associated with the self-fertility of this species (Dietz et al. 1999a; Denisow et al. 2016) and the reduced gene flow between populations in both the native and non-native ranges.

In numerous cases, Bayesian cluster analysis indicated that the non-native populations shared relatedness with native populations, which was characteristic of populations assigned to the light gray, white, and dark gray clusters (Fig. 3). The native populations grouped into the gray cluster did not show relatedness to any non-native population from the set of populations included in the STRUCTURE analysis. The non-native populations of the black cluster were also exclusive and did not exhibit relatedness to any native population, which implies that the studied native populations do not represent all of the existing genetic diversity in the native range. The relatedness between the non-native eastern Baltic (LT3 and LV8) and western Siberian population gene pools assigned to the black cluster (RUS3, RUS4 and RUS5) illustrates this situation. As the black cluster included only the non-native populations, it is conceivable that related native source populations were not included in our study. This point of view is supported by the prevailing differentiation between populations from the native and non-native regions. However, the lack of geographical clustering in non-native populations (Fig. 3) and the absence of an IBD pattern ($R = 0.11$; $P = 0.066$) indicate the significant influence of anthropogenic factors on the spreading of this species (Marrs et al. 2008; Shirk et al. 2014; Wang et al. 2015). STRUCTURE analysis revealed that the LT3 and LV8 non-native populations of the eastern Baltic region were highly mixed and showed a combination of genetic pools from genetically different source populations. Genetic recombination due to repeated introductions to the same location may increase genetic diversity, initiate adaptive evolution, and cause an alien species to spread by reducing the impact of genetic drift and generating genetic innovations (Lavergne and Molofsky 2007). This finding also implies that invasive populations probably have substantial amounts of adaptive genetic variation to reduce or avoid the effects of control measures (Allendorf and Lundquist 2003). However, recent genomic studies of outcrossing plant populations have revealed the accumulation of putative harmful mutations and the slight growth of genetic load in range-front populations compared to species core populations (González-Martínez et al. 2017). These revelations disclose the worth of native populations in the management of species genetic resources. In this aspect, our study is the first step toward the characterization of warty cabbage native populations and indicates that they possess rather different patterns of genetic diversity. The Siberian non-native populations included in our study appeared to be less mixed, possibly due to introductions from related gene pools at the same location (Okada et al. 2007).

In summary, our study did not reveal a decrease in genetic diversity in the non-native populations of *B. orientalis*. Similar tendencies of changes in the genetic structure of this species were detected in two geographically and ecologically distinct, non-native regions located in Europe and Asia. In both of these regions, the non-native populations exhibited higher genetic diversity than the native region populations, possibly due to multiple introductions of propagules from genetically distant sources. It appears that higher genetic diversity generated during multiple introductions of non-native populations may play a positive role in the spreading of *B. orientalis* to new areas. Moreover, the study of genetic polymorphism of native populations of *B. orientalis* has several implications not only for assessing the potential of invasive populations for the spread or the development of resistance to control measures but also for the characterization of local germplasm that can be used for breeding purposes.

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