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Research article

A wide range of abiotic habitat factors and genetic diversity facilitate expansion of *Trapa natans* within its native range

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ABSTRACT

Climate change and intense human activity are exacerbating changes in species' ranges. While the rapid spread of invasive alien species is well documented worldwide, the phenomenon of the spread of native species is poorly understood. To explain the problem of rapidly spreading species in the changing world, it is necessary to understand their ecology, genetic diversity and habitat limitation. The aim of our study was to analyze the ecological requirements and genetic diversity in the population of the macrophyte *Trapa natans* s. l., an invasive alien species in North America but native in Europe and Asia. We investigated the populations in its native range (Central and Northeastern Europe), where the species is defined as rare or extinct. We found the occurrence of *T. natans* in Northeastern Europe aquatic habitats where, up to now, it was described as an extinct species. The results of our environmental studies showed that the species has a wide range of tolerance to habitat conditions and lives in medium to highly nutrient-rich water with low and high salinity. Using Amplified Fragment Length Polymorphism (AFLP) analysis, we revealed high genetic variability within populations with relatively limited differentiation between populations. We showed that some populations are highly diverse (possibly refugia; Central Europe) and others are homogeneous (new sites, commercial reintroduction; Northeastern Europe). Conservation status of *T. natans* in its native range should be reconsidered, as the species has spread rapidly in recent decades and could be detrimental to aquatic habitats. The conclusion is that expansion/invasion can start from small populations, but under favorable conditions these populations spread rapidly. The introduction of species (even native) should be done carefully, if at all, as uncontrolled introduction to new locations, e.g. private ponds, could be the start of dispersal (native habitats) or invasion (non-native area).

1. Introduction

In the face of global climate change, one of the most important factors influencing the functioning of living organisms is the dynamic shifting of optimal life zone boundaries. This process, which is often observed in the context of non-native invasive species, has potentially negative consequences for the natural habitats (Pyšek et al., 2020). However, equally important are phenomena associated with "expanding native" species (Simberloff et al., 2012; Yazlık and Ambarlı, 2022), which can significantly affect the structure and composition of communities as well as interactions between species and ecosystems through the expansion of their ranges. These processes can result in a domino effect that can have far reaching consequences for ecological stability (Díaz et al., 2019). To explain the problem of fast spreading species in the changing world, understanding their ecology and genetic diversity is necessary, regardless of whether they are invasive alien or expansive native species.

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A special attention is paid to aquatic habitats and organisms dependent on them, because they are particularly vulnerable to climate changes (Weiskopf et al., 2020). One of the aquatic plants, which expansion in its native range is linked to climate change is *Trapa natans* s. l. (Walusiak et al., 2024). It is an annual, clonal macrophyte native to Europe, Asia and Africa (Phartyal et al., 2018; Palm et al., 2024) and an invasive alien species in North America (https://www.invasive.org/). Within its native range in Europe, it has been classified as rare in Bern Convention Appendix I (Convention on the Conservation of European Wildlife and Natural Habitats. Standing Committee, 1997) and in some countries (Lithuania, Spain) even as an extinct species (IUCN, 2022). However, in the last decades, a strong spread of this species has been observed in Europe (Marković et al., 2015; Merzlikin and Savitsky, 2021; Jusik et al., 2023; Ławicki et al., 2017; Walusiak et al., 2024; Herrmann pers. observation; https://www.iop.krakow.pl/kotewka). In some places, the spread is so vast that the species has been excluded from the national Red Book of Plants (Ukraine) (Merzlikin and Savitsky, 2021). The rapid expansion of *T. natans* in Europe raises questions about its genetic diversity and ecological requirements.

The taxonomic ambiguity of *Trapa* (Takano and Kadono, 2005 demonstrated up to 20 species) makes it difficult to determine the relationships between the ecological requirements of particular taxa. Clarification of these relationships would aid conservation efforts for endangered and regionally protected species.

The aim of our study was to analyzed the genetic diversity in the population of *Trapa natans* s. l. that have recently expanded in Central and Eastern Europe. We analyzed the genetic diversity within and between four river basins because some earlier studies, based only on the morphology of nuts, assumed that different species (e.g. *T. natans* and *T. conocarpa*) and their hybrids could co-occur in different river basins (Staszkiewicz and Wójcicki, 1981). Molecular analyses were combined with the studies of ecological conditions (chosen abiotic factors) of the habitats where species occurs. We want to explore whether *Trapa* expansion in Europe is cryptic, similar to United States case (Chorak et al., 2019), or this is only one species – *T. natans*. Other our questions concerned the genetic diversity of *T. natans*, whether it inhabits only specific habitats and its tolerance to abiotic factors. The results might help manage the invasions and expansions, which increases because of climate change and high human activity. Understanding the process of dispersal is crucial for the development of appropriate conservation plans for aquatic ecosystems and for specific species.

2. Material and methods

2.1. Study sites

The material of *Trapa natans* was collected from four river basins in Central and Eastern Europe: eastern basin of the Elbe river (NE Germany, 4 sites), the Oder river basin (W Poland, 8 sites), the Vistula river basin (S Poland, 6 sites) and the Dvina river basin (Lithuania and Latvia, 4 sites) (Fig. 1, Suppl. 1). Biological material was collected from reservoirs where *T. natans* was abundant, forming monospecific patches. The environmental analyzes were carried out at the same time and place. In total, the material from 22 sites was collected and analyzed (Suppl. 1). For molecular studies, at each sampling site, one leaf was collected from each of 10 individuals distanced at least 5 m from each other. Immediately after collection, each leaf was individually placed in a tube with silica gel, dried and stored at room temperature until DNA isolation.

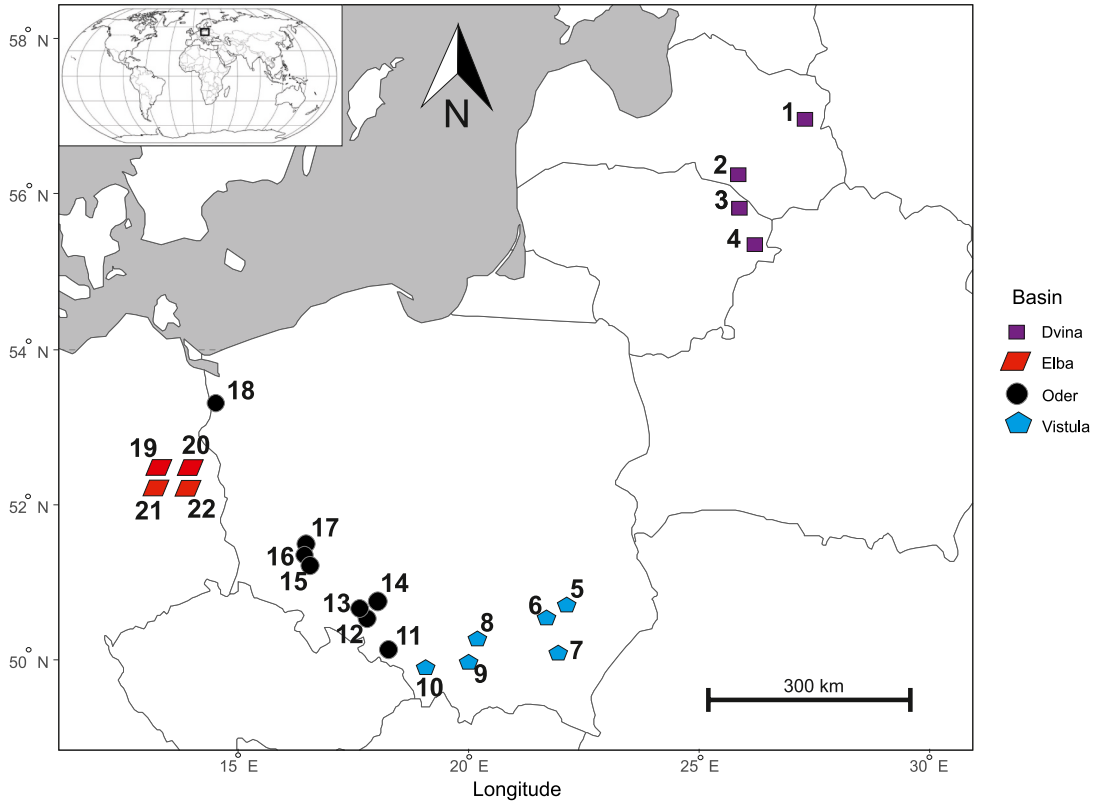

Fig. 1. Localities of sampled places: western Dvina basin (1–4), Vistula basin (5–10), Oder basin (11–18) and eastern Elbe basin (19–22).
Symbols correspond to individual populations: 1. LVP – Latvia, Lake Pokratas; 2. LVK – Latvia, Lake Klaucānu; 3. LTA – Lithuania, Lake Avilys; 4. LTS – Lithuania, Lake Šaminis; 5. PPN – Poland, Pniów; 6. PGP – Poland, Góry Pieprzowe; 7. PRZ – Poland, Rzeszów; 8. PPO – Poland, Podkamycze; 9. PTN – Poland, Tyniec; 10. POS – Poland, Oświęcim; 11. PLE – Poland, Łężczok Nature Reserve; 12. PGS – Poland, Gęsi Staw; 13. PST – Poland, Stobrawa; 14. PKR – Poland, Krogulna; 15. PSC – Poland, Ścinawa; 16. PLU – Poland, Lubów; 17. PTR – Poland, Tarchalice; 18. st2POD – Poland, Oder; 19. DEB – Germany, StandUpClub Berlin – Schmoeckwitz; 20. DEF – Germany, Fuerstenwalder Spree; 21. DET – Germany, Teupitz, Teupitzer See; 22. DEO – Germany, Oegelnscher See.

2.2. Environmental factors analyses

To determine the habitat conditions of *T. natans*, water temperature, pH and conductivity were measured *in situ* using YSI ProDSS probe. In order to perform chemical analyses (ions concentration) water samples were collected at the same time, stored in a field refrigerator and transported to the laboratory of the Institute of Nature Conservation of the Polish Academy of Sciences. Ion concentrations (phosphates, nitrate nitrogen, ammonia nitrogen, carbonates, sulphates) were measured using a Dionex ion chromatograph (DIONEX, IC25 Ion Chromatograph; ICS-1000, Sunnyvale, CA, USA). In order to visualize selected environmental variables with radar charts values of particular ones were scaled to values between 0 and 1, based on minimum and maximum value of each variable.

2.3. Molecular analyses: DNA extraction, Amplified Fragment Length Polymorphism (AFLP) fingerprinting and DNA sequencing

Total genomic DNA was extracted from 10 mg of dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. DNA quality and concentration were estimated on a 1% agarose gel stained with ethidium bromide against λ-DNA. The AFLP analysis of 220 individuals of *T. natans* was performed according to the procedure of Paun and Schönswetter (2012). After screening eight selective primer pairs, three combinations were selected that showed the highest level of polymorphism and reliability of AFLP profiles: *Eco*R-I-ACT/*Mse*I-CTG, *Eco*RI-AGG/*Mse*I-CAA and *Eco*RI-AGC/*Mse*I-CTG. To control the reproducibility of the method, we included duplicates of 5% of the individuals within and 3% between plates (Bonin et al., 2004). The final PCR products was prepared using POP7 polymer with a GeneScan 600LIZ internal size standard (Applied Biosystems, Foster City, CA, USA) on a 3130 Genetic Analyzer (Life Technologies, Carlsbad, California, US). After an initial check with the standard parameters, the size profiles of all individuals were checked and adjusted manually if necessary. Automated scoring was performed in the range of 50–500 bp using peak saturation, baseline subtraction, local southern peak call, spike removal, pull-up correction and a stutter peak filter of 5% (left and right) using GeneMarker® ver. 2.2.0 (SoftGenetics; Riley et al., 2014). The relative fluorescence unit (RFU) threshold was set at 30. All data was then were manually checked for accuracy. Only AFLP fragments that were repeatable, well separated and unambiguous included in subsequent analyzes, and single AFLP fragments were excluded. A panel of analyzable AFLP fragments was created for each primer combination, and fragments in the 50–500 bp range were scored for the presence (1) or absence (0) of fragments and finally assembled into a single binary matrix. Finally, AFLP fingerprint profiles of sufficient quality were obtained for 214 individuals from initial 220.

DNA sequence variation of 17 individuals of *Trapa natans* (10 populations) from the Dvina, Vistula, Oder and Elbe river basins were screened. The internal transcribed spacer (ITS) region was amplified and sequenced using ITS1A and ITS4 primers (White et al., 1990; Blattner, 1999). The composition of the PCR mixture with a total volume of 25.5 μL contained: 1U AmpliTaq360 DNA (Thermo Fisher Scientifc Inc.), 1 × PCR Bufer with the enzyme (Thermo Fisher Scientifc Inc.), 2.5 mM MgCl2 (Applied Biosystems, Thermo Fisher Scientifc Inc.), 0.3 μM of each primer, 0.12 mM dNTP (SigmaAldrich Co.), 0.8% BSA at a concentration of 1 mg/mL (New England BioLabs Inc.), and 1 μL of DNA template. The following touchdown PCR cycling profile was used: 3 min at 94 °C; 10 cycles of 30 s at 94 °C, 30 s at 60 °C (with a decrease of 1 °C per cycle), and 1 min at 72 °C; 25 cycles of 30 s at 94 °C, 30 s at 50 °C, and 1 min at 72 °C; final extension step of 7 min at 72 °C; cool down to 4 °C. Enzymatic purification of the PCR product was performed using a mixture of exonuclease I (10 U/μl; EURx) and alkaline phosphatase (1 U/μl; Afymetrix). Sequencing was performed using the BigDye Terminator ver. 3.1 Sequencing Kit (Thermo Fisher Scientifc Inc.) with buffer BDX64 (BigDye Enhancing Bufer; MCLAB) according to the BDX64 buffer manufacturer's recommendations for a 32-fold dilution of BigDye chemistry. Amplifications and incubations were performed using the GeneAmp PCR System and the VeritiTM 96-Well Thermal Cycler. Forward and reverse DNA sequences were automatically assembled and aligned using the Geneious Pro 6.0.2 program (Drummond et al., 2011) according to the ClustalW algorithm (Thompson et al., 1994; Larkin et al., 2007). In addition, the following 13 sequences of *Trapa* from Italy, Germany, Switzerland and Sri Lanka deposited in GenBank (KX098565–KX098577) were used for our analyzes. The original ITS sequences obtained in our study were deposited in GenBank (the deposit numbers: OR648250–OR648266).

2.4. Analysis of AFLP and ITS region data and statistical analysis

Statistical analysis was performed using the method based on the presence and frequency of alleles at AFLP loci (Bonin et al., 2007). Genetic diversity within populations was assessed by calculating the number (P) and proportion (%P) of polymorphic AFLP fragments and Nei's gene diversity (*h*). Because *T. natans* reproduces clonally, we also calculated parameters for clonal diversity, including the number of genotypes (*G*), genotype diversity (*D*) and the effective number of genotypes (*E*). Genetic parameters were calculated using the R-script AFLPdat (Ehrich, 2006). To identify isolated and genetically distinct populations, frequency down-weighed marker values (Schönswetter et al., 2005), were calculated using AFLPdat (DW2; Ehrich, 2006). In addition, as a measure of divergence, the number of private AFLP fragments (N_p) and the number of characteristic AFLP fragments (N_{ch}) were calculated using FAMD ver. 1.31 (Schlüter and Harris, 2006). The statistical significance of differences in genetic parameters between populations of specific river basins was tested using the non-parametric Mann-Whitney *U* test for independent groups (STATISTICA 8.0; StatSoft, Inc., 1984–2007, Tulsa, OK, USA).

The overall genetic pattern of relationships among individuals in AFLP datasets was investigated using Neighbour-Joining (NJ) analyses based on uncorrected p-distances, with bootstrapping determined from 1000 replicates, as implemented in SplitsTree ver. 4.14 (Huson and Bryant, 2006). In addition, spatial genetic relationships between individuals were investigated by Principal Coordinate Analysis (PCoA) based on a matrix of Nei & Li distances, using the program FAMD ver. 1.25 (Schlüter and Harris, 2006). For further study of the genetic structure of the populations, we used the model-based Bayesian multilocus assignment method described in the program STRUCTURE ver. 2.3.4 (Pritchard et al., 2000; Falush et al., 2007). Determination of the number of subpopulations exhibiting distinct genetic groups (ΔK) was performed using an *ad hoc* statistical method that takes into account the rate change within the log-likelihood of data between successive *K* values (Evanno et al., 2005). The analysis was performed by categorizing individuals into *K* groups based on their multilocus genotype. The estimated *K* value was set between 2 and 23 using 10,000 replicates and 50,000 burn-in steps for each *K*. All runs were repeated 10 times for each *K*, and the optimal *K* value was obtained by calculating ΔK with Structure Harvester ver. 0.6.92 (Earl and von Holdt, 2012).

To determine population structuring, a hierarchical analysis of molecular variance (AMOVA) was performed and genetic differentiation between populations and groups was tested – (i) comparison between populations from the Elbe, Oder, Vistula and Dvina river basins; (ii) between populations from the Vistula and Oder river basins; and (iii) between populations from the upper and lower Oder river, all using Arlequin software ver. 3.5.2.2 (Excoffier and Lischer, 2010). Gene flow (N_m) between populations was assessed using POPGENE ver. 1.32 software (Yeh et al., 1999).

The significance of the relationship between pairwise genetic (F_{ST} values) and geographic distances [km] was tested using the Mantel test (Mantel, 1967) with 1000 random iterations and performed with the Mantel Nonparametric Test Calculator ver. 2.0 (Liedloff, 1999). Approximate geographic distances [in km] between pairs of

populations, measured in a straight line, were calculated using their geographic coordinates.

The alignment of ITS region was analyzed using an unrooted maximum likelihood (ML) and generated using PhyML 3.0 software (Guindon et al., 2010). The analysis was performed on the ATGC bioinformatics server (http://www.atgc-montpellier.fr/phyml/) and the substitution models were automatically selected by SMS according to the Bayesian Information Criterion (BIC) (Lefort et al., 2017). To avoid interrupting the calculation at a local maximum of the likelihood function, five random trees were used as starting trees, which were estimated using the BioNJ algorithm (Gascuel, 1997). The nonparametric bootstrap method was used to calculate the support values for the internal branches with 1000 replicates.

3. Results

3.1. Environmental factors

Trapa natans inhabited low-current, slow-flowing or stagnant waters, meso-to highly eutrophic, with a wide range of environmental parameters (Fig. 2, Table 1, Suppl. 2).

- the water temperature ranged from 21.2 °C (3. LTA, Dvina river basin) to 26.5 °C (6. PGP, Vistula river basin);
- conductivity from 101.8 (1.LVP, Dvina river basin) to 1633.0 μScm^{-1} (9. PTN, Vistula river basin);
- water pH from 7.27 (20. DEF, Elbe river basin) to 9.10 (8. PPO, Vistula river basin);
- ammonia nitrogen from below detection limit (10. POS, Vistula river basin) to 0.5419 mgL^{-1} (11. PLE, Oder river basin);
- nitrate nitrogen concentration from below detection limit (5. PPN and 6. PGP, Vistula river basin), to 6.0455 mgL^{-1} (18. st2POD, Oder river basin);
- phosphates from below detection limit (several samples from Vistula, Oder and Elbe river basins) to 0.1014 mgL^{-1} (22. DEO, Elbe river basin);
- carbonates from 42.6521 (1. LVP, Dvina river basin) to 220.5028 mgL^{-1} (7. PRZ, Vistula river basin)
- and sulphates from 0.1177 (5. PPN, Vistula river basin) to 260.8528 mgL^{-1} (22. DEO, Elbe river basin).

The habitats in the northern part of Eastern Europe (Lithuania and Latvia, Dvina river basin) were characterized with the lowest values of conductivity, ammonia nitrogen, nitrate nitrogen, sulphates and phosphates (Table 1, Suppl. 2). Also, the lowest average values and SD for conductivity, ammonia nitrogen, nitrate nitrogen and sulphates were noted for the Dvina river basin (Lithuania and Latvia). In contrast, the habitats in Poland (Vistula and Oder river basins) were characterized with the highest values of the parameters studied, with the exception of

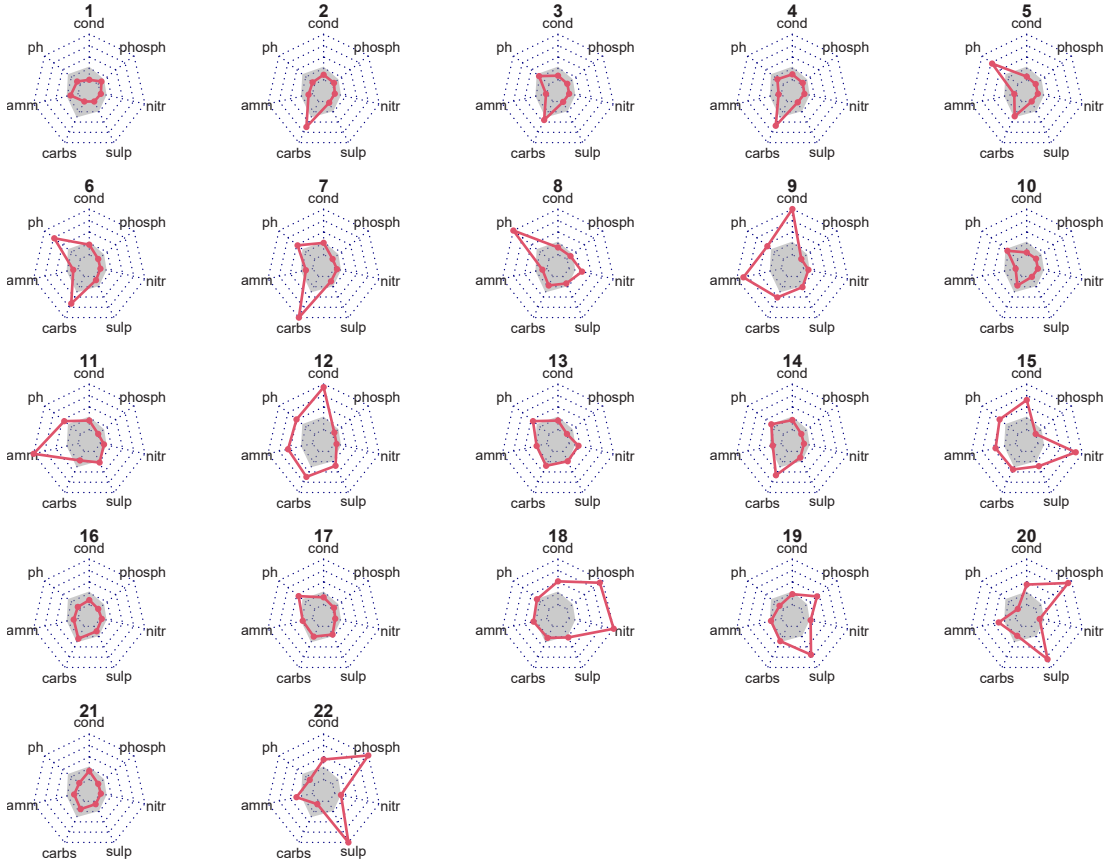

Fig. 2. The chemical and physical characteristics of the studied aquatic habitats: cond – conductivity; phosph – phosphates; nitr – nitrate nitrogen; sulp – sulphates; carbs – carbonates; amm – ammonia nitrogen; ph – water pH. The values are scaled in range 0–1.
Symbols correspond to individual populations of the western Dvina basin (1–4), Vistula river (5–10), Oder River (11–18) and eastern Elbe basin (19–22): 1. LVP – Latvia, Lake Pokratas; 2. LVK – Latvia, Lake Klaucānu; 3. LTA – Lithuania, Lake Avilys; 4. LTS – Lithuania, Lake Šaminis; 5. PPN – Poland, Pniów; 6. PGP – Poland, Góry Pieprzowe; 7. PRZ – Poland, Rzeszów; 8. PPO – Poland, Podkamycze; 9. PTN – Poland, Tyniec; 10. POS – Poland, Oświęcim; 11. PLE – Poland, Łężczok Nature Reserve; 12. PGS – Poland, Gęsi Staw; 13. PST – Poland, Stobrawa; 14. PKR – Poland, Krogulna; 15. PSC – Poland, Ścinawa; 16. PLU – Poland, Lubów; 17. PTR – Poland, Tarchalice; 18. st2POD – Poland, Oder; 19. DEB – Germany, StandUpClub Berlin – Schmoeckwitz; 20. DEF – Germany, Fuerstenwalder Spree; 21. DET – Germany, Teupitz, Teupitzer See; 22. DEO – Germany, Oegelnscher See.

Table 1
Chosen parameters of water habitats inhabited by *Trapa natans* in four river basins (Dvina basin – Lithuania and Latvia; Vistula and Oder basins – Poland; Elbe basin – Germany).

		Dvina basin	Vistula basin	Oder basin	Elbe basin
Water temperature [°C]	Min-max	21.2–23.9	23.7–26.5	22.5–24.6	22.8–24.2
	Av.	22.75	25.00	23.15	23.23
	SD	1.24	1.13	0.85	0.64
Conductivity [μScm^{-1}]	Min-max	101.8–286.0	175.0–1633.0	249.0–1530.0	388.0–783.0
	Av.	224.88	549.33	668.63	599.50
	SD	83.83	545.80	454.43	209.97
pH	Min-max	7.38–7.79	7.80–9.10	7.40–8.20	7.27–7.52
	Av.	7.52	8.21	7.90	7.39
	SD	0.18	0.46	0.26	0.12
Ammonia nitrogen [mgL^{-1}]	Min-max	0.0090–0.0909	0.0000–0.4625	0.0553–0.5419	0.0461–0.2052
	Av.	0.046	0.112	0.205	0.143
	SD	0.034	0.174	0.160	0.073
Carbonates [mgL^{-1}]	Min-max	42.6521–153.591	80.9831–220.5028	82.255–152.476	54.8199–106.2376
	Av.	116.39	130.34	110.12	80.48
	SD	51.01	53.69	26.76	21.23
Sulphates [mgL^{-1}]	Min-max	0.5098–7.3271	0.1177–67.2314	30.4531–93.6633	18.0872–260.8528
	Av.	4.35	28.01	63.76	166.53
	SD	2.97	25.77	22.66	104.61
Nitrate nitrogen [mgL^{-1}]	Min-max	0.0055–0.1133	0.0000–1.7271	0.0335–6.0455	0.0811–0.9438
	Av.	0.050	0.451	1.683	0.542
	SD	0.047	0.681	2.459	0.431
Phosphates [mgL^{-1}]	Min-max	0.0009–0.0089	0.0000–0.0097	0.0000–0.0935	0.0000–0.1014
	Av.	0.005	0.002	0.013	0.006
	SD	0.003	0.004	0.033	0.047

Abbreviations: Min-max (range of parameters), Av. (average) and SD (standard deviation).

phosphate concentration. The highest value and highest SD of phosphates concentration, were found in Elbe river basin (Germany) but the highest average value was found in Oder river basin (Poland) (Table 1, Suppl. 2). The lowest pH values, average and SD was found for Elbe river basin (Germany) but the highest for Vistula and Oder river basins (Poland) (Table 1, Suppl. 2).

3.2. AFLP and ITS region variation

The studied populations of *Trapa natans* exhibited varying levels of genetic diversity (Table 2). A total of 199 clearly identifiable AFLP fragments were yielded, of which 92 (46.2%) were polymorphic. The highest genetic diversity was found in the three populations from Polish

Table 2
Genetic diversity parameters of *Trapa natans* populations based on AFLPs and ITS region.

Code	N_{AFLP}/N_{ITS}	P/%P	N_{ch}	*h*	DW2	*G*	*D*	*E*	R
1. LVP	10	1/1	0	0	0.27	1	0	1	–
2. LVK	9/1	7/4	0	0.01	1.52	1	0	1	R1
3. LTA	10	1/1	1	0	1.27	1	0	1	–
4. LTS	10/2	15/8	3	0.02	5.61	4	0.64	2.38	R1
5. PPN	9/2	9/5	1	0.02	2.73	4	0.69	2.61	R1
6. PGP	10/2	11/6	0	0.02	2.13	5	0.67	2.5	R1
7. PRZ	9	33/17	2	0.07	11.61	9	1	9	–
8. PPO	10	23/12	0	0.03	6.96	4	0.53	1.92	–
9. PTN	9/2	9/5	1	0.02	2.79	4	0.75	3	R1
10. POS	9	30/15	1	0.06	7.59	9	1	9	–
11. PLE	10/2	16/8	0	0.02	4.05	5	0.67	2.50	R1
12. PGS	10	17/9	0	0.03	3.53	10	1	10	–
13. PST	10	14/7	0	0.03	4.08	8	0.96	7.14	–
14. PKR	10/1	12/6	0	0.02	2.56	6	0.84	4.17	R1
15. PSC	10	11/6	0	0.02	2.29	4	0.73	2.94	–
16. PLU	10/1	31/16	0	0.05	7.39	8	0.93	6.25	R1
17. PTR	10	10/5	0	0.02	1.50	5	0.67	2.50	–
18. st2POD	9	13/7	0	0.02	2.56	4	0.75	3	–
19. DEB	10	18/9	1	0.04	4.84	10	1	10	–
20. DEF	10	17/9	0	0.02	4.55	3	0.51	1.85	–
21. DET	10/2	17/9	2	0.02	6.14	4	0.64	2.38	R1
22. DEO	10/2	23/12	0	0.04	6.01	8	0.96	7.14	R1
Mean per population ±SD	9.7/1.7	15.36 ±8.59/ 8.05 ±1.50		0.03 ±0.02	4.18 ±2.67	5.32 ±2.82	0.68 ±0.32	4.24 ±3.07	
Total	214/17	92							

Abbreviations: N_{AFLP}/N_{ITS} – population sampling for AFLP analysis and nrDNA sequencing; P/%P – number and percentage of polymorphic AFLP fragments per population; N_{ch} – characteristic (rare) AFLP fragments; *h* – average gene diversity; DW2 – frequency down-weighted marker values; *G* – number of genotypes; *D* – genotype diversity; *E* – effective number of genotypes; R – ribotype variants of ITS region in population.
1. LVP – Latvia, Lake Pokratas; 2. LVK – Latvia, Lake Klaucānu; 3. LTA – Lithuania, Lake Avilys; 4. LTS – Lithuania, Lake Šaminis; 5. PPN – Poland, Pniów; 6. PGP – Poland, Góry Pieprzowe; 7. PRZ – Poland, Rzeszów; 8. PPO – Poland, Podkamycze; 9. PTN – Poland, Tyniec; 10. POS – Poland, Oświęcim; 11. PLE – Poland, Łężczok Nature Reserve; 12. PGS – Poland, Gęsi Staw; 13. PST – Poland, Stobrawa; 14. PKR – Poland, Krogulna; 15. PSC – Poland, Ścinawa; 16. PLU – Poland, Lubów; 17. PTR – Poland, Tarchalice; 18. st2POD – Poland, Oder; 19. DEB – Germany, StandUpClub Berlin – Schmoeckwitz; 20. DEF – Germany, Fuerstenwalder Spree; 21. DET – Germany, Teupitz, Teupitzer See; 22. DEO – Germany, Oegelnscher See.

rivers and their basins: the Vistula river (pop. 7. PRZ and pop. 10. POS) and the Oder river (pop. 16. PLU). The highest clonal diversity was found in the two Polish populations from the Vistula river basin (pop. 7. PRZ and pop. 10. POS) and one German population from the Elbe river basin (pop. 19. DEB). The lowest genetic and clonal diversity were found in the two Lithuanian – Latvian populations from the Dvina river basin (pop. 1. LVP and pop. 3. LTA; Table 2).

Frequency down-weighed marker (showed as DW2; Table 2) had low values with strong differences between populations, ranging from 0.27 in the Dvina river basin (pop. 1. LVP) to 11.61 in the Vistula river basin (pop. 7. PRZ), with an average of 4.18 (±2.67). The highest DW2 values (above 7) were observed in the two populations from the Vistula river basin (pop. 7. PRZ and pop. 10. POS), and one from the Oder river basin (pop. 16. PLU). It is also worth to indicate population 8. PPO from the Vistula river basin (Poland) and two populations: 21. DET and 22. DEO from the Elbe river basin (Germany) which had high DW rarity (6.96, 6.14 and 6.01 respectively; Table 2).

Private AFLP fragments were not detected and only eight populations contained characteristic (rare) AFLP fragments (N_{ch}). There were: two populations from the Dvina river basin (populations: 3. LTA and 4. LTS, both from Lithuania), two populations from the Elbe river basin (populations: 19. DEB and 21. DET, both from Germany), and four populations from the Vistula river basin (populations: 5. PPN, 7. PRZ, 9. PTN, 10. POS, all from Poland). The populations from the Oder river basin (Poland) had no characteristic AFLP fragments (Table 2).

The Mann-Whitney *U* test showed that the indexes of genetic diversity within the populations of the Dvina river basin differed significantly ($P < 0.05$) from those of the Vistula and Oder river basins (parameters: *h*, *G*, *D*, *E*) and from those of the Elbe river basin (%P). The populations of the Vistula and Oder river basins differ only in the number of private AFLP fragments ($P < 0.05$), while the populations of the Elbe river basin differ from those of the Oder river basin only in the DW value. The genetic diversity indexes did not differ significantly ($P > 0.05$) between the populations of the Vistula and the Elbe rivers (Table 2).

The Neighbour-Joining (NJ) analysis of *T. natans* showed the highest differences in some populations taken from the Vistula and Oder river basins (e.g. populations from the Vistula river basin: 7. PRZ, 10. POS, and from the Oder river basin: 13. PST, 16. PLU) (Fig. 3). At the same time, some individuals from populations, found in the Vistula, Oder and Dvina river basins, within different groups are scattered in the diagram (populations: 4. LTS, 5. PPN, 8. PPO and 16. PLU). Different groups in NJ have a low bootstrap support value (< 50%), although the genetic differences between river basins were found to be statistically significant in the AMOVA (Table 3). Majority of the Oder and Vistula river basins populations (Poland) were intermixed and formed one group; populations from the Elbe river basin (Germany) formed two groups: one of them was related to the Vistula and Oder river basin (Poland) and the second one to the western Dvina river basin (Lithuania and Latvia) (Fig. 3).

In the PCoA diagram (the first three axes explained of the total genetic variation: 19.19%, 16.94% and 8.78%, respectively), the individuals of *T. natans* were generally arranged in homogeneous groups. In majority there was lack a clear division between the all four river basins (Suppl. 3). However, individual populations showed varying degrees of genetic variation, ranging from genetically homogeneous populations, e.g. from the Dvina basin and the Elbe basin (pop. 20. DEF), to genetically distinctly variable populations from the Vistula basin (pop. 7. PRZ and pop. 10. POS), the Oder basin (populations: 11. PLE, 13. PST and 16. PLU) and second one from the Elbe basin (pop. 19. DEB) (Suppl. 3).

Based on the Bayesian analysis of the genetic structure of the

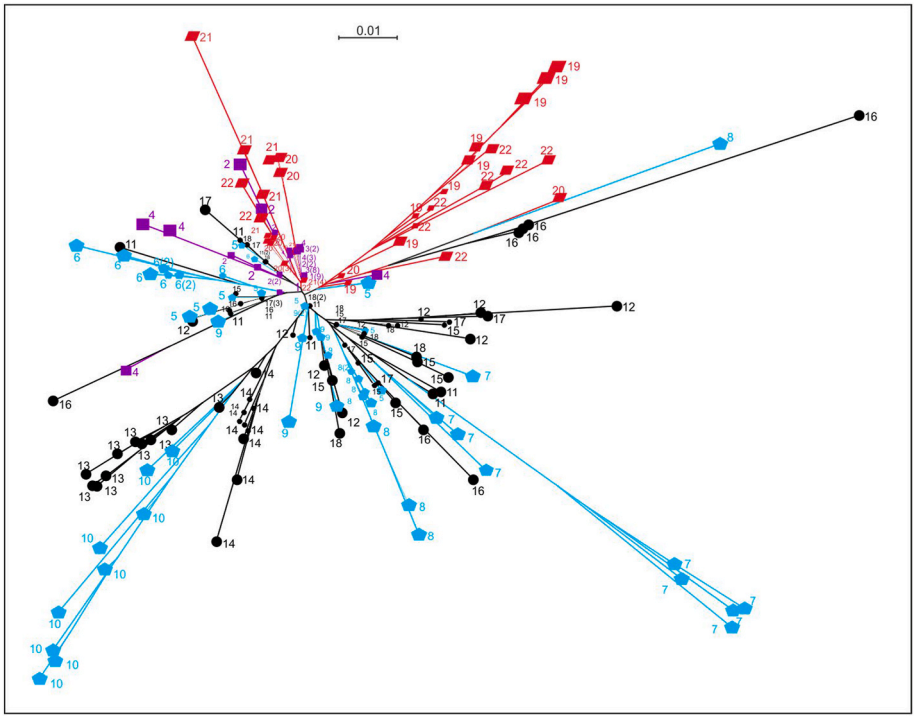

Fig. 3. Neighbour-Joining diagram based on uncorrected p-distances derived from AFLP data of *Trapa natans* s. l. from the northern European range limit, violet – the Dvina basin (1–4), blue – the Vistula basin (5–10), black – the Oder basin (11–18), red – the Elbe basin (19–22).
Abbreviations: 1. LVP – Latvia, Lake Pokratas; 2. LVK – Latvia, Lake Klaucānu; 3. LTA – Lithuania, Lake Avilys; 4. LTS – Lithuania, Lake Šaminis; 5. PPN – Poland, Pniów; 6. PGP – Poland, Góry Pieprzowe; 7. PRZ – Poland, Rzeszów; 8. PPO – Poland, Podkamycze; 9. PTN – Poland, Tyniec; 10. POS – Poland, Oświęcim; 11. PLE – Poland, Łężczok Nature Reserve; 12. PGS – Poland, Gęsi Staw; 13. PST – Poland, Stobrawa; 14. PKR – Poland, Krogulna; 15. PSC – Poland, Ścinawa; 16. PLU – Poland, Lubów; 17. PTR – Poland, Tarchalice; 18. st2POD – Poland, Oder; 19. DEB – Germany, StandUpClub Berlin – Schmoeckwitz; 20. DEF – Germany, Fuerstenwalder Spree; 21. DET – Germany, Teupitz, Teupitzer See; 22. DEO – Germany, Oegelnscher See.

Table 3
Summary of analysis of molecular variance (AMOVA) of *Trapa natans* s. l.

Source of variance	d.f.	Sums of squares	Variance components	% total variance	*F* statistics
(1) Dvina, Vistula, Oder, Elbe basins					
Among groups	3	116.963	0.444	10.12***	F_{CT} = 0.10
Among populations within groups	18	286.452	1.372	31.27***	F_{SC} = 0.35
Within populations	192	493.944	2.573	58.62	F_{ST} = 0.41
Total	213	897.360	4.389		
(2) Vistula and Oder basins					
Among groups	1	21.245	0.019	0.39^{NS}	F_{CT} = 0.004
Among populations within groups	12	241.900	1.782	37.40***	F_{SC} = 0.37
Within populations	121	358.811	2.965	62.22	F_{ST} = 0.38
Total	134	621.956	4.766		
(3) Upper Oder and Lower Oder basins					
Among groups	1	19.690	0.118	2.95^{NS}	F_{CT} = 0.03
Among populations within groups	6	89.997	1.252	31.18***	F_{SC} = 0.32
Within populations	71	187.756	2.644	65.87	F_{ST} = 0.34
Total	78	297.443	4.014		
(4) Central and Eastern Europe (all study populations of *T. natans* s. l.)					
Among populations	21	403.415	1.711	39.94***	F_{ST} = 0.40
Within populations	192	493.944	2.573	60.06	
Total	213	897.360	4.283		

Abbreviations: ***$P < 0.001$, ^{NS} – not significant ($P > 0.05$).

T. natans populations, an optimal division into two genetic groups $K = 2$ (i.e. the respective genetic pools) was found, which occur in different proportions in almost all populations in the entire study area (Fig. 4). Based on the determined mixing pattern, it was shown that the *Trapa* populations are characterized by different proportions of both genetic pools. All studied populations from Eastern Europe (the Dvina river basin, populations: 1. LVP, 2. LVK, 3. LTA, 4. LTS) proved to be almost completely genetically homogeneous, while the populations from the other studied river basins are characterized by a different level of mixing of both genetic pools. The two populations from the Elbe river basin (pop. 20. DEF and pop. 21. DET) are genetically homogeneous, while two others (pop. 19. DEB and pop. 22. DEO) are characterized by almost equal proportions of the two genetic pools. In the Vistula river basin, some populations (populations: 5. PPN, 6. PGP and 9. PTN) proved to be genetically homogeneous, while others (pop. 7. PRZ and pop. 10. POS) are the most different from the others, however population 8. PPO is the most variable. Within the Oder river basin, some populations are also homogeneous or nearly homogeneous (populations: 11. PLE, 12. PGS, 15. PSC, 17. PTR and 18. st2POD), while others show a varying degree of mixing of both gene pools (populations: 13. PST, 14. PKR and 16. PLU; Fig. 4). This statement is consistent with the calculated parameters of genetic variation within the populations of *T. natans* (Table 2).

With an assumed number of genetic groups $K = 3$, it was shown that part of the total genetic pool of *T. natans* occurs exclusively in the Vistula and Oder river basins, while it is absent in the Elbe and Dvina river basins (Fig. 4). It was also found that some populations from the Oder (pop. 13. PST and pop. 14. PKR) and Vistula (populations: 7. PRZ, 8. PPO and 10. POS) river basins have a distinct third gene pool, which is not present in the other populations (Fig. 4). This finding is consistent with the results of the hierarchical AMOVA, which revealed a small but statistically significant genetic difference ($F_{CT} = 10.12$, $P < 0.001$) between the river basins (Table 3). The hierarchical AMOVA revealed that the variance components were significantly split between the populations of the different river basins (10.1%, $F_{CT} = 0.10$, $P < 0.001$; Table 3). In the river basins, the vast majority of genetic differences was due to within-population variation (58.6%, $F_{SC} = 0.35$) compared to between-population variation (31.3%, $F_{ST} = 0.41$). A similar distribution of molecular variance was found between the Vistula and Oder river basins, where only 0.4% ($F_{CT} = 0.004$) of the genetic variation was between basins, but this value was not significant ($P > 0.353$; Table 3). Again, a greater proportion of diversity (62.2%, $F_{SC} = 0.37$) was found within populations but not between populations in the river basins (37.4%, $F_{ST} = 0.38$). Overall, AMOVA showed that genetic variation was mainly within populations (60.1%), and we found that the most genetically similar populations were often not geographically adjacent (Table 3). On the other hand, no significant differences in pairwise genetic distances (F_{ST}) ($P > 0.05$) were found for some populations within the Dvina river basin (pop. 1. LVP *vs.* 3. LTA and 4. LTS), the Oder river basin (populations: 11. PLE *vs.* 18. st2POD; 12. PGS *vs.* 17. PTR and 17. PTR *vs.* 18. st2POD), the Elbe and Dvina river basins (pop. 21. DET *vs.* pop. 4. LTS) and the Vistula and Oder river basins (populations 5. PPN *vs.* 11. PLE and 17. PTR). The remaining pairwise F_{ST} values between

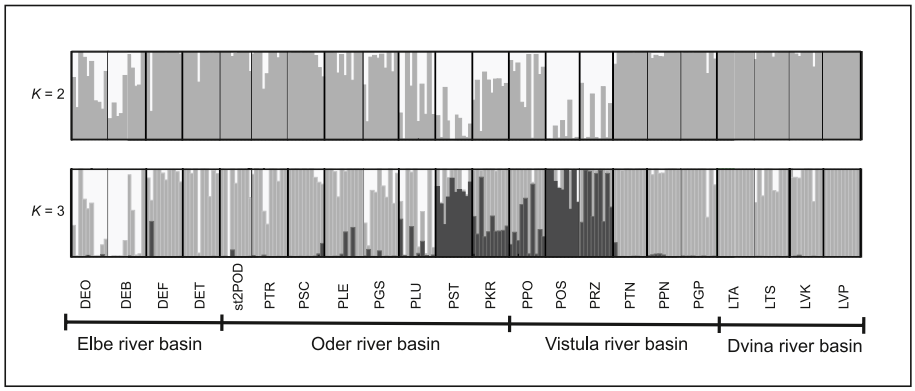

Fig. 4. Genetic structure of *Trapa natans* s. l. as resolved by the Bayesian spatial clustering (Structure software) of AFLP genotypes (214 individuals from 22 populations) at $K = 2$ and $K = 3$.
Abbreviations: DEO – Germany, Oegelnscher See; DEB – Germany, StandUpClub Berlin – Schmoeckwitz; DEF – Germany, Fuerstenwalder Spree; DET – Germany, Teupitz, Teupitzer See; st2POD – Poland, Oder 2; PTR – Poland, Tarchalice; PSC – Poland, Ścinawa; PLE – Poland, Łężczok Nature Reserve; PGS – Poland, Gęsi Staw; PLU – Poland, Lubów; PST – Poland, Stobrawa; PKR – Poland, Krogulna; PPO – Poland, Podkamycze; POS – Poland, Oświęcim; PRZ – Poland, Rzeszów; PTN – Poland, Tyniec; PPN – Poland, Pniów; PGP – Poland, Góry Pieprzowe; LTA – Lithuania, Lake Avilys; LTS – Lithuania, Lake Šaminis; LVK – Latvia, Lake Klaucānu; LVP – Latvia, Lake Pokratas.

populations differed significantly at $P < 0.05$ (Suppl. 4). The value of gene flow (assuming Hardy-Weinberg equilibrium) between all 22 populations from the four river basins was below 1 and amounted to $N_m = 0.73$. Similarly low values were also found between the populations in the Oder river basin with $N_m = 0.99$ and in the Vistula river basin with $N_m = 0.81$. In contrast, high N_m values were found in the Dvina basin ($N_m = 2.67$) and in the Elbe basin ($N_m = 2.45$). The Mantel test showed a very weak correlation between genetic differentiation and geographical distance for the entire set ($r = 0.20$, $P < 0.05$, $g = 1.680$, critical $g = 1.645$; Suppl. 5).

The resulting ITS region alignment was 405 bp long and had identical sequences in all individuals of *T. natans*, representing one rybotype. Comparison of the ITS region sequences of individuals labeled as *T. natans* (in GenBank) from different parts of the distribution area in Europe did not reveal any genetic differentiation between the individuals (diagram tree, not shown).

4. Discussion

Based on AFLP results, our study showed the high genetic variability within the populations with relatively low inter-population diversity of *Trapa natans*, which is mainly related to its high reproduction rate and efficient gene flow through seed dispersal (including human-mediated). Interestingly, previous studies of *T. natans* in the southern Alpine lake area (Insubria) showed no signs of strong genetic drift and associated loss of genetic diversity, despite a reduction of over 50% of local populations since the early 19th century (Frey et al., 2017). *T. natans* is not a typical annual plant and is not subject to typical extinction-colonization dynamics as it occurs in permanent water bodies (Barrett et al., 1993; Frey et al., 2017). Populations of water chestnut tend to have large census populations and a very efficient and long-term seed bank that reduces the level of genetic drift (Frey et al., 2017).

The expansion of *Trapa natans* within native range may be facilitated by its evolutionary potential, phenotypic plasticity, dispersal ability and climate change. Aquatic plants with a wide global distribution, such as *T. natans* s. l., have undergone evolutionary selection for stress resistance, with broad tolerance ranges. Selective advantages are provided by clonal growth, which increases plant tolerance to stress, genet survival and population viability. At the same time, broad plastic responses are common in aquatic species, facilitated by a combination of clonal growth, large local dispersal and temporal variability (Santamaría, 2002). Our molecular analyses indicate that the *Trapa* species expanding in Central and Northeastern Europe is exclusively *T. natans* s. l., which is able to adapt to different water habitats. In our study, no unique AFLP fragments were detected for potentially separate *Trapa* taxa, and the ITS region was found to be homogeneous, consistent with recent studies (Frey et al., 2017; ITS sequences of *T. natans* deposited in GenBank). This result contradicts the previous statement, based on observations in natural habitats and biometric analysis of nuts, that different *Trapa* species (i.e. *T. natans*, *T. conocarpa* and their hybrids) coexist in the river basins of Central Europe (Piórecki, 1980; Staszkiewicz and Wójcicki, 1981). Aquatic plants display a pronounced tendency to possess general-purpose genotypes that are capable of occupying large areas and exhibiting high clonal persistence. In most cases, they exhibit a considerable degree of intraspecific variation, which significantly constrains taxonomic resolution (Santamaría, 2002). However, in order to definitively establish the presence/absence of distinctive *Trapa* species, a genetic study of the material in the herbaria would have to be carried out. Moreover, studies of historical samples from herbaria, would better determine whether the *Trapa* lineages currently spread are historically native or they are more recently introduced from elsewhere. Populations of *T. natans* are able to adapt to different water habitats, resulting from the synchronization of the development cycle with climatic conditions and the hydrology of river systems (Piórecki, 1980).

We found presence of *T. natans* in Lithuanian aquatic habitats (Eastern Europe), where it has been described as an extinct species (Sinkevičienė, 2007; IUCN, 2022). If it persist and expands further, a new Lithuanian population will fill the species distribution gap between Polish and Latvian habitats. Generally, throughout the study area (Germany: eastern Elbe river basin; Poland: Oder and Vistula river basins; Lithuania and Latvia: western Dvina river basin), *T. natans* has been found in habitats with stagnant or weakly flowing waters, which is typical for this species (Herrmann pers. observation).

The results of our environmental studies showed that species has a wide tolerance spectrum of habitat conditions and inhabits medium to high nutrient-rich water (from meso-to highly eutrophic). Furthermore, the study showed that species is also able to dwell habitats with low and high salinity, in contrast to opinion of Hummel and Kiviat (2004), who claimed that *T. natans* does not inhabit aquatic ecosystems with high salinity. The broad tolerance spectrum (Groth et al., 1996), which was also proved in our studies, and the ability to inhabit non-specific habitats is certainly one of the characteristics that enable the species vast expansion or invasion. Also factors related to the climate change are important. Admittedly, we have not analyzed relationships between climate change and *T. natans* spreading in this article but it is unquestionable that linear temperature increasing in Europe during the last 70 years (Twardosz et al., 2021). Our previous studies indicated that the most important climatic factors, determining habitat suitability for *T. natans*, are temperature of the warmest quarter and the precipitation of the driest months (Walusiak et al., 2024). Therefore, we are convinced that the global factors (climate change: increase of temperature and more violent and often rivers floods) and the local factors (ability to inhabit a wide range of meso- and eutrophic aquatic habitats) are main factors in the rapid success of this species.

AFLP diversity data showed little geographical structure and high overall genetic variability of *T. natans*. We found significant variance between the studied populations from Central and Eastern Europe ($F_{ST} = 0.40$) and the predominant proportion of variability within populations (60.06%) of *T. natans*. Most of the genetic variation found within populations suggests significant gene flow, which has shaped the current genetic structure. This indicates that the mechanisms of gene flow in *T. natans* are surprisingly complex and occur despite barochoric dispersal and high self-pollination inbreeding. The relatively high level of intra-population variability is surprising, as facultative xenogamy was found in *T. natans*, based on pollen-ovule ratio (Sinjushin, 2018) and in agreement with the classification of Cruden (1977). It is assumed that gene flow through pollen is limited in *T. natans*. This weak gene flow was also confirmed by our research ($N_m = 0.73$), which is consistent with the statement that the more the populations differ genetically, the lower the gene flow is (Latta, 2003). However, gene flow among populations of *T. natans* may be frequent but not extensive, as relatively moderate differentiation within the groups was identified by AMOVA. Low gene flow is typical for species with low dispersal ability or low mobility that inhabit fragmented habitats, when populations are small and/or the distance between populations is large (Hastings and Harrison, 1994; Hamrick and Godt, 1996). This is an interesting finding especially in light of the rapid spread of *Trapa* in Europe during the last 20 years (Walusiak et al., 2024). We argue that the expansion might have started from the isolated habitats of particular *T. natans* populations, where the species has survived unfavorable conditions, which were present on the turn of XIX/XX century (Piórecki, 2014). Optionally, it might also be a result of some introductions in XX and at the beginning of XXI century as species conservation actions. When environmental conditions improved, species has been starting expansion from refugia which are well documented in the biogeographic context for different groups of species (cp. Stewart and Lister, 2001; Magri, 2008). An example of such refugium might be the population 10. POS from the Vistula river basin. In 2008 in the vicinity of this sampling area the species has been introduced (Śmieja and Ledwoń, 2013). In our studies, this population showed high diversity and all genetic parameters demonstrated its distinctiveness. Two others likely refugia are populations 7. PRZ and 16. PLU (the Vistula and Oder river basins), both showing high diversity and genetic

variability parameters. High rarity (measured as frequency down weighted marker values, DW2) is typical for refugia (Paun et al., 2008). The highest DW found in above mentioned three populations is the further evidence to identify these places as refugium where the prevailing environmental conditions and genetic features allowed the species to survive unfavorable times.

The highest genetic diversity of *Trapa natans* was found in the Polish populations from the two river basins (Oder and Vistula). Also, the highest clonal diversity was found in the Polish populations and additional in the populations from Elbe river basin (Germany). High genetic diversity in plants is usually associated with a large geographical range and with outcrossing and wind pollination systems (Hamrick and Godt, 1990). Species with high genetic diversity have a greater potential to adapt to changing or new environments (Holderegger et al., 2006; Teixeira and Huber, 2021). They have a greater chance of survival and are less at risk of extinction (Frankham, 2005). All of those features explain the rapid expansion of *T. natans* in the Elbe, Oder and Vistula basins. Clonal diversity is also controlled by factors other than proximity to the potential dispersal source (Wu et al., 2022). The Lithuanian – Latvian populations showed the lowest genetic and clonal diversity, what might be related to be the northern population. It is believed that for many organisms, genetic diversity decreases with latitude (Martin and McKay, 2004), meaning that less diversity was found in northern populations than in southern ones. Another explanation is that Lithuanian – Latvian populations are peripheral populations, which according to the central-marginal hypothesis, exibit low genetic diversity (Eckert et al., 2008).

A similar trend was found for clonal diversity, where northern populations was found to be less diverse (Stenström et al., 2001). However, in this case, the low level of clonality was sufficient for the establishment and successful persistence of the species in the Dvina basin. Lower genetic diversity (genetic homogeneity) means that northern populations have a lower chance of survival (Martin and McKay, 2004), therefore we might expect that Dvina river populations of *T. natans* are most vulnerable comparing all studied populations. However, genetic homogeneity was found not only in the Dvina basin but also in some populations from the Elbe basin. We think it might be a result of origin of *T. natans* from a small number of seeds or it might be an effect of founder population. New habitats can be opened up for species through recent introduction it by people, seed transport by animals (beavers, birds, etc.) or the dispersal of the seed-bearing rosettes with contact to flowing waters. Individual rosettes detach themselves from the association and can be transported over long distances by currents and wind without dying immediately. In the juvenile stage, the still weakly anchored plants can also be lifted out of the mud and drift away if the water level rises quickly. Under favorable conditions, they can then continue to grow elsewhere (Herrmann pers. observation).

High level of genetic homogeneity could indicate a common origin from commercial specimens for breeding in domestic ponds. One example is *T. natans* in Lake Šaminis (pop. 4. LTS, Lithuania). This population is of anthropogenic origin, *T. natans* was deliberately introduced to this lake by the owners (owner pers. comm.). It is also worth mentioning that the Lithuanian – Latvian population of *T. natans* has genetic peculiarities due to the presence of characteristic (rare) AFLP fragments. This is a new genetic quality compared to the nearest studied populations of *T. natans*, but its nature and origin cannot be determined at this stage of study.

The determination of genetic diversity showed the possible refugia from which the present *Trapa* expansion started. It also revealed areas of basins (in Poland and Germany) where the populations are considered 'strong' due to their genetic diversity and better adaptability for environmental changes, while the Dvina basin (in Lithuania and Latvia) are considered 'weak' due to their homogeneity and vulnerability to unfavorable conditions. We claim that conservation status of *T. natans* should be reconsidered, especially in Central Europe, where species rapidly spread, and may be harmful for aquatic habitats.

It should be emphasized that genetic variation reflects not only the genetic differences between populations, but also the ecological divergence between different habitats (Li et al., 2023). It would be also worth to study in the future, whether the differences between genetic variations between populations might be the result of habitat differences, e.g. whether mesotrophic conditions of Eastern Europe aquatic habitats may be responsible for higher homogeneity of *T. natans* but eutrophic habitats promote higher genetic diversity.

Our studies are important for expansion/invasion problem understanding. They demonstrate that expansion/invasion may start from small populations, but when favorable conditions arise such populations rapidly increase and spread.

5. Conclusions

The conservation efforts of species like *Trapa natans* should take into account different information: the genetic and clonal variability of the species, which is related to the probability of extinction or survival, the nature of environmental conditions, results of climate changes and the possible place of origin of the species (natural or commercial). Conservation policy should be tailored to the conditions of the species populations. In some places, species may be so numerous that they are detrimental to the ecosystem, while in other places they may be threatened with extinction. On the other hand, proving high potential to dispersion, even after long time of unfavorable period, the introduction of *Trapa* (and other similar species) into new habitats and/or private ponds should be done carefully or even should be forbidden in the areas where they are not native plants. We also postulate that red-listing and way of protection of *T. natans* require revision.

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CRediT authorship contribution statement

Edward Walusiak: Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Elżbieta Cieślak:** Writing – original draft, Validation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Elżbieta Wilk-Woźniak:** Writing – original draft, Validation, Funding acquisition, Data curation, Conceptualization. **Magdalena Szczepaniak:** Writing – original draft, Visualization, Formal analysis, Validation, Conceptualization. **Armin Herrmann:** Writing – original draft, Validation, Investigation. **Lukas Petrulaitis:** Writing – original draft, Validation, Data curation. **Valerijus Rašomavičius:** Writing – original draft, Validation, Investigation. **Domas Uogintas:** Writing – original draft, Validation, Investigation. **Wojciech Krztoń:** Writing – original draft, Validation, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.122468.

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