

VILNIUS UNIVERSITY

JURGITA BUTKUVIENĖ

BATRACHIUM IN LITHUANIAN
RIVERS: MORPHOLOGICAL AND
GENETIC DIVERSITY AND RELATED
FACTORS

Summary of doctoral dissertation
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Scientific supervisor – prof. dr. Donatas Žvingila (Vilnius University, biomedical sciences, biology – 01B)

Scientific consultant – dr. Donatas Naugžemys (Vilnius University, biomedical sciences, biology – 01B)

The doctoral dissertation will be defended at the public session of the Council of defence:

Chairman – prof. habil. dr. Juozas Rimantas Lazutka (Vilnius University, biomedical sciences, biology – 01B)

Members:

prof. dr. Darius Danusevičius (A. Stulginskis University, agricultural sciences, forestry – 04A)

dr. Iona Jukonienė (Nature Research Centre, biomedical sciences, biology – 01B)

dr. Carla Lambertini (Bologna University, biomedical sciences, biology – 01B)

prof. habil. dr. Eugenija Kupčinskienė (Vytautas Magnus University, biomedical sciences, biology – 01B)

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Address: Saulėtekio av., 7, Vilnius 10257, Lithuania

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VILNIAUS UNIVERSITETAS

JURGITA BUTKUVIENĖ

BATRACHIUM LIETUVOS UPĖSE:
MORFOLOGINĖ IR GENETINĖ
ĮVAIROVĖ BEI JĄ LEMIANTYS
VEIKSNIAI

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Mokslinis vadovas – prof. dr. Donatas Žvingila (Vilniaus universitetas, biomedicinos mokslai, biologija – 01B).

Mokslinis konsultantas – dr. Donatas Naugžemys (Vilniaus universitetas, biomedicinos mokslai, biologija – 01B).

Disertacija ginama viešame disertacijos gynimo tarybos posėdyje:

Pirmininkas – prof. habil. dr. Juozas Rimantas Lazutka (Vilniaus universitetas, biomedicinos mokslai, biologija – 01B).

Nariai:

prof. dr. Darius Danusevičius (A. Stulginskio universitetas, žemės ūkio mokslo, miškotyra – 04A),

dr. Iona Jukonienė (Gamtos tyrimų centras, biomedicinos mokslai, biologija – 01B),

dr. Carla Lambertini (Bolonijos universitetas, biomedicinos mokslai, biologija – 01B),

prof. habil. dr. Eugenija Kupčinskienė (Vytauto Didžiojo universitetas, biomedicinos mokslai, biologija – 01B).

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Adresas: Saulėtekio al., 7, Vilnius LT-10257, Lietuva.

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INTRODUCTION

The genus *Batrachium* (DC.) S.F. Gray. or *Ranunculus* L. sect. *Batrachium* DC. is one of the most complicated taxonomic groups of aquatic plants. The difficulties in species identification according to morphological features arise mainly from its extreme phenotypic plasticity, morphological reduction and frequent hybridization (Cook, 1966; Dahlgren, 1995; Lambertini et al., 2017). Simplified morphology and variable taxonomic interpretation of the group, often makes identification to species level very difficult. *Batrachium* is distributed almost worldwide, however its largest diversity is found in Europe. According to different authors (referred by Bobrov et al. 2015), the number of *Batrachium* species varies from 17 to 30. In the last taxonomic evaluation of section *Batrachium* Wiegand et al. (2017) recognized 30 species. White-flowered *Batrachium* species occurring in the rivers from Lithuania and other Baltic countries (Estonia, Latvia) have been poorly investigated so far. The authors (Laasimer et al., 1993) of the first version of “Flora of The Baltic Countries” indicated the possibility of the occurrence of *Ranunculus fluitans* Lam. in the southern part of Lithuania on the basis of old references and without herbarium evidence. *Ranunculus penicillatus* (Dumort.) Bab. was specified as not found in the Baltic countries. The presence of *B. fluitans* has been recorded in Lithuania since the late 18th century (Gilibert, 1782; Jundziłł, 1830; Kuprevičius, 1934; Snarskis, 1954; Lekavičius, 1989). *Batrachium penicillatum* was a neglected taxon and was occasionally simply associated with *B. fluitans* (Aparia, Lekavičius, 1961). Tzvelev (2012) recorded homophyllous *B. pseudofluitans* (Syme) Nym. in Lithuania and Latvia and heterophyllous *B. penicillatum* Dumort. in Latvia. Lansdown (2013) noted *R. penicillatus* (Dumort.) Bab. in Estonia. The taxonomic composition and genetic structure of *Batrachium* species in Lithuanian rivers have been unknown to date.

The use of molecular methods opens new possibilities to effectively tackle complicated problems of *Batrachium* systematics and provides a deeper insight into population biology of these plants (Telford et al., 2011; Bradley et al., 2013; Coppi et al., 2015; Zalewska-Gałosz et al., 2015; Lambertini et al., 2017).

It should be mentioned that river stretches containing *Batrachium* communities (habitat code 3260) are among the most endangered freshwater habitat types throughout Europe (European Commission, 2007). *Batrachium* patches, modifying the flow velocity, dynamics of nutrients and sediments, can provide a lot of ecological niches suitable for small invertebrates and fishes (Dawson, Robinson, 1984; Haury, Baglinière, 1996). The loss of this type of vegetation in watercourses throughout Europe as well as macrophyte diversity in general is related to pollution, eutrophication, increased turbidity and river channelization (Riis, Sand-Jensen 2001; Steffen et al. 2013). The retention of this habitat is closely related to the requirements on water quality according to Water Framework Directive (European Parliament, Council of the European Union 2000). *Batrachium* species can be considered as indicators of riverine ecosystem sustainability. In this aspect, the intraspecific variation of *Batrachium* as a habitat-forming species can have important ecological consequences at the population, community, and ecosystem levels. So, factors affecting sustainability of *Batrachium* species are important not only from the scientific point, but also in some practical aspects associated with ecosystem services.

The aim and main tasks of the research

The aim of this study is to determine the diversity of *Batrachium* species, the genetic and morphological variability of *Batrachium* populations in Lithuanian rivers and to assess the influence of factors determining the distribution of species. The following main tasks were set in order to achieve this aim:

1. To select the sampling sites according to the results of analysis of *Batrachium* species in the herbarium of Vilnius University (WI) and Nature Research Centre, Institute of Botany (BILAS).
2. To evaluate morphological and species diversity of genus *Batrachium* in some Lithuanian rivers.
3. To investigate the genetic structure of populations of riverine *Batrachium* species using the ISSR method, and to evaluate the factors that influence genetic structure of *Batrachium* populations.
4. To perform taxonomic analysis of *Batrachium* species using sequencing of *ITS* region of rDNA and *trnH-psbA* region of cpDNA.
5. To evaluate the ecological conditions of river stretches with *Batrachium* and their impact on the diversity of *Batrachium* communities.
6. To prepare the recommendations for the protection of the *Batrachium* species and their habitats.

Scientific novelty of the study

For the first time, combined studies of several *Batrachium* species' morphological and genetic diversity at population level were carried out. The variability of *Batrachium* plants was evaluated using the morphological and DNA markers (ISSR, cpDNA and rDNA). The complete sequences of *trnH-psbA* region of 24 individuals were deposited in GenBank[®]. The new association *Ranunculetum pseudofluitantis* has been described.

Statements to be defended

1. The average length and number of petals, the length of the second and sixth leaves, the hairiness of receptacle and whole plant are the main morphological characters of the *Batrachium* suitable for species identification in Lithuanian rivers.
2. Low DNA polymorphism and spread of clones indicate that vegetative propagation predominates in some Lithuanian *Batrachium* populations.
3. Studies of ISSR polymorphism suggest that *Batrachium* populations in different rivers have a specific genotypic diversity.
4. The analysis of *ITS* region of rDNA and *trnH-psbA* region of cpDNA indicates that *B. fluitans*, *B. pseudofluitans*, *B. trichophyllum* and *B. penicillatum* s.str. are spread in the studied rivers of Lithuania.
5. Morphologically similar species (*B. fluitans* and *B. pseudofluitans*) and their communities are spread in the habitats that differ in physical and hydrochemical parameters.
6. Water tourism has a negative impact on *Batrachium* plants and their communities.

Practical and scientific significance

Our study revealed that four *Batrachium* species (*B. fluitans*, *B. pseudofluitans*, *B. penicillatum* and *B. trichophyllum*) are found in the studied rivers of Lithuania. Low genetic diversity and lack of sexual reproduction imply that *B. fluitans* is an endangered species. *Batrachium* communities dominated by *B. fluitans* and *B. pseudofluitans* were recorded in the rivers that differ in physical and hydrochemical water parameters and were not found growing together. The measures concerning protection of *Batrachium* species were proposed and the recommendations were submitted to the Ministry of Environment of the Republic of Lithuania, to include *B. fluitans* in the list of protected species and to develop measures for the conservation of *Batrachium* communities.

The presentation and approbation of the results

One scientific paper related to the topic of the doctoral dissertation was published in the *Clarivate Analytic Web of Science* issue with the impact factor (IF). Also two research articles related to the topic of the doctoral dissertation are accepted for publication in the issue included in ISI Master Journal List and in the proceedings of international conference. One popular science article was published in Lithuanian periodical journal. The research results were presented at four international and one Lithuanian conferences.

MATERIALS AND METHODS

Plant material

Sampling sites of *Batrachium* plants were chosen based on the analysis herbaria (WI and BILAS) specimens. The revision of 56 sites revealed only 16 sites where *Batrachium* plants were found. Samples for morphological and molecular analyses were collected in June-July of 2012 and 2013 during the *Batrachium* flowering period, at the time of optimal development within the plant communities. In total, 185 *Batrachium* individuals were collected from 16 rivers stretches (Fig. 1.). The leaves from all 185 individuals were used for DNA extraction and ISSR-PCR analysis. Of the 185 tested specimens, 103 fully developed and flowering plants were used for morphological analysis. DNA samples of 34 individuals (4 from Tatula, 4 from Skroblus, 2 from Gauja, 4 from Ūla, 4 from Viešvilė, 4 from Merkys, 4 from Grūda, 2 from Vilnia, 4 from Verknė, and 2 from Žeimena) included in morphological and ISSR analyses were also used for *trnH-psbA* chloroplast region and rDNA *ITS* region sequencing.

A species-level taxonomic identification of individuals was performed using the keys described by Cook (1966), Webster (1988), Webster, Rich (1998), Dahlgren, Jonsell (2001), and Bobrov (2015). Morphological characters (presence of intermediate leaves, length of 2nd and 6th leaves, number of 2nd and 6th leaf furcations, length of 2nd and 6th leaf terminal segments, length of 2nd and 6th leaf petioles, hairiness of stipules, length and number of petals, and hairiness of receptacle) were evaluated for 103 individuals in this study. Leaf morphological features were assessed for the leaves from the second and sixth nodes (counting from the top of the plant) of each plant. In total, 192 leaves were examined. The number and the length of petals for all flowers of the plant were assessed. The number of flowers ranged from 1 to 9 per plant. In total, 120 flowers were measured and analyzed.

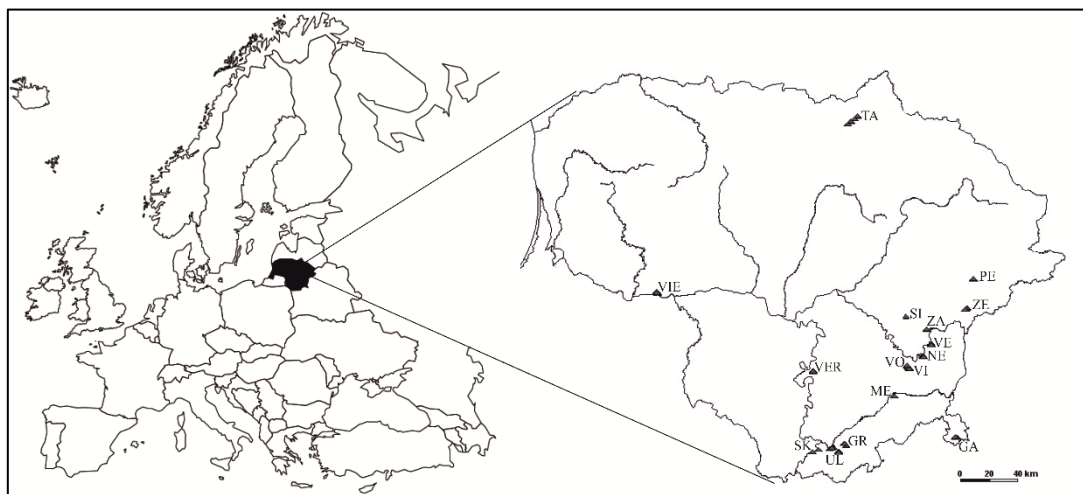


Fig. 1. Map of the location of sampled populations. Codes of populations names: TA – Tatula, SI – Širvinta, PE – Peršokšna, ZE – Žeimena, ZA – Žalesa, VE – Veržuva, NE – Neris, VO – Vokė, VI – Vilnia, ME – Merkys, GA – Gauja, GR – Grūda, UL – Ūla, SK – Skroblus, VER – Verknė, VIE – Viešvilė

The study of influence of water tourism was carried out in May-September, 2016. There were one control and three stationary experimental plots in the river Ūla. The

control plot was 1 m × 4 m in size and was set up at Mančiagirė (54° 7' 57.4" / 24° 27' 8.87"). Three experimental plots were chosen at Ūla's Eye (54° 8' 30.18" / 24° 26' 22.29"), where the influence of canoes was most intense. The plot size was 1 m × 4 m. The abundance of *Batrachium* species and other macrophytes was evaluated using the Braun-Blanquet (Braun-Blanquet, 1964) scale. The depth and the formation of generative structures (flowers or fruit) of the plants of *Batrachium* were also assessed. The data on the number of canoes rides each month was obtained from the Dzūkija National Park.

Materials of phytosociological analysis

According to herbarium and National Water Monitoring data, *Batrachium* rich sites were selected in 15 stretches of 10 rivers (Neris – NE (1), Peršokšna – PE (1), Širvinta – SI (3), Tatula –TA (4), Verknė – VER (2), Veržuva – VE (1), Vilnia – VI (1), Vokė – VO (1), Žalesa – ZA (1), Žeimena – ZE (1)). Field studies were carried out in 2013–2014, optimum development in *Batrachium* plant communities – between the months of June and July. The study sites correspond the conditions of habitat type 3260 “Water courses with *Ranunculion fluitantis* vegetation” and are located close to the sites of hydrochemical monitoring according to Water Framework Directive (data of river monitoring 2013, 2014). A total of 97 phytosociological relevés with *Batrachium* sp., were made at 15 sites in sampling plots not smaller than 4 m², after phytosociological Braun-Blanquet approach. Water depth and river bottom substrates in sampling sites were evaluated.

DNA extraction and ISSR-PCR analysis

The leaves of plants were used for DNA extraction. In total, 100–150 mg of plant material was used for DNA isolation. Total genomic DNA was isolated from fresh leaves that were ground in liquid nitrogen using a modified CTAB method (Doyle and Doyle, 1987). PCR amplification was carried out in a final volume of 20 µL containing 20 ng of template DNA, 2 µL of 10× PCR buffer (Thermo Fisher Scientific/Baltics), 200 µM dNTPs, 1 U of Taq polymerase (Thermo Fisher Scientific/Baltics), 300 µM MgCl₂, and each primer at 0.4 µM. ISSR-PCR was conducted as follows: 94°C for 7 min; 32 cycles of 94°C for 30 s, the temperature for each primer for 45 s, and 72°C for 2 min; and then 72°C for 7 min. Of the 20 tested primers, 6 were selected for further study (ISSR-39, ISSR-28, ISSR-50a, ISSR-34, ISSR-O and ISSR-C), and all reactions were run at least twice. Negative control tubes were included in each experiment. Following PCR, the products were visualized on 1.5 % TBE-agarose gels stained with ethidium bromide and imaged using a BioDocAnalyze system (Biometra, Göttingen, Germany). Amplified bands were scored in a size range from 200 to 1500 bp. A molecular weight standard, GeneRuler™ DNA Ladder MIX (Thermo Fisher Scientific/Baltics), was used. Samples of three or four populations were analyzed on the same gel. Negative control samples showed no amplification. Only reproducible and clear DNA bands were scored for data analysis. The reproducibility of the ISSR bands was assessed according to the method of Bonin et al. (2004). The test ISSR-PCR was performed on 20 samples. The difference between the test matrix and the main matrix for 20 samples was 66 bands of 1693 comparisons, and the error rate was 3.9 %.

Analysis of the *trnH-psbA* and *ITS1-ITS2* regions

The *trnH-psbA* region of the cpDNA was analyzed in 34 *Batrachium* plants representing different populations. We used primers developed by Shaw et al. (2005) for the amplification of the *trnH-psbA* region. The amplification reactions were performed as described by Vyšniauskienė et al. (2015). Additional analysis using *ITS* sequences (primers *ITS1* (5'-TCCGTAGGTGAACCTGCGG-3') and *ITS4* (5'-TCCTCCGCTTATTGATATGC-3')) was performed. The amplification of the *ITS1-ITS2* region was performed according to Telford et al. (2011). The PCR products were excised from 0.8 % agarose gel and purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific/Baltics) following the manufacturer's instructions. The purified DNA fragments were cloned using the InsTAclone PCR Cloning Kit (Thermo Fisher Scientific/Baltics) as described in manufacturer's instructions. Plasmid DNA was isolated from 2–3 clones per sample using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific/Baltics) in accordance with the manufacturer's protocol. Plasmids bearing inserts of suitable size were sequenced using M13/pUC sequencing primers at the BaseClear B.V. (Leiden, The Netherlands) with a ABI3730xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). cpDNA sequences representing different populations were deposited in GenBank[®] under accession numbers MF167609–MF167632.

Data analysis

The amplified DNA bands were scored as a binary data matrix, where the presence of a band in the ISSR profile of an individual was recorded as 1 and the absence of a band was recorded as 0. Principal Coordinate Analysis (PCoA) and Analysis of Molecular Variance (AMOVA) were carried out to assess the overall distribution of diversity among and within populations using GenAlEx v. 6.5 (Peakall, Smouse, 2012). Genetic relationships among the 16 *Batrachium* populations and 185 individuals within these populations were determined using STRUCTURE v. 2.3.4 (Pritchard et al., 2000), which implements a Bayesian model-based clustering method for inferring population structure. The number of possible clusters ranging from $K = 1$ to $K = 20$ was tested using the admixture model. The runs for each cluster were repeated 10 times. Overall, 500.000 burn-in iterations and 1000.000 MCMC (Markov Chain Monte Carlo) repetitions were used for the analysis. Following Evanno et al. (2005), the delta K method was used to infer the real number of clusters (K). The DISTRUCT program (Rosenberg, 2004) was used to show the results of this analysis. Genetic diversity was analyzed at the population and species level. Nei's gene diversity (H_j) was estimated on the basis of allele frequencies (Lynch, Milligan, 1994) using a band-based approach with AFLP-SURV v. 1.0 (Vekemans et al., 2002). The band richness (Br) and the proportion of polymorphic bands (PLP, 5 % level) for standardized sample sizes (Petit et al., 1998) were determined with AFLPdiv v. 1.1 (Coart et al., 2005). Clonal diversity of populations was assessed by calculating the proportion of distinguishable genotypes – $i = G/N$, where G is the number of genotypes and N is the total number of individuals sampled.

The sequencing data were evaluated as described in Vyšniauskienė et al. (2015) using the MEGA 7.0.25 (Kumar et al., 2016) software. Alignments were subsequently checked to correct obvious errors.

PAST v. 2.17 (Hammer et al., 2001) was used to perform nonmetric multidimensional scaling (NMDS) analysis, and nonparametric MANOVA was performed to check if the groups differed significantly. The Gower distance measure was used in both analyses.

STATISTICA v. 12.0 (StatSoft, 2014) was used to perform the nonparametric Mann-Whitney *U* test to assess if there were differences in morphological features and to compare the averages of environmental parameter in localities of different *Batrachium* species also to check for differences between population groups.

To determine the habitat conditions of *Batrachium* rich plant communities the following physical-chemical parameters of water from the study sites were used: flow velocity (m/s), pH, alkalinity (mmol/l), conductivity ($\mu\text{S/cm}$), ammonium (NH_4) (mg/l), nitrites (NO_2) (mg/l), nitrates (NO_3) (mg/l), total nitrogen (total N) (mg/l), total phosphorus (total P) (mg/l), phosphates (PO_4) (mg/l) and calcium (Ca^{2+}) (mg/l). Values of these parameters (mainly 4-month averages) were taken from the Environmental Protection Agency data base, available at <http://vanduo.gamta.lt/cms/index?rubricId=6adeeb1d-c902-49ab-81bb-d64b8bccefdd> (data of river monitoring 2013, 2014).

RESULTS

Morphological analysis of *Batrachium* populations

The NMDS analysis revealed that all *Batrachium* individuals clustered into two main groups according to their morphological characters (Fig. 2). The first group (Group I) included homophyllous plants from the nine river populations (NE, PE, SI, VE, VER, VI, VO, ZA, and ZE), tentatively identified as *B. fluitans*. The second group (Group II) included plants from the seven river populations (GA, GR, ME, SK, TA, UL, and VIE) and identified as *B. penicillatum* s.l. and *B. trichophyllum*.

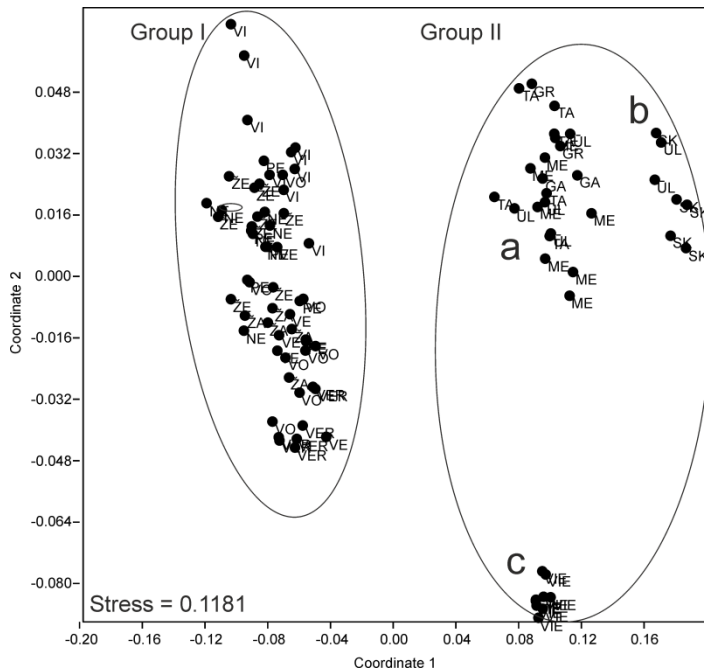


Fig. 2. Nonmetric multidimensional scaling (NMDS) analysis for all morphological data (two main groups are circled)

The plants of Group I were characterized by a sparsely hairy receptacle and 5–8 petals, whose length ranged from 0.4 to 1.1 cm; a densely pubescent receptacle with 5 petals ranging from 0.2 to 0.5 cm in length was characteristic of Group II. A Mann-Whitney U test showed that the petal length between these two groups differed significantly ($U = 21.50$; $p < 0.001$) (Fig. 3). Comparing the lengths of the second and sixth leaves also revealed significant differences between these groups ($U = 273.0$; $p < 0.001$ and $U = 201.0$; $p < 0.001$, respectively) (Fig. 3). The length of the second capillary leaf ranged from 4 to 9 cm in the plants of the Group I and from 3 to 7 cm in plants of the Group II. In addition, the length of the sixth capillary leaf ranged from 6 to 12 cm in the Group I and from 5 to 8 cm in Group II. Statistical analysis of the morphological features also revealed that not all them were equally important for *Batrachium* species delimitation. The differences of four characters, including the average length of petals, the length of the second and sixth leaves and the hairiness of the receptacle, were statistically significant and differed between individuals of Group I and Group II (Figs. 3 and 4).

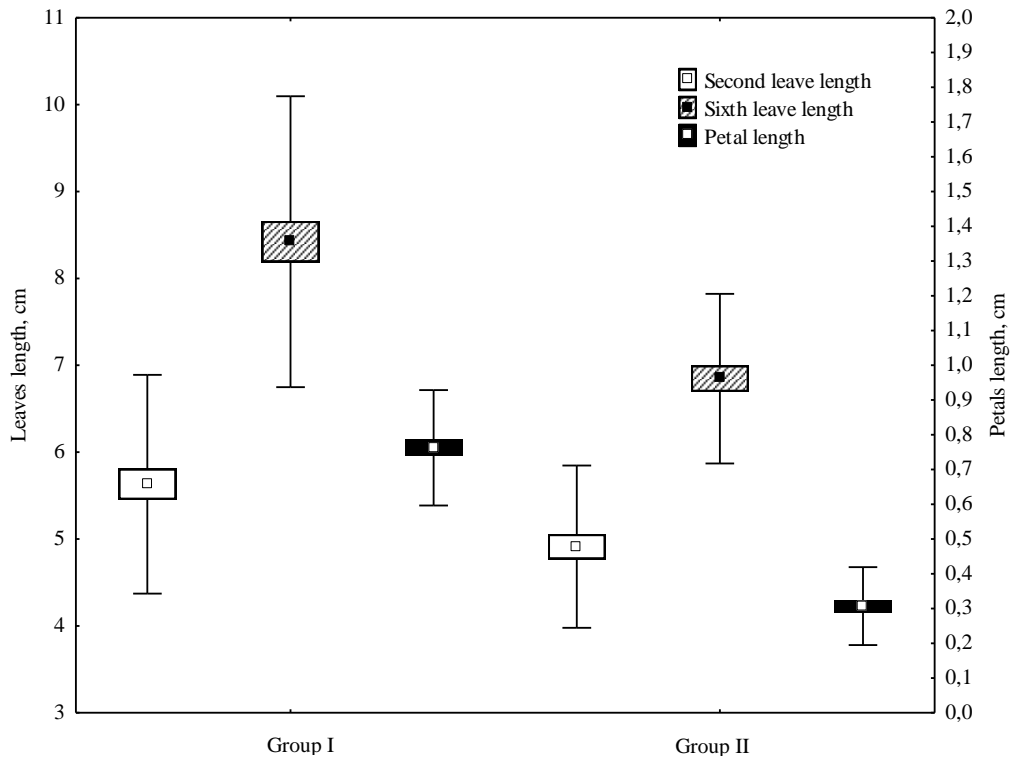


Fig. 3. Second, sixth leaf lengths and petal length of individuals from Group I and Group II

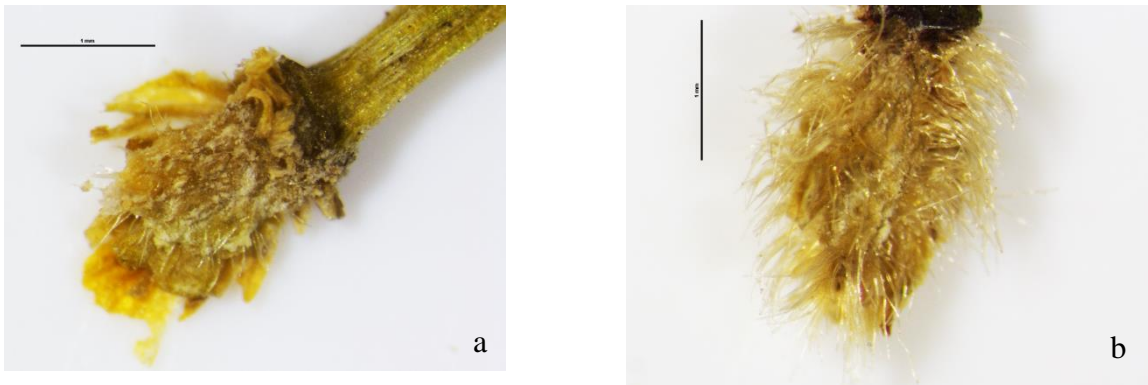


Fig. 4. The hairiness of receptacle of individuals from Group I (a) and Group II (b)

Group II was very heterogeneous, and individuals of this group were assigned to one of 3 subgroups (Fig. 2). The largest subgroup, *a*, included homophyllous plants from the six populations (GA, GR, ME, TA, UL, and VIE), while subgroup *b* included merged heterophyllous plants from the UL and SK populations. The small subgroup *c* included homophyllous plants from the VIE population (possible *B. trichophyllum*). Nonparametric MANOVA showed that the differences between these three subgroups were statistically significant ($p < 0.05$) (data not shown). It was revealed that only one feature – intermediate leaves – separated plants of subgroup *b* from those of subgroup *a* and that only hairy stipules distinguished plants of subgroup *c* from those of the other two subgroups.

DNA polymorphisms and genetic diversity of populations

Analyzed plants yielded 106 reproducible and clear DNA bands, 79.2 % of which were polymorphic; the remaining 20.8 % were monomorphic. The total number of

scorable bands per primer ranged from 13 (ISSR-O) to 21 (ISSR-C). The proportion of polymorphic loci at the 5 % level ranged from 0 to 0.308, and the expected heterozygosity (Nei's gene diversity) values varied from 0 to 0.06 (Table 1).

The GR population showed the highest level of band richness (1.21), whereas the PE population was monomorphic (Table 1). The proportion of distinguishable genotypes ranged from 0.083 (PE population) to 0.8 (SK and ME populations).

Principal coordinate analysis (PCoA) was carried out to assess the genetic diversity and relationships among the studied populations. Approximately 39.53 % of the total genetic variability was described by the first two coordinates (Coord1 explains 26.57 % of the total variation, and Coord2 explains 12.96 %). The PCoA revealed that all populations could be combined into two main groups (Fig. 5). Group I included all individuals from 9 populations (NE, PE, SI, VE, VER, VI, VO, ZA, and ZE), which were identified as *Batrachium fluitans* according to morphological characters.

Table 1

Polymorphism parameters of different *Batrachium* populations estimated using ISSR marker data. i – proportion of distinguishable genotypes; PLP 5 % [8] – proportion of polymorphic loci at the 5 % level with rarefaction to 8 individuals per population; H_j – expected, heterozygosity (Nei's gene diversity); Br [8] – band richness with rarefaction to 8 individuals per population

| Pop. No. | Population name | i | H _j | Br [8] | PLP 5 % [8] |
|----------|-----------------|-------|----------------|--------|-------------|
| 1. | GR | 0.583 | 0.059 | 1.21 | 0.308 |
| 2. | ME | 0.8 | 0.060 | 1.16 | 0.178 |
| 3. | SK | 0.8 | 0.022 | 1.06 | 0.065 |
| 4. | VIE | 0.417 | 0.017 | 1.06 | 0.075 |
| 5. | TA | 0.625 | 0.015 | 1.04 | 0.037 |
| 6. | GA | 0.417 | 0.012 | 1.03 | 0.037 |
| 7. | UL | 0.583 | 0.024 | 1.07 | 0.093 |
| 8. | ZA | 0.5 | 0.021 | 1.08 | 0.103 |
| 9. | NE | 0.727 | 0.046 | 1.14 | 0.168 |
| 10. | VO | 0.5 | 0.012 | 1.04 | 0.047 |
| 11. | VE | 0.333 | 0.007 | 1.03 | 0.037 |
| 12. | SI | 0.417 | 0.009 | 1.03 | 0.037 |
| 13. | VI | 0.667 | 0.017 | 1.04 | 0.037 |
| 14. | VER | 0.5 | 0.013 | 1.03 | 0.037 |
| 15. | ZE | 0.167 | 0.003 | 1.01 | 0.009 |
| 16. | PE | 0.083 | 0.0 | 1.0 | 0.000 |

Group II included 7 populations (GA, GR, ME, SK, TA, UL and VIE) and was morphologically defined as species of *Batrachium penicillatum* s.l. and *B. trichophyllum*. PCoA also indicated the number of distinguishable genotypes in each population and revealed that individuals from several populations (GA, NE, UL, VIE, VO and VI) formed discrete groups and did not overlap with other populations (Fig. 5).

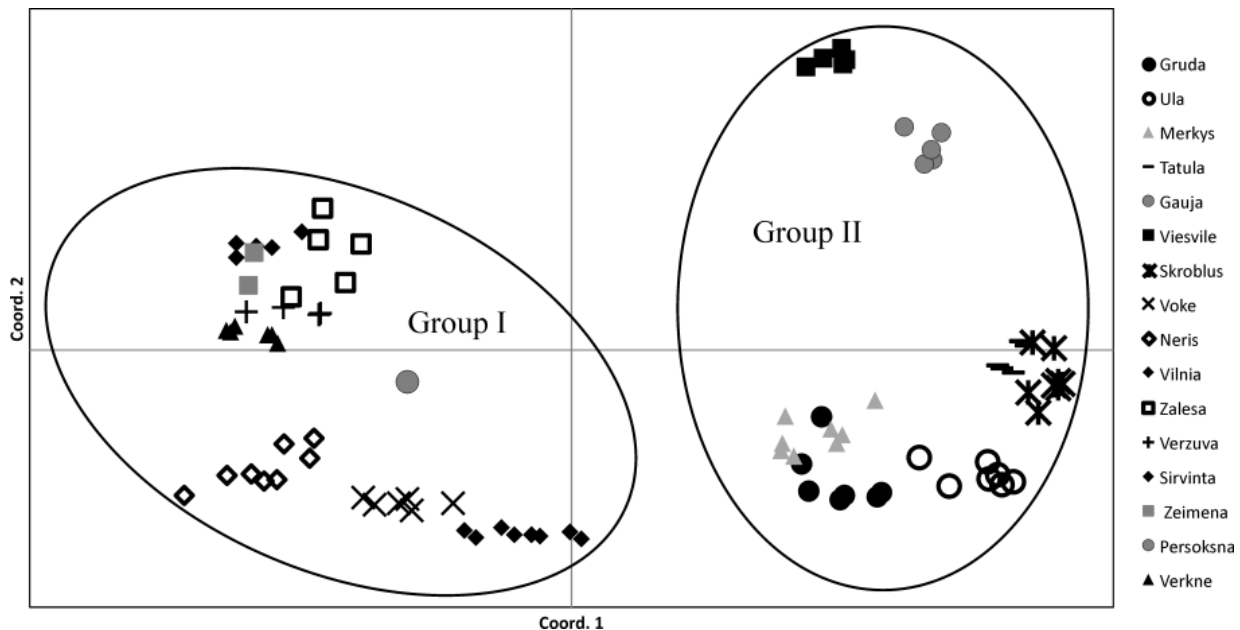


Fig. 5. Principal coordinate analysis of 185 *Batrachium* individuals from 16 populations

Higher clonal diversity was detected in Group II of *B. penicillatum* s.l. and *B. trichophyllum* populations (Table 2). The mean clonal diversity in this group was 0.604 ± 0.06 , whereas in *B. fluitans*, it was 0.433 ± 0.07 .

Table 2

Genetic diversity parameters of individuals from Group II (*Batrachium penicillatum* s.l., *B. trichophyllum*) and Group I (*B. fluitans*). PL – polymorphic loci; EL – exclusive loci; i – clonal diversity; P % – percentage of polymorphic loci; H_j – expected, heterozygosity (Nei's gene diversity); Br [8] – band richness with rarefaction to 8 individuals per population; PLP 5 % [8] – proportion of polymorphic loci at the 5 % level with rarefaction to 8 individuals per population

| | PL | EL | $i \pm SE$ | P % | $H_j \pm SE$ | Br [8] $\pm SE$ | PLP 5 % [8] $\pm SE$ |
|---|----|----|------------------|------|-------------------|------------------|----------------------|
| <i>Batrachium penicillatum</i> s.l. and <i>B. trichophyllum</i> | 71 | 1 | 0.604 ± 0.06 | 11.5 | 0.029 ± 0.008 | 1.09 ± 0.026 | 0.113 ± 0.037 |
| <i>Batrachium fluitans</i> | 59 | 2 | 0.433 ± 0.07 | 5.3 | 0.014 ± 0.004 | 1.04 ± 0.014 | 0.053 ± 0.017 |

Genetic variation was also analyzed within the Groups I (*B. fluitans*) and II (*B. penicillatum* s.l. and *B. trichophyllum*) (Table 2). The group of *B. penicillatum* s.l. and *B. trichophyllum* in comparison with *B. fluitans*, showed higher values of polymorphic loci number (71 versus 59), band richness (1.09 versus 1.04), expected heterozygosity (0.029 versus 0.014) and proportion of polymorphic loci (0.113 versus 0.053). Three group-specific ISSR bands were identified: 2 unique bands for *B. fluitans* and 1 for the group of *B. penicillatum* s.l. and *B. trichophyllum*. Band I-28₉₀₀ was observed for all studied *B. fluitans* individuals and was absent in plants of Group II. The other band specific for *B. fluitans*, ISSR-C₇₇₀, was detected in 89 % of individuals. The band I-28₉₅₀ was unique for *B. penicillatum* s.l. and *B. trichophyllum*, and 86 % of the plants from this group had this band. Only one population-specific band was detected (VIE population). GR populations stood out among the populations in terms of the highest number of rare bands. Three bands were observed that were encountered in 25 % or less of the populations, and 7 bands were present in 50 % or less of the populations.

A hierarchical analysis of molecular variance (AMOVA) was used to assess molecular variation partitioning among and within groups and populations. Of the total genetic diversity, 59 % was attributable to the among-population diversity within groups, and only 8 % was attributable to within-population differentiation (Table 3). The total genetic diversity among groups was 33 %. AMOVA also revealed that genetic differentiation among populations within *B. fluitans* was 91 % and between *B. penicillatum* s.l. populations – 84 %.

Table 3

Analysis of molecular variance (AMOVA) for populations of *Batrachium*. df – degrees of freedom; SS – sum of squared deviation; MS – mean squared deviation; Est. Var. – estimated variance; % – percentage of total variance

| Source | df | SS | MS | Est. Var. | % | p |
|---------------------------------|-----|----------|---------|-----------|-------|---------|
| Among two groups | 1 | 443.140 | 443.140 | 4.003 | 33 % | < 0.001 |
| Among populations within groups | 14 | 1225.878 | 87.563 | 7.566 | 59 % | < 0.001 |
| Within populations | 167 | 177.758 | 1.064 | 1.064 | 8 % | < 0.001 |
| Total | 182 | 1846.776 | | 12.634 | 100 % | |

The delta K approach (Evanno et al., 2005) showed that two clusters is an optimal cluster number at the uppermost level of genetic structuring in *Batrachium* populations (Fig. 6, A). A STRUCTURE analysis corroborated the results of the morphological analysis and PCoA and showed distinct clusters that separated the two population groups in the same manner as did NMDS and PCoA (Fig. 6, B).

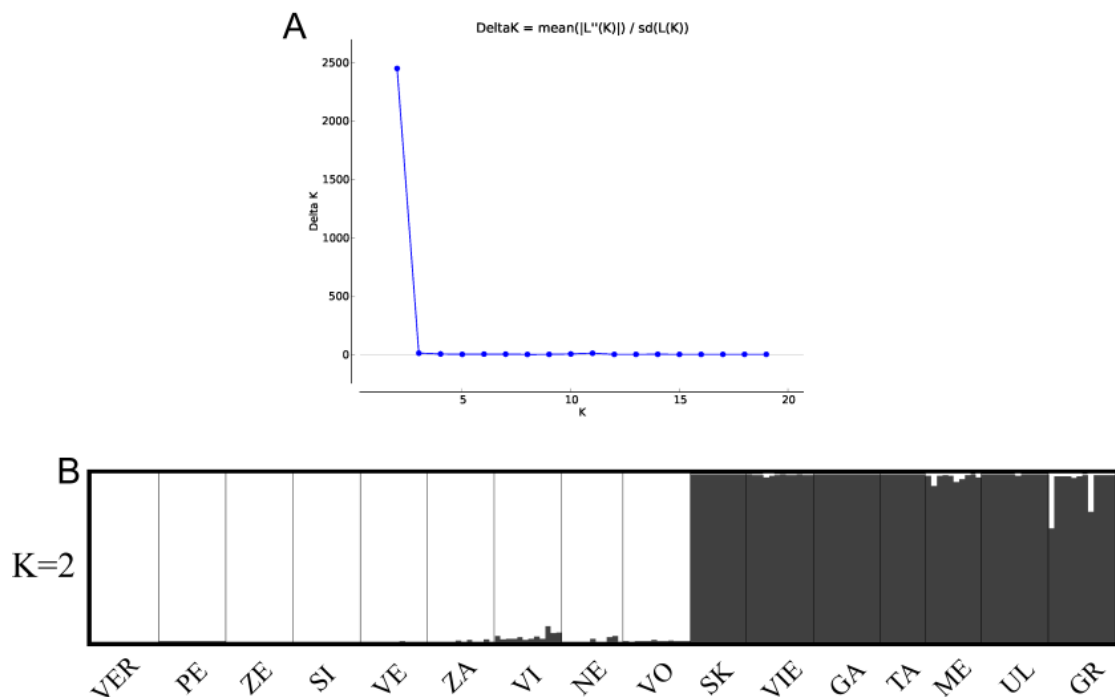


Fig. 6. Bayesian cluster analysis of the genetic structure of *Batrachium* spp. populations. A) Peak of ΔK -value at $K = 2$, B) Grouping probabilities of 185 *Batrachium* spp. individuals in two clusters (black and white). The 16 populations are separated by black vertical lines. Vertical lines represent individuals from certain populations. Population codes same as in Fig. 1

The white cluster combined nine populations (VO, NE, VI, ZA, VE, SI, ZE, PE and VER) and the black cluster included genetic groups from seven populations (GR, UL, ME, TA, GA, VIE and SK). Signs of admixture were detected in some populations (GR, ME, NE, and VI). However, the admixture level according to the results from the STRUCTURE analysis was low, and populations were rather homogeneous. Structure analyses performed under the same conditions within the identified clusters also detected substructuring within each cluster (Fig. 7). The white cluster (*B. fluitans* group) was divided in two subclusters of VO, NE, and VI and ZA, VE, SI, ZE, PE, and VER populations (Fig. 7, A). The black cluster (*B. penicillatum* s.l. and *B. trichophyllum* group) also split into a larger subcluster of the GR, UL, ME, TA and SK populations and a smaller subcluster including the GA and VIE populations (Fig. 7, B).

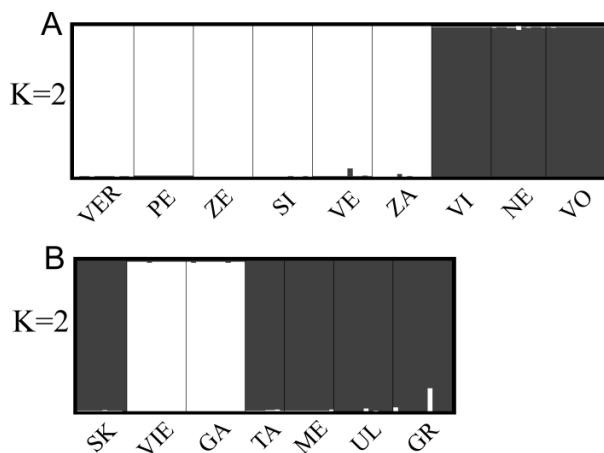


Fig. 7. Two graphs indicating the subgrouping of populations within A) white and B) black clusters representing Group I (*Batrachium fluitans*) and Group II (*Batrachium penicillatum* s.l. and *B. trichophyllum*) groups, respectively

The sequences of cpDNA determined from cloned fragments that represented full the *trnH-psbA* region were 435 bp long, and the aligned sequence was 440 bp in length. Four hundred thirty-one nucleotides were conserved, and 9 were variable. Two indels were detected. Individuals of Group I (GenBank[®] accession Nos. MF167629–MF167632) from VER and VI populations had the same sequence of the *trnH-psbA* region, and the sequences were identical to those of the GenBank[®] accessions (HQ894440.1) of *R. fluitans*. The analyzed individuals of Group II showed polymorphism in the *trnH-psbA* region. The individuals from the TA population (GenBank[®] accessions Nos. MF167609–MF167610) showed highest (99 %) similarity to GenBank[®] accession HQ894439.1 of *R. penicillatus* subsp. *pseudofluitans*. Other genotypes (GenBank[®] accession Nos. MF167611–MF167628) of this group included in the analysis were identical to GenBank[®] accession HQ894441.1 of *R. trichophyllum*.

The length of aligned sequence of rDNA determined from cloned fragments that represented complete *ITS1-ITS4* region was 699 bp. Six hundred sixty-four nucleotides were conserved, and 35 were variable. In total 9 *Batrachium* rDNA haplotypes were detected.

Floristic structure of communities

The described *Batrachium* rich communities were attributed to *Batrachion fluitantis* Neuhausl 1959 alliance, order *Callitricho hamulatae-Ranunculeitalia aquatilis* Passarge ex Theurillat et al. 2015 in class *Potamogetonetea* Klika in Klika et Novak

1941. The largest number (85) of phytosociological records was assigned to association *Ranunculetum fluitantis* (Allorge 1922) Koch 1926 (Table 4).

The coverage of *Batrachium fluitans* varied from approximately 40 to 95 % of the sampled vegetation plot. A total of 26 species co-occurring with *B. fluitans* were inventoried. Their number varied from one to eight in the sampling plot and their constancy was relatively low. Solitary characteristic species of *Batrachion fluitantis*, typical to fast flowing waters, *Stuckenia pectinata* f. *interrupta* as well as accompanying *Fontinalis antipyretica* and *Lemna trisulca*, reached the second class of constancy. In general, *Ranunculetum fluitantis* differed from *Batrachium pseudofluitans* rich communities by higher diversity of *Potamogetonaceae* species, however, their constancy as well as this of other differential species from *Batrachion* alliance, *Lemnetea* class or accompanying species usually not exceeded limits of the I class.

The hierarchical cluster analysis revealed four main clusters of *Ranunculetum fluitantis* that can be treated as variations of association. The first cluster A was characterized by the occurrence of *Elodea canadensis*, *Sparganium erectum* and *S. pectinata* f. *interrupta*. Cluster B was characterized by *Fontinalis antipyretica*. *S. pectinata* f. *interrupta* together with occasional *Fontinalis antipyretica* are typical for river rapids with coarse bottom. This variant of association was mainly distributed in the medium-sized rivers. Cluster C was characterized by such species as *Potamogeton crispus* and *Cladophora* sp., and cluster D was characterized by the occurrence of *Calitriche cophocarpa* (Fig. 8).

Table 4

Species composition and constancy/abundance in the communities with predominant *Batrachium* species
 (* submerged or floating forms of plants)

| Communities | <i>Ranunculetum fluitantis</i> | <i>Ranunculetum pseudofluitantis</i> |
|---|--------------------------------|--------------------------------------|
| Number of relevés | 85 | 12 |
| Total number of species | 26 | 17 |
| Characteristic species of communities: | | |
| <i>Batrachium fluitans</i> | 100 (3-5) | |
| <i>Batrachium pseudofluitans</i> | | 100 (2-3) |
| Ch. Batrachion fluitantis | | |
| <i>Sparganium erectum</i> * | 11 | 58 (1-3) |
| <i>Schoenoplectus lacustris</i> * | 2 | 50 (1-3) |
| <i>Agrostis stolonifera</i> * | 6 | 33 (+-1) |
| <i>Stuckenia pectinata</i> * | 37 (1-3) | 8 |
| <i>Berula erecta</i> * | 7 | 17 |
| <i>Veronica anagallis-aquatica</i> * | 9 | 17 |
| <i>Callitriche cophocarpa</i> * | 4 | 8 |
| <i>Sagittaria sagittifolia</i> * | 13 | |
| <i>Butomus umbellatus</i> * | 7 | |
| <i>Sparganium emersum</i> * | 7 | |
| <i>Glyceria fluitans</i> * | | 25 |
| <i>Hippuris vulgaris</i> * | | 17 |
| <i>Myosotis scorpioides</i> * | | 8 |
| Ch. Potamogetonion | | |
| <i>Potamogeton x nitens</i> | 1 | 42 (+-2) |
| <i>Elodea Canadensis</i> | 19 | |
| <i>Potamogeton crispus</i> | 15 | |
| <i>Myriophyllum spicatum</i> | 9 | |
| <i>Potamogeton perfoliatus</i> | 11 | |
| <i>Potamogeton lucens</i> | 5 | |
| <i>Potamogeton x nerviger</i> | 2 | |
| Ch. Lemnetea | | |
| <i>Lemna trisulca</i> | 21 (+-2) | 25 (+-1) |
| <i>Lemna minor</i> | 8 | |
| <i>Spirodela polyrhiza</i> | 5 | |
| Accompanying | | |
| <i>Cladophora sp.</i> | 14 | 33 (1-2) |
| <i>Fontinalis antipyretica</i> | 27 (+-2) | 17 |
| <i>Ranunculus lingua</i> | 1 | |
| <i>Rorippa amphibian</i> | 2 | |
| <i>Rumex aquaticus</i> | | 8 |
| <i>Sium latifolium</i> | | 8 |

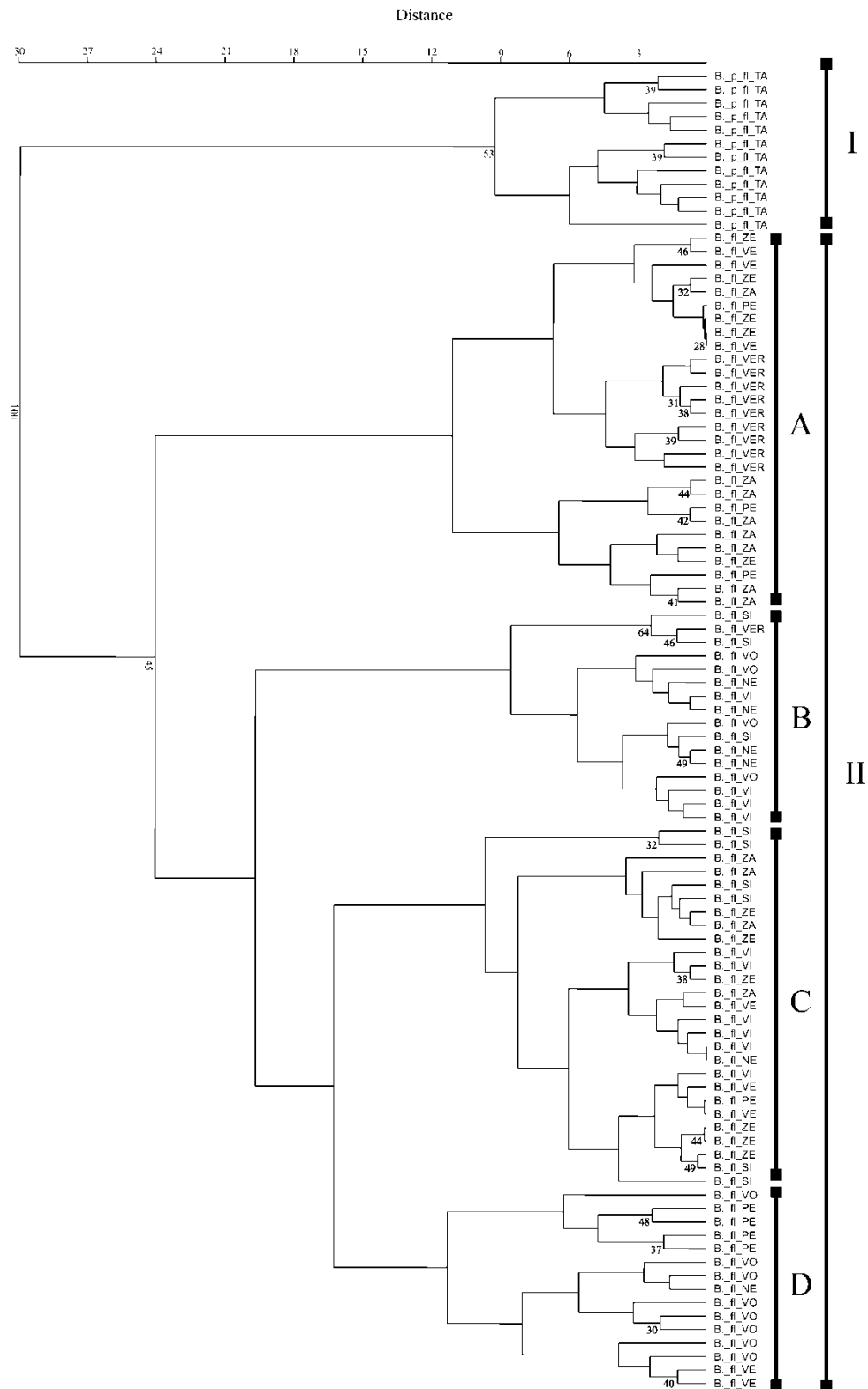


Fig. 8. Cluster analysis of *Batrachium* rich communities in Lithuanian rivers, using *Ward's* distance and *Neighbour* joining clustering method

The community with dominant *Batrachium pseudofluitans* (12 phytosociological records) only provisionally was named as *Ranunculetum pseudofluitantis* (Table 4). It was characterized by relatively high constant (II–III class) floating forms of helophytes (*Agrostis stolonifera*, *Glyceria fluitans*, *Sparganium erectum*, *Schoenoplectus lacustris*)

– characteristic taxa of *Batrachion fluitantis* alliance (Table 4). Other characteristic species of the alliance (*Berula erecta*, *Veronica anagallis-aquatica* and *Callitriche cophocarpa*) did not exceed I class of constancy. *Potamogeton* × *nitens* was a solitary species, characteristic of *Potamogetonion* alliance presented and reached III class of constancy. Typical to alkaline waters *Hippuris vulgaris* separated this community from *Ranunculetum fluitantis*, however, this species was noted only in several relevés.

A total of 17 species were inventoried in *Ranunculetum pseudofluitantis* community, their number ranged from two to five per sampling plot. One variant of this community was differentiated by semi-submerged forms of *Sparganium erectum* and *Berula erecta*. These phytocoenoses were distributed in the river stretches with relatively low flow and bottom covered by clayish sediments. The second variant of phytocoenoses was characterized by submerged form of *Schoenoplectus lacustris* and *Potamogeton* × *nitens*, mainly distributed in the river stretches with faster flow and coarse bottom.

Environmental parameters

The *Ranunculetum fluitantis* was distributed in the large, medium-sized as well as in the small rivers of the Neris River basin. The growing depth varied from 0.25 m to 1.1 m. The river beds usually were covered by mixed sand/gravel substrates, sometimes with different sized boulders (Table 5). The *Ranunculetum pseudofluitantis* was described in the medium-sized Tatula River located in the gypsum karst region. The growing depth varied from 0.3 m to 0.9 m. The river bed mainly was covered by mixed clay/gravel substrates, sometimes with different-sized boulders.

Table 5

Parameters of water (mean ± SE) in the locations with different *Batrachium* communities

| | <i>B. fluitans</i> N=20 | <i>B. pseudofluitans</i> N=10 |
|--------------------------------------|----------------------------|----------------------------------|
| Flow velocity, m/s | 0.54 ± 0.06 | 0.11 ± 0.04 |
| Conductivity, µS/cm | 433.21 ± 10.58 | 711.20 ± 30.36 |
| Alkalinity, mmol/l | 3.50 ± 0.126 | 6.28 ± 0.14 |
| pH | 8.1 ± 0.138 | 7.8 ± 0.098 |
| Ca ²⁺ , mg/l | 31.39 ± 7.266 | 47.58 ± 24.20 |
| NH ₄ ⁺ , mg/l | 0.07 ± 0.01 | 0.05 ± 0.01 |
| NO ₂ ⁻ , mg/l | 0.01 ± 0.001 | 0.02 ± 0.002 |
| NO ₃ ⁻ , mg/l | 0.71 ± 0.05 | 3.04 ± 0.25 |
| PO ₄ ³⁻ , mg/l | 0.04 ± 0.006 | 0.06 ± 0.01 |
| Total N mg/l | 1.18 ± 0.08 | 4.49 ± 0.28 |
| Total P, mg/l | 0.08 ± 0.006 | 0.08 ± 0.01 |
| Depth, m | 0.64 ± 0.05 | 0.61 ± 0.09 |
| Bottom substrates | sand/gravel (boulders) | clay/gravel (boulders) |

In the habitats of different *Batrachium* communities out of 11 analysed parameters of the water (Table 5), significant differences ($p < 0.05$) were revealed between six parameters: flow velocity, conductivity, alkalinity, concentrations of nitrite (NO_2^-), nitrates (NO_3^-) and total nitrogen (total N) (Fig 9).

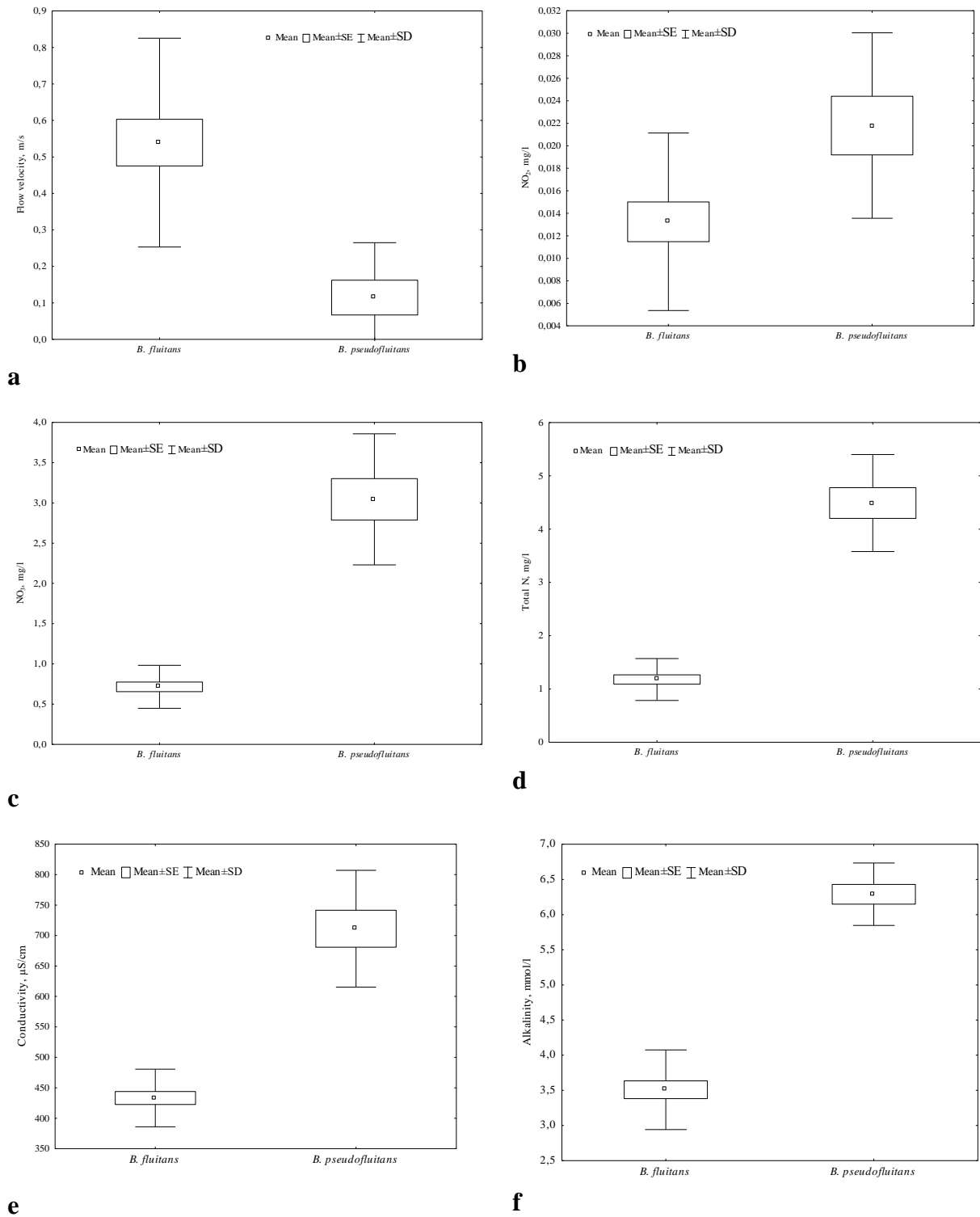


Fig. 9. Statistically significant differences of water physical and hydrochemical parameters in habitats of *B. fluitans* and *B. pseudofluitans* communities

Flow velocity in the locations of *B. fluitans* communities was significantly higher than in these of *B. pseudofluitans* (Table 5, Fig. 9). The waters in locations of *B. pseudofluitans* communities were characterized by significantly higher conductivity, alkalinity and concentrations of nitrite (NO_2^-), nitrates (NO_3^-) and total nitrogen (N) compared to waters inhabited by *Ranunculetum fluitantis* (Table 5). Concentrations of total phosphorus (P) were similar in the locations of both species communities.

The study of the impact of river tourism services on the sustainability of *Batrachium* communities was carried out in the river Ūla and showed that most intensive canoeing occurs in July and August during the main flowering of *Batrachium* plants (Fig. 10). In these months, the total plant cover in the studied plots was minimum (Fig. 11).

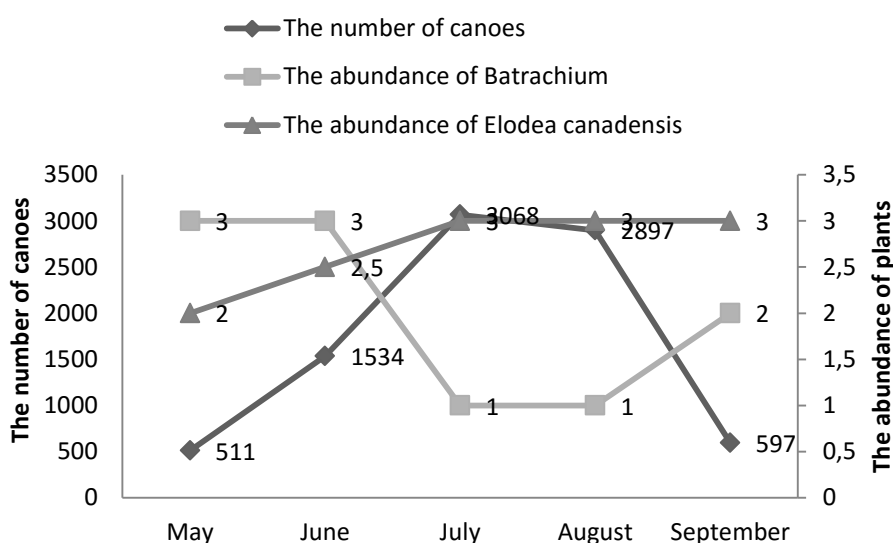


Fig. 10. Correlation between intensity of canoeing in summer months and abundance of *Batrachium* and *Elodea canadensis* in the study plots

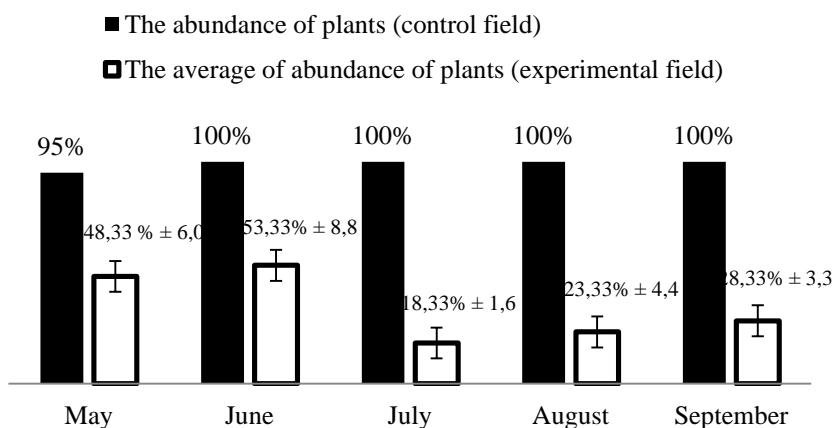


Fig. 11. The total plant abundance in different experimental sites

The negative influence of canoeing manifests itself through the mechanical disturbance of *Batrachium* plants (cut off flowers and fruits) (Fig. 12).

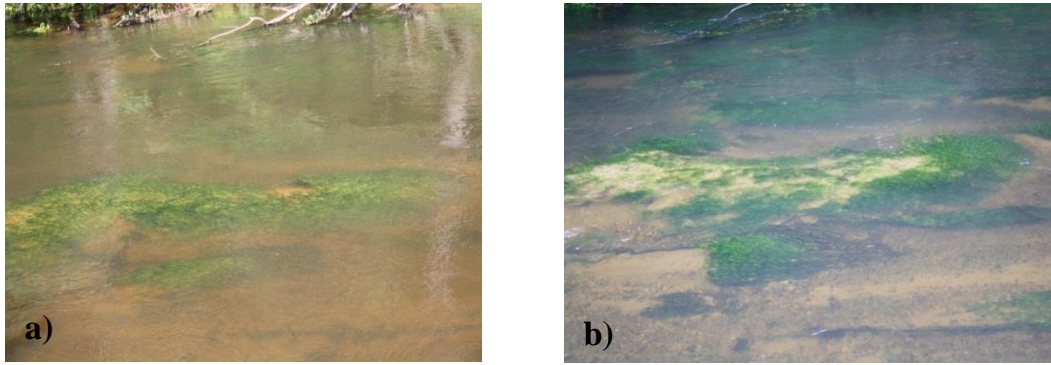


Fig. 12. The experiment site in May (a) and July (b) (apparent mechanically damaged plants)

Also, the canoeing brings the sediments which accumulate in the river onshore, which favours establishment of shade-tolerant plant species, such as *Elodea canadensis* in these sites. The negative correlation was detected between the intensity of canoeing and abundance of *Batrachium* plants in the experimental sites ($r = -0.8029$, $p < 0.01$).

DISCUSSION

Morphological and genetic diversity of *Batrachium*

The analysis of four morphological characters (the length of petals, the lengths of the second and sixth leaves and the hairiness of the receptacle) statistically significantly distinguished a large group (Group I) of homophyllous plants, identified as *B. fluitans* (Fig. 2). *Batrachium* identification keys (Cook, 1966; Webster, Rich, 1998; Bobrov, 2015) also indicate other morphological characters that may be important for the identification of these species, but the analysis of plants from studied populations showed that some of these characters were not fully expressed. Similar to other authors (Cook, 1966; Webster, Rich, 1998; Bobrov, 2003), we found that *B. fluitans* plants had a sporadically hairy receptacle. In our study, *B. fluitans* plants had 0.4–1.1 cm long petals, which were slightly smaller than described by Cook (1966) (0.7–1.3 cm), but the petal sizes were closer to that indicated for the northernmost population in Sweden by Dahlgren and Jonsell (2001) and reported by Webster and Rich (1998) – 0.6–1.0 cm and 0.5–1.0 cm, respectively.

All examined plants as well as specimens from herbaria material did not have ripe achenes. Ripe achene absence was also noted for Nordic material (Dahlgren, Jonsell, 2001). This can be explained, at least in part, by the presence of species on the edge of the distribution range.

The second distinguished group (Group II) was more heterogeneous (Fig. 2). It contained both homophyllous and heterophyllous plants and preliminarily was identified as *B. penicillatum* s.l. and *B. trichophyllum*. *Batrachium* specimens, having intermediate leaves (SK and UL populations), were the most different and were assigned to *B. penicillatum* s.str. In the SK population, which has been recorded since 1983, plants with fully developed floating leaves have never been observed; however, intermediate leaves have always occurred. Bobrov et al. (2015) noticed that floating leaves in North European *R. penicillatus* populations were only sometimes present, whereas intermediate leaves were frequently present. Englmaier (2016) noted that in the Austria and the Eastern Alps, floating and even intermediate leaves of *R. penicillatus* were not always present. In our case, the heterophyllous UL population was recorded in the same river as homophyllous plants.

We suppose that subgroups *a* and *c* (Fig. 2) of the homophyllous plants may consist of true homophyllous and heterophyllous species with undeveloped lobed or intermediate leaves. The sequencing results indicate the occurrence of homophyllous *B. pseudofluitans* (TA population). However, some populations, especially those distributed in the Merkys River basin (ME, UL, and GR), could include *B. penicillatus* with undeveloped floating leaves, *B. pseudofluitans* and even *B. trichophyllum* or *B. kauffmannii*. The last species has been recorded in Russia, Belarus and Poland (Bobrov et al., 2003; Sautkina, 2007; Zalewska-Gałosz, 2015).

The *Batrachium* genus is distinguished by its extreme phenotypic plasticity, and certain characters of these plants are strongly dependent on dynamic environmental conditions. Webster and Rich (1998) reported that *B. penicillatum* can have longer leaves in response to slower streams and shade and can thus mimic the phenotype of *B. fluitans*. However, if the flow rate increases and the habitat is sufficiently illuminated, *B. fluitans* leaves can be short and stiff. Growing in suboptimal conditions also changes leaf

morphology (e.g., leaf fragmentation and segment length). Although morphological characters analyzed in our study varied within a much narrower range than that indicated by descriptors of the species, the combination of four characters distinguished populations of the two main studied groups.

Neutral molecular markers are preferred in genetic variability studies at the intra- and interspecific levels (Weising et al., 2005; Kekkonen, 2015). ISSR is a popular technique for analyzing unexplored, non-sequenced genomes and is widely used in ecological, population or phylogenetic studies (Weising et al., 2005; Archibald et al., 2006; Coppi et al., 2015). In our study, molecular analysis based on ISSR markers confirmed the separation of *Batrachium* plants into two main groups, which were revealed using traditional morphological characters. We scored 106 ISSR bands, a number that, according to Fajardo et al. (2014), was sufficient to assess the genetic relatedness of species and detect polymorphism between individuals within populations. *Batrachium penicillatum* s.l. and *B. trichophyllum* populations showed higher values for average expected heterozygosity (H_j) and the percentage of polymorphic loci than did the populations of *B. fluitans*. A Mann-Whitney test showed that differences between H_j values for the two population groups were statistically significant. The expected heterozygosity and percentage of polymorphic loci calculated for Lithuanian populations of the Group II plants (*B. penicillatum* s.l. and *B. trichophyllum*) and *B. fluitans* were lower than those of previously published results (Bradley et al., 2013). The decline in the number of polymorphic loci and average heterozygosity in Lithuanian *Batrachium* populations is possibly determined by genetic drift, especially in the situation when number and size of populations are decreasing (Frankham, 1996; Shrestha et al., 2002; Ilves et al., 2016). Bradley et al. (2013) also did not detect signs of clonality in populations of *B. fluitans*. However, low values of genotypic and genetic diversity parameters revealed in our study indicate expressed clonality in the studied populations. The spread of clones was detected in all populations from Lithuanian rivers, and one population (PE) was monomorphic. This situation of reduced levels of genetic diversity and sexual reproduction suggests that populations possibly exist in suboptimal conditions (Beatty et al., 2008). *Batrachium fluitans* populations in Lithuania exist at the northeastern edge of its distribution range (Jalas and Suominen, 1989) and likely propagate only vegetatively. Although *B. fluitans* forms flowers, ripe seeds were not detected (Butkuvienė et al., 2014). This also implies that asexual reproduction predominates. Based on the ISSR marker analysis, we revealed that the proportion of distinguishable genotypes in populations was lower for *B. fluitans* ($i = 0.433 \pm 0.07$) than for group of *B. penicillatum* s.l. and *B. trichophyllum* (0.604 ± 0.06). Although sexual reproduction occurs in the populations of the *B. penicillatum* s.l. and *B. trichophyllum* group in Lithuania, our study also revealed the existence of identical genotypes in populations of this group, which suggests that seedling establishment is rather limited. It should be noted that most *Batrachium* species are self-compatible and often have a tendency toward cleistogamy, which is frequently dependent on environmental conditions (Zalewska-Gałosz et al., 2014). Considering that Lithuanian populations of studied *Batrachium* species are relatively fragmented and small, we suppose that inbreeding could play a role in maintaining a low level of genetic diversity in some populations of *B. penicillatum* s.l. The AMOVA results displaying the partitioning of genetic diversity support the opinion that *Batrachium* populations are isolated and genetically differentiated. The lack of common genotypes in these populations

corroborates this view, as does the observation that populations from different rivers are isolated. Small and isolated populations are frequently affected by founder effects and/or genetic drift that increase genetic differentiation (Nei et al., 1975; Durka et al., 2017). Similarly, high degrees of genetic differentiation were detected among populations of *Batrachium bungei* growing in rivers of the Hengduan Mountains in China (Wang et al., 2010). It should be noted that the high genetic differentiation of populations is also affected by taxonomic heterogeneity of *Batrachium* in Lithuania.

PCoA and STRUCTURE analyses provided results that agree with those of the morphological analysis. All the studied populations were clustered into two main groups that correspond to different *Batrachium* species or species groups (Figs. 2, 5 and 6). STRUCTURE, like the PCoA and NMDS analyses, also revealed substructuring within these two clusters, indicating the taxonomic complexity of *Batrachium* (Figs. 2, 5 and 7). In general, STRUCTURE analysis did not reveal clear signs of interspecific hybridization, except for a few admixed individuals from the GR, ME, NE and VI populations that could be considered a result of gene flow between species (Fig. 6). The results of the morphological analyses and DNA sequencing of previous studies indicate that hybridization between different *Batrachium* species is rather frequent (Cook, 1966; Zalewska-Gałosz et al., 2014; Wiegleb et al., 2017); however, Bradley et al. (2013) did not observe direct evidence of interspecific hybridization between *B. fluitans* and *B. penicillatum* in a study of water crowfoot in Ireland. Our results indicate a similar situation in the studied populations.

This study and a previous revision of the herbarium specimens (Butkuvienė et al., 2014) support earlier observations that these two species groups have a tendency to occur separately (Mony et al., 2006). We did not find genotypes spread among different populations. Because *B. penicillatum* s.l. is a heterogenic and insufficiently understood taxon (Zalewska-Gałosz et al., 2014), its taxonomic characterization requires the use of various methodical approaches. To obtain more information about the taxonomical status of the studied plant material, we also performed the analysis of the *trnH-psbA* of cpDNA region and *ITS* region of rDNA in plants assigned to different groups and subgroups. Previously, *trnH-psbA* region was characterized as one of the most informative in the barcoding of *Batrachium* species (Telford et al., 2011). The *trnH-psbA* region sequencing results mostly corroborated the results of other analyses. The sequences of the *trnH-psbA* region of the individuals from the *B. fluitans* group, identified in our study as Group I, showed identity with those of *R. fluitans* (GenBank[®] accession HQ894440.1). Two different haplotypes of this region were detected in the individuals of Group II, as defined in our study. One haplotype showed identity with *R. trichophyllum*, and the second showed high similarity to *R. penicillatus* subsp. *pseudofluitans*. Because the barcoding effectiveness of the *trnH-psbA* region in *Batrachium* is limited (Telford et al., 2011), the results of species identification should be treated with some caution. The sequencing of *ITS1-ITS4* region of rDNA of study plants revealed more complicated taxonomic situation. The analysis of this region clearly identified *B. pseudofluitans*. Two ribotypes were detected for *B. trichophyllum*, one in population from Rospuda (Poland) and the second in Viešvilė population. Bobrov et al. (2015) also noticed ribotype diversity of this species in populations from Scandinavia and Russia. In other studied plants of *B. penicillatum* group were detected six additional ribotypes. So, the results of *ITS* region sequencing support the view of other authors

(Zalewska-Gałosz et al., 2014) and our own data obtained by analyses of morphological characters and ISSR markers that *B. penicillatum* is taxonomically complicated group.

Our study detected a low level of genetic diversity in most of the studied *Batrachium* populations. These situations is threatening for plants with sexual reproduction, especially for those with a small population size, as harmful alleles that reduce fitness may become fixed in populations and increase the risk of extinction (Reed, Frankham, 2003). However, it is difficult to establish a baseline or reference point against which to measure the changes in population size and diversity (Taylor et al., 1994; Steinber, Jordan, 1998). Therefore, our study could help understand the current status of *Batrachium* species and populations in Lithuanian rivers and provide measures for evaluating the future risk of loss of diversity in certain regions of the range of this species.

A low degree of genetic diversity, high level of genetic differentiation and low level of gene flow between populations of riverine *Batrachium* species in Lithuania indicate that a conservation strategy should be implemented. Although the communities that contain *Batrachium* species are protected, specific attention to *Batrachium* species, the primary component of these communities, should be increased. During the flowering time of water crowfoot (from the end of June until the end of July), the most intensive canoeing occurs in Lithuanian rivers, which may disturb the ripening of *B. penicillatum* s.l. seeds. The influence of canoeing on the spread and genetic diversity of *Batrachium* species is poorly investigated. Therefore, it is difficult to give an unambiguous assessment of this issue. Nevertheless, our personal study of the impact of water tourism on aquatic plants suggest that the impact would be largely negative because tourism activities tear or break plants such that the plant parts cannot root or result in establishing in dark places unsuitable for flowering. Long-term observations should be carried out to evaluate the impact of anthropogenic activities on *Batrachium* species. Finally, some anthropogenic activities, particularly those in river stretches, should be regulated by legislation. Based on our results, we conclude that *B. fluitans* should be included in the list of protected species, and selected river stretches with *B. fluitans* should be protected. The species group *B. penicillatum* s.l. should be studied in more detail to identify the distribution of the species or other taxonomic ranks.

Floristic structure and environmental parameters of *Batrachium* communities

Batrachion fluitantis alliance communities are mainly characterized by dominance of *Batrachium* species, occurring in condition of the fast current. They usually are poor in species diversity due to prevailing vegetative propagation, stressful aquatic environment and shading of the river beds (Spink et al., 1997; Lumbreras et al., 2009). The fast flow affects the appearance of *Batrachium* plants as well as commonly associated taxa. Elongated forms of submerged plants (e.g. *Stuckenia pectinata* f. *interrupta*, *Potamogeton perfoliatus* f. *cordato-lanceolatus*), submerged forms of amphibian plants (*Berula erecta*, *Callitriche cophocarpa*, *Veronica anagallis-aquatica*) and fluvial forms of helophytes (*Agrostis stolonifera* f. *submersa*, *Butomus umbellatus* f. *vallisneriifolius*, *Sagittaria sagittifolia* f. *valisneriifolia*, *Schoenoplectus lacustris* f. *fluitans*, *Sparganium emersum* f. *fluitans*, *S. erectum* f. *fluitans*) are typical in such plant communities. In general, main part of characteristic species of *Batrachion fluitantis* alliance is similar in various streams and rivers (Rich, Jermy, 1998; Hatton-Ellis, Grieve, 2003; Haslam, 2006), however, Lithuanian communities clearly stand out by absence of

many *Callitriche* species, which distribution areas do not reach this territory. Only *C. cophocarpa* was recorded. The presence of *Sparganium* species as well as filamentous algae *Cladophora* usually indicates low water during vegetation period or increase of nutrient content.

According to the data on distribution of characteristic species (Butkuvienė et al., 2014), the association *Ranunculetum fluitantis* is the most widespread in the Lithuanian rivers, however, it is mainly concentrated in the River Neris and its different-sized tributaries. At the studied sites, this association mainly differs from that described in Europe (Oberdorfer, 1977; Dethioux, Noirfalise, 1985; Šumberová, 2011) by the absence of other *Batrachium* species (*B. penicillatum*, *B. trichophyllum* and hybrids of *B. fluitans*). Furthermore, this community was distributed in different rivers compared to other *Batrachium* communities. Fluvial forms of *Stuckenia pectinata* and *Potamogeton* species not always, but more often were associated with *B. fluitans* in communities of large rivers, whereas *Callitriche cophocarpa*, *Fontinalis antipyretica*, *Lemna trisulca* and *Elodea canadensis* were most often in the small-sized rivers. *Ranunculetum fluitantis* sometimes occupied the entire sections of the river, but more often it was found in mosaic patches of other plant communities.

The *Ranunculetum pseudofluitantis* phytocenoses recorded only in one river were not assigned to any other association described. The associations *Callitriche brutiae-Ranunculetum pseudofluitantis* Pizarro & Rivas-Martínez 2002 and *Callitriche lusitanicae-Ranunculetum pseudofluitantis* Melendo, Cano & Valle 2003 described for Iberian peninsula (Rivas-Martínez et al., 2001, 2002; Melendo et al., 2003) according to the species composition were significantly different from our described, first off all by the absence of *Callitriche* species (*C. lusitanica*, *C. brutia*), which distribution area is situated southwards of Lithuania. Moreover, *Callitriche brutiae-Ranunculetum pseudofluitantis* is characterized as oligotrophic neutral to acidophilous community of moving or running water (Rivas-Martínez et al., 2001, 2002). On the contrary, British *Ranunculus penicillatus* ssp. *pseudofluitans* community is confined to base-rich and moderately fertile waters. Only few common species such as *Stuckenia pectinata*, *Berula erecta* and *Elodea canadensis* were found in Lithuanian and British *Ranunculus penicillatus* ssp. *pseudofluitans* communities (Rodwell, 1995), despite their ecological conditions were similar. The abundance of submerged helophyte forms in Lithuanian *Batrachium* communities indicates on the one hand a low water level during the vegetation period, on the other hand, also can be related to the fertility of water and sediments. We suppose that northern *B. pseudofluitans* rich communities must be assigned to other association than southern, but type of the community could be described from less anthropogenically impacted location.

Flow is important ecological factor, affecting macrophyte species distribution and morphological appearance. Wade et al., (2002) characterized the flow as having two different effects on submerged macrophytes: a direct effect (via plant washout), and an indirect effect (through epiphyte removal). *Ranunculetum fluitantis* is most characteristic of larger rivers with swift flow (Cook, 1963; Rodwell, 1995), but can be found in small streams also (Pott, 1995). In Lithuania, this community was observed in different-sized rivers. We suppose that in our rivers a considerable water movement, necessary for *Batrachium fluitans*, is provided by a relatively stable water level also supported by underground waters, dominant in supply of rivers of sandy plains. In contrast, *Ranunculetum pseudofluitantis* was distributed in the river crossing watertight calcareous

moraine plain (gypsum karst region) with significant water level fluctuations during vegetation period. In general, flow rate was lower than in locations of *Ranunculetum fluitantis*, but it can significantly increase after abundant rainfalls.

Stable (gravelly, stony) river beds are preferable to *Ranunculetum fluitantis* (Rodwell, 1995). Even more, Cook, (1966) indicated that the physical nature of the substratum, but not hydrochemistry of the water, is a limiting factor in *Batrachium fluitans* distribution. In our case, *Ranunculetum fluitantis* communities were observed in the rivers with more stable (sandy, gravelly, stony) sediments than *Ranunculetum pseudofluitantis* in the rivers with sometimes soft clayish sediments mixed with crushed gypsum keystone.

Lithology is very important in water classification (Lumbreras et al., 2009) and subsequently for species and their communities' distribution. Environmental conditions in our case clearly separated two *Batrachium* rich communities. *Ranunculetum pseudofluitantis* was restricted to one northern river with calcareous catchment area and with waters of high alkalinity and conductivity. Additionally, they were enriched with nitrogen compounds, which may come from the surrounding areas of intensive agriculture. In Great Britain, *B. pseudofluitans* is typical species of the chalk streams with high values of conductivity and alkalinity, but generally only of moderately fertile waters (Rodwell, 1995; Spink et al., 1997; Hatton-Ellis, Grieve, 2003; Poynter, 2014). In Denmark, this taxon is restricted to similar swiftly flowing streams with moraine clay bottom (Dahlgren, Jonsell, 2001). Carr and Goulder (1990) have noticed that the potential for enhanced nutrient concentrations to influence the distribution and composition of aquatic macrophytes and filamentous/benthic algae is particularly high in naturally stable chalk streams. In Great Britain, *B. pseudofluitans* is only rarely represented in the communities of more base-poor watercourses with *Callitriche* and *Myriophyllum alterniflorum* (Rodwell, 1995). Thus, the occurrence of *B. pseudofluitans* rich communities in oligotrophic neutral to acidophilous waters of Iberian peninsula (Rivas-Martínez et al., 2001, 2002; Melendo et al., 2003) though can be explained by differences of Mediterranean and North European climate condition, but in any case is still controversial.

Ranunculetum fluitantis in Lithuania was mainly restricted to the eastern rivers with waters in about twice lower alkalinity and significantly lower concentrations of nitrogen compounds than *Ranunculetum pseudofluitantis*. According to Rodwell, (1995), the association *Ranunculetum fluitantis* usually is found in moderately fertile and moderately base-rich waters. Pott, (1995) indicated the considerably larger ranges of *Ranunculetum fluitantis* from calcareous to non-calcareous and from mesotrophic to eutrophic cold oxygen-rich waters. Cook, (1966) reported that *R. fluitans* is a species fairly tolerant to pollution as long as the water remains clear. In any case, the significant negative relationship was established between the concentration of nutrients and relative abundance of both *Batrachium* species in their communities. It means that nutrient enrichment directly or indirectly affects the abundance of *Batrachium* species and floristic structure of macrophyte communities in the rivers. Preliminary studies of influence of water tourism suggest that water transport can decrease *Batrachium* abundance in the river. The negative influence of canoeing comes through the mechanical disturbance of *Batrachium* plants (cut off the flowers and fruits). Also, the canoeing brings the sediments which accumulate in river onshore and by reason of this shade-tolerant plant species can establish in these sites. To get a more complete view of

the influence of water tourism on *Batrachium* communities, it should be carried out periodic surveys, and the strategy of protection must be treated.

CONCLUSIONS

1. Statistically significant diagnostic characteristics (the average length of petals, length of the second and sixth leaf, the hairiness of receptacle) of *Batrachium* plants from the studied populations were identified that reliably differentiate *Batrachium fluitans* from the taxonomically heterogeneous group consisting of *B. penicillatum* s.l. and *B. trichophyllum*.
2. The populations of the genus *Batrachium* are characterized by low genetic diversity and high genetic differentiation ($F_{ST} = 0.9$). This indicates that genetic drift overcomes gene flow between populations and impacts isolation of populations.
3. The very low genetic and genotypic diversity of *Batrachium fluitans* populations is characterized by the occurrence of a small number of genotypes or the spread of one genotype. This indicates that asexual reproduction predominates in this species. The results of our studies show that, although the *B. fluitans* plants form flowers, but they do not run to seeds. The lack of genetic variation and predominance of asexual reproduction show that *B. fluitans* populations exist in suboptimal conditions in Lithuania and could be considered as endangered species that should be included in the list of protected species.
4. The results of the ISSR polymorphism studies indicate that each *Batrachium* population from the studied Lithuanian rivers has a specific genotypic diversity, which can be considered as a consequence of the genetic drift.
5. The analysis of the morphological and molecular markers (ISSR) of *Batrachium* individuals and the results of the sequencing of chloroplast DNA (*trnH-psbA* region) and rDNA (*ITS1-ITS4* region) suggest that *B. fluitans*, *B. pseudofluitans*, *B. trichophyllum* and *B. penicillatum* s.str. are distributed in the studied rivers.
6. The communities of morphologically similar species *Batrachium fluitans* (*Ranunculetum fluitantis*) and *B. pseudofluitans* (*Ranunculetum pseudofluitantis*) varied according to floristic composition. *B. fluitans* communities are characterized by a greater variety of *Potamogetonion* alliance species. At the regional aspect both communities differed from those described in other countries by reduced diversity of *Callitriche* species.
7. Statistically significant differences in the ecological conditions of the habitats were revealed for both species and their communities. *Ranunculetum pseudofluitantis* communities are spread in waters with slower flow, higher level of alkalinity and conductivity and higher concentration of nitrogen compounds, characteristic of the northern region of Lithuania.
8. The evidence from this study suggests that water tourism exerts negative impacts on the sustainability of the *Batrachium* populations in the River Ūla, therefore it is recommended, where possible, to limit the canoeing activities in this river in July and August months.

Research papers

One scientific paper related to the topic of the doctoral dissertation was published in the *Clarivate Analytic Web of Science* issue with the impact factor (IF):

Butkuvienė J., Sinkevičienė Z., Naugžemys D., Patamsytė J., Žvingila D., 2017: Genetic diversity of *Batrachium* (Ranunculaceae) species reveals the necessity of their protection in Lithuanian rivers. – *Aquatic Botany*, 142: 61–70.

Two research articles related to the topic of the doctoral dissertation were published in the issue included in ISI Master Journal List and in the proceedings of international conference.

Butkuvienė J., Sinkevičienė Z., Naugžemys D., Patamsytė J., Žvingila D., 2017: Vandens turizmo poveikis kurklių (*Batrachium*) bendrijų stabilumui. – *Žmogaus ir gamtos sauga*, 2017:122–124.

Butkuvienė J., Sinkevičienė Z., Žvingila D., 2014: *Batrachium* (Ranunculaceae) in the rivers of Lithuania. – *Botanica Lithuanica*, 20 (1):46–56.

Conference presentations

Butkuvienė J., Sinkevičienė Z., Žvingila D., 2014. Genetic diversity of water crowfoot species in some Lithuanian rivers. – *Vital Nature Sign – Kaunas*.

Butkuvienė J., Sinkevičienė Z., Žvingila D., 2014. Peculiarities of water crowfoot distribution in the rivers of Lithuania. – Современное состояние, тенденции развития, рациональное использование и сохранение биологического разнообразия растительного мира – Minsk.

Butkuvienė J., Sinkevičienė Z., Žvingila D., 2014. Morfologiniai požymiai *Batrachium* (DC.) Gray taksonomijoje. – *Mokslas gamtos mokslų fakultete – Vilnius*.

Butkuvienė J., Sinkevičienė Z., Naugžemys D., Žvingila D., 2016. *Batrachium* species in different tropical state rivers. – *Ecological sustainability engineering change – France, Montpellier*.

Butkuvienė J., Sinkevičienė Z., Naugžemys D., Žvingila D., 2016. Morphological comparison of some riverine *Batrachium* species. – *Vital Nature Sign – Vilnius*.

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SANTRAUKA

Batrachium (DC.) Gray. – tai kurklės gentis, kuriai priklauso vienmečiai ir daugiamečiai vandens augalai paplitę stovinčio ir tekančio vandens telkiniuose. Sistematinio požiūriu, tai viena sudėtingiausių vandens augalų grupių, kurios sistematika iki šiol nenusistovėjusi tiek Europoje, tiek ir visame pasaulyje. Tikslus kurklių rūšių ir jų paplitimo nustatymas labai svarbus ne tik moksliniu požiūriu, bet ir sprendžiant jų apsaugos klausimus, nes upių sraunumos su kurklių bendrijomis yra įtrauktos į Europinės svarbos buveinių sąrašą. Tyrimus su *Batrachium* genties rūšimis atlieka ir kitų šalių mokslininkai, tačiau dėl hibridizacijos ir didelio morfologinių požymių varijavimo yra dar daug neatsakytų klausimų ir problemų. Manoma, kad šios genties rūšių morfologija labai priklauso nuo aplinkos sąlygų, todėl pagal kitose šalyse sudarytus apibūdinimo raktus sudėtinga identifikuoti Lietuvos sąlygomis augančias *Batrachium* rūšis. Išimtinai Lietuvos tekančiuose vandenyse paplitusios kurklės yra aprašomos įvairiuose literatūros šaltiniuose, tačiau dažnai jos „slepiasi“ po vienu – *Batrachium fluitans* vardu. Įvairiais laikotarpiais šios rūšies buvimas / nebuvimas Lietuvos upėse buvo traktuojamas skirtingai. Todėl tekančiuose vandenyse augančių kurklių rūšinė sudėtis ir tikslus jų paplitimas iki šiol nežinomi. Šio darbo metu buvo tiriami Lietuvoje augančių *Batrachium* rūšių morfologiniai požymiai, įvertinta genetinė įvairovė ir aprašytos augalų bendrijos su *Batrachium* rūšimis. Atlikti *Batrachium* augalų tyrimai parodė, kad ne visi užsienio apibūdinimo raktuose naudojami *Batrachium* morfologiniai požymiai yra informatyvūs ir tinkami Lietuvoje augančioms kurklių rūšims apibūdinti. Nors kai kurie morfologiniai požymiai yra labai svarbūs ir informatyvūs atskiriant *B. fluitans* nuo didelės ir heterogeniškos *B. penicillatum* s.l. grupės, tačiau vien jais pasikliauti negalima. Norint tiksliau atskirti tam tikras rūšis ir objektyviau tirti *Batrachium* populiacijas, būtina taikyti ir molekulinis metodus. Atlikus *Batrachium* populiacijų genetinę analizę ir nustatius mažą genetinę įvairovę, didelę genetinę diferenciaciją bei mažą genų srautą tarp populiacijų, galima teigti, kad vis tik šios genties rūšių populiacijos linkę mažėti. Juo labiau, kad kurklės yra pagrindinis saugomų bendrijų komponentas. Lietuvos upėse tyrimo metu buvo išskirtos dvi bendrijos su *Batrachium* rūšimis (*Ranunculetum fluitantis* ir *Ranunculetum pseudofluitantis*). Įvertinus vandens turizmo poveikį augalų bendrijoms su kurklėmis, buvo nustatyta neigiama vidutinė koreliacija tarp praplaukusių baidarių skaičiaus ir *Batrachium* augalų gausumo tyrimo laukeliuose. Apibendrinant galima teigti, kad kurklėms, kaip pagrindiniam Europinės svarbos buveinių komponentui, turėtų būti skiriamas didesnis dėmesys. Po atliktų tyrimų buvo pateiktos rekomendacijos Lietuvos Aplinkos ministerijai dėl *Batrachium fluitans* įtraukimo į saugomų rūšių sąrašus, o *Batrachium penicillatum* s.l. ir toliau tiriama, vykdoma tiksli rūšių identifikacija ir hibridų paieška

DARBO TIKSLAS

Nustatyti *Batrachium* rūšių, paplitusių Lietuvos tekančiuose vandenyse, įvairovę, populiacijų genetinį, morfologinį kintamumą ir įvertinti juos lemiančių veiksnių įtaką rūšių paplitimui.

PAGRINDINIAI TYRIMŲ UŽDAVINIAI

1. Remiantis *Batrachium* rūšių pavyzdžių, esančių Vilniaus universiteto herbariume (WI) ir Gamtos tyrimų centro Botanikos instituto herbariume (BILAS), analizės rezultatais parinkti augalų rinkimo vietas.
2. Įvertinti *Batrachium* augalų rūšinę įvairovę ir morfologinį kintamumą pasirinktose Lietuvos upių populiacijose.
3. ISSR metodu iširti *Batrachium* rūšių, paplitusių tekančiuose vandenyse, populiacijų genetinę sandarą ir įvertinti jai įtaką darančius veiksnius.
4. Atlikti taksonominę *Batrachium* analizę naudojant branduolio *ITS* ir chloroplastų *trnH-psbA* regionų sekoskaitą.
5. Įvertinti tekančiuose vandenyse augančių *Batrachium* rūšių augaviečių ekologines sąlygas ir jų įtaką bendrijų su *Batrachium* augalais įvairovei.
6. Parengti rekomendacijas dėl *Batrachium* rūšių ir jų buveinių apsaugos.

DARBO NAUJUMAS IR PRAKTINĖ REIKŠMĖ

Pirmą kart atlikti *Batrachium* augalų gamtinių populiacijų tyrimai, panaudojant morfologinius ir DNR (ISSR, cpDNR ir rDNR) žymenis, įvertinta šios genties augalų rūšių bei populiacijų įvairovė.

Sekoskaitos metodu nustatytos iširtų augalų cpDNR *trnH-psbA* regiono pilnos sekos buvo užregistruotos GenBank[®] duomenų bazėje.

Išskirta nauja augalų su kurklėmis *Ranunculetum pseudofluitantis* bendrija, kuri skiriasi nuo kitų autorių aprašytų bendrijų su šia rūšimi, nustatyti regioniniai bendrijų savitumai.

Pateiktos rekomendacijos Valstybinei saugomų teritorijų tarnybai prie Aplinkos ministerijos dėl kurklių buveinių išsaugojimo ir *B. fluitans* įtraukimo į saugomų rūšių sąrašus.

GYNIMUI PRISTATOMI TEIGINIAI

1. Vidutinis vainiklapių ilgis, antrojo ir šeštojo lapo ilgis, žiedsosčio plaukuotumas – pagrindiniai *Batrachium* augalų morfologiniai požymiai, kurie yra tinkami rūšiai Lietuvos upėse nustatyti.
2. Mažas DNR polimorfizmas ir klonų paplitimas rodo, kad kai kurių Lietuvos upių *Batrachium* populiacijose vyrauja nelytinis dauginimasis.
3. ISSR polimorfizmo tyrimai leidžia daryti prielaidą, kad kiekvienoje tirtoje Lietuvos upėje yra savita *Batrachium* genties rūšių populiacijų genotipinė įvairovė.
4. Chloroplastų *trnH-psbA* ir branduolio *ITS* regionų sekoskaita rodo, kad tirtose Lietuvos upėse auga *B. fluitans*, *B. pseudofluitans*, *B. trichophyllum* ir *B. penicillatum* s.str.

5. Morfologiškai panašios rūšys (*B. fluitans* ir *B. pseudofluitans*) ir augalų bendrijos su jomis paplitę skirtingose pagal fizikinius ir hidrocheminius rodiklius augavietėse.
6. Vandens turizmas daro neigiamą įtaką *Batrachium* sąžalynams ir bendrijoms su kurklėmis.

MOKSLINĖ IR PRAKTINĖ DARBO REIKŠMĖ

Tyrimo metu nustatyta, kad tirtose Lietuvos upėse paplitusios keturios *Batrachium* rūšys (*B. fluitans*, *B. pseudofluitans*, *B. penicillatum* ir *B. trichophyllum*). Taip pat identifikuotos augalų bendrijos su dvejomis skirtingomis *Batrachium* rūšimis. Valstybinėje saugomų teritorijų tarnyboje buvo prarastas seminaras apie kurkles, joms keliamas grėsmės ir galimą apsaugos strategiją, taip pat Lietuvos Respublikos Aplinkos ministerijai buvo pateiktos rekomendacijos dėl buveinių apsaugos ir *B. fluitans* įtraukimo į saugomų rūšių sąrašą.

Disertacijos tema paskelbtos dvi publikacijos: viena – ISI *Master Journal List* sąrašė esančiame žurnale (*Botanica Lithuanica*); kita – *Clarivate Analytic Web of Science* referuojamame, citavimo indeksą turinčiame, žurnale (*Aquatic Botany*). Parengtas ir išspausdintas vienas mokslo populiarinimo straipsnis ir vienas straipsnis recenzuojamame leidinyje. Tyrimų rezultatai buvo pristatyti vienoje respublikinėje ir keturiose tarptautinėse konferencijose.

IŠVADOS

1. Nustatyti statistiškai reikšmingi *Batrachium* genties augalų morfoliginiai požymiai (vidutinis vainiklapių ilgis, antrojo ir šeštojo lapo ilgis, žiedsosčio plaukuotumas), kuriais remiantis *Batrachium fluitans* patikimai atsiskiria nuo taksonomiškai heterogeniškos grupės, kurią sudaro *B. penicillatum* s.l. ir *B. trichophyllum*.
2. *Batrachium* genties rūšių populiacijoms būdinga maža genetinė įvairovė ir didelė diferenciacija ($F_{ST} = 0,9$). Tai rodo didesnę genų dreifo poveikį populiacijoms palyginti su genų srauto įtaka ir dėl to didėjančią populiacijų izoliaciją.
3. Labai maža genetinė ir genotipinė įvairovė, nedidelis genotipų skaičius arba net vieno genotipo paplitimas *Batrachium fluitans* populiacijose ir sėklų nesubrandinimas rodo, kad šios rūšies populiacijose vyrauja nelytinis dauginimasis. Tai rodo, kad Lietuvoje *B. fluitans* auga nepalankiomis sąlygomis ir ją rekomenduojama įtraukti į saugomų rūšių sąrašą.
4. ISSR polimorfizmo tyrimų rezultatai rodo, kad kiekvienoje ištirtoje Lietuvos upėje yra savita *Batrachium* populiacijų genotipinė įvairovė, kurią galima vertinti kaip genų dreifo pasekmę.
5. *Batrachium* genties augalų morfoloogiinių ir molekuliinių žymenų (ISSR) analizės, chloroplastų DNR (*trnH-psbA* regiono) bei branduolio rDNR (*ITS1-ITS4* regiono) sekoskaitos rezultatai rodo, kad tirtose Lietuvos upėse paplitusios *B. fluitans*, *B. pseudofluitans*, *B. trichophyllum* ir galimai *B. penicillatum* s.str. rūšys bei hibridai.
6. Bendrijos su morfologiškai panašiomis rūšimis *Batrachium fluitans* (*Ranunculetum fluitantis*) ir *B. pseudofluitans* (*Ranunculetum pseudofluitantis*) skyrėsi pagal floristinę sandarą – *Ranunculetum fluitantis*

pasižymėjo didesne *Potamogetonion* sąjungos rūšių įvairove ir platesniu paplitimu. Regioniniu požiūriu abi bendrijos skyrėsi nuo kitose šalyse aprašytų mažesne *Callitriche* rūšių įvairove.

7. Nustatyti statistiškai reikšmingi skirtumai tarp bendrijų su abejomis rūšimis. *B. pseudofluitans* ir bendrijos su šia rūšimi paplitusios vandenyse su žymiai mažesniu srovės greičiu, didesniu šarmingumu, savituoju elektriniu laidžiu ir didesne azoto junginių koncentracija, būdinga šiaurės Lietuvos regionui.
8. Vandens turizmas daro neigiamą poveikį *Batrachium* augalų gausumui Ūlos upėje, todėl rekomenduojama riboti vandens transporto srautą šioje upėje liepos ir rugpjūčio mėnesiais.

CURRICULUM VITAE

ASMENINIAI DUOMENYS

Vardas, pavardė: Jurgita Butkuvienė
Adresas: Ažuolų 19-oji g. 13, Kalikštiškės, Maišiagalos sen., Vilniaus raj.
Telefonas: +37062680059
El. paštas: jurgita.makaviciute@gmail.com
Gimimo data, vieta 01 01 1987, Trakai

IŠSILAVINIMAS

| | |
|-----------|--|
| 2012-2016 | Vilniaus universiteto doktorantė, Doktorantūros mokslinių tyrimų kryptis: <i>Batrachium</i> (DC.) Gray. genties rūšių genetinės ir morfologinės įvairovės tyrimai. |
| 2009-2011 | Vilniaus universitetas, Specialybė: Botanikas, Laipsnis: Biologijos magistras |
| 2005-2009 | Vilniaus universitetas, Specialybė: Biologas, Laipsnis: Biologijos bakalauras |

PROFESINĖ PATIRTIS

| | |
|------------------|---|
| 2011 – 2014 | Gamtos tyrimų centro Botanikos instituto Floros ir geobotanikos laboratorijos biologė |
| 2014 – iki dabar | Laborantė, Vilniaus Universitetas, Gyvybės mokslų centras |

MOKSLINIŲ INTERESŲ KRYPTIS

Vandens augalų rūšių tyrimai, ekologija, biologija, genetika.

CURRICULUM VITAE

Personal information

Name: Jurgita Butkuvienė
Address: Ažuolų 19 str. 13, Vilnius distr., Lithuania.
Phone: +37062680059
Email: jurgita.makaviciute@gmail.com
Date and place of birth 01 01 1987, Trakai, Lithuania

Education

| | |
|-----------|--|
| 2012-2016 | PhD student, Department of Botany and Genetics, Faculty of Natural Sciences, Vilnius University. |
| 2009-2011 | Master degree in Biology, Vilnius University. |
| 2005-2009 | Bachelor degree in Biology, Vilnius University |

Current position and workplace

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|-------------|---|
| 2011 – 2014 | Biologist at the Nature Research Centre, Institute of Botany. |
| 2014 – now | Laboratory assistant at the Department of Botany and Genetics, Faculty of Natural Sciences, Vilnius University. |

Scientific Interest

Aquatic plants, ecology, biology, genetics.