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New data on the *Austropotamobius torrentium* (Schrank, 1803) from the tributaries of the Bosna River

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Abstract

The crayfish *Austropotamobius torrentium* is a dominant species of the family Astacidae in the rivers of the Black Sea and Adriatic basins of Bosnia and Herzegovina. Molecular phylogenetic studies in Europe revealed the presence of eight phylogroups. No morphometric and meristic characteristics have been determined that would clearly separate them. The paper presents new data on the morphometric parameters species Austropotamobius torrentium with watercourses Presjenica, Jošanica and Crna Rijeka. This analysis is based on 22 parameters that were measured on 38 crayfish individuals, whose size was larger than 5 cm. Using Anova test, Bonfferoni test and discrimination analysis, it was determined that females differ significantly in a small number of parameters, while males showed greater morphological divergence. The great diversity of habitats of Austropotamobius torrentium in Bosnia and Herzegovina obliges the inclusion of the Dinarides region in molecular phylogenetic studies. This will result in more precise and complete data on Austropotamobius torrentium populations.

Keywords: stone crayfish, protect, morphometrics, distribution, divergence

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1. Introduction

The crayfish *Austropotamobius torrentium* is the smallest species of the Astacidae family that is still not well known. It is widely distributed in Bosnia and Herzegovina, and its area ranges vertically from 175 to 1100 m (Karaman, 1963; Trožić-Borovac, 2011; Roljić et al., 2021, Trožić-Borovac et al., 2022). As in other European countries, it dominates in hilly and mountainous areas, in sheltered and preserved habitats, with well-developed climatogenic coastal vegetation and lower water temperatures. It is believed that the origin of this species

is from Central and Southeast Europe (Manfrin et al., 2022). It is listed as a data deficient species (DD) on the IUCN Red List of Threatened Species Füreder et al. (2010). It is internationally protected by the Bern Convention (Appendix III, protected animal species), and by the EU Habitats Directive 92/43/EEC, where it is listed in Appendix II (the central areas of the species' habitat are designated as Areas of Community Importance (SCI) and included in the Natura 2000 network) and in Appendix V (member states can decide how to manage the population, but must ensure that their exploitation and collection from the wild is compatible with maintaining a favorable conservation status). This species is protected by national legislation in most of its range (Füreder et al., 2010, Vigneucs et al., 2002), and in Bosnia and Herzegovina it is included in the Red List of Threatened Species (Škrijelj et al, 2013). The results of previous research (morphology, molecular-genetic, evolutionary) of the species indicate a high genetic diversity represented by eight different mtDNA lineages/phylogroups that have been discovered so far (Klobučar et al., 2013; Petrušek et al, 2017; Lovrenčić et al., 2020b).

2. Materials and Methods

Sampling was carried out in Presjenica, Crna rijeka and Jošanica streams in the period from May 2016 to july 2022. The researched watercourses are tributaries of the Željeznica River (Crna Rijeka), the Vogošće River (Jošanica) and the Bijela River (Presjenica). The distance between Crna Rijeka and Presjenica is 1.5 km, and Jošanica is 25 km from the Presjenica River. The riverbanks in the researched sections have partially preserved climatogenic vegetation, without visible sources of degradation, but with the presence of anthropogenic influence (roads, tree felling, livestock, agriculture, cottages, settlements, etc.). All three investigated watercourse sections are in the hilly-mountainous zone with oak-hornbeam forests (table 1). The largest number of microhabitats was registered in the river Presjenica, while Crna rijeka and Jošanica have a smaller number of microhabitats. For water temperature and oxygen concentration, the average measurements were taken during the research (Oxi 3205 Set 3 2BA103 WTW), and the depth was measured several times during the research. Individuals were caught by hand, with a benthos net or hand-made traps that were left overnight. A large number of smaller individuals were observed on all three streams, which were only recorded. Only specimens larger than 50 mm were used for analysis.

2.1. Morphometric analysis

A total of 38 individuals (18 males and 19 females) were analyzed. Morphometric measurements were carried out according to the work of Sint.all. (2005) and all measured morphometric characteristics were normalized for size by dividing them by the corresponding postorbital length (POL = HEL + ARL). A total of 22 morphometric characters were used for analysis: claw length (CLL), claw width (CLW), claw height (CLH), length of the claw palm (CPL), length of the claw finger (CFL), rostrum length (ROL), rostrum width (ROW), head length (HEL), head width (HEW), areolar length (ARL), areolar width (ARW), abdomen length (ABL), abdomen width (ABW), abdomen height (ABH), telson length (TEL), telson width (TEW), carapace width (CPW), width at the cervical groove (CGW), width of the carapace at the hind edges (CEW), carapace height (CPH), and total length (TL). All the

characteristics were measured with a digital calliper with a precision of 0.01 mm. One-way ANOVA (Post-Hoc-test: Bonferroni) was applied to select morphometric parameters for each group (sex) that differed significantly from other populations. Males and females were analyzed separately because crayfish show sexual dimorphism after reaching sexual maturity (Grandjean et al., 1997, Vlach & Valdmanová, 2015). Multivariate discriminant analysis was used to reveal the morphometric parameters that best characterize different crayfish populations. IBM SPSS Statistics 25 and Excel 2016 software were used for statistical analysis.

3. Results

The average total body length (TL) of males from the Crna River was 74.79±1.16 mm, males from the Presjenica River are slightly longer 74.33 ±14.74 mm, and the smallest are from Jošanica 60.21± 12.55 mm. Using the Bonferoni test, a statistically significant difference was found in the width of the rostrum (p=0.002), head length HEL (p=0.002), (ARL) areolar length (p=0.000), carapace width CPW (p=0.001), width of the carapace at the hind edges (CEW) (p=0.006), abdominal length (ABL) and total body length TL (p<0.05). The results of the discriminant analysis determined that males are discriminated the most with the highest loadings in discriminant functions for body length (TL), head length (HEL), cheliped length (CLL) and rostrum width (ROW). Whereas if crayfish individuals have a longer head, a larger width of chelipeds (CLW), a smaller width of the rostrum, it is more likely that they are from the Presjenica River (Table 1-2, Figure 1).

Tabela 1. Values of measured abiotic parameters on the investigated watercourses

river	Altitude	depth	T °C	O_2 mg/l	Bottom substratet			
					Pebbles	Sand	Stone	fital
Crna rijeka	610	450	10	10,05	25	40	30	5
Presjenica	620	370	11	9,85	20	60	10	10
Jošanica	629	200	12	9,45	70	25	5	0

Table 2. Standardized canonical discriminant function coefficients for morphometric characteristics of Austropotamobius torrentium males for each discriminant function; TL total length; W weight, CEW carapace width at the hind edges; ROL rostrum length; ARW areolar width; ROW rostrum width; ARL areolar length; ABL abdomen length, HEL head lenght; CGW width at the cervical groove, CLL claw lenght, CPW carapace width; % expl.var. percentage of explained variance; cum. Prop., cumulative proportions; canonical r, canonical correlations

Characteristic	Function 1	Function 2
ROL	-3.903	-2.173
ROW	5.503	-0.545
HEL	-4.435	6.220
CGW	3.206	-3.682
CLL	1.769	-2.399
ARL	-6.295	3.189
CLW	-3.463	2.348
CPW	3.328	7.696
ARW	.379	-.132
CEW	5.415	3.937
ABL	-8.883	-8.804
TL	24.015	-8.611
W	-15.949	2.980
Eigenvalue	288.460	108.218
% of Variance	72.7	27.3
Cumulative %	72.7	100.0
Canonical Correlation	0.998	0.995

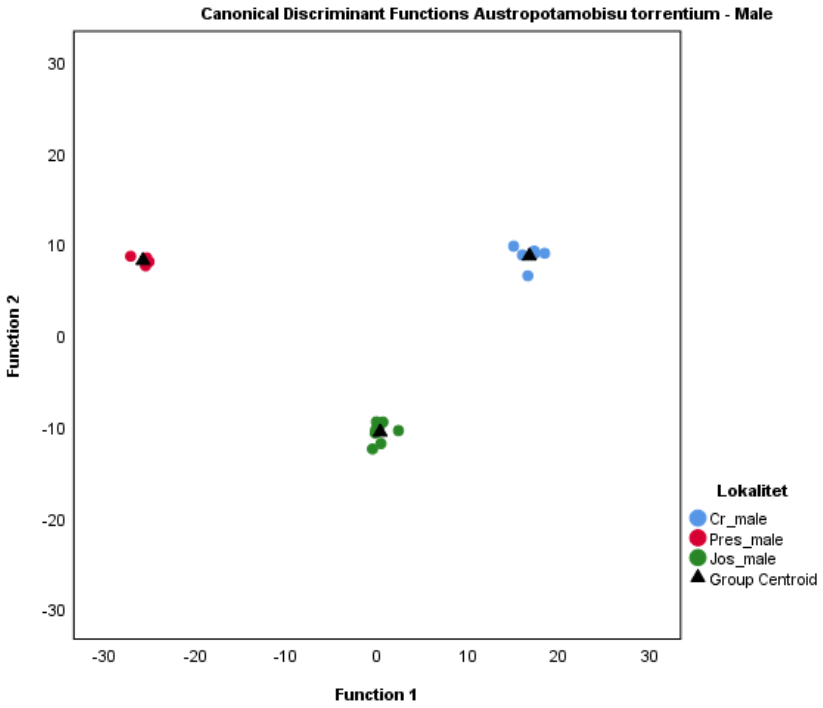

Figure 1: Discrimination of the different populations of males of austropotamobius torrentium by the first two discriminant functions

Anova test in females revealed a significant difference in the characters head lenght HEL (p=0.006), ARL (p=0.001), CEW (p=0.01). The Bonferroni test showed that there is a significant difference between the individuals of Presjenica and Crna Rijeka (p=0.002) and Presjenica and Jošanica 0.006 in the ARL parameter, in CEW Presjenica and Crna Rijeka (p=0.038) and Presjenica and Jošanica (p=0.034), and in HEL Jošanica and Presjenica (p=0.006). Discriminative analysis of females revealed that individuals are most discriminated by the parameters CLW, ARW and width at the hind edges of the carapace (CEW), and (ABL) abdomen lenght (Table 3, Figure 2).

Table 3. Standardized canonical discriminant function coefficients for morphometric characteristics of Austropotamobius torrentium females for each discriminant function; ROL, rostrum length; ROW, rostrum widt; CEW, carapace width at the hind edges; ARW, areolar width; ARL, areolar length; ABL, abdomen length; HEL, head lenght; CGW width at the cervical groove, CLL claw lenght, CPW, carapace width; % expl.var., percentage of explained variance; cum. Prop., cumulative proportions; canonical r, canonical correlations

Characteristic	Function 1	Function 2
ROL	0.173	0.454
ROW	-1.247	-1.354
HEL	-1.698	-1.491
CGW	10.154	11.430
CLL	-1.830	5.021
ARL	2.783	2.529
CLW	5.014	-2.746
CPW	-12.955	-16.889
ARW	-1.332	3.662
CEW	4.212	1.366
ABL	-2.299	-.563
Eigenvalue	11.110	62.2
% of Variance	62.2	37.8
Cumulative %	62.2	100.0
Canonical Correlation	0.958	0.933

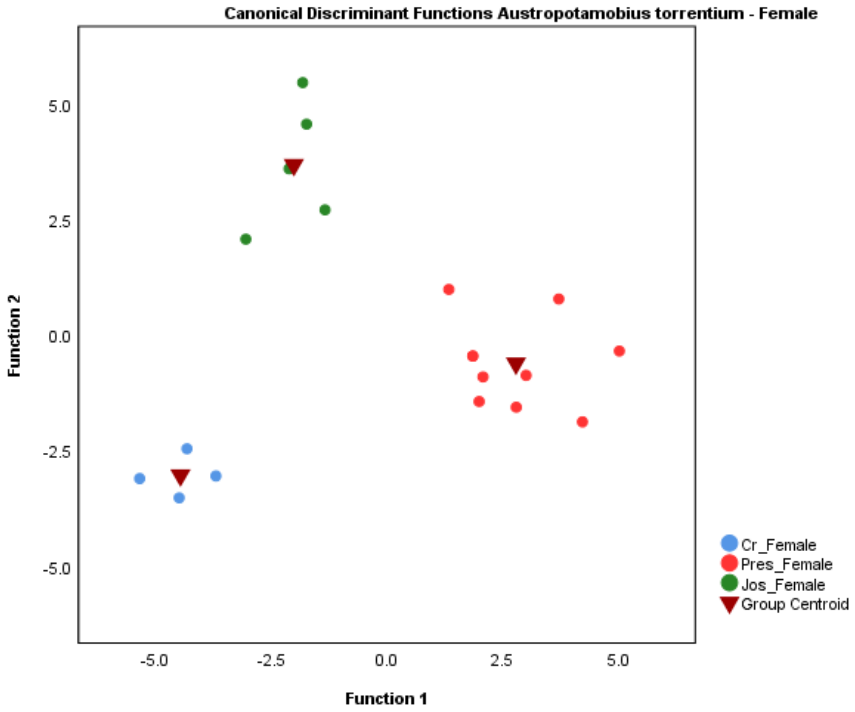

Figure 2: Discrimination of the different populations of females of Austropotamobius torrentium by the first two discriminant functions

4. Discusion

Based on the analysis, relatively stable crayfish populations were determined in Jošanica, Presjenica and Crna Rijeka. Morphometric measurements show statistically significant differences in females in CEW, ARL and HEL. In males, statistically significant differences ($p>0.05$) were observed for head length, TL - total body length and cheliped length (CLL). According to research (Maguire et al. 2017, Trožić-Borovac et al. 2022), male crayfish individuals show a statistically significant difference in body length and pincer length, as an adaptation to habitat conditions. Discriminative analysis confirmed that populations from geographically close areas show differences in morphometric parameters, as a response to ecological conditions and the degree of anthropogenic pressures. Research on males from the populations of Sopotski Slap, Zeleni Vir and Blato (Maguire et al., 2017) found that males differ significantly in rostrum length, CPH and telzone width (TEW). Unnamed stream, Preodac and the Korana river in the locality of Bosansko Grahovo (Lovrenčić et al, 2020b), and research area of this paper, Crna rijeka, Presjenica and Jošanica are inhabited by representatives of the phylogroup central and south-eastern Europe (CSE). Morphometric analysis within the same phylogroup showed diversity, which was stated for the area of Croatia (Maguire et. al. 2017, Lovrenčić et al. 2020a) and Romania (Pârvulescu et al., 2019). In similar studies, males from three different populations were discriminated based on the shape of the chelipeds (CLW, CLL, CFL, CPL, CPH) and rostrum length (ROL), rostrum width (ROW). (Maguire et. al., 2017). Males and females show different adaptations to environmental conditions, and on the other hand, ecological conditions result in different adaptations. Species delimitation requires integrative taxonomy, and an approach that combines molecular, morphological, ecological, and geographic data to build species hypotheses. Pârvulescu et al. (2019), have identified the existence of a new phylogroup

endemic to the Romanian Apuseni Mountain Region (APU). The molecular studies of this species included morphological data that resulted in the description of a new species of the genus *Austropotamobius*. It is necessary to implement the provisions for the protection of habitats of this species, which are also under significant anthropogenic pressure in the analyzed watercourses. The intensity of these impacts is particularly pronounced on the Jošanica watercourse.

5. Conclusion

Morphometric measurements of *Austropotamobius torrentium* populations, in two geographically close watercourses and one distant one, which include a large number of parameters, proved to be a powerful tool in the analysis of the similarity/difference of the conditions in which they exist. A lower degree of diversity, especially in female individuals, confirms their belonging to the same phylogroup but also to similar ecological conditions in which they exist. For more complete data, it is necessary to include new meristic parameters and intensive molecular phylogenetic research.

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