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First Insight into Distribution and Ecology of *Austropotamobius torrentium* (Schrank, 1803) (Decapoda: Astacidae) in the North-Western Bosnia and Herzegovina

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Abstract: In Bosnia and Herzegovina, four species of indigenous European freshwater crayfish have been identified: noble crayfish (*Astacus astacus*), narrow-clawed crayfish (*Pontastacus leptodactylus*), stone crayfish (*Austropotamobius torrentium*) and white-clawed crayfish (*Austropotamobius pallipes*). The aim of this study was to determine distribution and habitat of the stone crayfish in the north-western Bosnia and Herzegovina (Republika Srpska). Its presence was recorded in the waterbodies of both Black Sea and Adriatic basins at 16 sites. New data on distribution indicate its distribution in the tributaries of the Vrbas and Una rivers. Totally, 438 specimens were analysed (246 males and 192 females). The treatment included the measurement of 12 morphometric characteristics: total body length (TBL), weight (W), rostrum length (ROL), rostrum width (ROW), claw length (CLL), claw width (CLW), carapace length (CPL), carapace width (CPW), abdominal length and width (ABL, ABW) and telson length and width (TEL, TEW). T-test results indicate significant difference in measured morphometric features between sexes due to sexual dimorphism. Significant differences were also recorded between different populations. Linear regression analysis showed correlation between TBL and W as well as between W/CLL and CPW/TBL. Fulton`s Conditions Factor and Crayfish Constant indicate that males are in better condition. New data obtained during this study will be used for legislation corrections for crayfish protection in the Rebublic of Srpska.

Key words: stone crayfish, morphometrics, condition indices

Introduction

The family Astacidae includes four genera; *Pacifastacus* Bott, 1950 is native to North America while *Astacus* J. C. Fabricius, 1775, *Pontastacus* Bott, 1950 and *Austropotamobius* Skorikov, 1907 are native to the European continent (Crandall & De Grave 2017). This family is widely distributed in Europe. The distribution and zoogeography of the European crayfish species are well known compared to other aquatic invertebrates; however, this is not the case for all parts of the Balkan Peninsula.

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The diversity and distribution of crayfish in Slovenia, Croatia, parts of Bosnia and Herzegovina, Serbia, Romania, Bulgaria, Montenegro, Kosovo and Greece are relatively well studied (Simić et al. 2008, Trožić-Borovac 2011, Rajković 2012, Trichkova et al. 2013, Živić et al. 2014, Perdikaris et al. 2017, Maguire et al. 2018, Pârvulescu 2019, Lovrenčić et al. 2020b). However, almost no published scientific data are available from Republika Srpska (Bosnia and Herzegovina).

Four native species of the family Astacidae are known in Europe: *Astacus astacus* (Linnaeus, 1758), *Pontastacus leptodactylus* (Eschscholtz, 1823), *Austropotamobius torrentium* (Schrank, 1803) and *Austropotamobius pallipes* (Lereboullet, 1858) are distributed in the Balkan Peninsula (Karaman 1976, Obradović 1984, Maguire & Gottstein-Matočec 2004, Bedjanič 2004, Rajković 2007, Simić et al. 2008, Trožić-Borovac 2011, Živić et al. 2014). At the European level, populations of *A. astacus*, *A. pallipes* and *A. torrentium* are declining and endangered that they are included in the international list of threatened species (IUCN Red List) and protected by Annex III of the Bern Convention, Annex II and V of the Habitats Directive (92/43/EEC).

The genus *Austropotamobius* contains three species: *A. pallipes*, *A. torrentium* and *A. bihariensis* Pârvulescu, 2019, all of them threatened throughout their ranges. They have recently been classified as vulnerable and rare by the International Union for the Conservation of Nature and Natural Resources (IUCN). In the IUCN list, *A. astacus* is classified as a vulnerable species (VU), *A. pallipes* as an endangered species (EN), while *A. torrentium* is classified as a data-deficient species (DD) with a decreasing population trend (Edsman et al. 2010). The conservation status of the recently-described *A.bihariensis* has not been assessed (Pârvulescu 2019).

Populations of *A. astacus*, *A. pallipes* and *A. torrentium* are in such decline that they are now considered rare and vulnerable species and are listed as protected in the Appendix III of the Bern Convention (Taylor 2002). Red books and red lists are an internationally recognised method for determining the risk of species extinction and contribute greatly to the biodiversity conservation. All indigenous crayfish are listed in the Rulebook on the Proclamation and Protection of Strictly Protected and Protected Wild Species in the Republic of Srpska (Annonymous 2020).

The aim of this study is to record the presence of *A. torrentium* in the north-western part of the Republika Srpska (Bosnia and Herzegovina) and to contribute to the knowledge of its distribution and the state of its populations.

Materials and Methods

The study was carried out in between September 2018 and June 2021 in the north-western part of the Republika Srpska (Bosnia and Herzegovina). At 16 localities, 438 specimens of *A. torrentium* of different sizes were captured. All crayfish were sampled overnight, from 08:00 PM to 08:00 AM. Crayfish samples were collected manually and using nets (Machino & Füreder 2005). All collected individuals were identified at the species level using the illustrated key for identification of freshwater crayfishes of the family Astacidae (Maguire 2010).

According to Sint et al. (2005), the following morphometric characteristics were measured: total body length (TBL), weight (W), rostrum length (ROL), rostrum width (ROW), length of the claw palm (CPL), carapace width (CPW), claw length (CLL), claw width (CLW), abdomen length (ABL), width of the first abdominal pleura (ABW), telson length (TEL) and telson width (TEW). All the characteristics were measured with a digital calliper with a 0.01 mm precision. After recording all of the measurements, two crayfish condition indices were calculated: Fulton's Condition Factor (Ricker 1975) according to the formula $FCF = W / TBL^3$ and Crayfish Constant (Adegboye 1981) according to the formula $CC = W / (TBL \times CPL \times CPW)$.

After the examination, the crayfishes were released at the place where they were sampled. No specimens were injured or displaced from the habitat they were found during the fieldwork. On each site, we recorded GPS coordinates, elevation, water temperature, pH, turbidity and electrical conductivity using WTW 340i field water analyser. Macrozoobenthos samples were collected with Surber net, with a catchment area of 0.1 m^2 and mesh size of 250 μm. Individuals were stored in tubes containing 70% ethanol for later taxa identification. In the laboratory, samples were analysed using a stereomicroscope (Leica EZ4D at 45× magnification). Macroinvertebrates were identified, whenever possible, to the family or generic level, using the identification key by Kriska (2013). The saprobic index was calculated according to Pantle and Buck (Pantle & Buck 1955). The saprobity values were taken from the lists of indicator taxa (Wegl 1983).

Results

The results of this study indicated that the stone crayfish is widely distributed in the north-west area of Bosnia and Herzegovina, especially in the basins of the Vrbas and Una Rivers (Fig. 1). The habitats

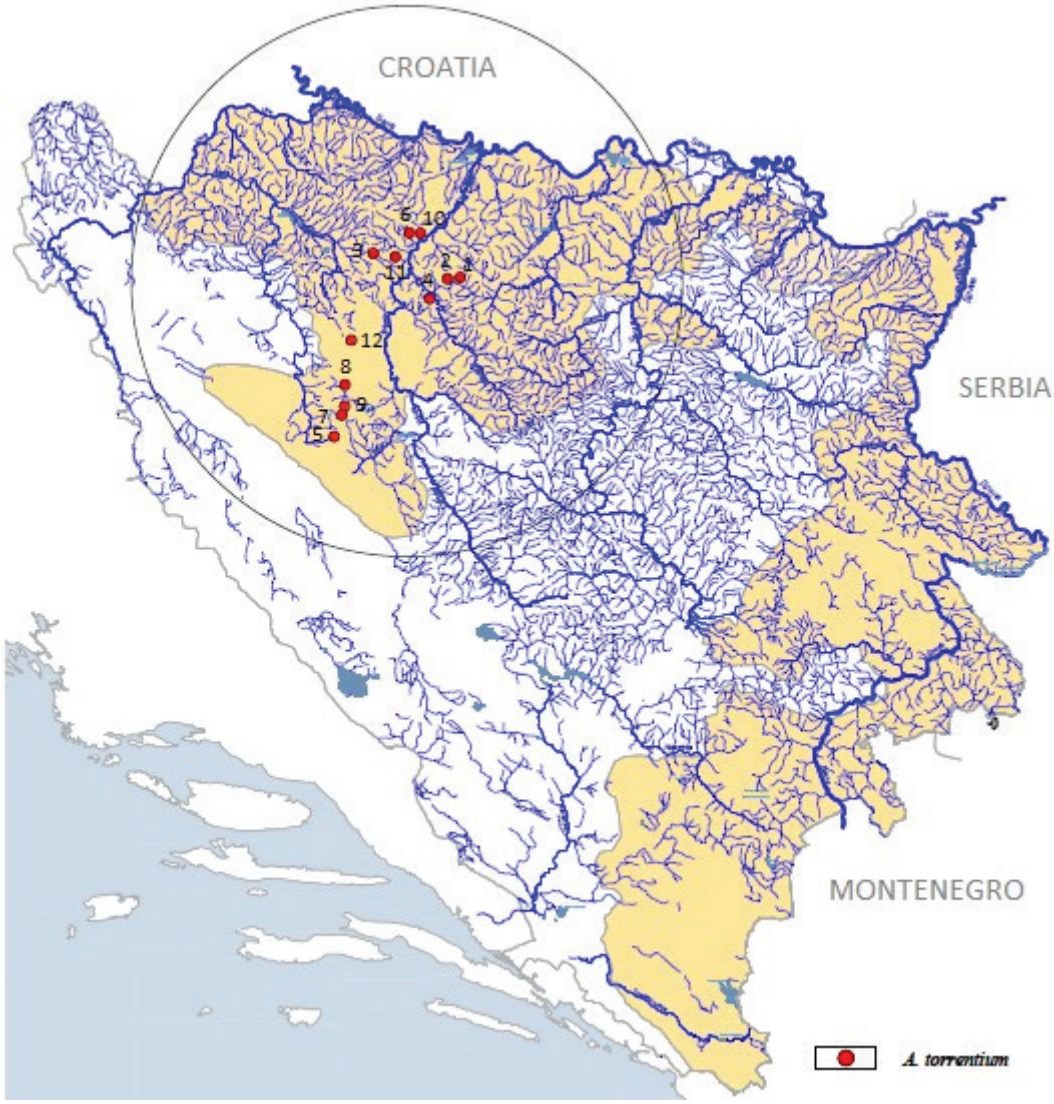

Fig. 1. Distribution of *Austropotamobius torrentium* in the north-western part of Bosnia and Herzegovina**.**

of the stone crayfish in the studied sites occur at the altitudes from 180 m to 846 m, with the majority (56.25%) being situated between 200 and 400 m a. s. l. Water temperatures ranged from 12 to 15.5°C. The dissolved oxygen concentrations were minimum 8 mg/l. BPK_5 values were similar on all sample sites (approximately 0.5 mg O_2/l). pH values ranged from 7.15 to 8.28. Water saprobity, according to macroinvertebrate indices, was oligosaprobic to beta-mesosaprobic (Table 1).

The average body length of males crayfish from the Vrbas River was 106.60 mm (±SD 14.44; min. 60.40 mm; max. 132.05 mm) and of females 96.31 mm (±SD 12.68; min. 57.87 mm; max. 128.09 mm). In the Una River Basin, the average body length of males was 74.03 mm (±SD 11.31; min. 51 mm; max. 94.64 mm) and of females 65.52 mm (±SD 8.28; min. 50.91 mm; max. 92.01 mm) (Table 2). There were significant difference between sexes in all measured morphometric characteristics ($p<0.05$), except for width of the first abdominal pleura ($p>0.05$), whereby higher mean values were observed for females (Table 3). Stone crayfish from the Vrbas River Basin have higher average and maximum values in all analyzed morphometric characters compared to crayfish from Una River Basin. T-test showed significant differences ($p<0.05$) between all analyzed characters except telson width (TEW) in males (Tab. 4). Strong positive correlation was recorded between TBL and W in the crayfish from the Vrbas River Basin (♂ R^2= 0.8776; ♀ R^2=0.9257) as well as in the Una River Basin (♂ R^2=0.9439; ♀ R^2=0.8019) (Fig. 2).

Two body condition indices were calculated. Male`s mean values of condition indices were higher compared to females in both waterbodies belonging to the Vrbas River basin and the Una River basin. This difference is statistically significant (Table 5). Namely, the analysis showed that specimens from

Table 1. Localities where the stone crayfish *Austropotamobius torrentium* was found in 2018–2021, with rates of males and females, altitude data, water temperature, saprobic index and GPS coordinates.

Drainage area	Habitat, locality	GPS coordinates	Elevation (m)	Temperature °C	Saprobity	♂/♀ rates
Vrbas River Basin	Stanikova River	44.73713°N 17.46004°E	328	12.2	1.53	1/1
	Mlinska Stream	44.75088°N 17.46322°E	267	15.5	1.63	1.72/1
	Rudnička River	44.82929°N 17.09777°E	262	12.8	1.57	1.71/1
	Marjanovića Stream	44.71530°N 17.29464°E	310	13.1	1.78	1.76/1
	Matića Stream	44.88761°N 17.22211°E	233	12.6	1.55	1.38/1
	Zelenikovac	44.394331°N 16.97244°E	846	12.2	1.73	1.41/1
	Ponor	44.435869°N 17.025834°E	757	12	1.56	1.5/1
	Dževerov Stream	44.41230°N 16.98980°E	767	12.4	1.62	1/1
	Dobraš	44.87569°N 17.24475°E	253	12.9	1.53	1.75/1
	Šargovački Stream	44.82323°N 17.16140°E	201	12.3	1.61	1/1
	Ledenac	44.61569°N 16.99916°E	841	12.1	1.57	1/1
	Ponir	44.4972°N 17.32304°E	785	12	1.71	1/1
	Rajnerov Stream	44.737692°N 17.175987°E	384	12.1	1.55	1.67/1
Una River Basin	Korana	44.316525°N 16.93559°E	637	13.6	1.65	1.34/1
	Vojskova	44.89264°N 16.29903°E	226	12	1.56	1/1
	Japrica	44.91906°N 16.37129°E	180	12	1.63	1/1

Table 2. Descriptive statistics for morphometric measures and weight of adult crayfish (males and females) in populations belonging to the Vrbas and Una River Basins.

	Tributary of the Vrbas River							
	Males				Females			
	Mean	Min	Max	SD	Mean	Min	Max	SD
TBL	106.60	60.4	132.05	14.44	96.31	57.87	128.09	12.68
W	30.87	10.9	57.01	8.33	26.98	9.5	42.6	7.46
ROL	11.04	6.05	15.12	1.76	10.56	5.2	14.49	1.71
ROW	8.49	3.74	11.60	1.42	7.82	4.1	10.64	1.41
CPL	59.06	26.13	93.92	11.55	52.55	27.47	67.59	8.86
CPW	31.83	13.65	41.55	5.82	27.64	15.25	34.14	4.29
ChL	40.01	18.83	58.8	7.61	28.95	19.58	41.71	4.45
ChW	16.89	9.09	26.71	3.56	12.44	8.55	17.55	1.99
ABL	38.17	25.27	49.24	5.49	35.55	19.55	51.52	6.42
ABW	25	12.51	31.77	3.75	27	12.75	35.43	4.44
TEL	13.28	7.05	17	1.92	11.45	7	15.77	1.96
TEW	9.54	5.3	16	2.41	8.96	5	15.43	2.31
	Tributary of the Una River							
	Males				Females			
	Mean	Min	Max	SD	Mean	Min	Max	SD
TBL	74.03	51	94.64	11.31	65.52	50.91	92.01	8.28
W	18.25	7.6	29	5.78	15.09	6.1	26	3.85
ROL	8.52	5	12.94	1.61	7.41	5	10.76	1.32
ROW	6.43	3.68	11.31	1.47	5.31	3.74	8.23	1.03
CPL	35.87	20.78	60.35	7.36	31.29	21	54.36	5.66
CPW	19.3	10.11	28.8	4.03	16.32	10	27.89	2.92
ChL	26.14	12.4	38	6.45	21.58	10.1	38.34	6.19
ChW	11.25	5	18.22	3.11	9.97	6	17.7	2.67
ABL	31.66	19.7	44.8	6.26	28.89	20.92	45.91	6.01
ABW	17.14	11	26.55	3.53	15.64	12.1	25.46	2.93
TEL	10.95	6.3	14.73	1.57	10.58	7.05	14.56	1.49
TEW	9.23	5	13.2	1.51	8.26	5.97	12.12	1.4

the Una River Basin have higher fitness indices compared to crayfish from the Vrbas River Basin.

The results obtained for the weight-length (W/TBL, W/CLL) and width-length (CPW/TBL) relationships, along with some descriptive statistics, are given in Table 6. Correlation tests showed that there are significant positive correlations between the measured parameters for both males and females in both basins.

Table. 3. The t-test values between sexes (statistically significant results are bolded)

Variable	t-value	p
TBL	3.85	**p < 0.05**
W	3.17	**p < 0.05**
ROL	3.41	**p < 0.05**
ROW	4.79	**p < 0.05**
CPL	2.95	**p < 0.05**
CPW	3.85	**p < 0.05**
ChL	7.82	p < 0.05
ChW	6.30	**p < 0.05**
ABL	3.48	**p < 0.05**
ABW	-0.77	p > 0.05
TEL	4.40	**p < 0.05**
TEW	4.42	**p < 0.05**

Table 4. The t-test between populations belonging to the Vrbas and Una River Basins for males and females

Variable	Males		Females	
	t-value	p	t-value	p
TBL	19.44	p < 0.05	20.35	p < 0.05
W	13.89	p < 0.05	14.44	p < 0.05
ROL	11.30	p < 0.05	14.34	p < 0.05
ROW	10.58	p < 0.05	14.23	p < 0.05
CPL	19.15	p < 0.05	20.28	p < 0.05
CPW	19.75	p < 0.05	21.76	p < 0.05
ChL	15.02	p < 0.05	9.04	p < 0.05
ChW	12.84	p < 0.05	6.97	p < 0.05
ABL	8.06	p < 0.05	7.35	p < 0.05
ABW	16.20	p < 0.05	21.36	p < 0.05
TEL	10.24	p < 0.05	3.50	p < 0.05
TEW	1.21	p > 0.05	2.63	p < 0.05

Discussion

The aims of this study were to obtain information about distribution, ecology and morphological differentiation of stone crayfish in the study area. Generally, *A. torrentium* is distributed in the waterbodies belonging to the Black Sea Basin but occurs also in some streams in Aegean and Adriatic drainage (Karaman 1929, Albrecht 1982, Maguire et al. 2011, Trichkova et al. 2013).

In this study, *A. torrentium* was recorded at altitudes ranging from 180 to 846 m a.s.l. In other studies, more frequently, it has been found at altitude range from 300 to 900 m and, less often, below 200 m and above 1200 m (Trichkova et al. 2013). Therefore, our findings are in accordance with known altitude records. The results of the ecological condition`s analysis, in which the this crayfish species lives in Europe, mostly coincide with the conditions in northwestern Bosnia and Herzegovina. This species is related to the hilly and mountain altitudinal belts of beech forests. It exists in waters of high quality (I / II), with a lower temperature (10 ºC) and a high oxygen contents. According to the values of the saprobity index, rivers and streams were oligosaprobic to β-mesosaprobic (unpolluted to low-polluted).

Indisputably, the life of stone crayfish is directly dependent on the specific characteristics of the aquatic ecosystem, especially on stony substrate (Lodge & Hill 1994). Heterogeneity of habitat conditions, quality of the waterbody substrate as well as the absence of contaminants are prerequisites for the existence and survival of the species (Bohl 1999, Streissl & Höedl 2002). It is clear that the stream morphology is vital for the occurrence of this species (Troschel 1997).

Table 5. Fulton's condition factor (FCF) and Crayfish Constant (CC) of *Austropotamobius torrentium* specimens from the Vrbas and Una River Basins with values of t-test for differences between males and females per river basin.

	The tributaries of the Vrbas River									
	Males				**Females**					
	Mean	**Min**	**Max**	**SD**	**Mean**	**Min**	**Max**	**SD**	**t-value**	**p**
FCF	0.03	0.02	0.05	0.01	0.03	0.02	0.05	0.01	-5.68	p < 0.05
CC	0.20	0.14	0.39	0.01	0.17	0.11	0.51	0.07	-3.32	p < 0.05
	The tributaries of the Una River									
	Males				**Females**					
	Mean	Min	Max	SD	Mean	Min	Max	SD	t-value	p
FCF	0.05	0.03	0.07	0.01	0.05	0.03	0.06	0.01	-11.22	p < 0.05
CC	0.46	0.19	0.71	0.10	0.37	0.18	0.74	0.09	-8.85	p < 0.05

Table 6. Descriptive statistics and estimated parameters of weight-length and biometric relationships for both sexes of *Austropotamobius torrentium* in north-western Bosnia and Herzegovina. (TBL, total length (mm); W, weight (g); CLL, chela length (mm); CPW, carapace width (mm).

Drainage area	Relation		Sex	
			M	F
Vrbas River Basin	TBL/W	*y*	$0.5268x^{-25.172}$	$0.5556x^{-26.881}$
		R^2	0.8776	0.9257
		Correl.	87.76	92.57
	W/CLL	*y*	$0.8436x^{+10.535}$	$0.5901x^{+13.244}$
		R^2	0.6896	0.9445
		Correl.	68.96	94.45
	CPW/TBL	*y*	$2.5312x^{+26.564}$	$2.809x^{+19.658}$
		R^2	0.9340	0.8990
		Correl.	93.40	89.90
Una River Basin	TBL/W	*y*	$0.4603x^{-16.71}$	$0.4666x^{-17.244}$
		R^2	0.9439	0.8019
		Correl.	94.39	80.19
	W/CLL	*y*	$1.1849x^{+5.4982}$	$1.2601x^{+4.7912}$
		R^2	0.9379	0.7745
		Correl.	93.79	77.45
	CPW/TBL	*y*	$2.6651x^{+23.978}$	$2.6087x^{+23.087}$
		R^2	0.8955	0.8846
		Correl.	89.55	88.46

In this study, a large number of morphometric characters were analysed. Totally, 12 characters were measured per crayfish on populations from two water basins (Vrbas River and Una River) in north-western part of Bosnia and Herzegovina. We have observed significant differences between the two sexes. The observed differences are consequence of sexual dimorphism that was previously reported for different crayfish species (Jelić 2014, Roljić et al. 2020, Lovrenčić et al. 2020a) and considered as reproductive advantage (Vlach & Valdmanova 2015). As already reported, males have longer chelae and body length while females have a larger abdomen, which is associated with the reproductive success (Lindqvist & Lathi 1983, Skurdal & Taugbøl 2002, Maguire et al. 2005).

Our results show that there is a significant difference between populations from the Vrbas River Basin and Una River Basin. The mean values of majority of measured characteristics (ROL, ROW, CPL, CLL, CLW) are significantly higher in the Vrbas River Basin populations. The variation in the morphometric characteristics could be a consequence of either environmental or genetic factors or their combination (Dakić & Maguire 2016, Rudolf et al. 2016).

In order to protect this endangered species, it is necessary to continue the monitoring of the populations. The monitoring of the natural populations of *A. torrentium* and its habitat is a necessary step to establish the conservation status of species and to help improving management decisions. In addition, the study needs to be extended to other stone crayfish habitats in Bosnia and Herzegovina and to other astacid species in fragile habitats.

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