Morphometric characteristics of spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) from the Danube River on the territory of Serbia

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Abstract: Twenty-three morphometric characteristics of spiny-cheek crayfish *Faxonus limosus* from Danube, Serbia, were analyzed to describe the general body shape. Forty-eight specimens were caught in May 2022 and January 2023 and measured for morphometric variation. Also, the values of two crayfish condition indices, Fulton's condition factor (FCF) and crayfish constant (CC), were determined. Most of the analyzed characteristics were moderately variable (CV 10-30%). Principal component analysis (PCA) was applied to the morphological measurements. All the variables make similar contributions to the variance of the first principal component. The highest contributions are from variables CEF and ARL. ARW, CPH, ABH, CPW, and ROW significantly contributed to the second principal component variance. The population of spiny-cheek crayfish at the investigated site has a relatively stable age structure.

Keywords: non-native crayfish; morphometric variation; body condition; Danube

INTRODUCTION

The following species of autochthonous crayfish from the Astacidae family are widespread in Serbia's freshwater ecosystems: the noble crayfish *Astacus astacus* (Linnaeus, 1758), narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823), stone crayfish *Austropotamobius torrentium* (Schrank, 1803) and three allochthonous, introduced species: the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817), Chinese mitten crab *Eriocheir sinensis (*H. Milne-Edwards, 1854) and signal crayfish *Pacifastacus leniusculus* (Dana, 1852).

F. limosus originated from North America and was introduced to Europe for commercial reasons in the late 19[th]century [1]: to Poland, back in 1890 [2], the river system of Oder River in Germany in 1894 [3], and then the Loar River in France in 1912 [4]. The Spiny-cheek crayfish has recently been reported from 22 European countries [5]. It is assumed to have been deliberately introduced for use in aquaculture [6]. Namely, a few years earlier, it was noticed that the number of crayfish had dropped, so they were imported from North America. It quickly turned out that their spread could not be controlled and that the impact on indigenous species was devastating [7].

F. limosus has been identified as an invasive species in Bulgaria and Serbia, with potential for further spread [89]. The first record of *F. limosus* in Serbia was reported in 2002 in the Danube near Apatin [10]. Since then, the species has spread along the entire section of the Danube River in Serbia, the Sava, Tisa, Velika Morava, and Tamiš, due to the absence of natural enemies, and it negatively affects indigenous species of crayfish and the non-biotic components of the ecosystem [11-13].

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Also, spiny-cheek crayfish is a vector of the pathogen *Aphanomyces astaci* that causes crayfish plague, a highly infectious disease of all crayfish of non-North American origin [14]. The invasiveness of crayfish indigenous to North America is significantly greater than that of crayfish from the rest of the world [15], and it has been estimated that allochthonous crayfish species will completely dominate indigenous populations in the next few decades [16].

Morphometric research based on data obtained by measuring morphological characters has proven to be very useful for analyzing intra-and interspecies variability in freshwater crayfish [17]. Most studies so far were based on traditional morphometric methods [18-20]. The results presented in this paper are the first attempt to obtain morphometric characteristics of spiny-cheek crayfish caught in Serbia.

MATERIALS AND METHODS

Ethics statement

This study was carried out on crayfish, which are considered invertebrates; therefore, ethics committee approval was not required.

Study area

Specimens of spiny-cheek crayfish were collected in May 2022 and January 2023, and a total of 48 crayfish were caught. The sampling from the Danube River was carried out at the Slankamen site (GPS 45°08'23.64" N 20°15'35.64" E) (Supplementary Fig. S1).

Sampling and identification

Most of the crayfish were trapped using two baited LiNi traps left in the water overnight [21], and some were collected manually. After sampling, individuals were determined to the species level according to Maguire's Manual for freshwater crustacean inventory and condition monitoring [22].

Measurements

Individuals with no signs of regeneration were measured for linear morphometry. Twenty-two morphometric characteristics of the collected crayfish were measured to describe the general body shape in more detail using the "point-to-point" method. The "point-to-point" method is common in morphometric studies where specific landmarks are measured to describe their shape. This method provides quantitative data and can help detect subtle differences in body shape among individuals or populations. Measurements taken from spiny-cheek crayfish were ABH – abdomen height, ABL – abdomen length, ABW – abdomen width, ARL – areolar length, ARW – areolar width, CEW – width at the hind edges of the carapace, CFL – claw finger length, CGW – width at the cervical groove, CLH – claw height, CLL – claw length, CLW – claw width, CPH – carapace height, CPL – claw palm length, CPW – carapace width, HEL – head length, HEW – head width, CEF – cephalothorax length, ROL – rostrum length, ROW – rostrum width, TEL – telson length, TEW – telson width, TL – total length, W – total weight according to Sint et al. [14]. For the determination of morphometric characteristics, a caliper was used (marked in mm, with an accuracy of ±0.02 mm). A Kern PFB Version 2.2 technical scale (maximum weight 1200 g with an accuracy of ±0.01 g) was used to measure body weight.

Condition indices

Two condition indices were used, Fulton's condition factor [23] and crayfish constant [24]. Fulton's index (FCF) was calculated using the formula:

$FCF=W/TL^3$ (W – crayfish weight, TL – crayfish total length).

The crayfish constant (CC) was obtained using the formula:

$CC = W/(TL+CPH+CPW)$ (CPH–carapace length, CPW–carapace width).

Statistical analysis

As the first step in analyzing the crayfish dataset, a graphical display in the form of a scatterplot is used (Supplementary Fig. S2). It gives an overall picture of the distribution of all variables and the relationship between each pair of 23 variables. In the next step, the Mahalanobis distance with the first two principal components was used to detect possible outliers in the dataset. Finally, the contribution of each crayfish to the variability of the principal components was calculated using the statistical program R4.3.2 with various packages for principal component analysis. Processing of the obtained data included descriptive statistics performed in STATISTICA 6.0 software.

Geometric morphometric (GM)method

Each specimen was photographed with a Nikon D3200 digital camera, with the cephalothorax positioned parallel to the photographic plane in the center of the optical field. Camera settings, position, and distance between the lens and the object were kept constant to minimize imaging errors related to distortion and parallax [25]. Fourteen two-dimensional landmarks were digitized on each specimen by the same person (AU) in TpsDig 2.17 software [26]. Eight landmarks (LM) on the rostrum tip, rostrum lateral spines, eye hollows, post-orbital spine tip, and cardiac socket were chosen because they were present on all specimens, represented the morphology of the studied structure, and agreed with the linear measures used in this study.

Generalized Procrustes analysis (GPA) was conducted to obtain a matrix of shape coordinates with the effects of position, size, and orientation removed [27]. The symmetric component of the shape variation, calculated from the averages of the original and mirrored landmark configurations for each specimen [28], was used as data for subsequent analyses. Centroid Size (CS), which is calculated as a square root of the summed squared distances of each landmark from the geometric center of the landmark constellation, was used as a measure of size [29]. We used principal component analysis (PCA) on a covariance matrix of the shape variables to explore the shape variation. To account for allometry, i.e., size-dependent shape differences in our sample, we conducted a multivariate regression of the symmetric shape scores onto the log-transformed CS. All geometric morphometric analyses were done in MorphoJ software [30].

RESULTS

A total of 48 individuals of the crayfish *F. limosus* were processed: 45 males (93.75%) and three females (6.25%, Fig. 1). Descriptive statistics were made separately for males (Table 1) and females (Table 2) for body weight and 22 morphometric characteristics. Most analyzed characteristics were moderately variable (CV 10-30%).

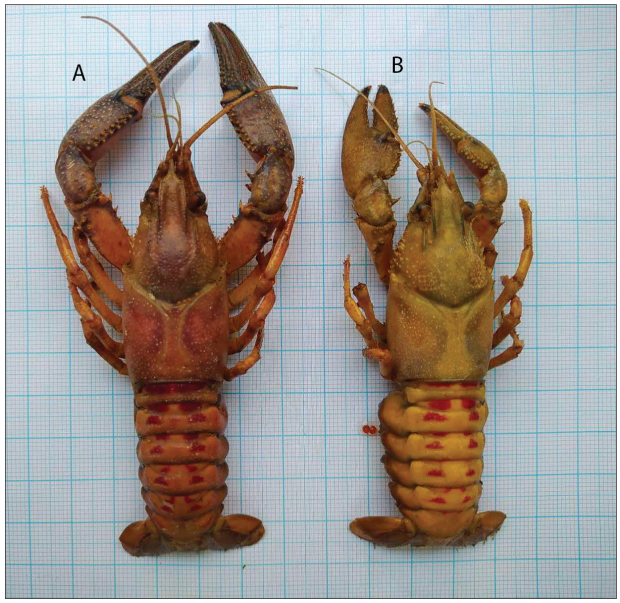

Fig. 1. Dorsal view of the whole male and female bodies of *Faxonius limosus*. **A** – male; **B** – female.

In males, the total length (TL) ranged from 55.95 to 108.90 mm (84.19±11.86). The range of measured weight was from 6.37 to 36.30 g, with a mean value of 21.83 g (SD=7.94). Most of the analyzed characteristics showed low variability (CV 10-20%); only weight (W) showed high variability (CV>30%). Two morphometric characteristics (CFL and ARW) were moderately variable (CV 20-30%).

In females, the total length (TL) ranged from 63.89 to 87.60 mm (76.29±11.89). The range of measured weight was from 7.7 to 17.5 g, with a mean of 12.57 g (SD=4.9). Stable character (CV coefficient of variation 5-10%) showed two morphometric characteristics, CFL and ARW. Low variable characteristics (CV 10-20%) included TBL, CLL, CPL, CLW, CLH, CEF, ROW, CEW, ABW, ABL, TEL, and TEW. Eight morphometric characteristics were moderately variable (CV 20-30%): ROL, HEL, HEW, CGW, CPW, CPH, ARL, and ABH. Only weight (W) showed high variability (CV >30%, Table 2).

Fig. 2A illustrates that the variables made similar contributions to the variance of the first principal

Table 1. Descriptive statistics of basic morphometric parameters in male spiny-cheek crayfish from the Danube River, Serbia

Variable	Mean	Min	Max	Std. dev.	CV
TL	84.19	55.95	108.90	11.86	14.09
W	21.83	6.37	36.30	7.94	36.37
CLL	36.15	22.04	48.00	6.77	18.73
CPL	12.31	8.07	18.90	2.40	19.50
CFL	19.82	10.17	27.30	4.23	21.34
CLW	13.37	8.24	19.40	2.33	17.43
CLH	8.14	5.40	13.90	1.55	19.04
CEF	41.63	27.36	51.30	6.05	14.53
ROL	12.15	8.56	15.51	1.86	15.31
ROW	5.69	3.54	7.00	0.82	14.41
HEL	14.98	9.43	19.30	2.22	14.82
HEW	12.99	8.73	16.20	1.76	13.55
CGW	17.05	11.50	23.00	2.42	14.19
CPW	20.89	11.76	27.00	3.49	16.71
CPH	16.14	9.13	21.24	2.69	16.67
ARL	14.18	8.00	17.67	2.30	16.22
ARW	2.10	1.12	3.17	0.49	23.33
CEW	16.63	11.22	22.90	2.32	13.95
ABW	18.74	12.88	23.40	2.43	12.97
ABH	13.22	9.34	17.00	2.23	16.87
ABL	32.69	20.00	41.00	4.86	14.87
TEL	12.30	9.11	15.00	1.76	14.31
TEW	9.88	7.12	12.40	1.34	13.56

Table 2. Descriptive statistics of basic morphometric parameters in female spiny-cheek crayfish from the Danube River, Serbia

Variable	Mean	Min	Max	Std. dev.	CV
TL	76.29	63.89	87.60	11.89	15.59
W	12.57	7.70	17.50	4.90	38.98
CLL	22.28	19.40	24.53	2.62	11.76
CPL	7.28	6.70	8.14	0.76	10.44
CFL	11.19	10.56	12.20	0.89	7.95
CLW	9.40	8.16	10.80	1.33	14.15
CLH	5.52	4.69	6.46	0.89	16.12
CEF	34.77	30.50	38.80	4.15	11.94
ROL	11.17	8.90	13.50	2.30	20.59
ROW	4.89	4.14	5.33	0.65	13.29
HEL	13.74	9.93	17.50	3.79	27.58
HEW	12.87	9.10	16.20	3.57	27.74
CGW	16.28	12.60	20.00	3.70	22.73
CPW	19.61	15.13	23.00	4.05	20.65
CPH	14.65	12.45	18.50	3.35	22.87
ARL	13.59	10.66	17.00	3.20	23.55
ARW	1.88	1.70	2.03	0.17	9.04
CEW	15.46	12.68	17.70	2.55	16.49
ABW	19.84	17.41	22.10	2.35	11.84
ABH	12.57	9.50	16.40	3.51	27.92
ABL	28.97	25.00	31.91	3.57	12.32
TEL	10.60	9.31	11.70	1.21	11.42
TEW	9.14	8.14	10.14	1.00	10.94

Table 3. The contributions of variables to the variability in a given principal component expressed in percentages

Variable	PC1	PC2	PC3	PC4
TL	4.87	0.06	4.17	0.61
W	4.85	0.04	0.02	0.99
CLL	4.61	0.01	1.67	4.59
CPL	4.21	0.68	1.01	10.60
CFL	4.55	0.54	0.72	2.91
CLW	4.65	0.02	1.48	0.65
CLH	4.48	0.02	1.67	0.11
CEF	5.06	0.11	0.30	0.26
ROL	4.23	0.47	1.16	1.61
ROW	4.02	1.65	4.91	14.35
HEL	4.69	0.67	0.02	0.00
HEW	4.54	0.40	5.29	0.15
CGW	4.36	0.77	0.10	5.01
CPW	4.57	2.72	2.21	5.47
CPH	3.58	3.21	27.59	11.85
ARL	5.04	0.00	0.38	0.26
ARW	1.85	85.61	0.23	1.96
CEW	4.31	0.01	2.46	7.61
ABW	4.86	0.03	1.20	3.12
ABH	3.24	2.90	33.23	25.90
ABL	3.65	0.02	9.47	0.76
TEL	4.88	0.08	0.68	0.23
TEW	4.88	0.00	0.03	1.00

Table 4. Descriptive statistics and estimated parameter of length-weight ratio and biometric parameters in males of spiny-cheek crayfish from Danube River, Serbia

Relation			
		M	
TL/W	*y* R^2 Correl.	y=0.613x-29.781 R^2=0.8389 83.89%	
W/CLL	*y* R^2 Correl.	y=0.7716x+9.311 R^2=0.8179 81.79%	
CPW/TBL	*y* R^2 Correl.	y=0.2632x-1.2601 R^2=0.8007 80.07%	

TL – total length (mm); W – weight (g); CLL – claw length (mm); CPW –carapace width (mm).

component. The highest contributions are those of CEF and ARL. The highest contributions to the second principal component variance were made by those variables far away from the horizontal line, i.e., the first principal component. These variables were ARW, CPH, ABH, CPW, and ROW. Fig. 2B shows how individuals are distributed in the space described with the first two principal components.

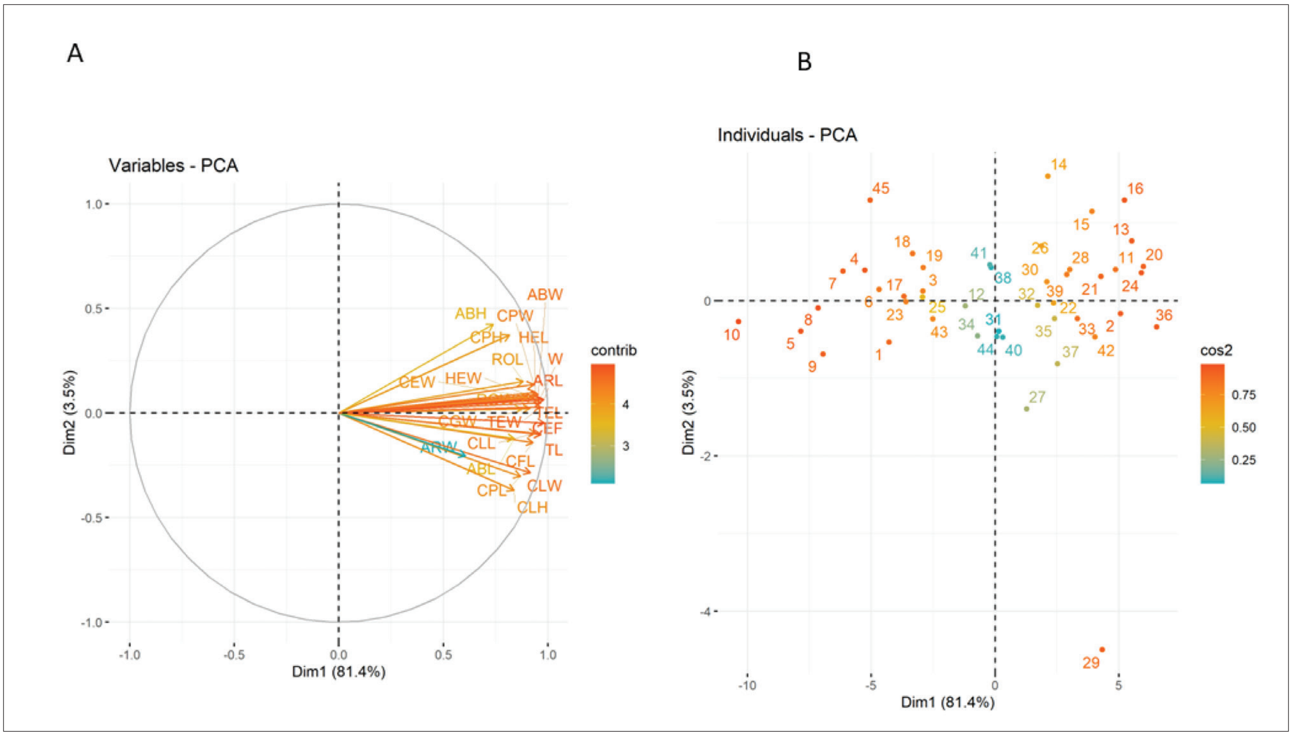

Fig. 2. Results of Principal component analysis (PCA). **A** – Contribution of each variable to the principal components. **B** – Contribution of individuals to the principal components.

Table 5. Value for the condition level for spiny-cheek crayfish from the Danube River, Serbia, and some autochthonous species from the Danube basin; FCF – Fulton's condition factor; CC – crayfish constant

Species	*Faxonus limosus*					Author [42]
Statistical parameters	Mean	Min	Max	SD	CV	
FCF (♂)	0.035	0.021	0.046	0.005	13.90	
CC(♂)	0.288	0.184	0.422	0.039	13.46	
Species	*Astacus astacus*					
Statistical parameters	Mean	Min	Max	SD	CV	
FCF(♂)	0.031	0.012	0.048	0.006	19.35	
FCF (♀)	0.029	0.014	0.039	0.005	17.24	
CC(♂)	0.241	0.096	0.399	0.049	20.33	
CC (♀)	0.229	0.088	0.494	0.050	21.83	
Species	*Pontastacus leptodactylus*					
Statistical parameters	Mean	Min	Max	SD	CV	
FCF(♂)	0.032	0.022	0.042	0.006	18.75	
FCF (♀)	0.029	0.026	0.036	0.003	10.34	
CC(♂)	0.337	0.286	0.389	0.025	7.42	
CC (♀)	0.300	0.187	0.454	0.084	28.00	
Species	*Austropotamobius torrentium*					
Statistical parameters	Mean	Min	Max	SD	CV	
FCF(♂)	0.053	0.033	0.066	0.008	15.09	
FCF (♀)	0.045	0.034	0.066	0.006	13.33	
CC(♂)	0.455	0.186	0.706	0.102	22.42	
CC (♀)	0.366	0.176	0.737	0.085	23.22	

Whereas correlation explains the strength of the relationship between an independent and a dependent variable, R-squared explains the extent to which the variance of one variable explains that of the second variable. R-squared is generally defined as the correlation or relationship between an independent and a dependent variable [31]. Using linear regression, a positive correlation was found between body length and weight (♂R^2=0.8389;), body weight and claw length (♂R^2=0.8179;), as well as carapace width and body length (♂R^2=0.8007;) (Table 4).

Results based on functions of length-weight ratios, the FCF and CC that were calculated specifically for males. In males, the Fulton condition index ranged from 0.021 to 0.046 with a mean of 0.035±0.005. The range of the crayfish constant in males ranged from 0.184 to 0.422 (0.288±0.039). Observing the results obtained (Table 5), spiny-cheek crayfish males are in good condition if we consider FCF and CC (Table 5). Results obtained using GM show that the

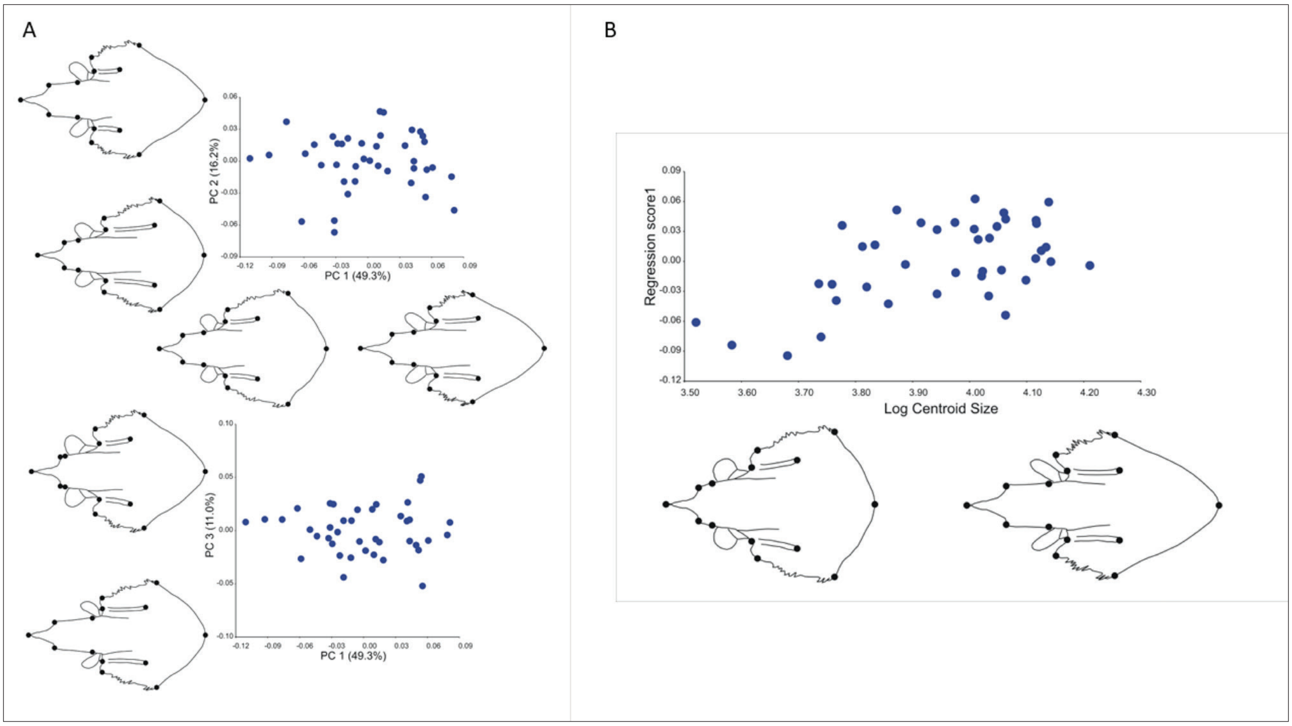

Fig. 3. A – Morphospace of the cephalon of *F. limosus* obtained by principal component analysis (PCA). Warped outline diagrams illustrate shape changes associated with the first three PC axes. **B** – Allometric shape changes of the cephalon of *F. limosus* assessed by the multivariate regression of the symmetric component of shape variation onto the log-transformed CS. Warped outline diagrams illustrate shape differences between the smallest and the largest specimens.

first three PC axes explained 76.5% of the total shape variation. The shape differences along the first PC axis consist of a gradient from the specimens in which the region described by LM 7, 8, and 9 is widened and shortened, the rostrum is shortened and widened, and the point described by LM 6 and 12 is reduced to the specimens in which the region described by LM 7, 8 and 9 is narrowed and elongated posteriorly, rostrum is narrower and elongated and the point described by LM 6 and 12 is increased. The shape differences along PC 2 consists of a gradient from specimens in which the region described by LM 2, 3, 13, and 4 is shortened, the structure described by LM 5 and 11 is elongated, and the region described by LM 7, 8 and 9 is shortened to the specimens in which the region described by LM 2, 3, 13 and 4 is elongated, the structure described by LM 5 and 11 is shortened and the region described by LM 7, 8 and 9 is elongated posteriorly. The third principal axis describes shape changes from specimens with elongated anterior and medial regions and slightly shortened posterior regions to the specimens with anteroposteriorly compressed anterior and medial regions and slightly elongated posterior regions (Fig. 3A). Allometry is statistically significant (p=0.0029) and explains 11.3% of the overall shape variation. Allometric shape changes are expressed as elongation and narrowing of the rostrum (LM 1, 2, 3, 13, and 14), an increase of the point described by LM 6 and 12, posterior elongation of the region described by LM 7, 8 and 9, and overall narrowing of the cephalothorax (Fig.3 B).

DISCUSSION

The first contemporary studies on the biogeography and taxonomy of freshwater crayfish on the territory of the Balkan Peninsula were performed by Karaman [32-34-25] and were based on morphometric research. After more than four decades, systematic research in the field of distribution, phylogeny, ecology, and conservation of crayfish in Serbia has resumed [35-39]. In general, morphological differences between individuals of the same species may occur because of genetic differences [40] or because of adaptations to specific ecological conditions in the habitat [41,42]. Research conducted by Sint et al. [17], Rajković [36], Đuretanović [39], and Roljić [42] showed that analyses of a large number of morphometric characteristics per individual were a suitable method for separating decapod groups, which could not be achieved by analyzing only a few morphometric characteristics.

Twenty-three morphometric characteristics of the 48 collected crayfish were measured to describe the general body shape in more detail using the "point-to-point" method to determine the variability of selected morphometric characteristics in adult individuals of both sexes. This is the first attempt to obtain morphometric characteristics of spiny-cheek crayfish caught in Serbia. The species is very tolerant to various environmental conditions, including highly eutrophic waters, decreased oxygen concentrations, low and elevated water temperatures, brackish water, and even drying of its habitats for several weeks [43-45]. Therefore, it has the potential for successful and rapid expansion of its range.

Our study shows that the sex ratio in the sample was significantly shifted in favor of males. This is a consequence of primarily reproductive behavior when sampling; in May and January, during the egg-laying/spawning period, males are significantly more numerous in the sample, and this ratio goes up to 3:1 [46-47]. The sex ratio of every population is important because only a uniform sex ratio is a sign of a healthy and stable population [48]. An uneven sex ratio can be a consequence of several biological factors, such as a lopsided sex ratio per hatching, differentiation of immigration and emigration of individuals of different sexes, as well as differences in activity between different sexes [44], differences in the age of reaching sexual maturity, different life spans of individuals of different sexes, but also sampling procedure [49]. The ascertained sexual dimorphism showed that higher mean values were found for most male characteristics due to the allometric growth of adults after reaching sexual maturity [49].

The population of spiny-cheek crayfish at the investigated site has a relatively stable age structure. The largest representation of individuals was in the group of 81-100 mm (56.25%), while in the group of 61-80 mm, the representation was 39.58%. The longest male was 108.90 mm, and the longest female was 87.60 mm, which is per the values given by Crandall [50]. Todorov [8] stated that the total length of adult spiny-cheek crayfish generally does not exceed 90-100 mm and rarely reaches 120 mm. The largest male weighed 36.30 g, and the female 17.50 g. The higher average weight of males can be attributed to the fact that males have larger claws than females, which contributes to their body weight. Spiny-cheek crayfish usually reach sexual maturity in the second year or at 15-16 months of age [51], but some juvenile specimens can mature and reproduce in the first year [52]. The length of the cephalothorax at sexual maturity ranges from 25-35 mm [51]. Based on these data, all the specimens we examined were sexually mature. Souty-Grosset [53] and Sint [17] support our data that higher mean values in the length and width of the claws were found in males, while in females, a higher mean value was found in the width of the first abdominal pleura, which represents the main sexual dimorphism characteristics in crayfish of this species.

According to the values of the calculated indices, it is evident that the males are in better condition, similar to the findings of Roljić [54]. Furthermore, according to Streissl and Hödl [55], this difference may be caused by the larger claws of males compared to females. This was also confirmed in noble crayfish *Astacus astacus* [56], narrow-clawed crayfish *Pontastacus leptodactylus* [57], stone crayfish *Austropotamobius torrentium* [56], and white-clawed crayfish *Austropotamobius pallipes* [58]. Research carried out by Streissl and Hödl [55] on freshwater decapod crustaceans revealed a positive correlation between crayfish body condition and habitat characteristics, namely, changes in water current intensity and the presence of various substrate types. These works coincide with our results if we consider the features of the specified locality where the crayfish were caught. The habitat abounds in various shelters where crayfish can be sheltered, thus avoiding aggressive contact and reducing activity in search of shelter, which ultimately results in a better body condition.

The studied population of *F. limosus* originates from the Danube, i.e., from a large lowland river into which it migrated 20 years ago. Numerous factors can be associated with morphological differences, such as sediment structure, and hydrological parameters (lentic and lotic microhabitats), i.e., minimum, and maximum flow velocity, water flow depth, and changes in temperature regime. In addition to the influence of abiotic parameters, the biotic component of the environment should also be considered, such as the amount of available food and the abundance of predators and parasites. The observed differences in the shape of morphological structures between individuals may result from developmental changes in response to different selection pressures.[46] Freshwater crayfish stand out in phenotypic plasticity – they react to changing habitat conditions, which often leads to the occurrence of specific life forms. Likewise, freshwater crayfish exhibit intraspecific morphometric variation that reflects environmental influence [59]. Thanks to its great phenotypic plasticity, this species successfully adapts to new habitats with considerable nutrition and reproductive biology differences.

CONCLUSIONS

The results presented herein contribute to the ecology of the spiny-cheek crayfish in the recipient habitat. This is the first attempt to obtain morphometric characteristics of spiny-cheek crayfish caught in Serbia to determine the variability of selected characters in adult males.

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Data availability: Data underlying the reported findings have been provided as a raw dataset, which is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Roljic%20et%20al_Dataset.xlsx

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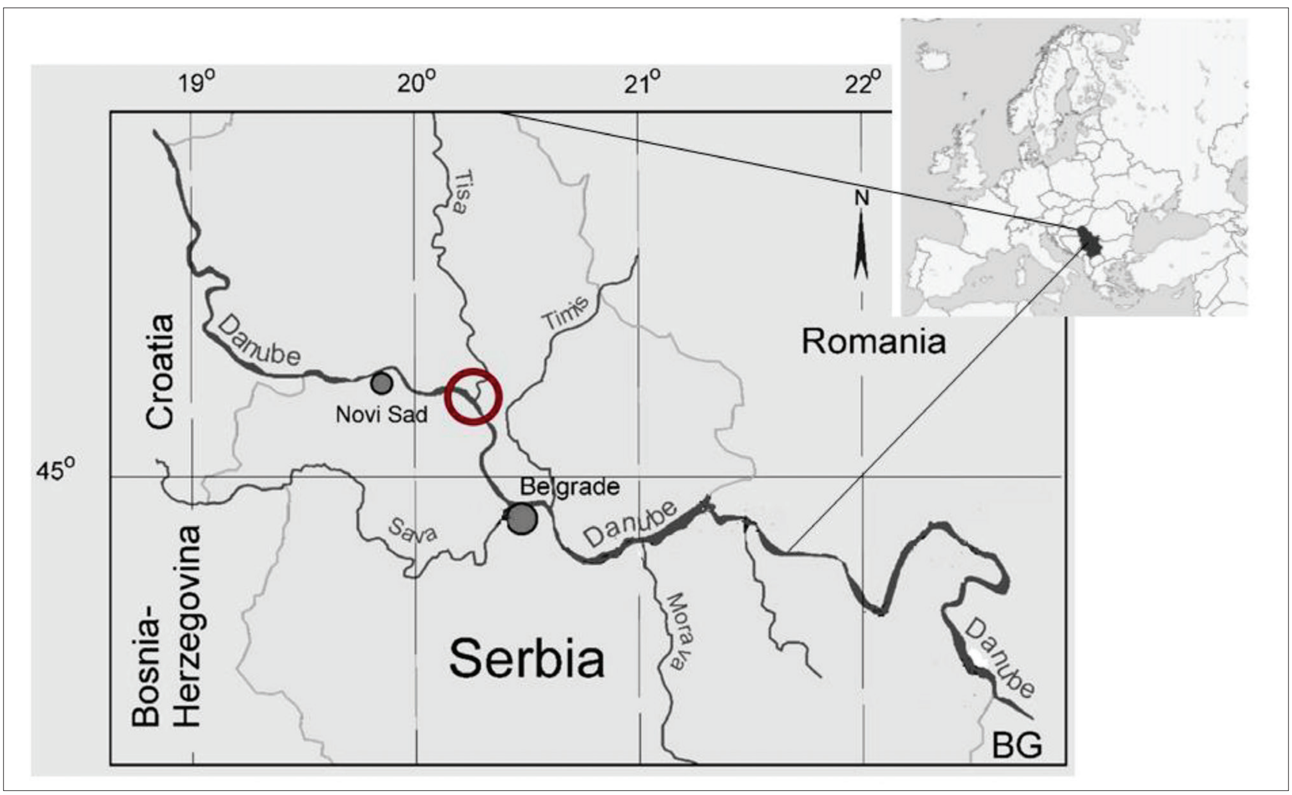
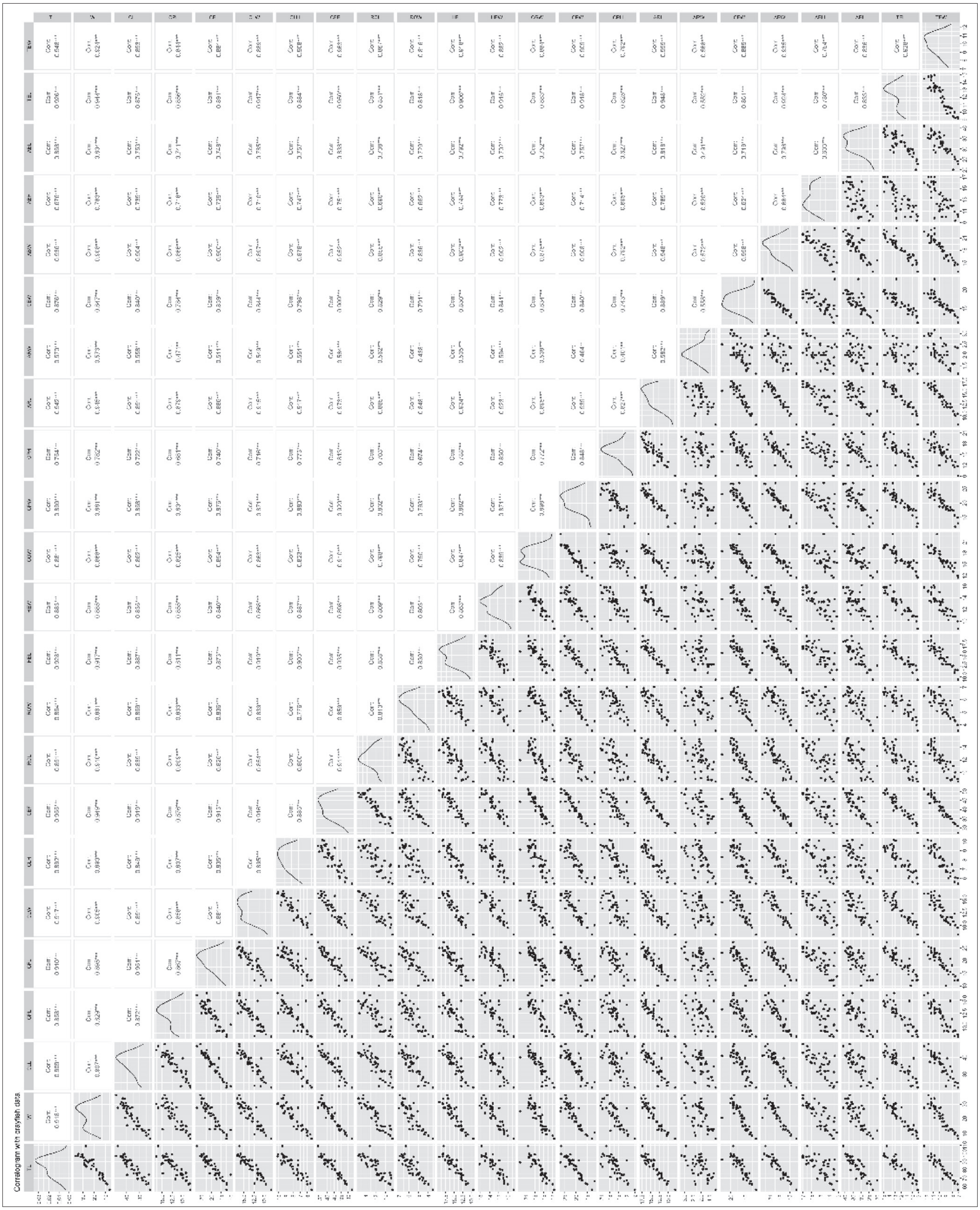
SUPPLEMENTARY MATERIAL



Supplementary Fig. S1. The sampling area (red circle) at the confluence of the Tisa and Danube near Slankamen.



Supplementary Fig. S2. Correlogram with spiny-cheek crayfish data. The asterisks represent significance at the10% (*), 5% (**), and 1% (***) confidence levels.