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Sexual dimorphism in *Branchipus schaefferi* Fischer, 1834 (Anostraca: Branchiopoda: Crustacea) from Serbia

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Abstract. Quantitative information regarding sexual dimorphism in large branchiopod crustaceans is insufficient, especially for the Balkans. Here we present the data obtained on several morphometric and meristic characters in six populations of *Branchipus schaefferi*. Male-biased sexual dimorphism was shown in the length of head and cercopods, whereas females had longer thorax and abdomen, absolutely and relative to the total body length. Among the meristic traits, significant difference between the sexes was found only in the number of hairs on the ventral side of the abdomen, with higher average value in females. The results differed among the analyzed populations, indicating geographic variability in morphology and degree of sexual dimorphism in this species. The exact roles of various body parts and sensory elements in mating process in the genus *Branchipus* are not yet fully explained. Results show that quantifiable differences between males and females exist, can be used as criteria for sex discrimination and are determined by the interplay of various types of selection.

Key words: Inter-gender differences, morphometry, PCA, *Branchipus*.

Differences in secondary sexual characteristics (sexual dimorphism, SD) in size (SSD) and shape of the body (SShD) result from the complex interactions of natural, intra- and intersexual, fecundity and ecological selections. In a number of animal taxa, growth rate and development time can vary between sexes (Beladjal et al. 2003, Blanckenhorn et al. 2007, Fairbairn et al. 2007). Intraspecific geographic variability in sexual dimorphism is also widespread (e.g. Blanckenhorn et al. 2007, Wellborn & Cothran 2007, Djordjević et al. 2011). In Anostracans (large branchiopods, Class Branchiopoda) sexual dimorphism was depicted mainly at a descriptive level. Statistical analyses for evaluation of SD were performed mainly on the genus *Artemia* Leach 1819 (Asem & Rastegar-Pouyani 2007, Asem et al. 2010, Ben Naceur et al. 2010), considering their importance for science and aquaculture. However, to our knowledge, similar analyses have not been conducted on the genus *Branchipus* Schaeffer, 1766 up to now. In this article we aimed to answer if in *Branchipus schaefferi* there are significant differences between sexes in body parts which are *a priori* unrelated to fertilization, and what role they could play in mating process.

A total of 109 individuals (57 males, 52 females) originating from six localities in Serbia were used for analysis (Table 1). Specimens were fixed in 70% ethyl-alcohol immediately after the capture. Five morphometric and ten meristic characters, as well as three morphological indices were processed (all characters are listed in Table 1). In order to check for significant inter-gender differences between the mean values of examined morphological traits, we used the paired t-test. Principal Component Analysis (PCA) was used to define sexual dimorphism in size and shape at multivariate level. All analyses were performed by Statistica 5.1. (Stat Soft Inc., Tulsa, USA). Measurements were obtained under a dissecting microscope (ZEISS Discovery V8 Stereomicroscope). For the endite setulation analysis, the first thoracopod (from the left side of the body) was dissected and mounted for light microscopy (Philips CM 12 Microscope). Morphological terminology is given according to Linder (1941).

Table 1 contains results of basic statistical analyses of morphometric and meristic characters and body indices in *B. schaefferi* from six localities in Serbia. Results of t-tests indicate substantial inter-population variability in the number and type of sexually dimorphic traits. In the samples from two localities (Titel and Trnjana), there are no differences between males and females in common morphological traits, while in the sample from Ogar genders differ in three morphological traits and two indices. Other samples display low (Banatsko Arandjelovo) or intermediate (Progar and Sutjeska) levels of sexual dimorphism.

Among the analyzed morphological traits, head length (HL) and cercopod length (CL) were sexually dimorphic in two samples, with higher values in males. Thorax length (TL) and abdomen

Table 1. Descriptive statistics, t-tests and statistical significance of analyzed morphological traits in males and females of *B. schaefferi* from different localities (significantly larger values given in boldface).

	Banatsko Arandjelovo (46°03′N 20°15′E)				Ogar (44°47′N 19°56′E)				Progar (44°42′N 20°09′E)			
	males (N=9)	females (N=8)			males (N=8)	females (N=9)			males (N=10)	females (N=7)		
	Mean±SD	Mean±SD	t-test	p	Mean±SD	Mean±SD	t-test	p	Mean±SD	Mean±SD	t-test	p
TBL	11.6±1.17	10.7±1.16	1.49	0.158	10.7±0.53	11.2±1.02	-1.36	0.194	13.1±2.17	13.0±1.45	0.15	0.880
HL	1.0±0.19	1.0±0.23	0.03	0.978	**1.0±0.15**	0.8±0.15	3.31	0.005	**1.2±0.13**	1.0±0.16	2.69	0.017
TL	4.5±0.65	4.0±0.23	1.97	0.068	3.9±0.21	**4.3±0.44**	-2.53	0.023	4.8±0.82	5.0±0.59	-0.65	0.527
AL	4.3±0.63	4.4±0.72	-0.33	0.743	4.2±0.17	**4.8±0.39**	-4.19	0.001	5.3±0.97	5.4±0.60	-0.25	0.804
CL	**1.8±0.31**	1.3±0.33	3.10	0.007	1.6±0.43	1.4±0.24	1.62	0.126	1.9±0.40	1.6±0.31	1.67	0.116
TBL/TL	2.6±0.23	2.7±0.29	-0.61	0.550	**2.8±0.12**	2.6±0.05	3.30	0.005	**2.8±0.12**	2.6±0.08	3.09	0.007
TBL/AL	2.7±0.38	2.5±0.19	1.82	0.088	**2.6±0.16**	2.3±0.04	4.10	0.001	2.5±0.13	2.4±0.10	1.45	0.168
TL/AL	1.1±0.25	0.9±0.19	1.22	0.240	0.9±0.05	0.9±0.03	1.58	0.135	0.9±0.07	0.9±0.06	-0.67	0.512
EN3 SS	1.8±0.44	1.8±0.46	0.13	0.901	1.9±0.35	2.1±0.33	-1.42	0.177	2.1±0.32	2.1±0.38	-0.25	0.803
EN3 LS	2.7±0.71	2.9±0.35	-0.75	0.464	2.8±0.46	3.0±0.00	-1.63	0.125	2.8±0.42	3.0±0.00	-1.24	0.233
EN4 SS	2.3±0.87	2.1±0.83	0.50	0.622	2.6±0.52	3.0±0.50	-1.52	0.150	3.3±0.82	3.6±0.53	-0.76	0.457
EN4 LS	2.0±0.00	2.1±0.35	-1.07	0.304	2.1±0.35	1.9±0.33	1.42	0.177	2.0±0.00	2.0±0.00	-	-
EN5 SS	2.7±0.50	2.5±0.76	0.54	0.596	2.1±0.83	2.1±0.78	0.04	0.972	2.9±0.57	2.4±0.53	1.72	0.105
EN5 LS	1.9±0.33	2.3±0.46	-1.86	0.082	1.9±0.35	2.0±0.00	-1.07	0.304	2.0±0.00	2.0±0.00	-	-
LHAB	1.9±0.33	1.9±0.35	0.08	0.935	2.0±0.00	2.0±0.00	-	-	1.8±0.42	2.0±0.00	-1.24	0.233
DHAB	1.0±0.00	1.0±0.00	-	-	1.0±0.00	1.0±0.00	-	-	1.1±0.32	1.1±0.38	-0.25	0.803
VHAB	3.1±0.78	3.4±0.74	-0.71	0.488	3.3±0.46	3.8±0.67	-1.87	0.081	2.7±0.67	**3.6±0.53**	-2.84	0.012
DLAB	6.0±0.00	6.0±0.00	-	-	6.0±0.00	6.0±0.00	-	-	5.7±1.34	6.0±0.00	-0.59	0.566
TBL	13.4±2.28	13.6±2.26	-0.24	0.816	11.7±4.07	9.49±3.86	1.23	0.237	11.7±1.39	12.2±1.74	-0.69	0.499
HL	1.2±0.36	1.0±0.19	0.92	0.369	1.0±0.26	0.88±0.47	0.71	0.485	1.0±0.18	1.0±0.21	-0.20	0.847
TL	5.0±1.08	5.3±0.85	-0.67	0.513	4.5±1.50	3.59±1.26	1.39	0.182	4.4±0.61	4.6±0.78	-0.52	0.609
AL	5.0±0.81	5.5±0.91	-1.24	0.229	4.6±1.75	3.78±1.68	1.00	0.330	4.6±0.50	5.1±0.69	-1.60	0.129
CL	**2.2±0.34**	1.8±0.39	2.48	0.023	1.7±0.76	1.24±0.47	1.47	0.159	1.7±0.42	1.5±0.34	0.71	0.488
TBL/TL	2.7±0.27	2.6±0.11	1.41	0.175	2.6±0.17	2.62±0.13	-0.12	0.902	2.7±0.20	2.7±0.19	-0.18	0.858
TBL/AL	**2.7±0.11**	2.5±0.12	3.52	0.002	2.6±0.14	2.53±0.07	1.26	0.226	2.5±0.21	2.4±0.08	1.67	0.114
TL/AL	1.0±0.13	1.0±0.08	0.62	0.542	1.0±0.09	0.97±0.07	0.81	0.428	1.0±0.14	0.9±0.08	1.04	0.311
EN3 SS	2.0±0.00	2.0±0.00	-	-	2.0±0.00	2.00±0.00	-	-	2.5±0.53	2.3±0.50	0.71	0.490
EN3 LS	3.0±0.00	3.0±0.00	-	-	3.0±0.00	3.00±0.00	-	-	3.0±0.00	2.9±0.33	1.06	0.305
EN4 SS	2.9±0.74	3.2±0.79	-0.88	0.391	2.9±0.57	2.89±0.60	0.04	0.967	4.3±1.06	4.0±0.87	0.67	0.511
EN4 LS	2.0±0.00	2.0±0.00	-	-	2.0±0.00	1.89±0.33	1.06	0.305	2.0±0.00	2.1±0.33	-1.06	0.305
EN5 SS	3.0±0.47	3.0±0.67	0.00	1.000	2.5±0.71	2.22±0.44	1.01	0.325	3.7±0.67	3.6±0.53	0.52	0.613
EN5 LS	2.0±0.00	2.0±0.00	-	-	2.0±0.00	1.89±0.33	1.06	0.305	2.1±0.32	2.1±0.33	-0.07	0.941
LHAB	2.0±0.00	2.0±0.00	-	-	2.0±0.00	2.00±0.00			2.3±0.67	2.0±0.00	1.33	0.201
DHAB	1.0±0.00	1.0±0.00	-	-	1.2±0.63	1.00±0.00	0.95	0.357	1.1±0.32	1.0±0.00	0.95	0.357
VHAB	3.1±0.57	3.0±0.82	0.32	0.754	2.3±0.48	2.78±0.67	-1.80	0.089	3.3±0.95	3.8±0.97	-1.08	0.294
DLAB	6.0±0.00	6.0±0.00	-	-	6.0±0.00	5.78±0.67	1.06	0.305	5.6±1.26	6.0±0.00	-0.95	0.357

Abbreviations: total body length (TBL), head length (HL), thorax length (TL), abdomen length (AL), length of cercopods (CL); number of shorter (SS), and number of longer setae (LS) on the first thoracopod endites 3, 4 and 5 (EN3, EN4, EN5); number of abdominal hairs: dorsal (DHAB), ventral (VHAB), lateral (LHAB), dorsolateral (DLAB); total body length to thorax length (TBL/TL), total body length to abdomen length (TBL/AL), thorax length to abdomen length (TL/AL).

length (AL) were sexually dimorphic in only one sample (Ogar), with higher values in females. Among the analyzed morphological indices, sexually dimorphic were the ratio of total body length to abdomen length, TBL/AL (in two samples), and the ratio of total body length to thorax length, TBL/TL (in two samples), with higher values in males. Values of other morphological indices were also higher in males, but differences were not statistically significant. Of all analyzed meristic traits, significant difference ($p<0.006$) was found only in the number of hairs on the ventral side of the abdomen (VHAB) in one of the analyzed samples, with higher average values in females.

Results of the Principal Component Analysis (PCA) showed that all morphological traits contributed to variability in size (PCA 1), while all morphological indices influenced variability in shape (PCA 2) (Table 2). The first and second principal component described 20.07% and 15.54% of the total variability, respectively. Relative positions of group centroids of males and females from different localities in the projection of the first and second principal axes are given in Figure 1. Differences in size (PC 1) were evident between males and females from Titel only. On the other hand, differences in shape (PC 2) were most prominent between the genders in Banatsko

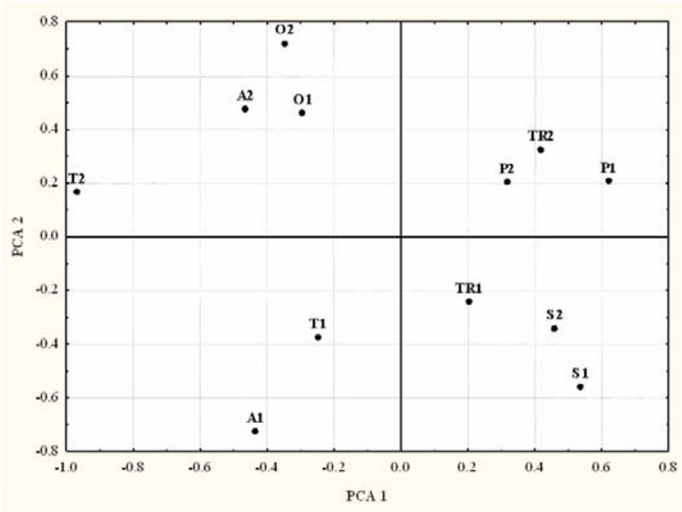

Figure 1. Relative position of group centroids of males and females from different localities in the projection of the first and the second principal axes. Abbreviations: A – Banatsko Arandjelovo; O – Ogar; P – Progar; S – Sutjeska; T – Titel; TR – Trnjana.

Table 2. Results of Principal Component Analysis. Significant loadings are given in bold face. Abbreviations as in Table 1.

	PCA 1	PCA 2
HL	**0.772**	-0.124
TL	**0.773**	-0.493
AL	**0.939**	0.008
CL	**0.783**	-0.313
TBL/TL	0.447	**0.697**
TBL/AL	-0.246	**-0.825**
TL/AL	-0.392	**-0.903**
EN3 SS	0.049	-0.013
EN3 LS	-0.039	-0.053
EN4 SS	0.370	-0.243
EN4 LS	0.167	0.061
EN5 SS	0.309	-0.207
EN5 LS	0.062	-0.019
BDAB_BR	0.089	-0.221
DDAB_BR	-0.115	0.194
VDAB_BR	0.095	0.330
DLAB_BR	0.103	-0.006

Arandjelovo, but were also present in Titel and Trnjana samples.

At univariate level, our study pointed to a significant inter-population variability in sexually dimorphic traits of *B. schaefferi*. These rather preliminary observations indicate a certain difference of specimens from localities Titel and Trnjana, and Banatsko Arandjelovo as well. Taxonomic status of these samples should be considered further, as some open questions regarding the systematics and phylogenetic relationships within mentioned areas have already been discussed in the literature (Petrov & Marinček 1991, Cvetković-Miličić et al. 2005). The overall body size of analysed specimens did not differ in a significant manner between genders in the entire sample. Obtained data comply with report of Beladjal et al. (2003), who found that, in natural populations of *B. schaefferi*, growth rate and development time do not vary between sexes. Both larger head and cercopods in males are in accordance with previous reports (Dumont & Negrea 2002, Asem et al. 2010). Our analysis also indicates a larger thorax and abdomen in females compared to males. At the multivariate level, however, variability in the shape between genders was shown to be greater than in size, which emphasizes the importance of external morphology of body parts responsible for sex recognition.

Results of both univariate and multivariate analyses can be discussed in light of the specific mate recognition system that may considerably enhance reproductive success of individuals. Larger abdomen in females (that carries a spacious brood pouch with eggs) is particularly supposed to be under fecundity selection, since the number of eggs/litter increases with increased body and/or abdomen size (Ghiselin 1974, Fairbairn et al. 2007, Kaliontzopoulou et al. 2007). Its larger proportion can provide selective advantage in unpredictable environments, like ephemeral and unstable water bodies, habitats common for *B. schaefferi* (Brendonck 1995). On the other hand, larger head with well-developed processes and appendages in males can be crucial in female monopolization during the complex (pre)copulatory interactions (Brendonck & Belk 1997, Dumont & Negrea 2002, Sugumar 2010). According to Rogers (2002), compatibility of the males' second antennae patterns with female genital morphology plays a significant role during amplexus and therefore can be explained by sexual selection.

Considering that doubts on degree of geographical and environmental influence on morphology and taxonomic determination of large branchiopods still exist, studies based on larger samples of individuals should delineate the effects of sexual, fecundity and ecological selection in these crustaceans. Behavioral studies that illuminate the role of morphological traits confirmed as sexually dimorphic should also be added to analysis.

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