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Sexual dimorphism of five *Cobitis* species (Cypriniformes, Actinopterygii) in the Adriatic watershed

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Abstract. Morphological differences between female and male spined loaches belonging to five species from the Adriatic basin were examined. Besides the presence of the Canestrini scale and the length of the pectoral fins, sexual dimorphism is also visible in the position of the pectoral fins, as well as in the length of the pelvic fins. Both pectoral and pelvic fins are significantly longer in males than in females, whereas pectoral fins of females are located more anteriorly than in males. Differences in the length of pectoral and pelvic fins among species are also revealed. The role of the secondary sexual characters in males is probably connected with their special mating behaviour. Furthermore, a hypothesis that habitat conditions have greater impact on body dimensions of females than males is proposed.

Key words: loaches, morphological differences, females, males, reproduction

Introduction

Secondary sexual characters play a significant role in reproductive behaviour of fishes so describing morphological differences between females and males of different fish species can help us to understand their reproductive biology. Furthermore, knowledge on variation in sexual dimorphism is very important in taxonomic investigations, since characters that depend on sex have to be analyzed separately for females and males in order to obtain reliable results. In this investigation we have compared morphometric and external morphology characters of females and males from five *Cobitis* species distributed in the Adriatic basin, in order to find differences between them and to compare results with already reported sexual dimorphism characters of other spined loach species.

Spined loaches of the genus *Cobitis* are a group of primarily freshwater fishes, widely distributed in waters of the Palearctic region (Nalbant et al. 2001) and represented with more than 60 species (Kottelat 2012) with similar morphological characters. They all have small, elongated, characteristically coloured bodies, with dark spots and blotches on paler basic

colour. Investigations on several *Cobitis* species revealed that females are generally significantly larger than males of the same age (Boroń & Pimpicka 2000, Erős 2000, Przybylski & Valladolid 2000, Schneider et al. 2000b, Kottelat & Freyhof 2007, Bohlen et al. 2008, Boroń et al. 2008, Valladolid & Przybylski 2008). The assumed reason for such difference is faster growth of females, since during the first year of life females utilize all their energy for growth and it is not before their second year that they start using energy for reproduction, whereas males use energy for reproduction already in the first year of life (Erős 2003). Previous researches showed that, besides in body length, females and males differ also in the size of their pectoral fins and presence of the Canestrini scale. Pectoral fins are usually significantly larger in males than in females of the same species, although all other body measures are larger in females (Freyhof & Serov 2000 for *Cobitis laoensis* Freyhof & Serov, 2007, Freyhof & Stelbrink 2007 for *Cobitis illyrica* Freyhof & Stelbrink, 2007, Mustafić et al. 2008 for *Cobitis jadovaensis* Mustafić & Mrakovčić, 2008). Furthermore, in males of the majority of *Cobitis* species (with the exception of *Cobitis elongata* Heckel

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& Kner, 1858 and *Cobitis calderoni* Băcescu, 1962) on dorsal side of pectoral fins one or two Canestrini scales (*laminae Canestrini*) are located (Kotusz 2000, Schneider et al. 2000a, Perdices & Doadrio 2001). They appear together with sexual maturation of males (Robotham 1981) and it has been hypothesized that they play a role during mating behaviour of male loaches (Bohlen 2008). Since sexual dimorphism has not been particularly investigated in the Adriatic spined loaches, the present paper aims to contribute to better understanding of sexual, but also interspecific variation of the genus *Cobitis*.

Material and Methods

Observations were made on *C. bilineata* Canestrini, 1865; *C. dalmatina* Karaman, 1928; *C. narentana* Karaman, 1928; *C. illyrica* and *C. herzegoviniensis* Buj & Šanda, 2014, from waters of the Adriatic watershed in Croatia and Bosnia and Herzegovina. Specimens were collected by electrofishing on 11 localities (Table 1). This material was also used for systematic revision of spined loaches in the Adriatic basin (Buj et al. 2014) in which morphological and genetic data were used to resolve the taxonomic status of *Cobitis* populations. Investigation presented in this paper is based on comparison of morphological characters between females and males of five *Cobitis* species in order to reveal their sexual dimorphism. Sex was determined based on the presence of the Canestrini scale in males. A total of 24 morphometric characters was measured using an electronic caliper to the nearest 0.01 mm: total length (TL), standard length (SL), lateral head length (c), preanus distance

(Pan), preanal distance (aA), prepelvic (preventral) distance (aV), prepectoral distance (aP), predorsal distance (aD), caudal peduncle length (lpc), length of dorsal (ID), anal (IA), caudal (IC), pectoral (IP) and pelvic (ventral, IV) fins, distance between pelvic (ventral) fins and anal aperture (Van), head depth (measured in the level of eyes) (hc), maximum body depth (H) and caudal peduncle depth (or minimum body depth, h), head width (laco), maximum body width (lac), distance between eyes (io), eye diameter (o), preorbital distance (prO), postorbital distance (poO). All measurements were made point to point. Standardization of morphometric characters was conducted by calculating morphometric ratios, i.e. percentage ratios of morphometric characters in relation to SL, c and H. For statistical comparison of morphometric features between females and males Student's t-test was employed and comparison was conducted between all females and males belonging to a certain species. Normal distribution of characters was confirmed by Shapiro-Wilk W test. In order to determine the correlation between each morphometric character and SL, the correlation analysis was conducted separately for females and males. For statistical analyses Statistica 7.1 software package was employed.

In addition to morphometric characters, overall external morphology was examined in details to check for differences between two sexes of each species. Morphological analysis included comparison of the external colouration (Gambetta zones, spots on the caudal fin base and dorsal blotches), shape and colouration of the head, scale coverage, structure of

Table 1. Taxonomy, geographic origin and the number of the investigated specimens.

Species	Locality	River system	Country	Number of specimens	
				females	males
<i>C. bilineata</i>	Zrmanja	Zrmanja	Croatia	19	7
<i>C. dalmatina</i>	Blato na Cetini	Cetina	Croatia	7	11
<i>C. narentana</i>	Mislina	Neretva	Croatia	5	4
	Norin	Neretva	Croatia	15	2
	Modro oko	Neretva	Croatia	15	4
	Hutovo b.	Neretva	Bosnia and Herzegovina	11	4
	Trebišnjica	Neretva	Bosnia and Herzegovina	15	6
	total <i>C. narentana</i>			61	20
<i>C. illyrica</i>	Matica	Neretva	Croatia	13	7
	Prološko blato	Imotski field	Croatia	18	1
	Krenica	Bekijsko field	Bosnia and Herzegovina	9	9
	total <i>C. illyrica</i>			40	17
<i>C. herzegoviniensis</i>	Mostarsko blato	Neretva	Bosnia and Herzegovina	8	15

Table 2. The length of the pectoral fin (IP) and the ratio between the length of the pectoral fin and the distance between pectoral and pelvic fins bases (IP/(aP-aV)) in females and males of investigated species. Values in the table are minimal and maximal measured values. Exceptional values (excep.) were found in only one specimen.

	females		males	
	IP (mm)	IP/(aP-aV)	IP (mm)	IP/(aP-aV)
<i>C. bilineata</i>	6.56-11.25	0.32-0.51 (excep. 0.56)	8.51-10.35	0.59-0.71
<i>C. dalmatina</i>	9.16-12.43	0.40-0.44	10.46-13.78	0.64-0.91
<i>C. narentana</i>	6.9-11.58	0.36-0.56	7.42-12.83	0.62-0.84 (excep. 0.56)
<i>C. illyrica</i>	5.89-11.25	0.37-0.52 (excep. 0.57)	8.34-12.15	0.62-0.84
<i>C. herzegoviniensis</i>	5.22-8.64	0.28-0.45	8.69-12.85	0.60-0.78

Table 3. Prepectoral distance (aP) and its relation to standard length (aP/SL) in females and males of investigated species. Exceptional values (excep.) were found in only one specimen.

	females		males	
	aP (mm)	aP/SL	aP (mm)	aP/SL
<i>C. bilineata</i>	10.18-16.16	0.193-0.241	10.16-11.84	0.223-0.252
<i>C. dalmatina</i>	13.81-19.15	0.197-0.216	10.93-15.79	0.231-0.273 (excep. 0.214)
<i>C. narentana</i>	11.51-17.48	0.187-0.248	10.24-14.25	0.226-0.274
<i>C. illyrica</i>	10.15-17.51	0.199-0.253	11.58-15.43	0.221-0.266
<i>C. herzegoviniensis</i>	11.75-14.03	0.210-0.235	9.75-14.02	0.221-0.275

Table 4. The length of pelvic fins (IV) and its relation to standard length (IV/SL) in females and males of investigated species.

	females		males	
	IV (mm)	IV/SL	IV (mm)	IV/SL
<i>C. bilineata</i>	6.06-10.24	0.120-0.141	6.18-8.11	0.136-0.164
<i>C. dalmatina</i>	7.48-9.55	0.111-0.122	7.05-9.04	0.132-0.179
<i>C. narentana</i>	5.92-10.01	0.098-0.144	5.63-8.55	0.126-0.178
<i>C. illyrica</i>	4.74-9.35	0.101-0.130	6.37-8.64	0.122-0.169
<i>C. herzegoviniensis</i>	5.49-6.67	0.098-0.108	5.02-8.08	0.108-0.156

Table 5. Minimal and maximal values of the caudal fin length and head length in relation to standard length (IC/SL and c/SL, respectively).

	IC/SL		c/SL	
	females	males	females	males
<i>C. bilineata</i>	0.168-0.208	0.172-0.192	0.196-0.220	0.203-0.231
<i>C. dalmatina</i>	0.146-0.180	0.159-0.216	0.187-0.205	0.199-0.244
<i>C. narentana</i>	0.151-0.204	0.163-0.231	0.185-0.222	0.206-0.237
<i>C. illyrica</i>	0.132-0.177	0.135-0.191	0.181-0.219	0.192-0.239
<i>C. herzegoviniensis</i>	0.125-0.154	0.141-0.167	0.199-0.218	0.196-0.230

scales, length of barbels (mandibular, maxillary and rostral) and length of urogenital papilla between females and males of each species.

It is important to mention that ploidy level of samples has not been investigated so the possibility of presence of hybrid biotypes cannot be completely excluded, even though they have not been recorded in the Adriatic basin.

Results

One Canestrini scale was formed as an outgrowth of the second pectoral fin ray in males. The smallest male in whom the Canestrini scale was already developed had 49 mm of TL and 42 mm of SL.

Besides the pronounced difference in the total and standard body lengths, the pectoral fins are visible and significantly longer in males than in females

Table 6. T-test results (p-values) between females and males of investigated species based on their morphometric ratios. Bold values represent statistically significant difference in certain character.

	c/SL	Pan/SL	aA/SL	aV/SL	aP/SL	aD/SL	lpc/SL	Van/SL
<i>C. bilineata</i>	0.0084	0.7643	0.6903	0.1851	0.0054	0.5427	0.1245	0.1275
<i>C. dalmatina</i>	0.0001	0.8492	0.5966	0.5056	0.0000	0.7776	0.0540	0.7573
<i>C. narentana</i>	0.0000	0.5640	0.7557	0.1345	0.0000	0.1226	0.6006	0.2525
<i>C. illyrica</i>	0.0000	0.3215	0.6226	0.1570	0.0000	0.2920	0.4696	0.9732
<i>C. herzegoviniensis</i>	0.0276	0.0099	0.0038	0.6507	0.0027	0.6486	0.6650	0.0663
	lD/SL	lA/SL	lC/SL	lP/SL	lV/SL	laco/SL	lac/SL	H/SL
<i>C. bilineata</i>	0.2043	0.4846	0.9850	0.0000	0.0000	0.1182	0.1564	0.0826
<i>C. dalmatina</i>	0.1954	0.2432	0.0153	0.0000	0.0000	0.6059	0.9715	0.6060
<i>C. narentana</i>	0.4066	0.0002	0.0015	0.0000	0.0000	0.3710	0.4823	0.0832
<i>C. illyrica</i>	0.5716	0.0266	0.0012	0.0000	0.0000	0.0000	0.5989	0.2007
<i>C. herzegoviniensis</i>	0.1484	0.6381	0.0446	0.0000	0.0000	0.0627	0.1191	0.1164
	h/SL	hc/H	h/H	io/c	o/c	prO/c	poO/c	h/lpc
<i>C. bilineata</i>	0.6316	0.0015	0.0626	0.1723	0.1276	0.0092	0.1494	0.4416
<i>C. dalmatina</i>	0.0137	0.0093	0.0021	0.2176	0.0010	0.0052	0.1889	0.2518
<i>C. narentana</i>	0.0003	0.1478	0.2624	0.2199	0.0002	0.0000	0.0002	0.0057
<i>C. illyrica</i>	0.0002	0.0660	0.0095	0.0710	0.0007	0.0571	0.0741	0.1969
<i>C. herzegoviniensis</i>	0.4200	0.9798	0.3100	0.1018	0.0012	0.0971	0.4143	0.9635

Table 7. Pearson's correlation coefficient of each morphometric character in relation to standard length for females from investigated species. Bold values represent significant correlation.

	c	Pan	aA	Van	aV	aP	aD	lpc	lD	lA	lC
<i>C. bilineata</i>	0.94	0.98	0.98	0.84	0.98	0.81	0.94	0.81	0.84	0.82	0.85
<i>C. dalmatina</i>	0.95	0.99	0.99	0.97	0.98	0.94	0.98	0.86	0.48	0.74	0.70
<i>C. narentana</i>	0.93	0.97	0.97	0.76	0.97	0.86	0.97	0.69	0.75	0.65	0.85
<i>C. illyrica</i>	0.95	0.99	0.99	0.93	0.96	0.89	0.98	0.67	0.84	0.81	0.83
<i>C. herzegoviniensis</i>	0.92	0.98	0.97	0.42	0.98	0.89	0.97	0.18	0.78	0.70	0.55
	lP	lV	hc	H	h	laco	lac	io	o	prO	poO
<i>C. bilineata</i>	0.74	0.88	0.86	0.82	0.80	0.76	0.77	0.48	0.54	0.78	0.81
<i>C. dalmatina</i>	0.84	0.80	0.94	0.96	0.97	0.75	0.94	0.96	0.44	0.76	0.99
<i>C. narentana</i>	0.79	0.82	0.87	0.71	0.83	0.73	0.60	0.48	0.60	0.90	0.86
<i>C. illyrica</i>	0.84	0.87	0.87	0.76	0.81	0.77	0.77	0.36	0.75	0.92	0.92
<i>C. herzegoviniensis</i>	0.77	0.86	0.88	0.66	0.55	0.75	0.65	0.18	0.15	0.77	0.89

(Table 2). In males from all investigated species they considerably exceed half of the distance between bases of pectoral and pelvic fins (59-91 %). On the other hand, pectoral fins of females reach maximally to the half of the distance between pectoral and pelvic fins bases, or, very rarely, only slightly pass that distance (up to 56 %). This can be noticed for all species, even though the absolute and relative lengths of pectoral fins differ among species. Accordingly, the length of the pectoral fins in relation to SL was significantly different between females and males of all species (t-test, $p < 0.05$; Table 6). It is noteworthy that several differences in this feature were recorded among

species (Table 2). In *Cobitis dalmatina* males the ratio between the pectoral fin length and the distance between pectoral and pelvic fin bases attains larger values than in the remaining species. On the other hand, this ratio was the smallest for *Cobitis bilineata*. Since all the Adriatic *Cobitis* species are quite similar in their external morphology (Buj et al. 2014), this difference could be useful in species determination. Position of the pectoral fins also differs between females and males – in females the bases of pectoral fins are located more anteriorly on their bodies (Table 3). This was confirmed by t-test that found statistically significant differences in the prepectoral

Table 8. Pearson's correlation coefficient of each morphometric character in relation to standard length for males from investigated species. Bold values represent significant correlation.

	c	Pan	aA	Van	aV	aP	aD	lpc	ID	IA	IC
<i>C. bilineata</i>	0.82	0.96	0.97	0.78	0.95	0.74	0.64	0.40	0.85	0.49	0.87
<i>C. dalmatina</i>	0.75	0.97	0.96	0.82	0.96	0.80	0.89	0.76	0.22	0.43	0.21
<i>C. narentana</i>	0.82	0.96	0.97	0.65	0.93	0.76	0.97	0.45	0.77	0.36	0.73
<i>C. illyrica</i>	0.71	0.96	0.98	0.75	0.93	0.77	0.95	0.73	0.20	0.28	-0.16
<i>C. herzegoviniensis</i>	0.92	0.98	0.99	0.93	0.97	0.74	0.92	0.39	0.78	0.86	0.87
	IP	IV	hc	H	h	laco	lac	io	o	prO	poO
<i>C. bilineata</i>	0.93	0.84	0.61	0.45	0.51	0.17	0.53	0.34	0.28	0.60	0.24
<i>C. dalmatina</i>	0.34	0.39	0.57	0.85	0.19	0.58	0.53	0.35	0.31	0.83	0.79
<i>C. narentana</i>	0.77	0.74	0.85	0.78	0.78	0.52	0.81	0.77	0.54	0.91	0.81
<i>C. illyrica</i>	0.12	0.18	0.76	0.68	0.34	0.82	0.41	0.41	0.42	0.74	0.70
<i>C. herzegoviniensis</i>	0.87	0.70	0.70	0.78	0.77	0.84	0.78	0.65	0.33	0.93	0.86

distance in relation to SL in all investigated species (Table 6). Due to different position, the impression of greater size of pectoral fins in males is even more pronounced. Prepectoral distance in females ranges between 18.7 and 25.3 % of SL, whereas in males it is 22.1-27.5 % of SL. This feature is quite uniform among species.

Between females and males of all species statistically significant differences in the length of their pelvic fins were also determined, although not so pronounced (t-test, $p < 0.05$; Table 6). The situation with the pelvic fins is the same as with the pectoral ones – in relation to SL they are longer in males than in females, although the difference is smaller. In females pelvic fins were 9.8-14.4 % of SL, in males their length was 10.8-17.9 % of SL (Table 4). However, inside the majority of species investigated there is almost none or only small overlap in this feature between females and males. Therefore, this character should also be considered as sexual dimorphism.

Besides mentioned features, also the head length in relation to SL turned out to be statistically significantly different between females and males of all species, whereas statistically significant differences in the caudal fin length in relation to SL were found in four species (with the exception of *C. bilineata*). Both characters were also larger in males, as were pectoral and pelvic fins, but with much smaller differences (Table 5). Differences in several other morphometric characters between sexes were also noticed for some species, but not consistently for all species and much less pronounced.

Other features of external morphology were the same among females and males inside certain population. Even though significant amount of intraspecific variability regarding body colouration and the scale

coverage has been recorded in several *Cobitis* species from the investigated area (Buj et al. 2014), none of those differences represent sexual diversity. Likewise, we did not find any difference among females and males in the appearance of scales on the body surface, the length and development of barbels, nor in the appearance of the urogenital papilla.

The results of the correlation analysis of each morphometric character with SL are presented in Tables 7 (for females) and Table 8 (for males). With the exception of *C. herzegoviniensis*, for all species more morphometric characters are significantly correlated with SL in females than in males.

Discussion

Based on the obtained results we can conclude that sexual dimorphism of spined loaches is visible in several morphometric features and the presence of the Canestrini scale in males. Besides differences in the body and pectoral fin lengths, this study confirms that sexual dimorphism of the species investigated also includes the position of the pectoral fins, as well as the length of the pelvic fins. Difference in the length of pelvic fins has already been noticed in the investigation of Jeleń et al. (2008) on *C. taenia*. Taking into account our results, it is likely that this sexual dimorphism pattern is also more widely represented inside the genus *Cobitis*. Jeleń et al. (2008) found statistically significant differences of linear correlation coefficients between females and males of *C. taenia* also in interorbital and predorsal distances, body depths, as well as fin lengths. We have found statistically significant difference between females and males in the length of pectoral and pelvic fins in all investigated species, in anal and caudal fins in some species, whereas dorsal fin length did not

differ among sexes. We have not found differences in the interorbital distance, but in the eye diameter and preorbital distance, so it is likely that some sexual dimorphism characters are species specific.

Mentioned differences in the length and position of the pectoral and pelvic fins are most probably connected with the sexual behaviour of *Cobitis* species. Although their exact function cannot be explained without thorough investigation of the reproductive behaviour of spined loaches, it can be suspected that they serve for stronger pressure of male on the female's body during mating, similarly as Bohlen (2008) proposed for the Canestrini scale. Namely, Bohlen noticed that during mating of several *Cobitis* species male wraps his body around female directly posterior to female's dorsal fin (Bohlen 2000). On the females of the majority of the species observed, after spawning he noticed whitish areas, so called spawning marks, presumably appearing due to male scratching female's body most probably with its Canestrini scale (Bohlen 2008). However, it is interesting that spawning marks were most visible on *C. taenia* Linnaeus, 1758 specimens; not so obvious, but also present in *C. elongatoides* Băcescu & Maier, 1969 and *C. melanoleuca* Nichols, 1925; whereas in the remaining species (including *C. bilineata*) spawning marks were not visible, although the spawning has occurred (Bohlen 2008). It is also noteworthy that, in the cases where spawning marks were present, they were located on both sides of the female's body, although male's head as well as his pectoral fin were reclined against one side of the female's body. Therefore, it is possible that the Canestrini scale is not responsible for spawning marks, but that male with his pectoral and pelvic fins presses and grazes female's body on both sides, inducing such marks in the species where fins are harder and longer. Our conclusion is in accordance with findings of Halačka et al. (2010) who rejected hypothesis that spawning marks are caused by abrasion and connected their appearance with nondestructive mechanical stimulation of the female's epidermis by the male during mating. Halačka et al. (2010) suggested mechanism of the formation of the spawning marks, but did not identify structures responsible for it and we find pectoral and pelvic fins of males as possible candidates. Considering that a

possible role of such pressure and grazing is for male to signalize and stimulate female to release eggs (with the aim to synchronize release of gametes in both partners, Bohlen 2008), we can suspect that larger surface of pectoral and pelvic fins in males can serve for stronger pressure and stronger signalization.

The correlation analysis revealed that a higher number of characters is significantly correlated with SL in females (Table 7), then in males (Table 8). Since the total and standard lengths are greatly influenced by the habitat conditions, especially food availability, all other body measures that are significantly correlated with SL are also greatly influenced by those conditions. Thereafter, based on the correlation analysis it is possible that ecological features have greater impact on the body dimensions of females, probably because they are more efficient in exploitation of the available resources. This hypothesis is in accordance with previous findings that morphological differences between sexes are ultimate consequences of differences in their reproductive roles, so that females are under selection to acquire, process and store more energy than males for production of offspring (Casselman & Schulte-Hostedde 2004). Henderson et al. (2003) explained higher growth rates of female *Sander vitreus* as consequences of greater food consumption and higher growth efficiency. Morita et al. (2015) found sex-dependent differences in the effect of the food availability on somatic growth of *Pleurogrammus azonus*. In light of the mentioned investigations and our results, it is possible that spined loaches are yet another example of different impact of environmental conditions on body sizes of females and males – a phenomenon that requires further attention.

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