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CONTRIBUTION TO THE MORPHOLOGY OF THE BALKAN LOACH, *COBITIS ELONGATA* HECKEL & KNER, 1858 IN CROATIA

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In Croatia, the Balkan loach, *Cobitis elongata* inhabits the Sava River basin. We found significant morphological differences between specimens inhabiting the Kupa River and those from its right tributary the Petrinjčica River. Results of ANOVA analysis were statistically significant for 17 measurements and all statistically significant parameters were considerably higher at the Petrinjčica River sites. These differences are probably caused by the spatial isolation of these two populations due to artificial and natural barriers on the Petrinjčica River and different ecological features of habitats in the Kupa and the Petrinjčica River.

Key words: *Cobitis elongata,* morphometric, meristic, Croatia

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U Hrvatskoj balkanski vijun *Cobitis elongata* naseljava sliv rijeke Save. Pronašli smo signifikantne morfološke razlike između primjeraka prikupljenih u rijeci Kupi i njenoj pritoci rijeci Petrinjčici. Rezultati ANOVA analize su statistički signifikantni za 17 mjera i svi statistički signifikantni parametri su značajno viši kod rijeke Petrinjčice. Razlike su vjerojatno uzrokovane izolacijom između tih dviju populacija uvjetovane umjetnim i prirodnim barijerama na rijeci Petrinjčici ali i različitim ekološkim značajkama staništa u rijekama Kupi i Petrinjčici.

Ključne riječi: balkanski vijun, morfometrija, meristika, Hrvatska

INTRODUCTION

The faunistic features, morphology, karyology, systematics, taxonomy, phylogeny, genetic diversity, feeding ecology, ethology, distribution and conservation of members of family Cobitidae are well studied (e.g. Bohlen, 2003; Bohlen *et al*., 2008, Boroń, 1999,

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2000; Boroń *et al.*, 2003; Boroń & Kotusz, 2000; Buj *et al.* 2008a, 2008b, 2014; Delić *et al.* 2003a, 2003b, 2009; Erk'akan *et al.*, 2008; Jelić *et al.*, 2012; Koščo *et al.*, 2008; Mičetić *et al.* 2008; Marić, & Pavlović, 2005; Mousavi-Sabet *et al.*, 2012; Mrakovčić *et al.*, 2000, 2004, 2006; Mustafić *et al.*, 2008; Özeren & Erk'Akan, 2000; Povž & Šumer, 2000, 2003; Šanda *et al.*, 2008; Valladolid & Przybylski, 2003; Vasil'eva & Vasil'ev, 2006, 2008). One of the most interesting genera in the family Cobitidae is the genus *Cobitis* (spined loaches). It has a relatively high diversity with approximately 60 freshwater species described (Buj *et al.*, 2014; Kottelat & Freyhof, 2007). The most recently described species is *Cobitis herzegoviniensis* Buj & Šanda, 2014 distributed in Bosnia and Herzegovina (Mostarsko blato, Herzegovina) (Buj *et al.*, 2014).

Croatia hosts an exceptionally high number of loaches, altogether nine species (Mrakovčić *et al.*, 2008). Several papers about different species of loaches in Croatia were published in the last decade (e.g. Buj *et al.*, 2008a; Delić *et al.*, 2009; Freyhof & Stelbrink, 2007; Mičetić *et al.*, 2008; Mrakovčić *et al.*, 2000; Zanella *et al.*, 2008). In those papers morphological studies of seven species from the family Cobitidae were conducted, for: *Cobitis bilineata* Canestrini, 1866, *Cobitis dalmatina* Karaman, 1928, *Cobitis jadovensis* Mustafić & Mrakovčić, 2008, *Cobitis illyrica* Freyhof & Stelbrink, 2007, *Cobitis narentana* Karaman, 1928, and *Sabanajewia balcanica* (Karaman, 1922) (Buj *et al.*, 2008b, 2014; Delić *et al.*, 2003b; Freyhof & Stelbrink, 2007; Mustafić *et al.*, 2008), but not for *Cobitis elongata* Heckel & Kner, 1858 and *C. elongatoides* Bacescu & Maier, 1969.

In Croatia, the Balkan loach is an indigenous species occurring in the Sava River basin (Danube River drainage) (Ivelić *et al.*, 2007; Mrakovčić *et al.*, 2008; Povž & Šumer, 2000). Further, it occurs in Montenegro, Romania, Slovenia & Serbia (Bănărescu, 1964; Kottelat & Freyhof, 2007, Marić & Pavlović, 2005: Mrakovčić *et al.*, 2006; Povž & Šumer, 2000). It is endemic for the Danube River drainage and its *terra typica* is the upper part of the Sava River (Bănărescu & Nalbant, 1957). Ivelić *et al.* (2007) published meristic data for *C. elongata* for the River Sava in Croatia. The first data about this species in Croatia came from the Kupa River (Medić, 1905). According to the IUCN and the Red List of Fishes of Croatia, *C. elongata* is listed as vulnerable (VU) because of its small distribution area, specific habitat preferences, as well as the constant river engineering and pollution (Mrakovčić *et al.*, 2000; 2004; 2006).

Data concerning both the distribution and the morphology of this species are still scarce, apart from the original description of Heckel & Kner (1858). Additional data may be found in papers by Bănărescu & Nalbant (1957), Povž & Šumer (2003) and (Šorić, 1985). Filling this gap is the task of the present paper. We are also interested in finding if there are some differences in the morphological characters between two spatially isolated populations inhabiting two closely located streams, the Kupa River and its right tributary, the Petrinjčica River. In addition, this is a contribution to the morphology of the Balkan loach in Croatia in which we compare our results with literature data from the rest of the distribution area: Romania (Bănărescu, 1964), Slovenia (Povž & Šumer, 2003) and Serbia (Šorić, 1985).

MATERIAL AND METHODS

Study area

The Kupa River basin, located in the central part of Croatia, extends over an area of 10.236 km^2 (Fig. 1). In the border area between Croatia and Slovenia, the Kupa River has

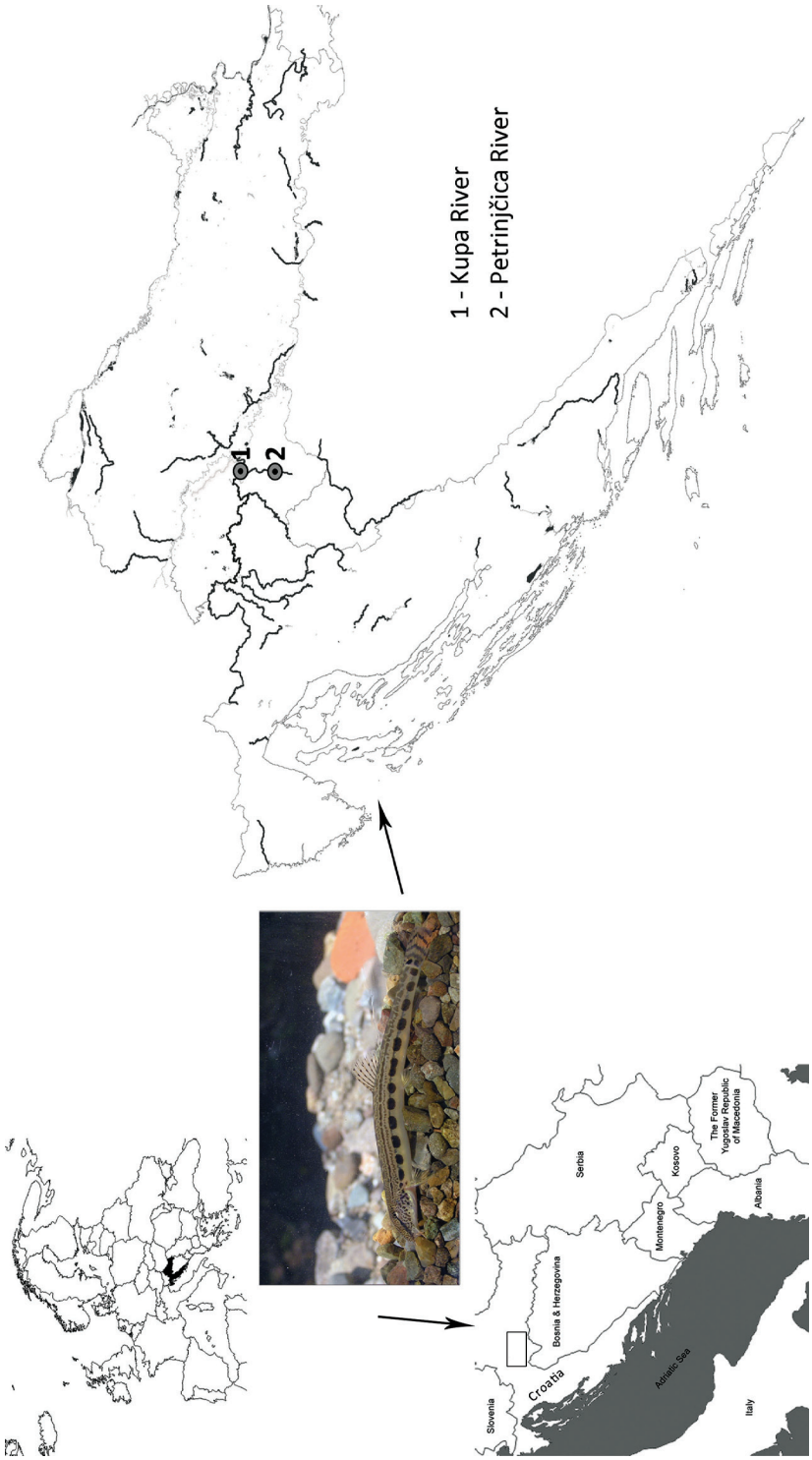
1 - Kupa River
2 - Petrinjčica River
1
2
Slovenia
Croatia
Bosnia & Herzegovina
Serbia
Montenegro
Kosovo
The Former Yugoslav Republic of Macedonia
Albania
Adriatic Sea
Italy

Fig. 1.

Tab. 1. Measurements of *Cobitis elongata* – our data, compared with the data of Bănărescu (1964), Šorić (1985) and Povž & Šumer (2003).

	Bănărescu n = 35 Nera River	Šorić n = 55 Gruža River			Povž & Šumer n = 31 Kupa River				Present study, Kupa River n = 22			Present study Petrinjčica River n = 23		
Characters	min-max	M	SD	min-max	M	SD	min-max	r	M	SD	min-max	M	SD	min-max
Tl mm	113.0-165.0				141.9	9.95	126.0-163.0		120.3	15.29	100.6-148.0	127.7	20.24	98.0-164.0
W g					10.5	2.46	6.0-14.0							
Sl mm									106.1	13.60	88.2-130.3	112.3	17.92	85.8-144.6
Sl in % ***Tl***		89.2	1.06	86.5-92.4	90.1	0.61	88.9-91.4							
in % of ***Sl***														
lc	16-19.6	17.7	0.66	16.4-19.5	17.0	0.64	16.2-17.9	0.86	18.2	0.63	16.6-19.2	18.5	1.27	14.4-20.6
prO									8.4	0.49	7.4-9.5	9.0	0.90	7.6-10.5
ina									2.3	0.21	1.9-2.7	2.4	0.25	2.0-2.8
io									2.7	0.25	2.1-3.0	2.9	0.25	2.3-3.3
poO									7.7	0.41	7.0-8.7	11.0	15.56	6.9-8.2
lpc	14.6-18.4	16.7	0.92	14.8-18.8	14.3	0.78	13.0-15.8	0.69	14.7	0.76	13.1-15.7	14.5	0.56	13.4-15.6
hc					8.5	0.28	7.9-9.2	0.88	9.1	0.40	8.5-9.8	9.7	0.51	8.6-10.9
lac									7.4	0.52	6.5-8.7	8.1	0.59	6.8-9.3
laco									7.9	0.65	6.3-9.0	8.7	0.50	7.8-9.8
hpc									6.4	0.36	5.7-7.2	6.9	0.39	6.2-8.0
lapc									4.1	0.24	3.7-4.6	4.2	0.29	3.5-4.6
H	9.7-12.1	12.2	0.89	10.9-14.8	9.7	0.54	8.7-10.7	0.80	10.7	0.71	9.1-12.5	11.2	0.64	9.9-12.5
h	5.9-7.4	6.9	0.44	5.9-8.5	5.8	0.39	5.0-6.2	0.71	6.1	0.33	5.6-6.8	6.6	0.37	6.2-7.8
Oh	2.2-3.4				2.1	0.16	1.9-2.3	0.85	2.6	0.27	2.2-3.1	2.8	0.29	2.4-3.4

	Bănărescu n = 35 Nera River	Šorić n = 55 Gruža River			Povž & Šumer n = 31 Kupa River				Present study, Kupa River n = 22			Present study Petrinjčica River n = 23		
pD	50.0-55.0	51.9	1.25	48.8-54.9	50.5	0.92	49.0-52.7	0.97	53.2	0.91	51.3-54.9	53.1	0.96	51.2-55.2
pV	50.5-55.5				53.8	1.04	52.0-55.9	0.97	54.4	0.98	51.4-55.5	55.0	0.92	53.3-57.0
pA	74.0-80.5	77.7	1.52	74.3-82.8	78.8	1.08	77.1-82.2	0.98	79.8	0.87	77.8-81.9	80.3	2.05	79.1-89.1
pP									19.4	0.71	18.1-21.1	18.9	1.10	14.8-20.5
P-V									35.7	1.25	33.8-38.0	36.5	1.06	33.9-38.7
V-A									26.0	1.05	24.6-28.4	25.2	0.78	23.8-27.2
lP	10.6-13.1				9.5	0.68	8.6-11.0	0.93	11.8	0.68	10.5-13.0	11.2	0.80	9.8-12.8
lV	9.0-11.3				9.2	0.42	8.6-10.4	0.77	10.3	0.61	9.5-11.7	10.1	0.40	9.4-10.9
lD					8.8	0.59	7.8-10.0	0.79	8.1	0.77	6.6-9.6	8.6	0.59	7.5-9.7
lA					5.5	0.29	5.2-6.7	0.88	5.5	0.52	4.0-6.3	5.9	0.43	5.1-6.6
hD					10.9	0.46	10.1-12.1	0.89	12.2	0.76	11.0-13.5	12.4	0.80	10.6-13.7
hA					9.6	0.89	8.3-11.0	0.77	10.1	0.71	8.8-11.4	10.3	0.65	9.3-11.5
IC									13.4	0.78	11.4-14.8	13.3	0.67	11.5-14.5
In % of lc														
Oh	11.3-18.6	16.4	1.48	13.4-18.8	12.5	1.20	11.0-14.3		14.6	1.49	11.6-16.6	15.4	2.05	12.6-19.9
hc		55.9	2.14	50.7-61.5	50.3	2.31	46.2-54.5		50.2	1.62	46.7-52.8	52.8	4.00	47.0-67.2
In % of H														
h					60.7	6.16	50.0-70.0		57.5	4.78	47.2-65.2	59.4	4.03	51.3-71.3
In % of lpc														
h					40.9	3.56	33.3-47.1		42.0	3.82	35.9-48.8	45.8	3.09	40.6-52.8

Abbreviations: n, sample size; min-max, minimum and maximum of a set of sample values; M, mean value; SD, standard deviation; r, Simple linear correlation coefficient (Pearson's).

all the characteristics of a mountain river, while downstream from Karlovac and all the way to its mouth into the Sava River, Kupa is a typical lowland river. Sampling of the material was done in June and July 2003 by electro fishing in the Kupa River at the sampling site of Brest (N 45°27′23.1′′, E 16°15′6.67′′, 96 m a.s.l.). The width of the stream at the sampling site is 60 to 70 m and the river bottom is mostly muddy, apart from small areas covered by sand and gravel.

The Petrinjčica River (Fig. 1) is a right tributary of the Kupa River, with a length of 33.5 km. The Petrinjčica River basin occupies an area of 135.2 km^2 (Fig. 1). The sampling site on the Petrinjčica River at Dodoši (N 45°26′28.6′′, E 16°16′23.1′′, 350 m a.s.l.) (Fig. 1) is 30 km distant from the confluence with the Kupa River (110 m a.s.l.). Between those two sampling areas seven cascades (three natural and four artificial) prevent upstream fish migration. The Petrinjčica River is from three to six meters wide at the sampling site with the riverbed covered with large stones, and the riffles with gravel and sand. Sampling of the material was done in June and July 2003 by electro fishing.

Morphometric and meristic characters

Morphometric and meristic data were taken following the standard methods (Holčik, 1989; 1998) on 23 specimens of *Cobitis elongata* (Fig. 1) from the Petrinjčica River and 22 specimens from the Kupa River (Tab. 1). Morphometric characteristics were measured using mechanical calliper measuring straight-line distances directly on the specimens.

The following morphometric characteristics were measured: *Tl*, total body length; *Sl*, standard length - the distance between the most anterior part of the head and the border of the outwardly visible base of the caudal fin; *lc*, head length; *prO*, preorbital distance; *ina*, distance between nostrils; *Oh*, horizontal diameter of the eye; *io*, interorbital distance; *poO*, postorbital distance; *hc*, head height; *lac*, head width; *pD*, predorsal distance; *pV*, preventral distance; *pA*, preanal distance; *pP*, prepectoral distance; *H*, body height – at base of D (maximum); *laco*, body width; *lpc*, caudal peduncle length; *hpc*, caudal peduncle height; *lapc*, caudal peduncle width; *h*, minimum body height; *P-V*, distance between pectoral fin base and ventral fin base; *V-A*, distance between ventral fin base and anal fin base; *lD*, length of dorsal fin base; *lA*, length of anal fin base; *lC*, length of caudal fin; *lP*, pectoral fin length; *lV*, ventral fin length; *hD*, length of dorsal fin; *hA*, length of anal fin.

Meristic characteristics were analysed by number on branched and unbranched rays on: dorsal fin - D, anal fin - A, pectoral fin - P and ventral fin – V. Number of branched rays is marked with Roman numbers and number of unbranched rays is marked with Arabic numbers.

In defining meristical features in species from the Cobitidae family there are certain concerns to be addressed as discussed in Schneider (Schneider, 1998). In most species of Cypriniformes groups, including the family Cobitidae, the first unbranched fin rays are not well seen as they are small and covered with skin (pers. comm. R. Šanda). Therefore, a layer of skin was removed in order to determine the presence of these small unbranched fin rays. In branched fin rays, in both dorsal and anal fins, the last two branched fin rays were counted as one as they grow from one pterigiofor.

We compared morphometric and meristic data of *Cobitis elongata* from the Petrinjčica River and the Kupa River with morfometric and meristic data of this species from the papers of Bănărescu (1964), Povž & Šumer (2003) and Šorić (1985). Because of different ways of measuring we did not compare our results with those of *C. elongata* from the

Tab. 2. Morphometric comparation of *Cobitis elongata* (sexes combined) from the Kupa River and Petrinjčica River. Measurements marked by* are statistically significant at α 0.05.

Kupa – Petrinjčica	ANOVA	
	F	p
In % of Sl		
lc	1.169	0.286
prO	6.288*	0.016
ina	3.606	0.064
io	4.608*	0.038
poO	1.003	0.322
lpc	0.518	0.475
hc	19.181*	0.000
lac	15.325*	0.000
laco	20.705*	0.000
hpc	20.678*	0.000
lapc	1.911	0.174
H	6.575*	0.014
h	24.057*	0.000
In % of lc		
Oh	5.223*	0.027
pD	0.072	0.789
pV	5.348*	0.026
pA	1.233	0.273
pP	3.074	0.087
P-V	5.474*	0.024
V-A	8.266*	0.006
lP	6.763*	0.013
lV	3.054	0.088
lD	6.273*	0.016
lA	9.031*	0.004
hD	0.625	0.434
hA	1.480	0.230
IC	0.191	0.664
Oh	2.490	0.122
hc	7.837*	0.008
In % of H		
h	1.906	0.175
In % of lpc		
h	13.709*	0.001

Abbreviations: F, variance analysis; p, degree of significance.

paper by Ivelić *et al*. (2007). Ivelić *et al*. (2007) did not include meristic features of *C. elongata* in their investigation.

Pearson's correlation matrices were used to determine the degree of correlation between fundamental morphometric measurements and stepwise multiple regression analysis in order to explain the total variation of *Sl*. ANOVA was used to test the differences in measurements between the Kupa River and the Petrinjčica River specimens. Direct measurements (mm) were used for calculating correlations and regression analysis. The SPSS 12.0 software package was used for all statistical procedures.

RESULTS AND DISCUSSION

This paper presents the measurements and counts of *Cobitis elongata* from the Petrinjčica River (n=23) and the Kupa River (n=22) (Tab. 1). Analysing the data assigned to the total body length (*Tl*) we have found considerable differences in the specimens collected from both rivers.

The highest measured value of total body length (*Tl*) of specimens measured from the Kupa River (148.0 mm) was low compared with the data for the Petrinjčica River (164.0 mm), the Nera River in Romania (165.0 mm) (Bănărescu, 1964) or the Slovenian part of the Kupa River (163.0 mm) (Povž & Šumer, 2003). The lowest value of *Tl* of specimens measured from the Petrinjčica River (98.0 mm) was low compared with the data for the Kupa River (100.6 mm) Nera River (113.0 mm) (Bănărescu, 1964) or the Slovenian part of the Kupa River (126.0 mm) (Povž & Šumer, 2003) (Tab. 1).

ANOVA analysis revealed statistically significant differences in both counts and measurements between Kupa and Petrinjčica populations. ANOVA was statistically significant for 17 measurements

Tab. 3. Meristic characteristics for *Cobitis elongata* from the Kupa River, the Petrinjčica River and from literature: Banarescu (1964), Heckel & Kner (1858), Karaman (1952), Povž & Šumer (2003) and Šorić (1985).

Literature and our research	n	D	A	P	V
Heckel & Kner (1858)	2	III/7	III/5-6	I/7	I/6
Karaman (1952)	2	II/7	II/6	II/7	I/6
Banarescu (1964)	35	II-III/7	II/6		
Šorić (1985)	55	III/6-8	III/4-6	I/6-8	II/5-6
Povž & Šumer (2003)	31	II/7-8	I-III/5-6	II/6-8	I-II/6-7
Kupa River (our data)	22	II-III/7-8	II-III/5	I/7	I-II/6
Petrinjčica River (our data)	23	II-III/7-8	II-III/5	I/7	I-II/6

Abbreviations: n - sample size; D - dorsalia; A - analia; P - pectoralia; V - ventralia.

(Tab. 2). These results imply that populations from both rivers are probably partially isolated and specific ecological features in those habitats could have an influence on morphological features of species inhabiting those rivers, including *C. elongata*.

In order to determine the correlation between *Sl* and other measurements, Pearson's correlation coefficient was calculated. For the Kupa River, all measurements were significantly positively correlated, and for the Petrinjčica all measurements except *poO* were significantly positively correlated with *Sl*. In order to explain the variation of *Sl*, stepwise multiple regression analysis was conducted. For the Kupa River population, 99.7% of *Sl* variance was explained. Predictors that explained the most variance were *pA* (β=0.825), *lpc* (β=0.109) and *IA* (β=0.082). For the Petrinjčica River population, 99.6% of *Sl* variance was explained. Significant predictors were *pV* (β=0.640), *V-A* (β=0.301) and *lapc* (β=0.071).

Comparison of meristic characteristics for the species *Cobitis elongata* from Heckel & Kner (1858); Bănărescu (1964); Šorić (1985), Povž & Šumer (2003) and the data from this study indicates some differences in the number of branched and unbranched fin rays as well as in the boundaries of variability in populations (Tab. 3). Results of meristic characteristics according to Heckel & Kner (1858) and Karaman (1952) must be taken with precaution because they do not reflect variability in analysed populations, as only two specimens were considered. The least variability was found between our results and those of Povž & Šumer (2003), which is to be expected due to the geographical position and proximity of the areas.

Morphometric and meristic differences in populations of *Cobitis elongata* in the Kupa and Petrinjčica rivers (Tabs. 1, 3) as we have already noted, are most probably caused by the spatial isolation of these two populations due to the artificial and natural barriers on the Petrinjčica River and the different ecological features in these two rivers. During high water levels on both rivers, communication of fish populations is still possible (unpublished data M. Bučar), causing minor morphometric differences, while meristic features are the same.

Results of molecular analyses of specimens collected in the Sava and Kupa Rivers showed that *Cobitis elongata* in Croatia are contained in the "Elongata" clade with two separate lineages (p-distance: in the range of 1.0-1.1 %) (Buj *et al.*, 2008a). These data indicate only the intraspecific variability of this species. Genetic data of the species *Sa-*

banejewia balcanica in several rivers of the continental part of Croatia display the same pattern. These data also show similar genetic features of populations of *S. balcanica* in the rivers Ilova, Petrinjčica, Rijeka and Una (Marešova *et al.*, 2011).

Research on caddisflies (Trichoptera) from the Banovina region (Kučinić *et al.*, 2010, 2013) displayed interesting processes of speciation in this group in this part of Croatia (Kučinić *et al.*, 2013). Similar processes may possibly be found in some species of the family Cobitidae and other fishes in the Banovina region. In the future, the family Cobitidae in the Banovina region and adjacent areas should be nvestigated from an ecological point of view after detailed distribution of species is revealed. These studies should also included genetic research into different fish species from the Petrinjčica River and other small streams in the Banovina region and adjacent areas.

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SAŽETAK

Prinos poznavanju morfologije balkanskog vijuna, *Cobitis elongata* Heckel & Kner, 1858 u Hrvatskoj

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U radu se daje prilog poznavanju morfoloških značajki vrste *Cobitis elongata* Heckl & Kner, 1858 u Hrvatskoj, prikupljenih na području rijeke Petrinjčice i rijeke Kupe. Analiza tih značajki ukazuje na određene razlike u usporedbi s literaturnim navodima Bănărescu (1964), Povž & Šumer (2003) i Šorić (1985), kao i na manje razlike između populacija u rijekama Kupi i Petrinjčici. Razlike između populacija u Petrinjčici i Kupi najvjerojatnije su uvjetovane izolacijom tih dviju tokova zbog 7 barijera (3 prirodne i 4 umjetne) koje se nalaze na rijeci Petrinjčici, kao i različitim ekološkim značajkama na lokacijama prikupljanja materijala u tim dvjema rijekama. Tijekom visokih voda rijeke Kupe i Petrinjčice moguća je i direktna komunikacija riba iz tih dvaju vodotokova što uvjetuje manje razlike između morfoloških značajki vrste *C. elongata*, dok su merističke značajke iste. Buduća istraživanja vrsta iz porodice Cobitidae, ali i ostalih ribljih vrsta na području Banovine treba usmjeriti na utvrđivanje ekoloških značajki, detaljnije rasprostranjenosti, kao i određenih genetskih značajki pojedinih populacija na tom i susjednim područjima.