

## Distribution and taxonomic relationships of spined loaches (Cobitidae, *Cobitis*) in the River Neretva basin, Bosnia and Herzegovina

Radek ŠANDA<sup>1,6,\*</sup>, Ivan BOGUT<sup>2,9</sup>, Ignacio DOADRIO<sup>3</sup>, Jan KOHOUT<sup>4,7</sup>, Anabel PERDICES<sup>3</sup>, Silvia PEREA<sup>3</sup>, Alena ŠEDIVÁ<sup>4,8</sup> and Jasna VUKIĆ<sup>5</sup>

<sup>1</sup> National Museum, Václavské náměstí 68, 115 79 Praha 1, Czech Republic; e-mail: rsanda@seznam.cz

<sup>2</sup> Institute of Fisheries, Zoology and Water Protection, Faculty of Agronomy, University of Mostar, Biskupa Čule 10, 88000 Mostar, Bosnia and Herzegovina

<sup>3</sup> Museo Nacional de Ciencias Naturales, C/José Gutiérrez Abascal 2, 28006 Madrid, Spain

<sup>4</sup> Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, v.v.i., Rumburská 89, 277 21 Liběchov, Czech Republic

<sup>5</sup> Department of Ecology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic

<sup>6</sup> Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 44 Praha 2, Czech Republic

<sup>7</sup> Research Institute of Fish Culture and Hydrobiology at Vodňany, University of South Bohemia Česká Budějovice, Zátíší 728/II, 389 25 Vodňany, Czech Republic

<sup>8</sup> Institute of Zoology of the Slovak Academy of Sciences, Dúbravská cesta 9, 845 06 Bratislava, Slovakia

<sup>9</sup> Institute of Special Zootechniques, Faculty of Agriculture, Josip Jujar Strossmayer University of Osijek, Trg Sv. Trojstva 3, 31000 Osijek, Croatia

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**Abstract.** In July 2004 and 2006, tributaries of the River Neretva and surrounding karstic fields (polje) in Bosnia and Herzegovina were sampled, and new data on spined loaches (*Cobitis*) and accompanying freshwater fish fauna gathered. Spined loaches were found in the River Bregava and Hutovo blato wetland, which are directly connected to the River Neretva, in the River Trebišnjica in Popovo polje, in Lake Krenica and the River Matica in Imotsko polje, and in the River Lištica drainage in Mostarsko blato. However, *Cobitis* were not found in karstic fields situated more westward (Livanjsko polje, Duvanjsko p., Kupreško p. and Glamočko p.), nor in karstic fields situated northward from the River Trebišnjica (Nevesinjsko polje, Fatničko p., Dabarsko p. and Gatačko p.). Neither were they found in the River Trebižat, which lies closer to the River Neretva than does Imotsko polje. Based on morphological and molecular characters, spined loaches inhabiting the rivers Trebišnjica and Bregava, and Hutovo blato were identified as *Cobitis narentana* Karaman, 1928, while specimens from Imotsko polje and Mostarsko blato were classified as *Cobitis* sp. Until now, a single species of spined loach, *C. narentana*, had been recorded from the lowermost part of the River Neretva basin. Our new findings suggest that the species diversity of the spined loaches in the Neretva basin in Bosnia and Herzegovina might be underestimated and that a detailed taxonomic study is required to determine the *Cobitis* diversity in this area.

**Key words:** *Cobitis*, Adriatic Sea basin, conservation, diversity

### Introduction

Spined loaches (Cobitidae) comprise a large family of cypriniform fishes inhabiting Eurasia and northern Morocco in Africa (Nalbant et al. 2001). These freshwater fishes are typical bottom burrowing dwellers (Slavík & Ráb 1996). Due to this habit and the fact that they have no commercial value, they are easily overlooked. Thus, knowledge of the distribution of cobitid fishes is still insufficient in many areas of their range. In particular, data on their

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\*Corresponding author

distribution in Bosnia and Herzegovina are very scarce. There are only old or general notes from the Danube drainage (Zaplata & Taler 1933, Karaman 1963, Vuković 1977) and a very general note from the River Neretva drainage, which belongs to the Adriatic Sea watershed (Vuković 1977). The Neretva drainage in Bosnia and Herzegovina deserves special attention, as this basin is well known for a high degree of endemism of freshwater fishes (Mrakovčić et al. 1995, Banarescu & Herzig-Straschil 1998, Bogutskaya & Zupančič 1999, Zupančič & Bogutskaya 2002). It is noteworthy that several new fish species have been described from this drainage during the last decade (Mrakovčić et al. 1996, Bogutskaya & Zupančič 2003, Kováčič 2005).

*Cobitis narentana* Karaman, 1928 is the only *Cobitis* species that has been mentioned from the Neretva drainage (Vuković 1977, Mrakovčić et al. 1995, 2000), and exact data on distribution of *Cobitis* populations are recorded only for the lowermost part of this drainage in Croatia (Schneider et al. 2000). Although Vuković (1977) considered the lower Neretva in Bosnia and Herzegovina as a distribution area for loaches, they have never been reported here (Kosorić & Vuković 1966, Aganović 1969, Kosorić 1974, 1977, 1978, Kosorić et al. 1989).

The taxonomy of spined loaches in the wider Adriatic Sea drainage is also insufficiently known. In areas adjacent to the Neretva drainage, *Cobitis dalmatina* Karaman, 1928 occurs in the River Cetina (Mrakovčić et al. 2000) and *Cobitis ohridana* Karaman, 1928 in the Ohrid-Drin-Skadar system (Bohlen et al. 2003). Recently, new *Cobitis* mtDNA lineages were identified in waters that ultimately drain into the Adriatic Sea, in isolated karstic fields in the vicinity of the Neretva drainage and in northern Dalmatia (Buj et al. 2008). It is likely that the taxonomic diversity of *Cobitis* in the Neretva drainage, or maybe in the whole Dalmatian ichthyological district as defined by Bianco (1990), is underestimated.

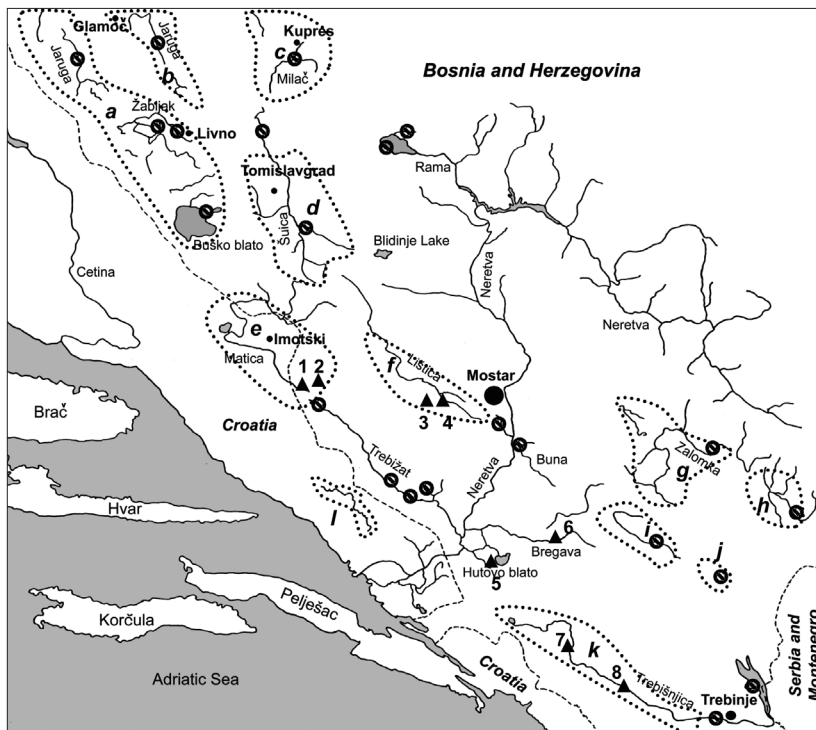
The aims of this study were therefore to provide new data on the distribution of *Cobitis* in the River Neretva drainage in particular, and in related hydrological systems in Bosnia and Herzegovina, and to discuss their taxonomic relationships with other populations of *Cobitis* from surrounding areas.

## Material and Methods

In July 2004 and 2006, field research was conducted in the River Neretva basin and surrounding karstic fields in Bosnia and Herzegovina (see Fig. 1). Portable engine or battery electrofishing gear and, where suitable, a 10 m long seine net, with mesh size of 10 mm, were used. Specimens were individually tagged and preserved in 5% formaldehyde, and a small piece of tissue was preserved in ethanol. Voucher specimens were deposited in the Museo Nacional de Ciencias Naturales in Madrid, Spain, and in the National Museum in Prague, Czech Republic. Geographic coordinates were measured with a Garmin eTrex personal navigator. In some localities, pH, temperature and conductivity were measured with a Combo HI-98130 multimeter (Hanna Instruments).

## Results

In all, 30 localities were sampled in southern Bosnia and Herzegovina (Fig. 1). *Cobitis* specimens were found only in eight localities, of which two (River Bregava and Hutovo blato) are connected directly with the River Neretva, while other localities represented isolated drainages in karstic fields



**Fig. 1.** Distribution of *Cobitis* in the River Neretva drainage, Bosnia and Herzegovina. ▲ indicates place where *Cobitis* was recorded; numbers correspond to those of localities in Table 1. ⊙ indicates place with no *Cobitis* caught. Dotted lines delimit independent karstic fields (a, Livanjsko polje; b, Glamočko polje; c, Kupreško polje; d, Duvanjsko polje; e, Imotsko polje; f, Mostarsko blato; g, Nevesinjsko polje; h, Gatačko polje; i, Dabarsko polje; j, Fatničko polje; k, Popovo polje; l, Jezero polje).

(local name polje): River Trebišnjica in Popovo polje, Lake Krenica and River Matica in Imotsko polje, and River Lištica and channels in Mostarsko blato. A basic description of the sample locations is reported in Table 1. In general, *Cobitis* was much more abundant in places with a soft bottom and low or no water current (Lake Krenica, Hutovo blato, Trebišnjica near Ravno, Mostarsko blato) than in flowing water with a hard bottom (River Matica, Trebišnjica at Dobromani, Bregava), where only very small numbers or a single specimen was recorded (see Table 1).

Identification of *Cobitis* specimens, based on morphology and coloration patterns, was difficult. Specimens from Rivers Bregava and Trebišnjica and Hutovo blato with a robust body, a short head with a steeply inclined anterior, overall intensive coloration, and a conspicuous black spot in the upper part of the caudal fin base were preliminarily identified as *Cobitis narentana* (its type locality is River Neretva near Metković). Those from Imotsko polje and Mostarsko blato with a rather slender body, elongated head, overall less intensive coloration and a less intense dark spot (sometimes even absent) in the upper part of the caudal fin base, were preliminarily classified as *Cobitis* sp.

## Discussion

The distribution of *Cobitis* is documented here for tributaries of the River Neretva and surrounding karstic fields in Bosnia and Herzegovina (Fig. 1), from where spined loaches

**Table 1.** Description of localities with occurrence of *Cobitis*. Sample location numbers correspond to those in Fig. 1.

Locality	Coordinates and altitude	Width and depth	Flow characteristic, pH, conductivity, temperature, substrate, vegetation	Species composition; number of <i>Cobitis</i> specimens caught in 2004/2006 in parentheses; NS, not sampled
1. River Matrica in Imotsko polje	N 43° 22' 17.1" E 17° 16' 38.6" 251 m a.s.l.	5–8 m ≤2 m	flowing water, hard bottom, mostly gravel with sand, wooden debris, no vegetation	<i>Cobitis</i> sp. (1/NS), <i>Rutilus basak</i> , <i>Delminichthys adpersus</i>
2. Lake Krenica in Imotsko polje	N 43° 22' 41.3" E 17° 19' 98.3" 257 m a.s.l.		stagnant, pH = 8.44, 220 $\mu\text{S}\cdot\text{cm}^{-2}$ , 26°C, gravel or fine sediment in the shallows, scattered macrophytes	<i>Cobitis</i> sp. (67/18), <i>Cyprinus carpio</i> , <i>Carassius gibelio</i> , <i>Rutilus basak</i> , <i>Squalius microlepis</i> , <i>Delminichthys adpersus</i>
3. Channels in Mostarsko blato	N 43° 19' 21.6" E 17° 43' 5.94" 218 m a.s.l.	2–6 m ≤1 m	stagnant, pools, pH = 7.39, 540 $\mu\text{S}\cdot\text{cm}^{-2}$ , 22.4°C, fine sediment, dense macrophytes	<i>Cobitis</i> sp. (NS/20), <i>Phoxinellus pseudalepidotus</i>
4. River Lištica in Mostarsko blato	not measured	7–10 m ≤2 m	flowing water, gravel and small stones, fine sediment around banks, locally dense macrophytes	<i>Cobitis</i> sp. (66/0), <i>Phoxinellus pseudalepidotus</i>
5. Channel in Hutovo blato wetland	N 43° 03' 86.0" E 17° 45' 29.8" 31 m a.s.l.	15–25 m ≤2 m	almost stagnant, pH = 7.63, 790 $\mu\text{S}\cdot\text{cm}^{-2}$ , 23.9°C, fine sediment, locally dense macrophytes, floating leaves of <i>Nymphaea</i>	<i>Cobitis narentana</i> (20/30), <i>Carassius gibelio</i> , <i>Rutilus basak</i> , <i>Alburnus</i> sp., <i>Scardinius plottizza</i> , <i>Tinca tinca</i> , <i>Lepomis gibbosus</i> , <i>Pseudorasbora parva</i> , <i>Squalius squalus</i> , <i>Gambusia affinis</i> , <i>Knipowitschia croatica</i> , <i>K. radovici</i> , <i>Gasterosteus gymnotus</i> , <i>Chondrostoma kneri</i>
6. River Bregava in Stolac	N: 43° 04' 98.4" E 17° 57' 15.2" 51 m a.s.l.	5–7 m ≤3 m	flowing water, rocks and gravel, no vegetation	<i>Cobitis narentana</i> (3/NS), <i>Alburnus</i> sp., <i>Squalius squalus</i> , <i>S. svallize</i> , <i>Phoxinus lumaireul</i> , <i>Salmo farioideus</i>
7. River Trebišnjica near Ravno	N 42° 53' 02.4" E 17° 58' 94.3" 237 m a.s.l.	20–50 m ≤1 m	almost stagnant to flowing, pH = 8.23, 180 $\mu\text{S}\cdot\text{cm}^{-2}$ , 22°C, concrete bottom covered with fine sediment and filamentous algae	<i>Cobitis narentana</i> (132/35), <i>Cyprinus carpio</i> , <i>Squalius squalus</i> , <i>Tinca tinca</i> , <i>Alburnus</i> sp., <i>Phoxinus lumaireul</i> , <i>Lepomis gibbosus</i>
8. River Trebišnjica near Dobromani	N 42° 47' 26.2" E 18° 09' 07.2" 278 m a.s.l.	20 m ≤1.2 m	flowing water, concrete bottom, scattered filamentous algae	<i>Cobitis narentana</i> (NS/1), <i>Squalius</i> sp.

have never previously been reported (Kosorić & Vuković 1966, Aganović 1969, Kosorić 1974, 1977, 1978, Kosorić et al. 1989). Their existence here highlights the issue of their correct taxonomic classification and their phylogenetic relationships, particularly as some of the *Cobitis* populations investigated in this study are isolated in karstic fields.

Recent molecular analysis of these *Cobitis* populations has revealed that they do not form a monophyletic clade, but instead represent two distinct mitochondrial groups (*C. narentana* and *Cobitis* sp. A) phylogenetically related to the Adriatic species *C. dalmatina* and *Cobitis bilineata* Canestrini, 1866 (Perdices et al. 2008). Nevertheless, all taxa belong to the Adriatic spined loach lineage as defined by Bohlen et al. (2006). Furthermore, a recent study on the Croatian *Cobitis* populations from the Neretva drainage, including specimens from the karstic field Jezero, shows that the Croatian populations also do not form a monophyletic group (Buj et al. 2008). Therefore, it seems that the *Cobitis* diversity in the Neretva basin is probably underestimated and further detailed taxonomic research is needed to resolve this issue.

Another important issue is the conservation status of the *Cobitis* taxa in the studied area. Although from the data obtained it is not possible to make definitive conclusions, some facts are remarkable. Firstly, all sampled populations inhabit relatively small areas with very rare suitable habitats. For example, most of the River Trebišnjica is canalised, with no cover and with minimum sediment. In this river, *C. narentana* was abundant only under the bridge near Ravno, where the water was almost stagnant and with a layer of fine sediment covered by filamentous algae (see Table 1). In Mostarsko blato, *Cobitis* inhabits the main stream (River Lištica), as well as minor channels. These channels can completely dry out during summer, while the habitats with fine sediment in the main river are also probably changeable, depending on flooding regime. Variability in the distribution of *Cobitis* is here documented by the fact that in the same place in this river, where *Cobitis* sp. was abundant in July 2004, not a single specimen was found in July 2006 (see Table 1). In Imotsko polje, *Cobitis* sp. was found in higher numbers only in the shallow part of the small Lake Krenica (5.8 ha, max. depth 78 m), while in the River Matica only a single specimen was found. This river generally does not provide suitable habitats for spined loaches, as it is quite fast flowing, with a hard bottom and cold water. Surprisingly, *Cobitis* was found in the upper part of the River Bregava, in habitats more suitable for salmonids. The best conditions for *Cobitis* exist in Hutovo blato, a large wetland with numerous suitable habitats.

Most of the populations of spined loaches sampled here should be treated as vulnerable due to their limited area of distribution and fragmentation of their habitat. Detailed research is necessary to obtain precise estimates of their population sizes, life histories and biology.

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