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Taxonomic diversity of spined loaches (Cobitidae: Cobitis) in the Caucasus

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The spined loaches from the genus *Cobitis* Linnaeus, 1758 represent a species-rich group of small fishes of fam. Cobitidae distributed in Europe, Northern Africa, and Asia, where new species are still discovering. Despite of high species diversity in this group, the only species *Cobitis satunini* Gladkov, 1935 was recognized in Azerbaijan, Armenia, and Georgia until the description of *C. amphilekta* Vasil'eva & Vasil'ev, 2012 from the Lankaran region of Azerbaijan and the discovery of the Iranian loach *C. saniae* Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh & Esmaeili, 2017 in Azerbaijan, Armenia, and Georgia (Freyhof et al., 2018). The aim of our study was to define the number of *Cobitis* species in the Caucasus, to determine their phylogenetic relationships and recent distribution.

METHODOLOGY AND MATERIALS

We analyzed the fragment of cytochrome c oxidase gene (COI) of mitochondrial DNA and the exon of recombination activating gene (RAG1) of nuclear DNA in the samples collected in Azerbaijan, Iran and Georgia from 2010 to 2018. We used primers and protocols described earlier (Ivanova et al., 2006; Perdices et al., 2016). Totally 69 individuals from 23 sampling sites in the Black and Caspian Sea basins with vouchers stored in the Zoological Museum of the Moscow State University (ZMMU) and in Papanin Institute for Biology of Inland Waters Russian Academy of Sciences in Borok were included in the phylogenetic analyses, as well as 30 sequences of COI and 8 sequences of RAG1 of *Cobitis* spp. from Genbank; sequences of *Sabanejewia baltica* Witkowski, 1994 and *S. balcanica* (Karaman, 1922) were used as outgroups. Bayesian phylogenetic analysis was performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003), with examining the likelihood plots using TRACER v 1.5 (Rambaut & Drummond, 2007).

Morphological characters accepted in the descriptions of *Cobitis* species (shape of lamina circularis, suborbital spine, body scales, and mouth, position of barbels and suborbital spine, etc.) and main color features were examined in all voucher samples from the Caucasus stored in ZMMU, as well as in previously collected samples of the ZMMU collection. Thus, these characters were analyzed in 362 individuals from different rivers and lakes of the Caspian Sea basin and in 50 specimens from the Black Sea basin of Georgia. Colour pattern was characterized as organized in four zones accepted from Gambetta (1934); we distinguished two pigment layers for spots at the base of the caudal fin, namely, the surface (in the dermis) and a deeper layer, following Saitoh and Aizawa (1987). The last two branched rays articulating to the same pterygiophore in the dorsal and anal fins were counted as "1½". Total body length (TL), standard length (SL) and 23 morphometric characters (following Bănărescu et al., 1972 and Vasil'eva and Vasil'ev, 1998) were measured in voucher samples showing significant genetic divergence. For comparative morphological analysis we calculated mean values and standard deviations and used data from our previous studies (Vasil'eva and Vasil'ev, 2012, 2019).

RESULTS

The length of the obtained fragments of COI was 684 bp, of RAG1 – 909 bp; fragments of COI contained more phylogenetically informative positions (118 positions or 17.3%) compared to nuclear gene RAG1 (26 positions or 2.9%). Both mtDNA and nuDNA trees are well-resolved, but have some differences. The analysis of COI reveals the presence of three major clades. The most basal clade consists of monophyletic *Cobitis avicennae* Mousavi-Sabet, Vatandoust, Esmaeili, Geiger & Freyhof, 2015, whereas all other phylogenetic lineages are organized in two clades with high support (PP = 0.94 and 0.91). First one includes two subclades: 1) *C. satunini* s. stricto from the Black Sea basin and 2) *C. fahirae* Erk'akan, Atalay-Ekmekçi & Nalbant, 1998 from the rivers of the Eastern Aegean Sea. The second clade comprises of two highly supported subclades: *C. meridionalis* Karaman, 1924 from the Prespa Lake (in Greece, Macedonia and Albania) and the subclade included all studied haplotypes from the Caspian Sea basin. The last subclade is represented by two distinct phylogenetic lineages: 1) Eastern Caspian *C. faridpaki* Mousavi-Sabet, Vasil'eva, Vatandoust & Vasil'ev, 2011 and 2) the haplotypes from the southwestern Caspian Sea basin which are divided into two groups. The first group combines most of revealed haplotypes with haplotypes of type specimens of *C. saniae* from Genbank, and the second group includes the

haplotypes of individuals from the Lower Kura drainage only (Agstafa River and Kara-Su River system). These results presume the presence of two cryptic species: *C. saniae* and undescribed species, designated as *Cobitis* sp. in Fig. 1. The analysis of RAG1 demonstrates existence of four clades branching off in the following order: 1) *C. meridionalis*, 2) *C. fahirae*, 3) *Cobitis* sp. and 4) mixed clade which combines the haplotypes from *C. saniae* and *C. faridpaki*. Thus both mtDNA and nuDNA analyses confirm independent specific status of *Cobitis* sp.

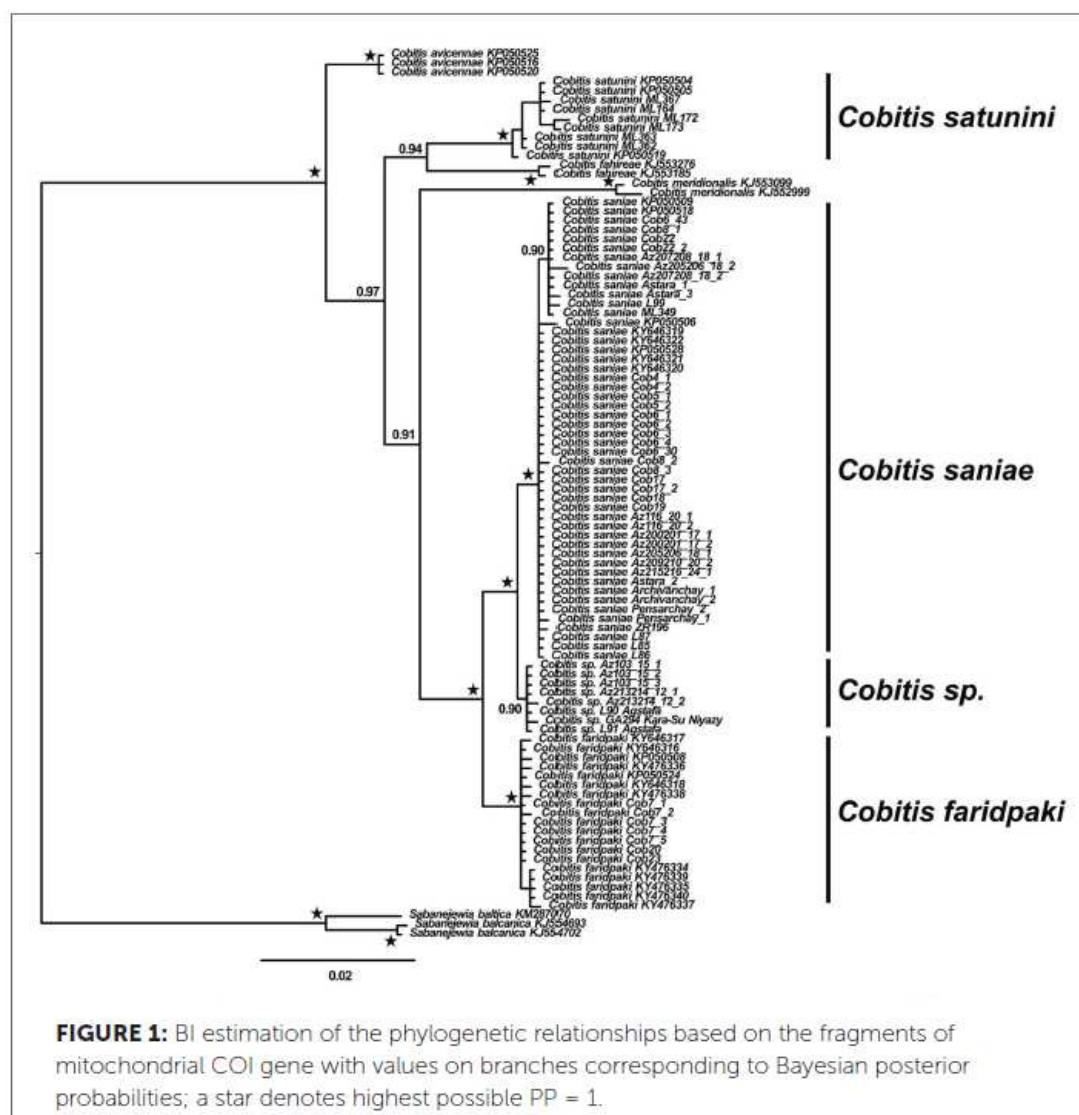




FIGURE 2: *Cobitis saniae* from the Lankaran, Azerbaijan.

Based on the morphological study of genetically identified specimens we developed a system of diagnostic features for spined loaches from the Caspian Sea basin and used them analyzing the ZMMU collection. Most of the specimens in this collection were identified as *C. saniae* (Fig. 2), a species that demonstrates the high variability of many external features. It was also found in the Kumbashi River, typical locality of *C. amphilekta*, but not revealed in the samples from the Black Sea basin, where *C. satunini* occurs. The spined loaches conspecific to *Cobitis* sp. were discovered in the Mingechaur reservoir basin.

MAJOR CONCLUSIONS

The evidences presented in this study suggest that historically at least four species of the genus *Cobitis* were distributed in the Caucasus, namely *C. saniae*, *C. amphilekta*, *C. satunini*, and *Cobitis* sp. Nowadays, *C. saniae* is the most common spined loach in the Caspian Sea basin. Probably this species completely displaced *C. amphilekta* which previously has been dominated in the Lankaran region, but both morphological and genetic data do not confirm the presence of *C. saniae* in the Black Sea basin.

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Keywords: taxonomy, RAG, COI, cryptic species, phylogenetic analysis, morphology, distribution

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