

## Craniological Features of the Italian Spined Loach *Cobitis bilineata* (Cobitidae)

E. D. Vasil'eva<sup>a, \*</sup> and V. P. Vasil'ev<sup>b, †</sup>

<sup>a</sup> Zoological Museum, Moscow State University, Moscow, Russia

<sup>b</sup> Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia

\*e-mail: vas\_katerina@mail.ru

Received September 23, 2023; revised December 9, 2023; accepted December 13, 2023

**Abstract**—The first craniological description of the Italian spined loach *Cobitis bilineata*, originally described as a variety of *C. taenia*, and a comparative analysis of its characteristics with previously studied *Cobitis* species are presented. In terms of the general structure of the skull, the number of bones and their shape, the Italian spined loach is closest to the previously described *C. melanoleuca*, the species turned out to be very phylogenetically distant. Thus, craniological features do not show correspondence with the genetic divergence and demonstrate significant stability within the genus *Cobitis*. Therefore, further molecular studies are of great importance to discover new cryptic species in these fish.

**Keywords:** skull and bone morphology, *Cobitis*, species divergence

**DOI:** 10.1134/S003294522470022X

### INTRODUCTION

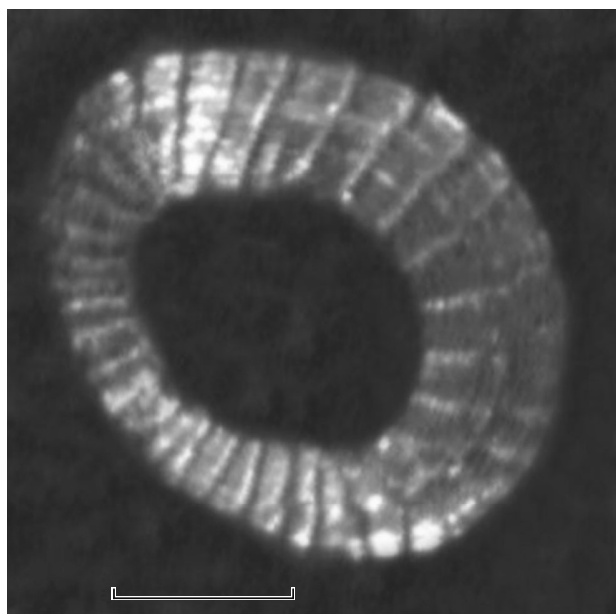
The Italian spined loach *Cobitis bilineata* Canestrini, 1865 belongs to the species of the family Cobitidae, in which adult males are characterized by a single lamina Canestrini (= lamina circularis) at the base of the first branched ray of the pectoral fin. It differs from other representatives of the genus *Cobitis* in Europe by a set of color features, including the presence of two black spots at the base of the caudal fin and the specificity of the so-called Gambetta's pigmentation zones (Gambetta, 1934). *C. bilineata* is distributed in the Northern Adriatic, from Po to Soca drainages (Italy, Slovenia, Switzerland), Durance (Rhône system, France) and Zrmanja (Croatia) basins. It has also been introduced to most of Italy, northeastern Spain, and the upper Rhine drainage in Switzerland. In these areas the Italian spined loach occurs in a wide variety of habitats, including lakes, rivers, streams, irrigation canals with stagnant to moderately flowing water, sandy, gravel or muddy bottom, often with vegetation (Kottelat and Freyhof, 2007).

The Italian spined loach was originally described as a variety of *Cobitis taenia* Linnaeus, 1758 (Canestrini, 1865), and later accepted as its subspecies *C. taenia bilineata* (Lelek, 1987; Bianco, 1988), and then as a separate species. According to current molecular studies, this species is not a phylogenetically close relative of the *C. taenia* species group. Phylogenetic analysis of the combined sequences of the complete mitochon-

drial cytochrome *b* (*cyt b*) and ATPase 8/6 genes for the main European Cobitid groups showed that *C. bilineata* is separated into a distinct lineage along with *C. elongata* Heckel et Kner, 1857 and *C. zanandrei* Cavicchioli, 1965 (Perdices and Doadrio, 2001). This strongly supported monophyletic lineage consists of two endemic Italian species belonging to the subgenus *Cobitis* s. stricto (with a lamina Canestrini) and the type species of the subgenus *Acanestrinia* *C. elongata* (without a lamina Canestrini), and is well isolated from the other five identified phylogenetic lineages. Later, this lineage was also confirmed by *cyt b* analysis and named the Adriatic lineage (Bohlen et al., 2006). In another mitochondrial DNA analysis, *C. bilineata* is completely separated from the subgenus *Cobitis* s. stricto and, together with *C. elongata*, form the basal group for all other *Cobitis* species (Ludwig et al., 2001). However, in a further study (Buj et al., 2014), phylogenetic analyses based on the *cyt b*, *Rag 1*, and first intron S7 haplotypes showed that in all of them, *C. bilineata* is related to *C. narentana* Karaman, 1928, but is well differentiated from both *C. elongata*, and from *C. zanandrei*. In general, similar results were obtained for other phylogenetic trees (Perdices et al., 2016, 2018; Eagderi et al., 2017a, 2017b).

Due to the observed uncertainty of the position of *C. bilineata* in the system of phylogenetic relations in the genus *Cobitis* s. lato, the divergence of the species from congeneric taxa according to different groups of characters is of undoubted interest. In this paper, we present the first craniological description of *C. bilineata*

<sup>†</sup> Deceased.



**Fig. 4.** Scale of large *Cobitis bilineata* female, ZMMU P-24581 SL 78.5 mm. Scale: 0.2 mm.

ous dark stripes in adult males and Z3, which is a narrow line of small spots, usually ends below or in front of the beginning of the dorsal fin in males (Kottelat and Freyhof, 2007; present study). Equally poorly dif-

ferentiated Gambetta's zones, often represented by continuous dark stripes, usually are observed in *C. melanoleuca* (Vasil'eva et al., 1989). But this species is characterized by a narrow, bottle-shaped lamina circularis and elongated, oval scales with a very large focal zone (its length is about 70% of the length of the scale).

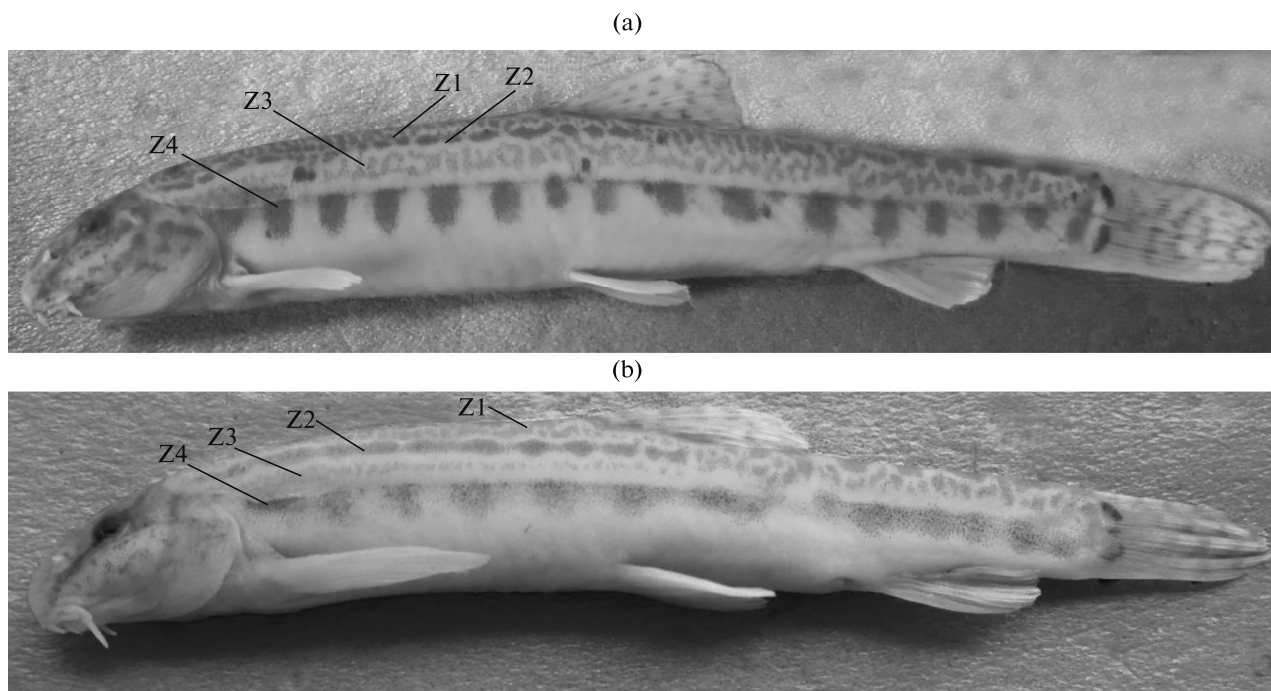
Despite the observed craniological similarities between *C. bilineata* and *C. melanoleuca*, they are certainly proven to be phylogenetically very distant species (Šlechtová et al., 2008; Perdices et al., 2016, 2018). Thus, we do not observe a direct correlation between the morphological divergence of individual species of spined loach and their divergence in some of the studied genetic markers. At the same time, the discrepancies in external morphology do not correspond to craniological differentiation. Therefore, when analyzing a wide range of markers and characteristics in this group of fish, one can expect the identification of new cryptic *Cobitis* species. And, of course, further molecular studies of local populations are of great importance.

#### ACKNOWLEDGMENTS

We are very grateful to anonymous reviewers for the analysis of our MS and helpful comments.

#### FUNDING

Scientific investigations of E.D. Vasil'eva are supported by the State Project of ZMMU No. 121032300105-0.



**Fig. 5.** *Cobitis bilineata* from the Canale Moneta, NW Italy, ZMMU P-24581: female SL 78.5 mm (a) and male SL 50.5 mm (b): Z1–Z4—Gambetta's zones.