



Research Article

## HISTOLOGY OF THE DIGESTIVE TRACT OF THE STENO-ENDEMIC *COBITIS HERZEGOVINIENSIS* (TELEOSTEI: COBITIDAE) FROM MOSTARSKO BLATO (NERETVA RIVER BASIN, BOSNIA AND HERZEGOVINA)

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### ABSTRACT

The study of endemic fish species is of great importance, considering that extensive knowledge is a prerequisite for adequate protection and conservation. This study investigated the histology of the digestive tract of the steno-endemic fish *Cobitis herzegoviniensis* Buj & Šanda 2014, from Mostarsko Blato (Neretva River basin, Bosnia and Herzegovina). Histology of the digestive tract of this species was investigated, since there is a scarcity of information regarding its digestive biology. Specimens (average total length 78 mm, average standard length 69 mm, mean weight 3,836 g) of the species *C. herzegoviniensis* were caught in the lower course of the Lištica River in the area of Mostarsko Blato in June 2019. The tissue of selected organs of the digestive system was used as the basic material. Using basic histological techniques, microscopic preparations were made from the tissue. This species has only esophagus and intestine. When studying the microscopic structure of the wall of the digestive tract, it was determined that the wall consists of three layers: mucosa (*tunica mucosa*), muscular layer (*tunica muscularis*) and outer layer (*tunica serosa*). The basic building block of the liver is the liver cell or hepatocyte.

**Keywords:** *Cobitis herzegoviniensis*, Ecology, Histology, Mostarsko Blato, Bosnia and Herzegovina.

### INTRODUCTION

The spined loaches genus (*Cobitis*) is widespread in the rivers and lakes of Europe and Asia. However, the area of the Adriatic Sea catchment area in Croatia, together with the area of Herzegovina, is considered the so-called hot spot of the spined loaches diversity in Europe, given the particularly large number of species distributed in that area. It is also interesting that most of the species of the Adriatic catchment area have an extremely small area of distribution and are considered stenoendemic. Such abundance of spined loaches in a small area of Dalmatia and Herzegovina and an extremely large number of endemics is explained by the interesting and complex geological history of the area and the isolation of the rivers (Čaleta *et al.*, 2015).

The genus *Cobitis* includes small, slender-bodied benthic freshwater fishes. The genus is characterized by the following features: the suborbital spine is erectile; the

mouth is small and inferior with three pairs of barbels; body pigmentation is organized in one dorsal and four lateral longitudinal lines or rows of blotches; and the presence of the lamina circularis at the base of the pectoral fin in adult males (Nalbant, 1963; Kottelat and Freyhof, 2007; Kim, 2009). Spined loaches typically inhabit shallow areas of stagnant and slow-flowing waters, where the bottom is covered with fine sediment. However, in the early stages of life, the young will prefer a different microhabitat, since the survival of eggs and young fish depends on the presence of dense vegetation. It is considered that the selection of such habitat, i.e. their attachment to muddy and sandy sediments, and sediments rich in organic particles, is caused by a specialized way of feeding - sifting the substrate. They take in food in a very unusual way - using a filtering apparatus with zones of sticky mucus in the pharyngeal cavity (Čaleta *et al.*, 2015).

A large part of their diet consists of small invertebrates that live in the upper layers of the sediment, such as shrimps, insect larvae and other invertebrates, but also organic matter and single-celled algae. These little fish are extremely nocturnal and feed and swim at night, while they are mostly buried in the substrate during the day (Ćaleta *et al.*, 2015). Morphological and histological studies are very useful for the characterization of the digestive tract, which provides important information for understanding feeding habits and digestive physiology of fish (Morrison and

Wright, 1999). Histological analysis of the digestive tract of fish is essential since it provides information which can formulate models which explain the trophic structure of the ecosystems and quantitative knowledge of the biological mechanisms of interaction between species (Fugi *et al.*, 2007). The present work investigated the histology of the digestive system of *C. herzegoviniensis*. The histological description of the digestive system of *C. herzegoviniensis* is compared with other teleost fish, to know whether they conform to a standard pattern.



**Figure 1.** *Cobitis herzegoviniensis* Buj & Šanda, 2014 (Photo: M. Jelić).

## MATERIAL AND METHODS

### Study Site and Fish Sampling

The Mostarsko Blato field is located in Western Herzegovina, in the basin of the middle course of the Neretva River, between the Čabulja mountain and the Polog ridge (Jastrebinika, 1138 m) in the north and lower limestone elevations (Trtla ridge, 690 m) in the south. The direction of stretching is northwest-southeast. It is located on a medium-altitude terrace (from 223 m above sea level to 245 m above sea level) between the upper terrace with the Ružovo, Trnopolje and Mokro fields and the lower terrace with the Mostarsko Polje field. The field is closed in relief, i.e. surrounded by limestone hills. On the north side, it is the Polog ridge (part of the Čabulja mountain), whose southern slopes are slightly pedimented, and the transition from the fields is narrow. In indentations, there is a glacia with a somewhat arable area outside the bottom of the fields. On the south side, the field is surrounded by slopes of the Trtla. The northern slopes of Trtla form a part of the mountain ridge that is pedimented, and the thickness of the pediment is often over 10 m (Bognar and Miličević, 2007). In the pediment there are sorted rounded pebbles covered with a loose layer of soil suitable for growing vines. The western part of the field has an alluvial character. The River Lištica, flowing into the field, spills over and deposits the brought material there. This alluvium is slightly inclined towards the southeast and is also suitable

for viticulture. Further in the southeast direction, the slopes are smaller, which is why the land occasionally floods due to the accumulation of rainwater (Miličević, 2009).

### Laboratory procedures

The taxonomic identification of the fish species was confirmed (Buj *et al.*, 2014). Each fish was dissected to expose the digestive tract, which was removed from the coelomic cavity. Tissue samples were fixed in 10% formalin for 24 hours. After fixation the digestive tract was submitted to microtomy to obtain fragments of the digestive organs. The fragments were submitted to routine histological techniques, such as dehydration, clearing, infiltration and paraffin embedding. They were micro sectioned at 6  $\mu\text{m}$ . Samples were stained with Haematoxylin-eosin (HE) (Suvarna *et al.*, 2019).

### Microscopy

Histological study of the organs of the digestive system of *C. herzegoviniensis* species was performed with an Olympus DP71 light microscope (Figure 3).

## RESULTS AND DISCUSSION

Total body length (mm), standard body length (mm) and the total weight of body (grams) of 5 fishes were measured. Mean total length, mean standard length and weight of the

fishes were 78 mm, 69 mm and 3,836 g respectively. The digestive tract of *C. herzegoviniensis* is a thin continuous straight tube. As such, it was not possible to distinguish externally anatomical organs, such as, the esophagus, stomach and intestine. The esophagus is lined by a mucous membrane that forms high folds. By looking through the microscope, it was observed that the lumen of the esophagus has a star-like appearance in cross-section. The esophagus wall of *C. herzegoviniensis* is constructed of three different histological layers: mucosa (*tunica mucosa*), muscular layer (*tunica muscularis*) and outer

layer (*tunica serosa*) (Figure 4). Mucosa (*tunica mucosa*) is built of epithelium (*lamina epithelialis*) and connective tissue (*lamina propria*), while the muscle layer of the mucosa (*lamina muscularis mucosae*) is not noticed. The epithelium of the esophagus is single-layered and cylindrical. Goblet cells are inserted between the epithelial cells. Goblet cells secrete mucus onto the surface of the esophageal mucosa. The *lamina propria* is a layer of connective tissue located just below the epithelium and permeated with blood vessels.



**Figure 2.** Sampling location - Pološki gaz (Photo: M. Jelić).

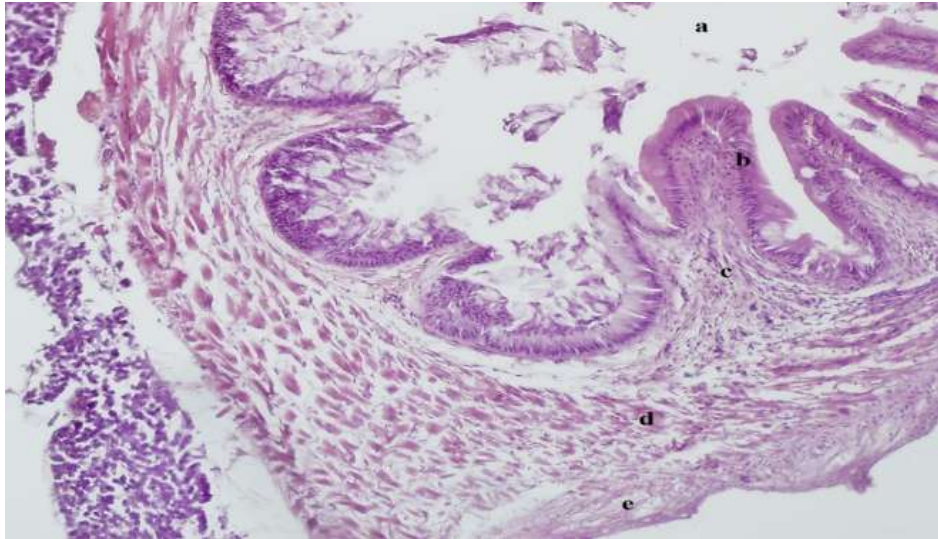
Specimens of the species *C. herzegoviniensis* (Figure 1) were caught in the lower course of the Lištica River in the area of Mostarsko Blato (Pološki gaz; N 43°20'36,2", E 17°41'36,4", 226 m n.v.) (Figure 2) in June 2019.



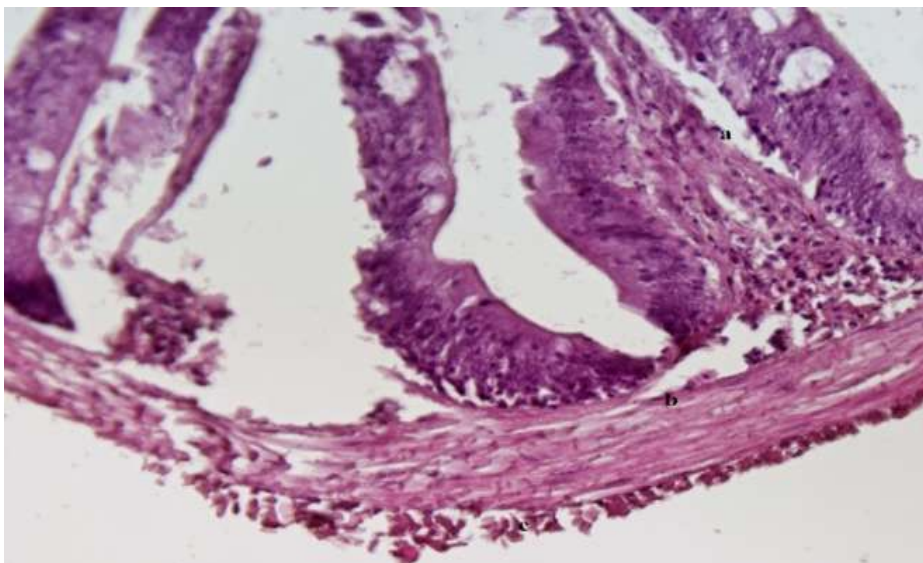
**Figure 3.** Light microscope Olympus DP71 (Photo: M. Jelić).

Analysis of light microscopy permitted differentiation of the layers and cells of the intestinal region. In the anterior part of the intestine, *tunica mucosa* had villi. The epithelial tissue lining was simple and cylindrical with microvilli, indentations, absorptive cells and goblet cells (Figure 5). The *lamina propria* is a very thin layer formed by loose connective tissue, without presence of glands and *muscular mucosa*, hence there was no *submucosa*. The muscular layer is formed by smooth muscles. Light microscopic observations revealed that the wall of posterior intestine

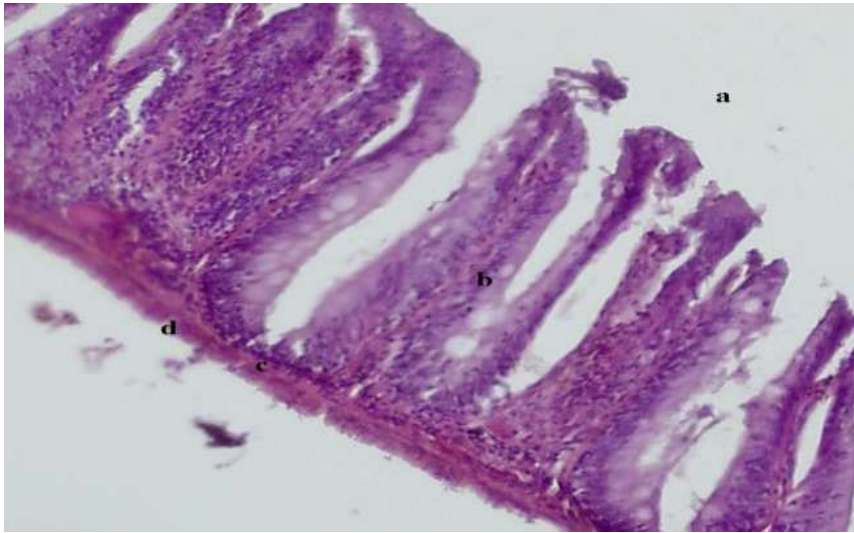
was composed of *tunica mucosa*, *tunica muscularis*, and *tunica serosa* (Figure 6). The liver is a digestive gland consisting of parenchymal cells and lattice fibers. The liver parenchyma is not divided into different lobes. The liver is a complete organ that is histologically constructed of liver cells, hepatocytes. Hepatocytes are arranged in radial arrays around the central vein. Sinusoidal capillaries were noticed between rows of hepatocytes. The melanomacrophage center was not observed, nor was the section through the bile ducts (Figure 7).



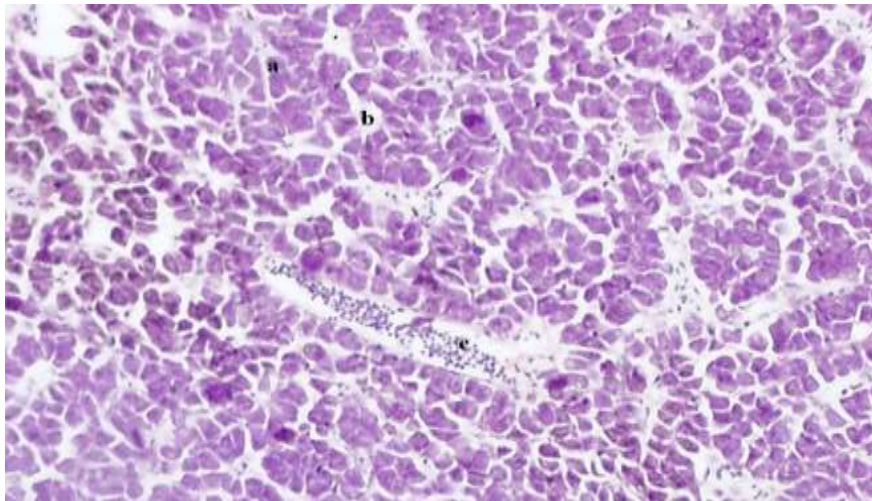
**Figure 4.** Cross-section through the esophagus of *Cobitis herzegoviniensis*: a) lumen, b) epithelium, c) *lamina propria*, d) *tunica muscularis*, e) *tunica serosa* (HE, 10x).



**Figure 5.** Cross-section through the anterior part of the intestine of *Cobitis herzegoviniensis*: a) *tunica mucosa*, b) *tunica muscularis*, c) *tunica serosa* (HE, 40x).



**Figure 6.** Cross-section through the posterior part of the intestine of *Cobitis herzegoviniensis*: a) lumen, b) *tunica mucosa*, c) *tunica muscularis*, d) *tunica serosa* (HE, 40x).



**Figure 7.** Histological structure of the liver of the species *Cobitis herzegoviniensis*: a) hepatocytes, b) liver sinusoids, c) blood vessel (HE, 40x).

The digestive system of fish is constructed of oropharyngeal cavity, esophagus, stomach, intestine and anal cavity. Digestive glands, liver and pancreas are also associated with the digestive system (Treer *et al.*, 1995). The oral cavity of fish is covered with a stratified squamous epithelium. Salivary glands are missing in the oral cavity of fish, but they are replaced by goblet cells that secrete mucus (Bogut *et al.*, 2006). The digestive system of *C. herzegoviniensis* species consists of oropharyngeal cavity, esophagus, intestine and anal cavity. The short esophagus in the study species is directly connected to the intestine. The wall of the esophagus of this species is constructed of three different histological layers: mucosa, muscular and outer layer. The mucosa (*tunica mucosa*) is made of epithelium (*lamina epithelialis*), connective tissue (*lamina propria*), while the muscle layer of the mucosa (*lamina*

*muscularis mucosae*) is not visible. These results are compliant with the histological structure of the esophagus as reported by Junqueira *et al.* (1999). The intestine wall of fish consists of a mucosa, a muscularis and a serosa (Bogut *et al.*, 2006). The intestine wall of *C. herzegoviniensis* contains mucosa, muscularis and serosa. The short esophagus indicates that the food items are directly taken to the intestine which has no anatomical boundaries. This pattern is similar to that of the lampreys, which are fish without stomach, where the esophagus is directly connected to the intestine (Stevens and Hume, 1995). In small and omnivorous species such as *Roeboides xenodon*, *Orthospinus franciscensis* (Barcellos *et al.*, 2014), *Anablepsoides urophthalmus* (Nascimento *et al.*, 2018) and also in *Phoxinellus pseudalepidotus* (Putica, 2019) the esophagus is short and this portion of the digestive tract

does not offer obstacle to the passage of food, similar to the observation in the study species. There is no histological demarcation for the regions of the intestine for the study species. Gastric gland liver was found along the digestive tract of the study species. The basic building block of the liver is the liver cell or hepatocyte.

## CONCLUSIONS

The digestive system of the species *C. herzegoviniensis* consists of the oropharyngeal cavity, esophagus, foregut and hindgut, rectum, and associated digestive glands. By histological research of the digestive wall of the species *C. herzegoviniensis* it was established that the wall consists of three layers: mucosa (*tunica mucosa*), muscular layer (*tunica muscularis*) and outer layer (*tunica serosa*). The mucosa (*tunica mucosa*) is made of epithelium (*lamina epithelialis*) and connective tissue (*lamina propria*), while the muscle layer of the mucosa (*lamina muscularis mucosae*) is not visible. In the study species (*C. herzegoviniensis*), it was observed that the liver parenchyma is not divided into different lobes. The basic building block of the liver is liver cells or hepatocytes, which are arranged in strips surrounding the central vein.

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