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Research Article

HISTOLOGY OF THE DIGESTIVE SYSTEM OF *PHOXINELLUS PSEUDALEPIDOTUS* (TELEOSTEI: CYPRINIDAE), AN ENDEMIC AND ENDANGERED SPECIES FROM MOSTARSKO BLATO (NERETVA RIVER BASIN, BOSNIA AND HERZEGOVINA)

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ABSTRACT

Eight specimens of the species *Phoxinellus pseudalepidotus* (Bogutskaya & Zupančič, 2003) were sampled to study the morphology of the digestive system. Hematoxylin-eosin staining was used in order to elucidate the histological features of the digestive organs in *P. pseudalepidotus*. *P. pseudalepidotus* is a stomachless fish whose digestive system is a hollow tube made of esophagus and intestines. The esophagus consists of three distinctive layers: the mucosa, the muscular layer and the outer layer. The esophageal epithelium contains abundant goblet cells. All observed parts of the intestines consisted of three-layered wall containing mucosa, muscular layer and outer layer. The liver as an associated digestive gland consists of the anastomosing plates of hepatocytes separated by sinusoidal capillaries. The results show that the histology of the digestive system in *P. pseudalepidotus* is congruent with its feeding habits.

Keywords: *Phoxinellus pseudalepidotus*, Ecology, Histology, Mostarsko Blato, Bosnia and Herzegovina.

INTRODUCTION

All the species of the genus *Phoxinellus* have very limited areas of distribution. They mainly inhabit only very small, isolated, karst watercourses in Croatia and Bosnia and Herzegovina. These fish survive adverse conditions during summer droughts by retreating to springs, caves and subterranean watercourses (Ćaleta *et al*., 2015). The Dinaric minnow, *Phoxinellus alepidotus* (Heckel, 1843), has so far been recorded in the Livno, Duvno and Glamoč karst fields, in the lakes of Buško and Blidinje and in the Korana River near Bosansko Grahovo in Bosnia and Herzegovina. In Croatia, it has only been recorded in the Cetina River in the Sinj karst field and in Stipančevo Lake. Until recently, it was thought that it also inhabits the watercourses of the Mostarsko Blato (Ličtica) in Bosnia and Herzegovina, but this population was described as a separate species, the Mostar minnow (*P. pseudalepidotus*). The Dalmatian minnow, *Phoxinellus dalmaticus* (Zupančič & Bogutskaya, 2000) is described as a species in 2000 and is stenoendemic to the Krka River basin because it only inhabits the rivers of Čikola and Krka (Ćaleta *et al*., 2015).

Previously, the genus *Phoxinellus* included a larger number of species, but recent analyses have revealed significant morphological and genetic differences, and some species have been moved to the separate genera *Delminichthys* and *Telestes*. Species that now belong to the genus *Phoxinellus* have a naked body with very few scales and do not have a pronounced urogenital tubercle. These are very small fish. They generally grow to a maximum of 12 centimeters, with the exception of the minnow, which reaches up to 14.5 centimeters. Their body is elongated and slightly laterally flattened. The dorsal side is darkly colored and sprinkled with small black dots, while the ventral side is lighter in color. A dark stripe extends from the head along the sides, but it is usually very poorly visible. These fish have very reduced scales, placed only in the lateral stripe and in several rows around it and immediately behind the head (Ćaleta *et al*., 2015). Very little is known about the lifestyle of these species. They inhabit clean, karst waters,

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with slow-flowing and stagnant water, as well as associated wetland habitats. During summer droughts and during the winter months, they retreat to subterranean water. In particular, the Dalmatian minnow can be found in larger schools when entering or exiting subterranean water. Precisely the adaptation to survive underground has enabled these fish to survive in karst areas (Ćaleta *et al*., 2015). Spawning takes place in smaller groups from April to June in shallower parts of the stream. Females lay their eggs on stones and gravel or in dense vegetation. During spawning, males acquire more intense color and spawning tubercles on the head and front of the body and along the margin ray of the pectoral fin (Ćaleta *et al*., 2015).

They feed on small aquatic invertebrates (Ćaleta *et al*., 2015). Mostar minnow, *P. pseudalepidotus*, is a freshwater fish endemic to the Neretva River basin. Present data suggest that the species is restricted only to wetland of the Mostarsko Blato but it is considered to be distributed more widely in the River Neretva basin (Bogutskaya & Zupančič, 2003). According to IUCN Red List, *P. pseudalepidotus* is listed as Vulnerable D2 ver. 3.1 (Crivelli, 2006; IUCN). It is endangered because of the extremely limited range of distribution, river regulation and influence of non-indigenous species (Mihinjač *et al*., 2014). *P. pseudalepidotus* inhabits streams or shallow channels with slow current and clean water (Crivelli, 2006; Bogutskaya & Zupančič, 2003). During unfavorable periods, it lives in subterranean waters (Markotić, 2013). This species is omnivorous and mostly feeds on water invertebrates (Insecta, Gastropoda) and herbal material (Markotić, 2013; Markotić *et al*., 2019).

Figure 1. *Phoxinellus pseudalepidotus* Bogutskaya & Zupančič, 2003 (URL1).

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 & Wright, 1999). The present study is the first record on the morphology of the digestive system in *P. pseudalepidotus*, and since some morphological and functional differences of the digestive system in fishes depend usually on whether they are herbivorous or carnivorous, it is important to elucidate whether the morphology of the *P. pseudalepidotus* digestive system is in accordance to its feeding habits.

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 slopes of Čabulja Mountain and the Polog ridge (Jastrebinka, 1138 m) in the north and lower karst plateau (Trtla ridge, 690 m) in the south. The runs northwest – southeast. It is situated on a medium-altitude terrace (altitude between 223 m and 245 m above sea level), between the upper terrace with the Ružovo, Trnpolje and Mokro fields and the lower terrace with the Mostarsko Polje Field. The field enclosed, i.e. surrounded by limestone hills. On the north side, there is the Polog ridge (part of the Čabulja mountain), which southern slopes are slightly pedimented, and the transition from the fields is narrow. In recessions, there is a glacis with a somewhat arable area outside the bottom of the field. On the south side, slopes of the Trtla surround the field. The northern slopes of Trtla form a part of the mountain ridge that is pediment, and the thickness of the pediment is often over 10 m (Bognar & Milićević, 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 Lištica River, flowing into the field, spills over and deposits the brought material there. This alluvium is slightly inclined towards the southeast and is 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). Specimens of the species *P. pseudalepidotus* (Figure 1) were caught in the lower course of the Lištica River in the area of Mostarsko Blato (Položki

gaz; N 43°20'36,2", E 17°41'36,4", 226 m above sea level) (Figure 2) in June 2019 by gill nets and "krtol", a traditional fishing tool.

Laboratory Procedures

The taxonomic identification of the fish species was confirmed (Bogutskaya & Zupančič, 2003). Eight specimens of the species *P. pseudalepidotus* were sampled to study morphology of the digestive system. The average total body length (TL) of the samples was 61 mm, and the average standard body length (SL) of the samples was 55 mm. The parts of the digestive system, from the cranial end of the esophagus to the caudal end of the rectum, were fixed in 10% formalin immediately after collection. The tissues were first dehydrated in an ascending series of ethanol and cleared with xylene, and then embedded in paraffin. Tissues sections were cut transversally at 6 µm and mounted on glass slides. The sections were then deparaffinized with xylene and stained with hematoxylin–eosin staining to present the basic morphology of the digestive organs (Sheehan & Hrapchak, 1980).

Microscopy

The sections were observed using an Olympus DP71 light microscope (Figure 3).

Figure 2. Sampling location - Pološki gaz (Photo: I. Putica).

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

RESULTS AND DISCUSSION

The average weight of the eight investigated individual fish of *P. pseudalepidotus* was 3.819 g, while the average standard length (SL) was 55 mm, and the average total length (TL) was 61 mm. The digestive system of the species *P. pseudalepidotus* consists of the alimentary canal with associated digestive glands. The alimentary canal is a hallow tube distinguished into the esophagus and the intestine, respectively**.** The esophageal wall of the species *P. pseudalepidotus* is composed of three distinct histological layers: mucosa, muscular layer, and outer layer. The mucosa (*tunica mucosa*) is composed of epithelium (*lamina epithelialis*) and connective tissue (*lamina propria*), while the muscularis mucosae (*lamina muscularis mucosae*) is not visible. The epithelium of the esophageal mucosa is a single-layered and cylindrical. Goblet cells are interspersed between the epithelial cells. The *lamina propria* is a layer of connective tissue that lies beneath the epithelium and is permeated with blood vessels (Figures 4 and 5).

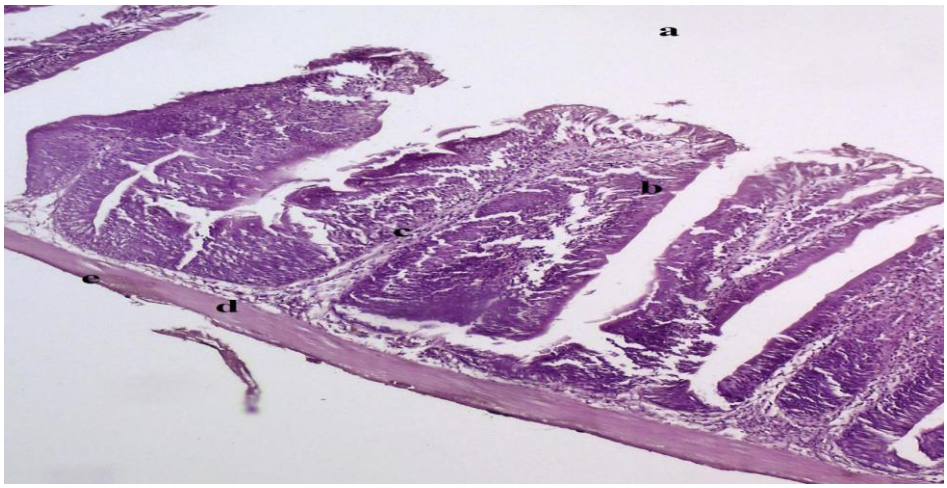

Figure 4. Cross-section through the esophagus of *Phoxinellus pseudalepidotus:* a) lumen, b) epithelium, c) *lamina propria*, d) *tunica muscularis*, e) *tunica adventitia* (HE, 40x).

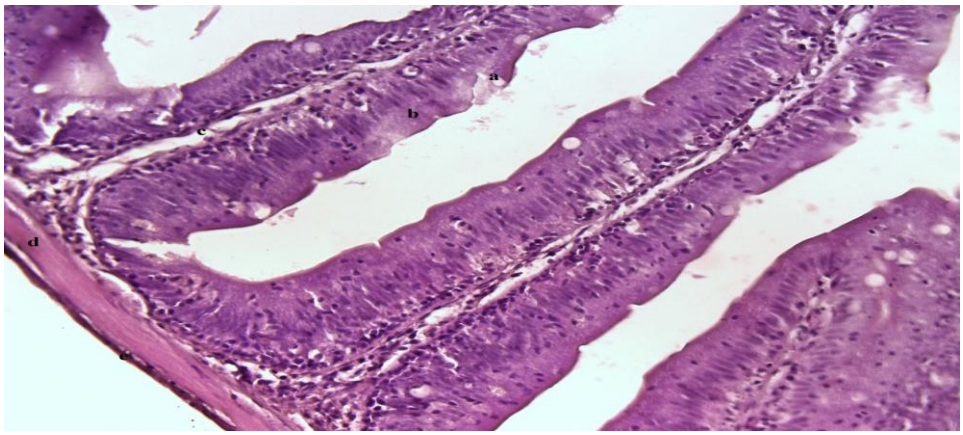

Figure 5. Cross-section through the esophagus of *Phoxinellus pseudalepidotus*: a) goblet cells, b) epithelium, c) *lamina propria*, d) *tunica muscularis*, e) *tunica adventitia* (HE, 40x).

The intestinal wall of *P. pseudalepidotus* consists of three distinct histological layers: mucosa, muscular layer and outer layer. The surface epithelium of the mucosa of the anterior and posterior regions of the intestine consists of enterocytes with goblet cells. The *lamina propria* of the intestinal mucosa does not contain multicellular glands (Figures 6, 7, and 8).

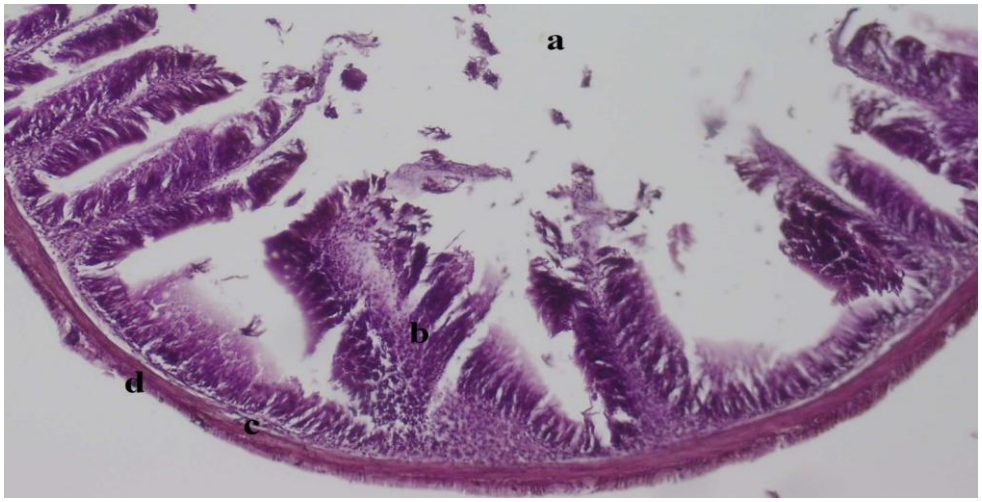

Figure 6. Cross-section of the anterior part of the intestine of *Phoxinellus pseudalepidotus*: a) lumen, b) *tunica mucosa*, c) *tunica muscularis*, d) *tunica serosa* (HE, 10x).

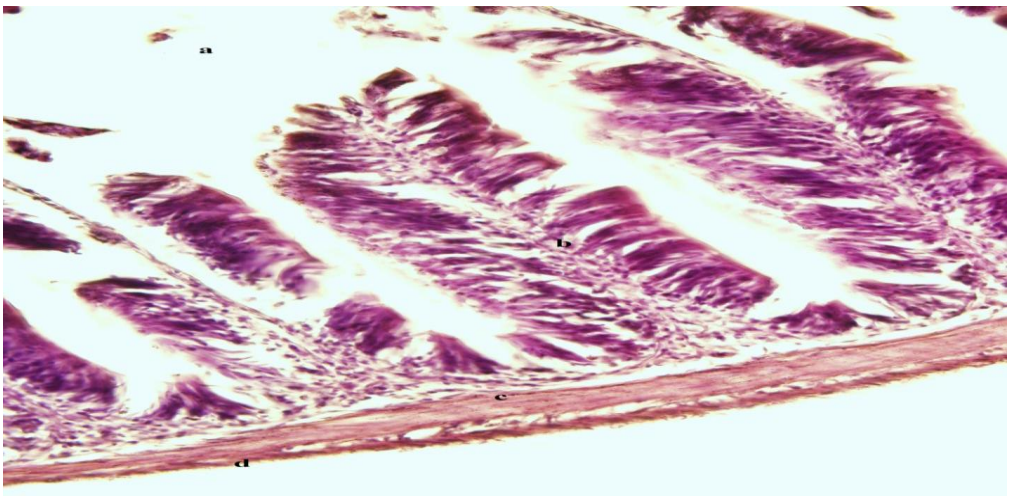

Figure 7. Cross-section of the anterior part of the intestine of *Phoxinellus pseudalepidotus*: a) lumen, b) *tunica mucosa*, c) *tunica muscularis*, d) *tunica serosa* (HE, 40x).

Figure 8. Cross-section of the posterior part of the intestine of *Phoxinellus pseudalepidotus*: a) lumen, b) *tunica mucosa*, c) *tunica muscularis*, d) *tunica serosa* (HE, 10x).

The liver in species *P. pseudalepidotus* is a simple organ that surrounds the upper part of the intestines. Liver parenchyma consists of the anastomosing plates of hepatocytes separated by sinusoidal capillaries. The sinusoidal capillaries open into the central vein (Figure 9).

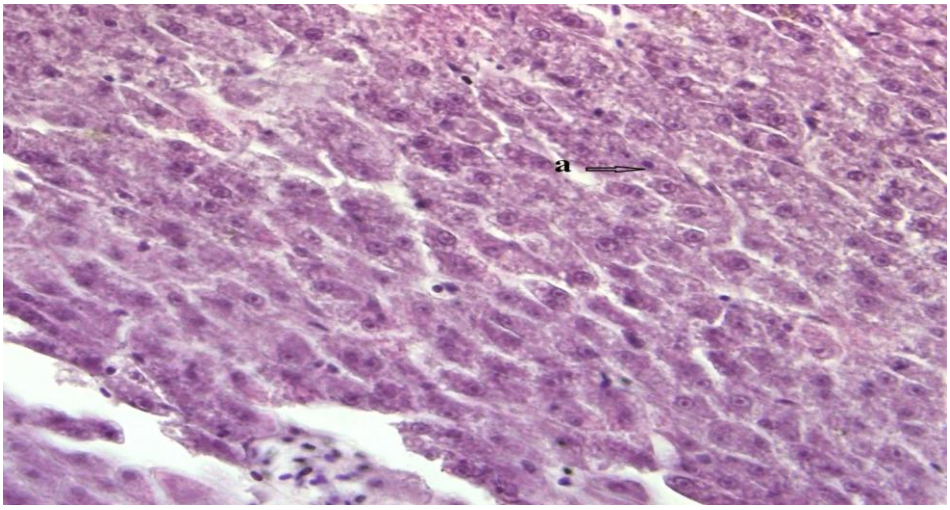

Figure 9. Histological structure of the liver of *Phoxinellus pseudalepidotus*: a) hepatocyte (HE, 40x).

DISCUSSION

The digestive system of fish consists 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 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 histological structure of the digestive tract of numerous fish species generally consists of mucosa, submucosa, muscularis, and serosa. Some differences of histological structures among fish digestive tracts are related to feeding habits, food, age, body shape, and weight (Mokhtar *et al*., 2017). The digestive system of *P. pseudalepidotus* consists of oropharyngeal cavity, esophagus, intestine and anal cavity. The digestive system of the studied species also includes digestive glands. The esophagus of *P. pseudalepidotus* is directly connected to the intestine. The esophageal wall of *P. pseudalepidotus* consists of three different histological layers: mucosa, muscular layer and outer layer. The mucosa (*tunica mucosa*) consists of epithelium (*lamina epithelialis*) and connective tissue (*lamina propria*), while the muscularis

mucosae layer (*lamina muscularis mucosae*) is not visible. The intestinal wall of fish consists of mucosa, muscular layer and serosa (Bogut *et al*., 2006). The intestinal wall of *P. pseudalepidotus* also consists of mucosa, muscular layer and outer layer. The liver is the highest glandular organ associated with the digestive system (Bogut *et al*., 2006). The liver is a large digestive gland that is composed of parenchymal cells and lattice fibers. The fish hepatic parenchyma is not arranged into distinct lobules. Three patterns of organization of fish hepatic parenchyma are recognized. The first pattern is composed of hepatocytes, which are radially arranged around the central vein. The second pattern, called tubular pattern, in form of tubules and the sinusoids form network around the tubules. The third pattern is present in some fresh and marine teleost, the hepatocytes lie in anastomosing lamina around the central vein (Mokhtar, 2017). The liver of *P. pseudalepidotus* is a simple organ that surrounds the upper part of the intestines. Liver parenchyma consists of the anastomosing plates of hepatocytes separated by sinusoidal capillaries. The sinusoidal capillaries open into the central vein.

CONCLUSION

In conclusion, the present study is the first record on the digestive system histology of *P. pseudalepidotus*, suggesting that its histological features are mostly similar to those of other omnivorous fishes, and congruent with its feeding habits.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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