

Original Article

Morphohistological characteristic of digestive tract of an endemic cichlid fish, *Iranocichla hormuzensis* Coad, 1982 (Teleostei: Cichlidae)

Mina Hojat Ansari¹, Mehregan Ebrahimi^{*2,3}, Hamid Reza Esmaeili²

¹Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz.

²Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran.

³School of Biological Sciences, Flinders University, Australia.

Abstract: Objective of the current study was to investigate morphohistology of digestive tract of *Iranocichla hormuzensis*, one of two native cichlid fishes in Iran. The species present omnivorous feeding habits and restricted in shallow, high temperatures and high salinity water in the Mehran River and its drainages. This species has a terminal mouth with several rows of serrated incisive teeth, which covered the upper and lower lips. Esophagus is a short tubular between pharynx and stomach, stomach is relatively small and sac-shape, and intestine is long. Gastric wall, through the digestive tube, consists of four layers of mucosa, submucosa, muscularis, and serosa with some characters specialized for each organ, such as a well-developed mucosa layer and numerous goblet cell in the ventral section of stomach. Morphohistology of digestive tract of *I. hormuzensis* supports its generalist diet.

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Introduction

Histological and anatomical study of digestive tract in fish is essential to understand feeding physiology, structure of digestive tract, and adaptation in response to feeding habits. There is an increasing number of studies focus on characterising morphohistology of digestive system in fish (e.g. Canan et al., 2012; Rønnestad et al., 2013; Purushothaman et al., 2016; Arman and Ucuncu, 2017; Kalhor et al., 2018). However, anatomical and histological aspects of digestive system are poorly characterised in many fishes, hindering an understanding of feeding dynamics and habitat occupation of the species. The cichlids (family Cichlidae) particularly remain underrepresented despite their high species richness among vertebrates.

The Iranian cichlid fish *Iranocichla hormuzensis* Coad, 1982, is an endemic fish restricted to a small river tributary in the Persian Gulf basin (Esmaeili et al., 2016; Schwarzer et al., 2017). This fish is distributed in shallow, high temperature and high salinity water of the Mehran River and its drainages

(Esmaeili et al., 2016; Schwarzer et al., 2017). Diet of *I. hormuzensis* is mainly detritus, cyanobacterium, diatoms and green algae, but zooplankton and small fishes have also been observed (unpublished data). This trophic plasticity attributed to seasonal variation, breeding strategies and a higher protein needs for reproduction and potential growth in adults (unpublished data). *Iranocichla hormuzensis*, beside of its natural beauty, interesting mouth-brooder behavior and importance as nutritional food for local people, is the only cichlid fished in Iran. This endemic species link to trophic dynamics of the Mehran River ecosystems makes it as an important species that its morphohistological data is missing. Therefore, the present study aimed to provide data on morphohistology of *I. hormuzensis* digestive tract.

Materials and Methods

Four specimens of *I. hormuzensis* (two males and two females) were collected from the Mehran River near the city of Bastak (27.04889N, 54.28360E). These specimens were collected under the appropriate

*Correspondence: Mehregan Ebrahimi
E-mail: m.ebrahimi@shirazu.ac.ir

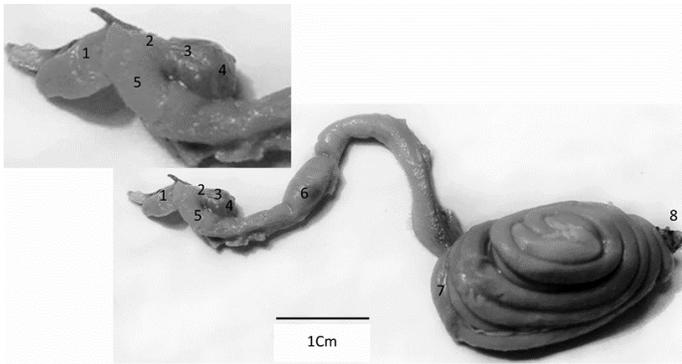


Figure 1. The structure of the digestive tract in *Iranocichla hormuzensis*. A fragment of the eight highlighted positions was used for preparing transverse and longitudinal sections. 1= esophagus; 2, 3, and 4= stomach; 5= transition region; 6= anterior intestine; 7= intermediate intestine; 8= posterior intestine.

permits (SUBC-840388) and transferred alive to the laboratory. In the laboratory, the structures of the digestive tract, position, and size of the mouth, dentition, and shape of the gill rakers were morphologically described in both sexes. The pharyngeal jaw, position, and size of mouth and structure of skull have been drawn using camera lucida coupled with a stereomicroscope (Zeiss SV 11). The teeth structure examined using the scanning electron microscope (SEM) following Monsefi et al. (2010). Briefly, samples were dehydrated in series of 30, 50, 70, 90 and 100% ethanol, fixed, mounted on metallic stubs, coated with a layer of gold (Sc7640 Sputter coater Fisons) and photographed (Leica Cambridge).

For histological description, a fragment of the pharynx, esophagus, stomach, and intestine (anterior, intermediate, and posterior portions) from two specimens (one male and one female) were examined through transverse and longitudinal sections (Fig. 1). In summary, the tissue samples initially were fixed in Bouin's fluid and then following the routine histological methods described in Bancroft and Stevens (1990), the tissue blocks were obtained. These tissue blocks were submitted to a microtome (microtome Spencer 820, U.S.A) to obtain micro section at 5-7 μm . Then micro sections were stained with hematoxylin-eosin (HE) based on Gurr (1962) and finally examined using a Zeiss microscope coupled to a Canon Eos digital camera.

Results

The structure of the digestive tract in *I. hormuzensis* is given in Figure 1. This fish has a terminal mouth that in female being smaller in size, although they have a bigger mouth cavity than males. The upper and lower lips include a stratified epithelium, with several rows of the serrated teeth sets on jaws. The upper jaw has three to four rows of tricuspid teethes, and the lower one often is observed with one more row of tricuspid teethes (Fig. 2A, B). The individual jaws are also including bicuspid teeth forms but just in the outer line (Fig. 2A). Pharyngeal jaw is covered with papilliform teethes in both upper and lower parts (Fig. 2C, D). The upper pharyngeal jaw consists of two oval shape bones and the lower jaw (LPJ) is triangle in shape (Fig. 2E, F). There is no external structure at the ventral LPJ when the two halves of the LPJ meet.

The esophagus is a short tubular drive from the posterior end of the pharynx extending towards the anterior part of the stomach. The esophagus consists of several concentric tissue layers of tunica; mucosa, submucosa, and muscularis (which formed by a thin inner longitudinal and an outer circular) (Fig. 3A). The mucosal epithelium has numerous goblet cells near the surface. The mucosa consists of many longitudinal folds along with stratified epithelium, which includes a large number of mucous cells (Fig. 3B). It then follows by lamina propria, which is formed by loose connective tissue with many fibers of the striated muscles (Fig. 3C). The submucosa, a thin layer of the dense connective tissue, support the mucosa and connects to the muscular.

The stomach is sac-shape and relatively small with lateral position to the intestine. The transitional region between esophagus and stomach simply detected when the cubic epithelium of the esophagus reduced and replaced by the cylindrical epithelium characterized for stomach. In addition, the strained muscular seen in the transitional region is replaced with smooth muscle fibers (a thick inner circular and an outer thin longitudinal layer) later in the stomach (Fig. 4A). At the transition region (base of the stomach), a confluence of the striated muscle and collagen fibers exist, which is similar to a sphincter

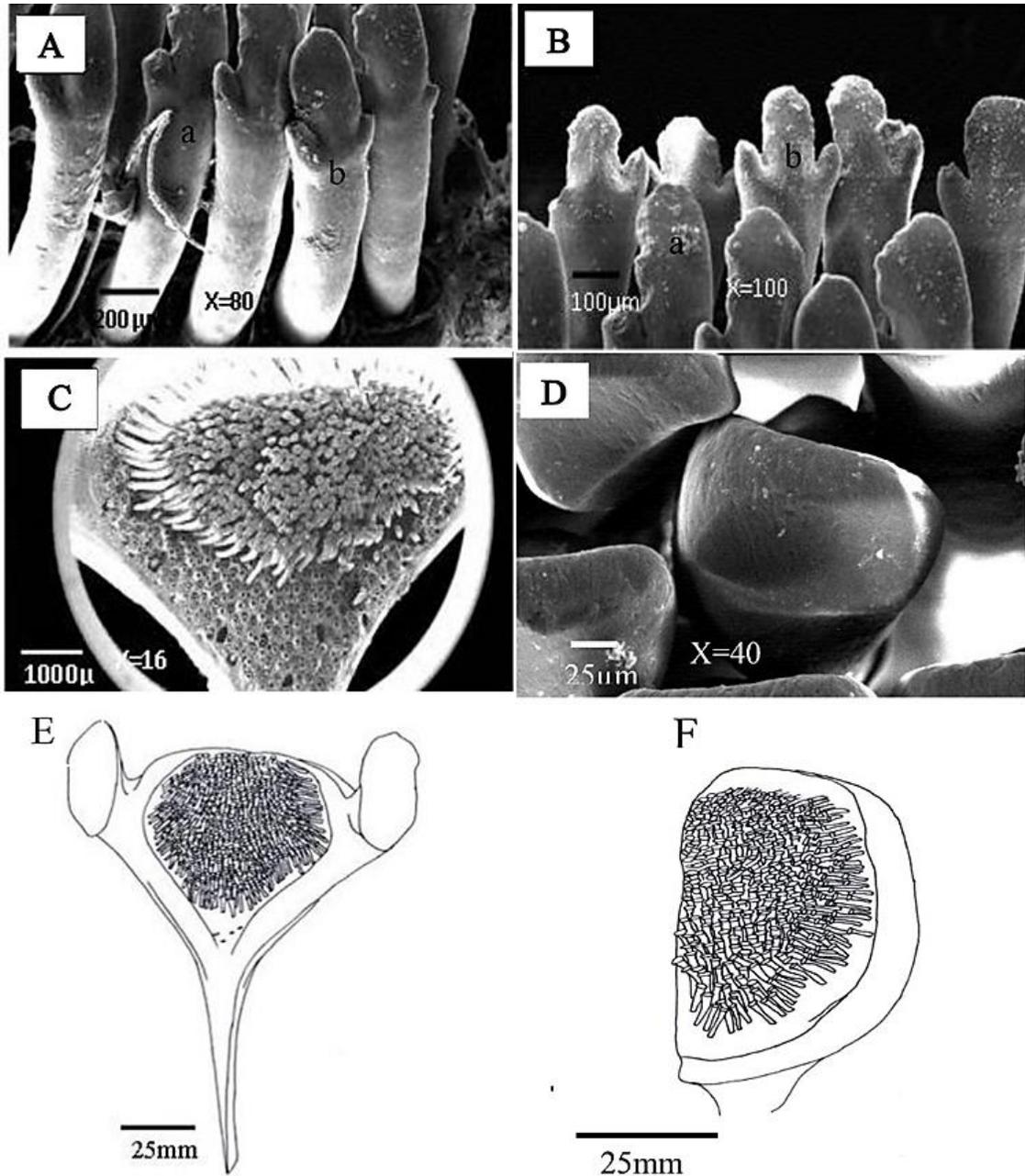


Figure 2. Shape and the structure of teethes in *Iranocichla hormuzensis*. A, B, C, D, E, and F. Figure A represents the scanning electron microscope (SEM) of the jaw showing the outer line including both bicuspid (a) and tricuspid (b) teethes. Figure B showing outer and inner lines of teethes that bicuspid (a) just seen in outer line and tricuspid (b) in all lines. Figure C demonstrates the SEM scan of the pharyngeal jaw and their papilliform teethes. Figure D showing the papilliform teethes cover all over the upper and lower pharyngeal jaw. Figure E represents a schematic view of the lower pharyngeal jaw. Figure F showing a schematic view of half of the upper pharyngeal jaw.

delimiting operating as a control mechanism (Fig. 4B). Presence of the fibromuscular folds of wall, including both striated and smooth muscle, that facing towards the intestinal lumen provided stronger support to existence of a true pyloric sphincter in the base of stomach. The stomach includes a thin layer of smooth muscle, which is approximately one in third of its

epithelium layer. The thickness of muscular layer reduced towards the end (bottom of the sack) and instead of that, the elastic fibers often are observed. In the stomach, the gastric glands predominated in lamina propria and often presents in tubular forms towards the end. The cell composition is often varied in dorsal and ventral sections, and across the wall

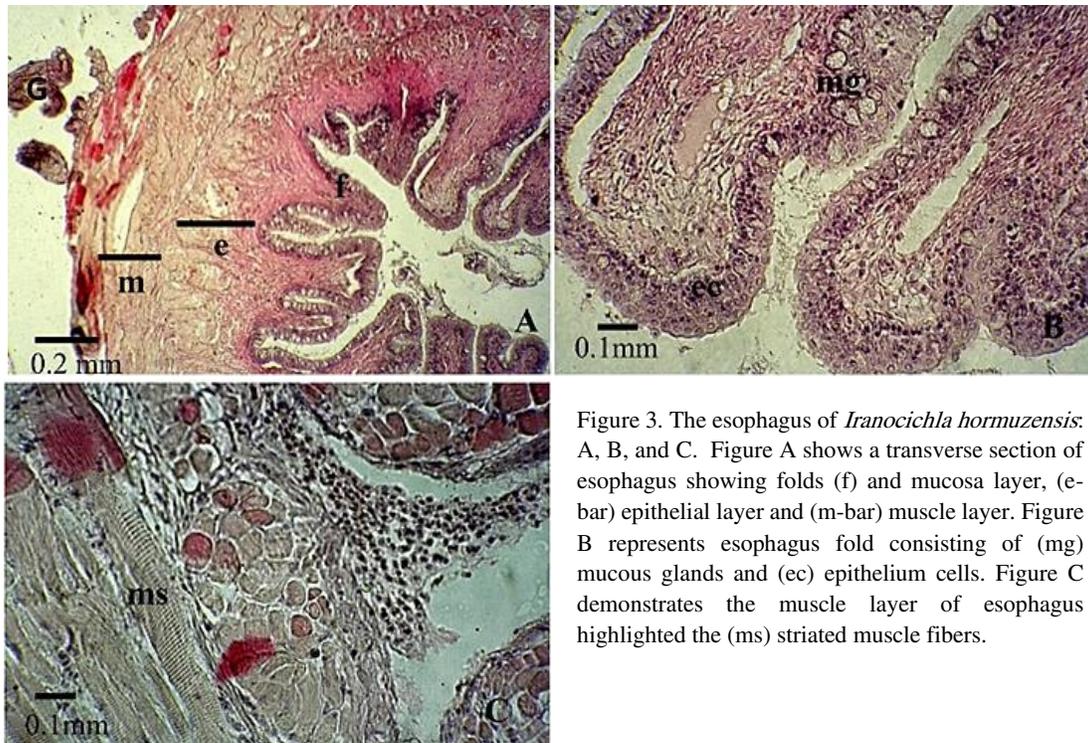


Figure 3. The esophagus of *Iranocichla hormuzensis*: A, B, and C. Figure A shows a transverse section of esophagus showing folds (f) and mucosa layer, (e-bar) epithelial layer and (m-bar) muscle layer. Figure B represents esophagus fold consisting of (mg) mucous glands and (ec) epithelium cells. Figure C demonstrates the muscle layer of esophagus highlighted the (ms) striated muscle fibers.

extensions of stomach (Fig. 4C, D, E). The beginning part of the stomach exhibits average and branched folds mucosa, which then replaces with uniform and tall mucosa through the middle and finally reduced in number and size at the fundus. In particular, the dorsal mucosa exhibits patulous, short and uniform folds, with single goblet cell and presence of a single layer of simple cylindrical epithelium. The mucosa in the ventral section shows tall and branched folds that are completely glandular and presents a much thicker layer of simple cylindrical epithelium compare with the dorsal section (Fig. 4D). The goblet cell seen in the beginning part of the dorsal section and often disappears towards fundus whereas in ventral section they increased by number (Fig. 4F).

The intestine is a long and thin tube, which forms several spiral loops joined by adipose tissue. This adipose tissue is significantly thicker in female, especially during the reproductive seasons. Average length of intestine tube measured at 45.73 mm approximately half (58.25%) of total body length. Anterior intestine has tall, thin and numerous folds, but they reduced in number and height posteriorly (Fig. 5A, B).

The epithelium of the intestine is cylindrical with nucleus positioned in basal region forming a border

facing lumen. Goblet cells exhibit across entire range of intestine (Fig. 5C), although their frequency is decreased at posterior part. After lamina propria is smooth muscular fibers, which arranged internally with a circular tunica and an outer longitudinal tunica, and overall muscular layer is ticker in the posterior part of the intestine (Fig. 5D). The tunica of the intestine wall lastly followed by serous.

Discussions

Principal objective of this study was to provide a description of the digestive tract of *I. hormuzensis*. Current findings indicated that histomorphological and anatomical structure of digestive tract of *I. hormuzensis* is well-adapted to its trophic plasticity (unpublished data). *Iranocichla hormuzensis* has a terminal mouth with serrated incisive teeth. We observed a smaller mouth size but a bigger mouth cavity in female. Digestive tract of teleost fishes often well-adapted to the feeding behavior exhibits certain characterised variation in morphological and functional features (Banan Khojasteh, 2012; Ghosh and Chakrabarti, 2015). Consequently, shape and position of mouth, dentition, and form and type of gill rakers can present feeding behavior of species (Motta, 1984; Albrecht et al., 2001; Monsefi et al., 2010). In

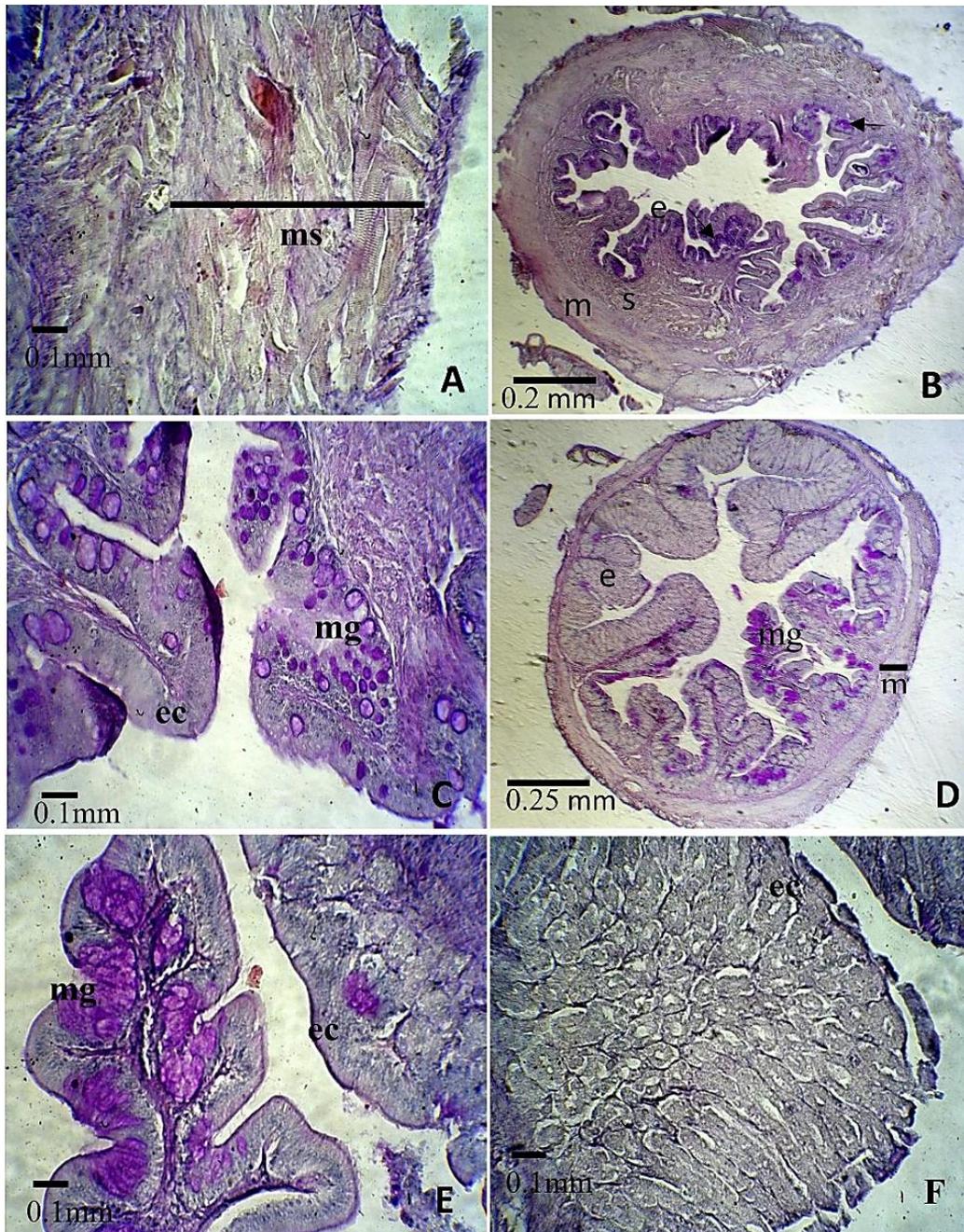


Figure 4. The stomach of *Iranocichla hormuzensis*: A, B, C, D, E, and F. Figure A represents a transition region that highlights the strained muscle (ms-bar). Figure B demonstrates a transverse section of the first part of the stomach (close to transition region), showing cell variation between dorsal and ventral (curly bracket), including epithelium (e), submucosa (s), muscularis layers (m-bar), and gastric glands in lamina propria (arrows). Figure C show dorsal folds in the stomach showing the epithelium cells (ec) and mucous glands (mg). Figure D represents an intermediate part of the stomach that showing clear variation in the dorsal epithelium and epithelium cells compare with the first part. Figure E shows folds in the ventral section of the stomach. Figure F represents the epithelial and epithelial cells of the stomach from the dorsal region from intermediate towards the end of the stomach.

this context, mouth size or gap is stated to be a proportion to the prey size consumed by the species (Canan et al., 2012). However, this condition may not totally be supported in *I. hormuzensis*, because more than 80% overlap in food items have been recorded in male and female (unpublished data). On the other

hand, it was indicated a larger size prey (a small size fish) consumed by male that might indicate the ability of male to swallow bigger prey. However, having a bigger mouth cavity observed in female of *I. hormuzensis* potentially is a specific adaptation related to the mouth broader behavior of female

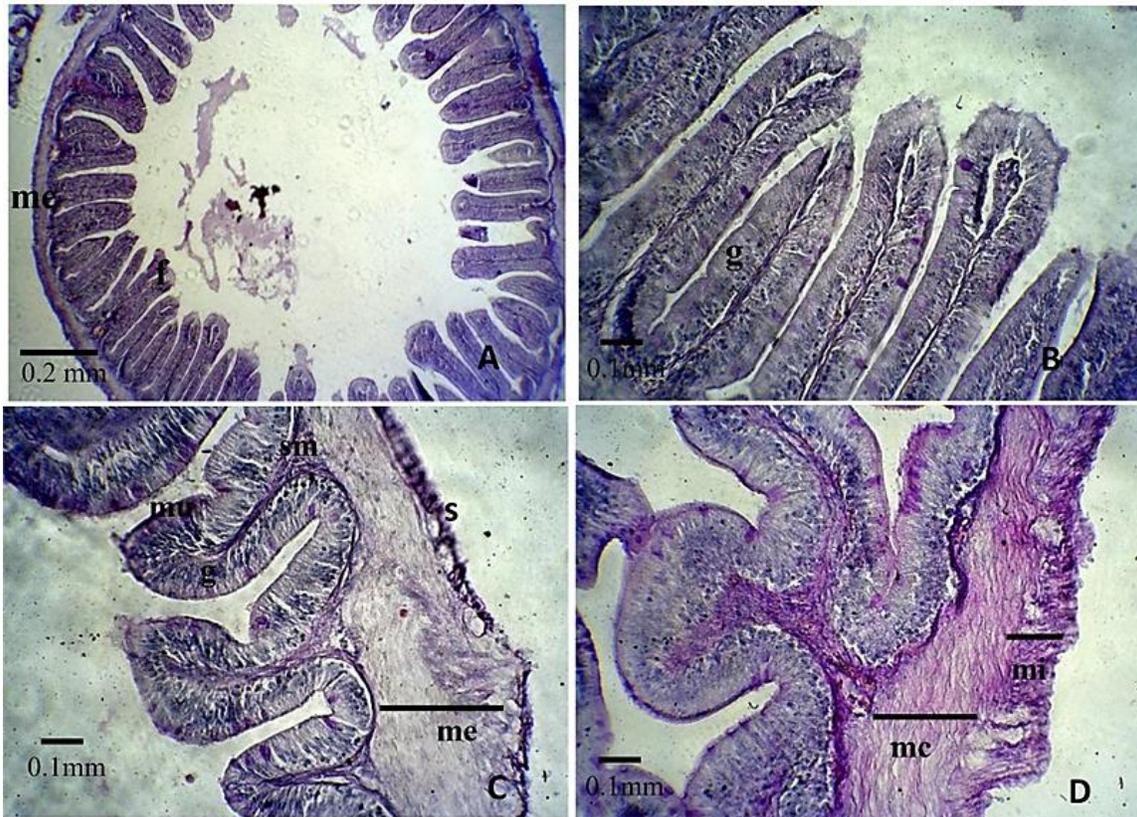


Figure 5. Intestine of *Iranocichla hormuzensis*: A, B, C, and D. Figure A represent anterior intestine with muscularis externa (me) and tall folds (f) that are very close to each other. Figure B shows anterior intestinal folds formed by a simple cylindrical epithelium including goblet cells (g). Figure C represents an intermediate section of the intestine with short folds and a thick muscularis externa (me), goblet cells (g), serosa (s), mucosa (mu) and submucosa (sm). Figure D demonstrates posterior intestine contains very short folds and smooth muscular fibers, arranged internally with a circular tunica (mc) and an outer longitudinal tunica (mi).

(Esmaeili et al., 2009).

Several unicuspid and serrated rows of teeth observed respectively in pharyngeal and oral jaws of *I. hormuzensis*. The dentition of both oral and pharyngeal jaws are highly correlated with the foraging behavior of cichlid fishes (Kocher, 2004; Streelman and Albertson, 2006). For example, the papilliform pharyngeal teeth in *Herichthys minckleyi* are specialized for herbivores to shred plants (Darrin Hulsey, 2006). In overall, morphology of oral jaw tooth ranged from unicuspid [observed in poscivorous, planktivorous and insectivorous species (e.g. *afra* cichlid, *Cynotilapia afra*)] to tricuspid [stated for algal scrapers (e.g. blue mbuna, *Labeotropheus fuelleborni*)] in cichlid fishes (Kocher, 2004; Streelman and Albertson, 2006). Likewise, the morphology of teeth in *I. hormuzensis* with papilliform pharyngeal teeth and bicuspid and tricuspid forms of teeth in the oral jaw is the norm for

cichlid fishes. *Iranocichla hormuzensis* have an outer row of bicuspid and tricuspid teeth followed by several tricuspid rows as similar as described for zebra mbuna, *Metriaclima zebra* (Streelman and Albertson, 2006) with exception of missing third cusp on the outer row of teeth in *M. zebra*. The morphology of teeth noticed in *I. hormuzensis* from both oral and pharyngeal jaw potentially justified by the fact that these parts assist in its generalist feeding habits.

In esophagus, it is generally assumed that striated fibers predominate in anterior part, which then replaces with smooth muscle in posterior region (Canan et al., 2012). Nevertheless, this structure seems to be evolved with certain variation in some species (see Canan et al., 2012), particularly in cichlid fishes, that esophagus consists of striated muscles across the entire region of its full length (e.g. Gargiulo et al., 1996; Morrison and Wright, 1999; da Silva et al., 2012). In support of our finding, a similar pattern

previously described for esophagus morphology in other cichlid fishes (Gargiulo et al., 1996; Morrison and Wright, 1999; da Silva et al., 2012).

Position and histological structure (that the strained muscle predominated at the externa tunica) of the sphincter in *I. hormuzensis* suggests that this region potentially prevents direct food passes into intestine before chemical digestion in the stomach. In general, stomach in fish with huge variation in shape and structure is an organ that serves chemical food digestion (Canan et al., 2012). The fact that *I. hormuzensis* has a small size stomach probably associated with two scenarios: first this organ provides a brief passage of food for chemical digestion (see da Silva et al., 2012), and second presence of pharyngeal plates (as described by Hahn and Cunha, 2005).

Wall of stomach in *I. hormuzensis* showed specific structure that is the most interesting variation compare with previous studies, which perceived either three histologically distinct areas [cardiac, intermediary and pyloric (e.g. Osman and Caceci, 1991; Morrison and Wright, 1999) or a unique structure across the whole organ (see da Silva et al., 2012). The presence of well-developed folds from the mucosa layer as well as their structural variation across dorsal and ventral sections, observed in the present study, may suggest an exclusive evolution towards adaptation in this species. As described by Osman and Caceci (1991) the mucosal folds would slow down the food passage and therefore provide sufficient time for acid digestion.

The length of intestine in *I. hormuzensis* seems to be in the intermediate range and possibly related to its feeding habits. It is in accordance with other studies that highlighted a correlation between relative length of intestine and diet of species, which is in order of carnivores < omnivores < herbivores and detritivores (Al-Hussaini, 1949; Fryer and Iles, 1972; Fugi et al., 2001; Rodrigues and Menin, 2008). The quantitative feature of digestion and food absorption are an expression of length of intestine as well as advanced structure of intestinal mucosa (Manjakasy et al., 2009; Canan et al., 2012). The complex and developed structure of intestinal mucosa with long and numerous folds, observed especially in the anterior part of

I. hormuzensis intestine, probably involved in final digestion and also increase absorption processes in line with previous studies (Manjakasy et al., 2009; Canan et al., 2012; da Silva et al., 2012). In addition, our finding that characterised a higher proportion of goblet cells in the first part of intestine (especially approximate part), support description by Takashima and Hibiya (1995). High variation in histological structure of digestive tract wall in fish compared with other vertebrates, illustrate their dynamic food habits (Santos et al., 2011) constant environment changes (da Silva et al., 2012) and different behavior and anatomical changes related to the feeding mechanism (Kullander, 2003). Consequently, different species of fish adapted to their habitat in response to environmental variation to increase their ability to take available food, thus optimize their fitness regarding environmental changes in their habitat.

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