

Study of the digestive tract of a rare species of Iranian blind cave fish (*Iranocypris typhlops*)

ALI EBRAHIMI

Department of Biology, College of Science, Malard Branch, Islamic Azad University, Malard, Tehran, Iran. Tel./Fax.: +98-21-65672964 email: ebrahimibal@gmail.com

Manuscript received: 7 April 2015. Revision accepted: 16 July 2015.

Abstract. Ebrahimi A. 2015. Study of the digestive tract of a rare species of Iranian blind cave fish (*Iranocypris typhlops*). *Biodiversitas* 16: 173-178. The Iranian blind cave fish (*Iranocypris typhlops*) is a unique taxon which only lives within a cave in Lorestan Province in southwest of Iran. Regardless of its enormous genetic interest, this species faces an imminent risk of extinction as no conservation efforts have been done for its protection. This study aimed to analyze the morphology of the digestive tract of this interesting fish using a histological approach. Detailed examinations of the fishes showed that the mouth is horseshoe-shaped and located in an inferior situation (ventral side) of the head. In it, there are three rows of pharyngeal teeth including inner, middle and outer rows, which bear 5, 3 and 3 teeth respectively. The esophagus is very short and lined with an epithelium containing numerous mucous folds. The stomach is not present. The anterior segment of the intestine is S-shaped which comprises about one half the gut. In this part, there are mucosal folds which show different sizes and mucous layer is thicker than other layers (submucosa, muscle layer and serosa). The distal portion of the intestine is straight (rectal) and terminates to the anal region. In general, the ratio of intestine length is 1.2, as compared to the body length. This study showed that the liver in this taxon is composed of two lobes, the right lobe being two-parts and bigger than the left one. The gall bladder is clear and spherical in appearance. The pancreas is red or orange and observed as scattered masses of cells on the mesentery of the digestive system. To our best knowledge, this is the first study to analyze the morphological characteristics of the digestive tract of the Iranian blind cave fish, and its unique characteristics here found confirmed its singularity and so, the urgent need for its conservation.

Keywords: Blind cave fish, digestive system, digestive tract, histology, morphology

INTRODUCTION

The fishes are very diverse taxa; weighing from a few grams to several tones and with a length of several centimeters to several meters. As well as, the variety of fish is abundant in Iran. Some species are not yet fully known. For example, there are few sources about one of the most unique species in Iran called the “Iranian blind cave fish” – *Iranocypris typhlops* (Figure 1) – which is limited to a cave in “Lován” Village located at Lorestan Province, Iran.

This species is threatened to extinction mostly because no conservation actions were taken to the moment and virtually no attentions have been paid to it. The Iranian blind cave fish (its characteristics are the same as the taxon) was first identified by Bruun and Kaiser (1944). This is the only blind fish species known in Iran and its unique morphology, grants it an enormous genetic interest. The Iranian blind cave fish is mentioned in the 1996 Red List of IUCN as one of the fish species threatened to extinction. The information on the species is scarce and many of its biological aspects are still unknown (IUCN 2015).

The fish’s digestive tracts are as diverse as the species themselves, sometimes with differences occurring within the same species (Coad 1996; Sheybani 2005). Knowing the feeding ecology of a species is a key aspect to understand its role and positioning in the prey/predator relationships, therefore, this study aimed to analyze the morphology of the digestive tract of Iranian blind cave fish as a step ahead to the knowledge of this evasive species.

MATERIALS AND METHODS

After obtaining the necessary permits from the Environmental Protection Organization of Iran as a necessity for fishing, ten fishes were fished collected from the wild habitat and were transferred to laboratory. The water temperature was about 17 C at the time of fishing. The average of total length of fish’s body was 41 mm and the average of their weight 1.5 g. The fishes were immediately processed and placed in a saline Formalin 10% fixative solution. Through a processing and embedding process in Paraffin, tissue sections were then prepared from the obtained samples. The thickness of sections was about 7 μ m. The sections were stained through Hematoxylin & Eosin techniques (Slack 1995). After they were prepared, tissue sections were examined through Leica microscope and then the required pictures with various microscopic magnifications were prepared by a digital “Canon” camera attached to the microscope. This study included the morphological and histological studies analysis of the digestive tract.



Figure 1. A total dorsal view of Iranian blind cave fish

RESULTS AND DISCUSSION

Morphology of digestive tract

The mouth of fish is located in lower position (Figure 2) and the mandibles have no teeth in it.

Three rows of pharyngeal teeth located at 5th gill arch (Figure 4) are present. These teeth are extremely small and can only be seen using a loupe. Pharyngeal teeth are situated in 3 inner, middle and outer rows and the numbers of teeth are: 4 in 3 specimens, 5 in 7 specimens on the inner row, 3 in middle row in all specimens, and 3 in outer row in all specimens (Figure 3).

The esophagus is quite short between 1 to 2mm in length of and it is not as thick as the intestine. This species is similar to carp fishes as they have no stomach.

The intestine consists of three distinct sections of variable thickness between them. The first section located next to esophagus is darker in contrast to the other sections and comprises half of the intestine. This section has an S-

shaped form and the intestine is thicker in this part. The joint part of bile duct is also located in this part. In the second third, the intestine is less thick and lighter in color. In the last third, the intestine becomes thicker. The total ratio of intestine to body length in this species is about 1.2 and the pylorus appendage is absent.

Liver, gallbladder, pancreas and swim bladder are the attached and joint parts of digestive tract. The liver consists of two separate lobes. The right lobe is larger and consists of two parts. The color of the liver is slightly different in specimens but its usual color is light cream. Gallbladder has a spherical shape and has a thin and clear wall. It is located at right part and under the liver.

Pancreas has a scattered structure located on mesentery peritoneum. Its color is between orange and red. In average, 3 to 7 scattered parts of pancreas can be found at this species locating on mesentery and extends from esophagus to intestine last part (Figure 7).

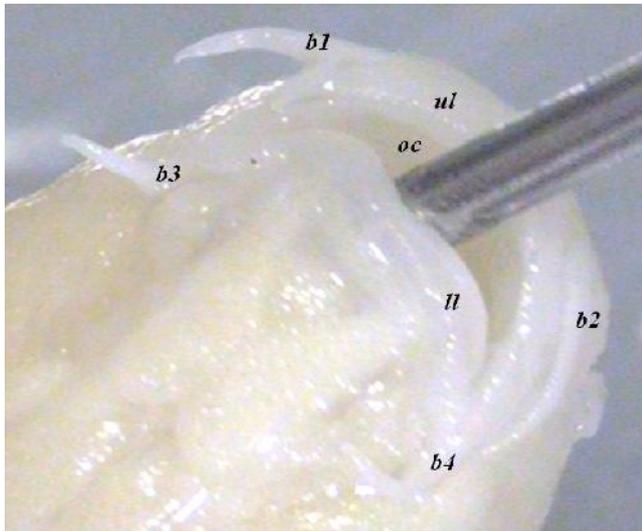


Figure 2. Mouth and Barbells position in cave fish, (ventral view), oc (oral cavity), ul (upper lip, ll (lower lip), b 1-4 (barbells)

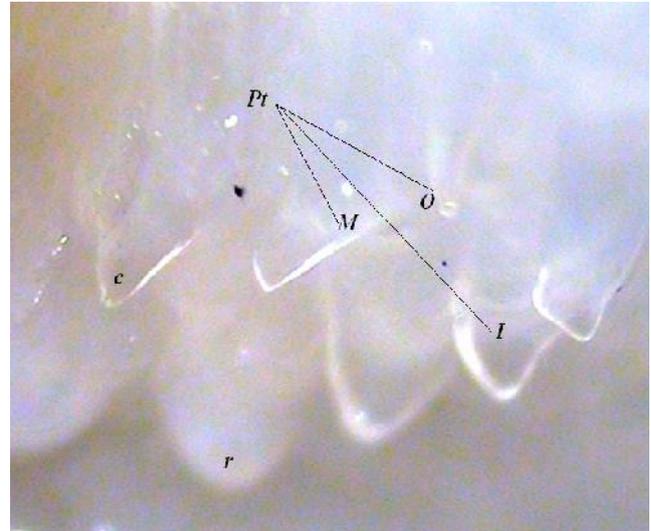


Figure 3. Pharyngeal tooth, inner row (I), middle row (M), outer row (O). Tip of tooth is cone or round

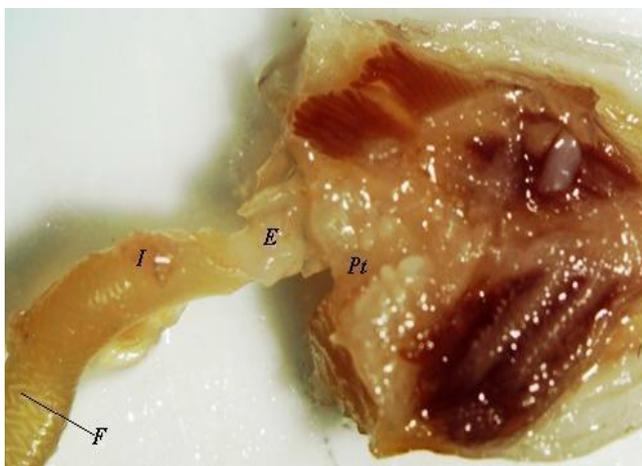


Figure 4. Initial part of digestive tract: Pharyngeal tooth (Pt), Esophagus (E), Intestine (I) and folds of mucous (F)

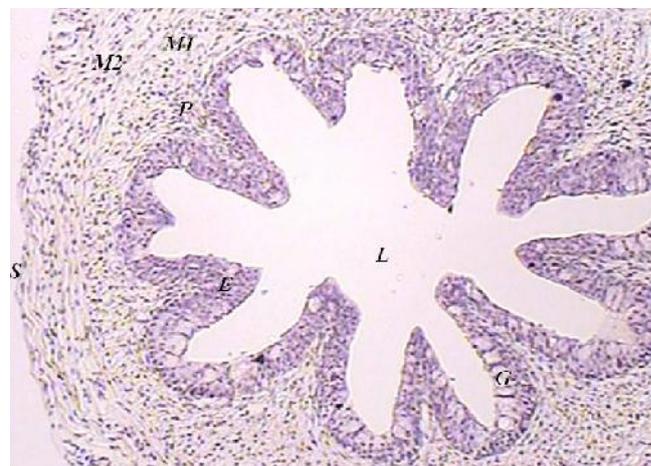


Figure 5. Cross-section of esophagus; Lumen (L), Epithelium (E), Parine (P), Longitudinal muscles (M1), Circular muscles (M2), Serous (S), H & E, $\times 10$

Digestive tract histology

Regarding histology, esophagus structure is clearly distinguishable from the other parts of the digestive tract. The esophagus mucosal contains many longitudinal mucosal folds that extend into lower cavity (Figure 5) but esophagus is thinner than other parts of digestive tract. Its epithelium is simple, stratified, squamous, and the number of cellular layers differ in various areas.

The longitudinal mucosal folds vary, both in shape and length. Two flat inner and outer muscle layers can be found which are longitudinal and circular respectively.

Esophagus muscular layers are thicker than other parts of digestive tract. Squamous cells of serous membrane surround the esophagus (Figure 5). The cells located on the base membrane of the esophagus contain one row of cylindrical cells with oval nucleus. A row of polyhedral cells are located on the surface of them. The cells of top layer are squamous and include elongated nucleus. In some parts of esophagus, between epithelium cells and the cells directed to the top of epithelium, there are some round, large and sub mucosal secretory cells that include foamy and nuclear cytoplasm near to base. These cells are similar to goblet cells and are not evenly distributed (Figure 6).

By means of serial sections of intestine, three parts of tissue can be distinguished. Part 1 is from beginning of intestine, the part to which the bile duct is attached to end of dilatation and includes half of intestine length. In this part, there are intestine mucosal folds in various sizes and the mucosal layer is thicker than other layers and muscular layers (inner and circular, outer and longitudinal) are thinner (Figure 7). Intestine epithelium is simple cylindrical and Paryn loose connective tissue is clearly recognizable in the mucosal folds (Figure 8).

In second part, intestine is thinner but there are more mucosal folds. In this part, the mucosa is quite thick and there is a thin muscular layer that is present but cannot be seen as a double layer.

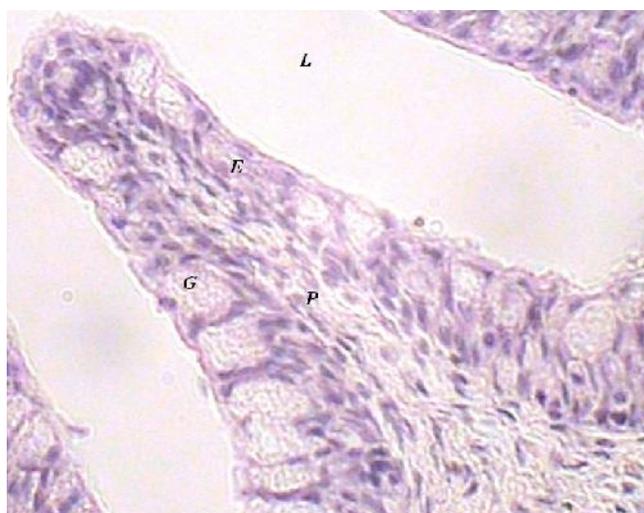


Figure 6. Cross-section of esophagus; Lumen(L), Epithelium (E), Parine (P), Goblet cell (G), H & E, ×20

Regarding histology, the distal part of intestine has a few intestine mucosal folds; the mucosa, sub mucosa and muscular layer which are thinner. In addition, the diameter of intestine increases in this part.

In liver, cells are arranged like irregular columns and form a mixed matrix which is not similar to mammals liver structure. There are no central veins. Liver exposures are seen between liver irregular columns in various shapes and Kupffer cells are located in their wall (Figure 10).

Gallbladder has some distinguished layers including simple cylindrical epithelium, Paryn connective tissue, very delicate muscular layer and serous membrane (Figure 9). In this specimen, the gallbladder is so clear because the constituent layers of its wall are so thin.

Pancreas is located throughout the digestive tract from esophagus to end of intestine and next to digestive tract; it is as scattered mass of cells on the peritoneum (Figure 7). The cells of pancreas exocrine part include a round nucleus and acidophilic cytoplasm that imply the presence of Zymogen granules. Among Acinar cells, there are a mass of cells containing smaller nucleus which are endocrine part of the glands (Figure 11).

Discussion

The mouth location in the fish is soleus and when located in a lower and more ventral position, it is a clear indication that it is a bottom feeding fish. Pharyngeal teeth in carps are different in various species regarding shape, number and their location on 5th gill arch. Therefore, they are used to identify the different carp species (Vosoghi and Mostajir 1994; Coad 1996; Bastani 1999).

Regarding the number of rows of pharyngeal teeth of blind cave fish, 2 rows (Bruun and Kaiser 1944) and 3 rows have been reported. In each row, inner, middle and outer teeth were reported to vary between 1-3, 3-4, 3-5 teeth (Humason 1979). The present study agrees with those finding as 3, 3 and 5 teeth were respectively found in those rows.

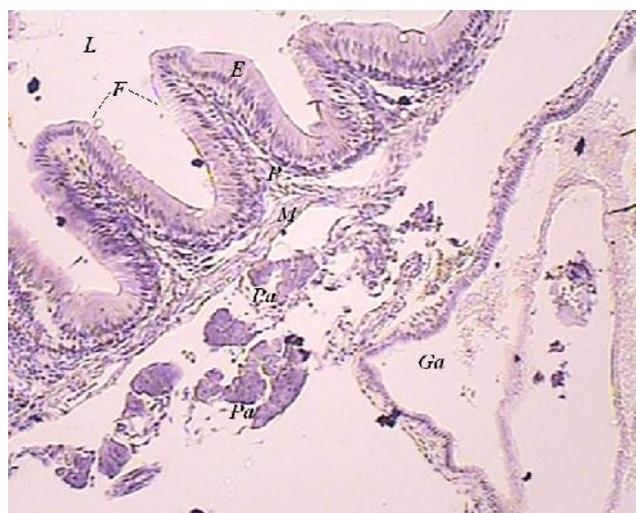


Figure 7. Cross-section of intestine in first part; Lumen(L), Epithelium (E), parine (P), muscular layer (M), Pancreas (Pa), Gall bladder (Ga), H & E, ×10

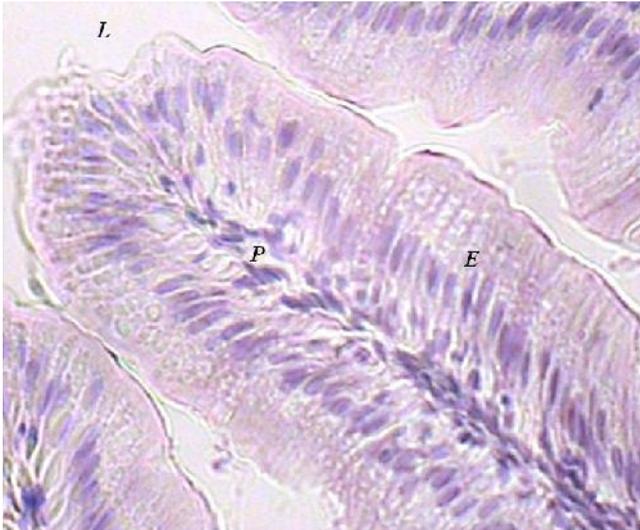


Figure 8. A plica mucous of midgute; Lumen(L), Epithelium (E), Parine (P), muscular layer (M), Pancreas (Pa), Gall bladder (Ga), H & E, ×10



Figure 9. Cross - section of Gall bladder; Lumen (L), Epithelium (E), Parine (P), muscular layer (M), serous (S) , H & E, ×40

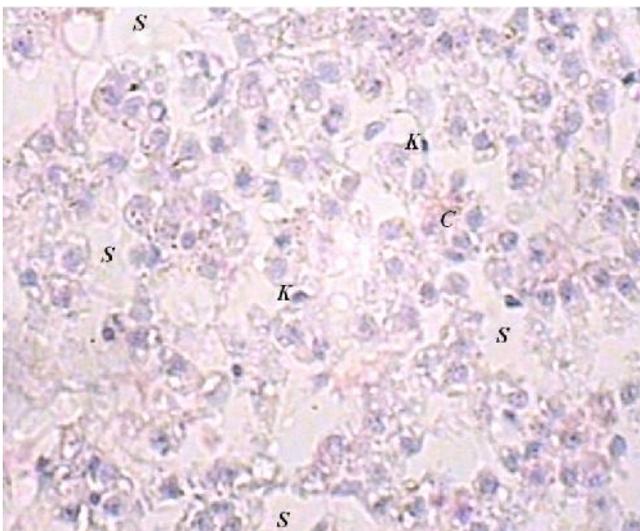


Figure 10. Liver tissue; cords of liver cells(C) are very erratic and kupffer cells (K) are seen in sinusoids, H & E, ×40

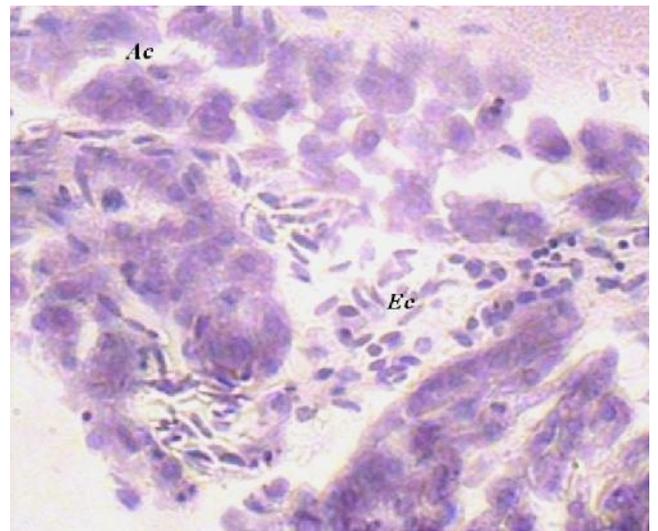


Figure 11. Pancreas tissue; Acini cells (Ac) and Endocrine cells (Ec), H & E, ×40

In the carp fishes, esophagus is shorter and morphologically in some of the species (e.g. Amur fish, silver carp and common carp) there is no difference between esophagus and intestine and it is hard to distinguish between them (Bastani 1999). In the present study, due to small size of fish, it was impossible to visually identify the esophagus which was only possible through histology. In some fish species such as white fish (Cyprinidae family), esophagus wall includes a thick muscular layer and it is easy to distinguish between esophagus and intestine (Bastani 1999). Regarding the esophagus epithelium cells, large and round secretory cells are identifiable. The same is true of *Acipenser stellatus* esophagus (*Acipenser stellatus* is one species of sturgeon that produces the caviar) (Ghavami 2000). The carp fish

esophagus are a simple and twisty tube in the abdominal cavity (Bastani 1999) a characteristic also seen in Iranian blind cave fish.

Intestine is in S shaped curve and intestines' epithelium is a simple cylindrical structure. The existence of brush border in intestine epithelium is a main mechanism in absorbing and consuming food and intestinal absorption process in fishes and mammals (Kapoor et al. 1975).

Since the body of Iranian blind cave fish is virtually transparent, intestines can be seen from the outside. Depending on the fish diet, the length and diameter of the intestine differ. In carnivorous fishes, the esophagus length is shorter than herbivorous fishes (they have no stomach) that have long esophagus including many folds (Coad 1996).

The ratio of intestines' total length to body length varies in Cyprinidae. For example, this ratio in silver carp is approximately 6 (Vosoghi and Mostajir 1994), in *Aspius aspius*, the ratio is 0.7 to 0.9 (Bastani 1999). In Persian sturgeon, the esophagus constitutes 40-50% of the digestive tract length (Sheybani 2005). In blind cave fish, the average ratio of intestine length to body length was 1.2.

The fish's intestine is divided into four parts: ventral, anterior, middle, posterior. The ventral part includes oral cavity and gill (pharyngeal). Anterior part is from gills' posterior edge to esophagus, stomach and pylorus (Coad 1996). In Persian Sturgeon, the intestine constitutes two distinguished parts including anterior intestine and posterior intestine (Sheybani 2005). In carp fishes which have no stomach or pylorus (Vosoghi and Mostajir 1994), middle intestine begins from back of pylorus to posterior esophagus with no clear border. Middle intestine includes a number of pyloric caeca which are not present in fishes which have no stomach. Middle intestine is the longest part of intestine and since it is longer than body length, is in shape of complex loops (Coad 1996). This loop is also seen in S shape in the blind cave fish.

Main function of liver as a digestive gland is producing and secreting bile. Color of fishes' liver ranges from dark brown to light cream (Navarro et al. 2006) and blind cave fish liver is light cream (Alboghhabish and Khaksari 2005). The liver of blind cave fish is divided into two (right and left) lobes. Carp species have two-part liver. In the blind cave fish, like the carp species, a large part of liver is located in the right part of abdominal cavity (Alboghhabish and Khaksari 2005). Liver parenchyma is surrounded by a delicate capsule of loose connective tissue (Alboghhabish and Khaksari 2005; Navarro et al. 2006). Liver cells secrete bile which flow to extracellular capillary tubes. The liver cells are hepatocytes containing spherical and central nucleus including different quantities of heterochromatin (Abbasi and Gharzi 2000).

Herbivorous carps as well as African lungfish have liver cells with two nucleuses (Alboghhabish and Khaksari 2005), but in present study, those were not identified. In this taxon the liver cells are arranged like columns and form liver cords a morphological characteristic also reported in Japanese salmon, too. The fish liver cells willingness to create liver cords is lower in contrast to mammals (Alboghhabish and Khaksari 2005). Liver cells including this particular morphology were identified in the present study, but they do not form regular columns as liver cords.

The main stored materials in fish's liver are glycogen and fat. The fat is present in organelles of cellular cirrhosis forming small to average drops which occupy the cytoplasm of these cells. Due to this fact, these cells are commonly referred as fat storage cells. Since fat and glycogen are not so chromophil through Hematoxylin and Eosin staining techniques, many vacuole structures are seen in the hepatocytes (Poosti and Sedighmarvdasti 2000; Navarro et al. 2006). These two substances can however be distinguished regarding the vacuole shape. Fat drops are spherical, single or in a mass, while glycogens are irregular

in form (Poosti and Sedighmarvdasti 2000). These vacuoles were also identified in the liver of blind cave fish.

Kupffer cells belong to macrophage cells which comprise reticuloendothelial system (Navarro et al. 2006). Some researchers believe that fish liver has no Kupffer cells, but these cells were reported in catfish (Alboghhabish and Khaksari 2005) and carps (Alboghhabish and Khaksari 2005). Kupffer cells including triangle nucleus and heterochromatin were observed among the endothelial cells of the liver exposure wall of the blind cave fish.

Gallbladder is present in many fishes and is divided into three parts: mucosa (epithelium and Paryn connective tissue), muscular part and serous part (Abbasi and Gharzi 2000). In the present study, the blind cave fish gallbladder appeared to be crystalline, spherical and clearly segmented in the showing the three tissue parts of gallbladder. Nonetheless, its muscular part was particularly thin.

In many fishes, pancreas tissue gradually lies next to portal vein branches and the result is called hepatopancreas tissue (Abbasi and Gharzi 2000; Dabrowski et al. 2003; Dyk et al. 2005). A part of pancreas of carp herbivorous fish is located within the liver (Alboghhabish and Khaksari 2005) a singularity that was not observed in blind cave fish. The pancreas of teleosts is an organ is scattered around the digestive tract peritoneum or other limbs and is present among fat tissues (Dyk et al. 2005). Exocrine cells in immature fish as well as that part of pancreas which scattered in fat tissue of adult fish have acinar form (Poosti and Sedighmarvdasti 2000; Dyk et al. 2005).

In the study on blind cave fish it was observed that the pancreas is present in the mesentery peritoneum as scattered parts in red to orange. Observing the pancreas parts through light microscope, it was found that secretory cells include a strongly basophilic cytoplasm and acidophilic spherical particles called zymogen (Poosti and Sedighmarvdasti 2000; Dabrowski et al. 2003; Alboghhabish and Khaksari 2005; Dyk et al. 2005). This can be easily observed in the tissue section of blind cave fish pancreas. Islets of Langerhans were identified forming light cellular masses in exocrine part of pancreas, but for more accurate identification of these organelles it is necessary to use specialized staining methods to identify all types of present cells.

ACKNOWLEDGEMENTS

I have taken efforts for accomplishing this project. However, it would not have been possible without the kind support and help of many individuals and organizations. I would like to express my sincere thanks to all of them, in particular, the Mallard Branch of Islamic Azad University, Iran.

REFERENCES

- Abbasi M, Gharzi A. 2000. Veterinary Comparative Histology, Preclinical Relation. Partoovaghee Publisher, Tehran.
 Alboghhabish N, Khaksari R. 2005. Structural study of liver and pancreas of Cyprinidea family fishes. Iranian Vet J 11: 25-33.

- Bastani P. 1999. Comparative study of digestive system in Cyprinidea fish with different diet. [M.Sc. Thesis]. Tarbiat Modares University, Tehran.
- Bruun AF, Kaiser EW. 1944. *Iranocypris typhlops* n.g., n.sp., the first true cave fish from Asia., Danish scientific investigations in Iran. Part 4. Copenhagen, 4: 1-8.
- Coad BW. 1996. Threatened fishes of the world: *Iranocypris typhlops* Bruun & Kaiser, 1994 (Cyprinidae). Environ Biol Fish 46: 374.
- Dabrowski K, Lee K, Rinchar J. 2003. The smallest vertebrate, Teleost fish, can utilize synthetic dipeptide-based diets. J Nutr 133: 4225-4229.
- Dyk J, Pieterse G, Vuren J. 2005. Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. Ecotoxicol Environ Saf 66: 432-440.
- Ghavami SM. 2000. Study of Pharynx, Esophagus and Prestomach, of Oozoonbooroon Fish. [Ph.D. Dissertation]. Veterinary Department, Tehran University, Tehran.
- Humason GL. 1979. Animal Tissue Techniques. 4th ed. W. H. Freeman, New York.
- IUCN. 2015. *Iranocypris typhlops* (Zagros cave garra), Status: Vulnerable D2 ver 2.3. In: The IUCN Red List of Threatened Species. Version 2015.2. www.iucnredlist.org. [13 July 2015].
- Kapoor B, Smith H, Verghina A. 1975. The alimentary canal and digestion in teleosts. Adv Mar Biol 13: 109-239.
- Navarro M, Lozano M, Agulleiro B. 2006. Ontogeny of the endocrine pancreatic cells of the gilthead sea bream, *Sparus aurata* (Teleost). Gen Comp Endocrinol 148: 213-226.
- Poosti I, Sedighmarvdasti S. 2000. Fish Histology Atlas. Tehran University Publisher, Tehran.
- Sheybani M. 2005. Microscopic Study of Iranian Sturgeon Fish, *Acipenser persicus* [Ph.D. Dissertation]. Department of Veterinary, Tehran University, Tehran.
- Slack J. 1995. Developmental biology of the pancreas. Development 121: 1569-1580.
- Vosoghi G, Mostajir B. 1994. Freshwater Fishes. Tehran University Publisher, Tehran.