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Anatomical and histological investigation of the pyloric caeca in beluga (*Huso huso*)

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Abstract: The objective of this paper was to study anatomy and histology of pyloric caeca in 2 years old beluga (*Huso huso* (Linnaeus, 1758)). In this study 12, 2-year old male and female *H. huso* were included. Anatomical position of pyloric caeca was studied through dissection. Histological specimens were fixed in 10% formalin buffer phosphate for 72 h. The specimens were processed through paraffin embedding and 7 micrometer sections were cut and stained by Hematoxylin and eosin and observed under light microscope. It was shown that pyloric caeca in *H. huso* were attached and formed a large mass between stomach and duodenum. The capsule was a smooth muscle that had dispatched trabeculae into the organ and formed lobules. There were numerous villi with simple columnar epithelium in different sizes in each lobule. It was demonstrated that pyloric caeca in *H. huso* like other sturgeon fish were attached to each other and formed an organ called pyloric caeca with a long leaf-shaped villi.
Key words: Anatomy, histology, pyloric caeca, *Huso huso.*

Introduction. Sturgeons (Acipenseridae) are prehistoric fish that have evolved over 80 million years ago. Beluga (*Huso huso* (Linnaeus, 1758)) has a wide distribution. It occurs in the Caspian Sea, Black Sea, and the Sea of Azov and many of the tributaries of these seas. Beluga is the largest species of Acipenseriformes reaching a length of six meters and a weight of more than one tone (Berg 1948, Sudagar et al 2010). The function of the pyloric caeca of fish has been uncertain since their detailed description by Aristotle. He suggested three hypotheses about their function: (1) to store up the food, (2) putrify it up and (3) concoct it (i.e., storage, fermentation and digestion) (Buddington & Diammond 1986; Yoshinaka et al 1978; Yoshinaka et al 1973; Mankura et al 1984; Nimmo 1986). Teleosts have blind tubes, so called pyloric cecum that is connected with the anterior end of the small intestine (Hideo et al 2003). Pyloric caeca are embryological derivation of the anterior intestine and possess a lumenal epithelium that is structurally similar to other segments of intestine (Yasutake & Wales 1983; Sodelin et al 2000; Veillette & Young 2005). Although the pyloric caeca are known to be a major site for nutrient absorption (Collie 1985; Buddington & Diammond 1987), little is known about their potential role in major functions of intestines and salt and water balance (Collie 1985; Buddington & Diammond 1987). Pyloric caeca is seen on intestine of a great number of Osteichthyes (Beorlegui et al 1992; Hideo et al 2003). At the end of pylorus there is one or several blind pouches called pyloric caeca. In some fishes such as *Ictalurus sp.*, topminnows and Esocidae there are no pyloric caeca. In *Polypterus sp.* there is only one pyloric caeca and in Percidae three of them and in pleuronectiforms several of them are present. In Scombridae, Salmonidae and Liparidae number of these processes get up to 200 or more (Getile 1989; De Groot 1971). In Acipenseridae the pyloric caeca join together and form a large mass which bears a single duct that opens to

beginning of intestine. In Salmonidae each of pyloric caeca opens to intestine separately (Hideo et al 2003).

Materials and methods. Twelve *H. huso* (male = 6 and female = 6) 2-years of age were included in this study. Anatomical studies were performed on 6 (male = 3 and female = 3) fish. Topographic position, attachments to adjacent organs, weight, length, width and thickness of pyloric caeca were studied. To locate the duct, 2 mL of colorful gelatin was injected into the pyloric caeca and after hardening of gelatin the organ was dissected. For histological studies of pyloric caeca the same number were used. The specimens were taken in 0.5 cm size and fixated for 72 h in 10% formalin buffer phosphate. The specimens were processed through routine paraffin embedding and cut at 7 micrometer then, stained with Haematoxylin and Eosin (H&E) technique. Statistical analyses were performed using t-test and one way ANOVA.

Results.

Anatomy: It is demonstrated that pyloric caeca processes joined together and formed a large organ called pyloric caeca in *H. huso* (Fig. 1). The pyloric caeca was attached to duodenum by a delicate ligament in the left side and to stomach in the right side. The pancreas was attached to pylorus by a flaccid connective tissue. The pyloric caeca has a convex dorsal surface, rather flat in ventral surface with a serrated edge (Fig. 1, 3). Pyloric caeca opens to the duodenum with a distinct duct (Fig. 2). Mean weight, length, width and thickness values of pyloric caeca were measured (Table 1).

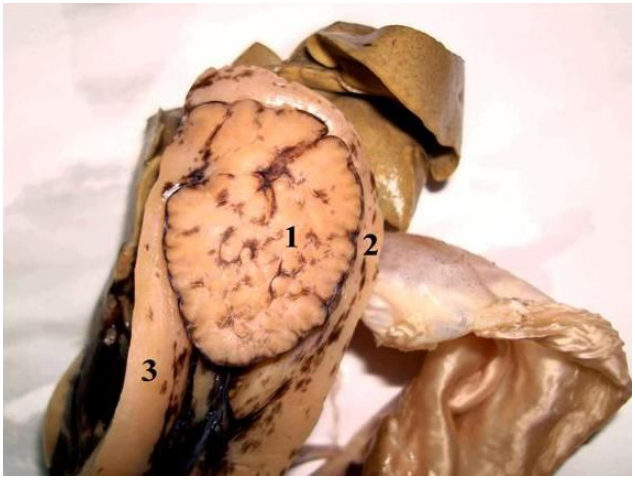

Fig. 1: (1): Pyloric caeca, (2): Stomach, (3): Duodenum.

Fig. 2: Right lateral view - (1): Pyloric caeca, (2): Duct of the pyloric caeca, (3): Stomach (4): Longitudinal section of the duodenum.

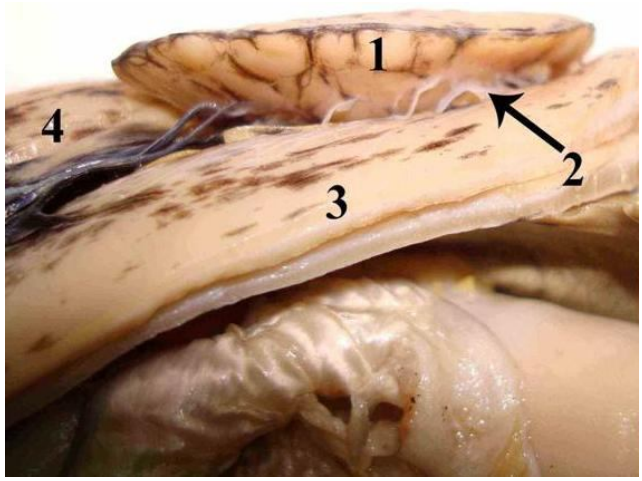

Fig. 3: (1): Left lateral view of pyloric caeca, (2): Delicate ligament in the left side that attached pyloric caeca into stomach, (3): Stomach, (4): Duodenum.

Table 1

Mean weight, length, width and thickness values of the pyloric caeca (M+SE)

Parameter / H. huso	*Weight (gr)*	*Length (cm)*	*Width (cm)*	*Thickness (cm)*
Male	15.40±0.46	3.15±0.09	2.00±0.06	1.07±0.03
Female	5.18±0.44	3.07±0.08	1.85±0.05	1.11±0.04

Histology: Pyloric caeca is encapsulated by a thick circular smooth muscle in which the nucleoli are oval and euchromatic. Among the muscular cells there were a great number of blood vessels including arteries, arterioles, veins, venouls and capillaries. A single layer of simple squamous cells (mesothelium) cover the external surface of the capsule. The muscular trabecula has divided pyloric caeca into several lobules in various sizes. There was less connective tissue between the trabecules (Fig. 4 and 5). The thickness of the capsule increased among lobules and formed a triangular region. Lobules of pyloric caeca were in different sizes and there were numerous polymorphic primary villi in each lobule.

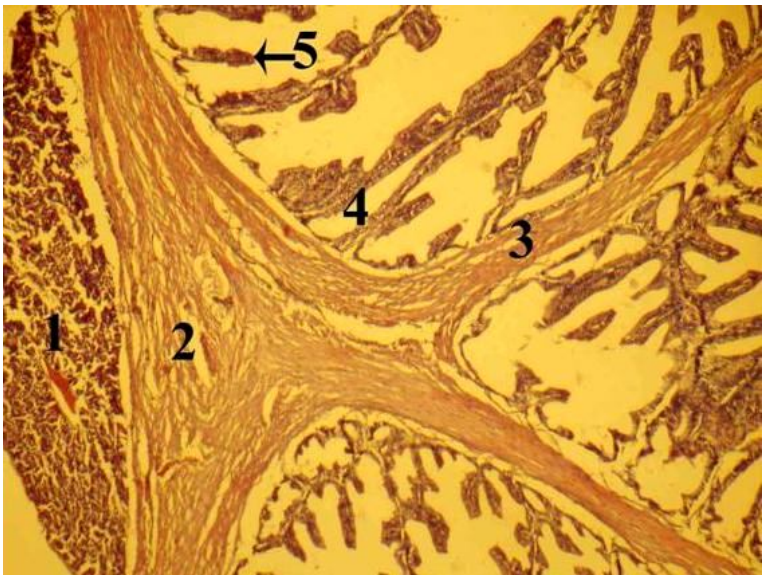

Fig. 4: (1): Pancreas, (2): Capsule of the pyloric caeca, (3): Trabecula, (4): Villi (5): Short villi between long villi (H and E, ×250).

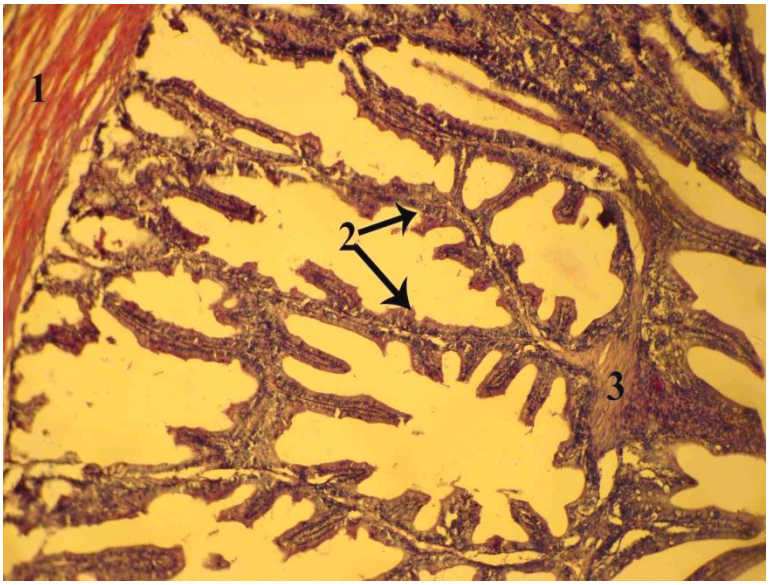

Fig. 5: (1): Trabecula, (2): Villus, (3): Smooth muscle (H and E, ×400).

The primary villi were leaf-like and slender. They had special outline so that between each two long primary villi there were one or two short ones (Fig. 4 and 7). The secondary villi were observed in the lateral side of the primary villi. In some parts of lobules villi were fused from distal parts and formed a region on which many short villi were present (Fig. 5). It should be noted that there were much smooth muscles where the villi attached to each other. Inside each villi one or two layers of smooth muscle were observed longitudinally. This study revealed that, there was a very thin layer of connective tissue beneath the villi (lamina properia). The epithelium of villi of pyloric caeca were simple columnar with eccentric nucleus and euchromatic. The nucleolus was heterochromatic and predominantly eccentric. Some goblet cells were observed among simple columnar cells. They were bowel-shaped and the cytoplasms were cloudy with a heterochromatic tinge in nucleus (Fig. 6).

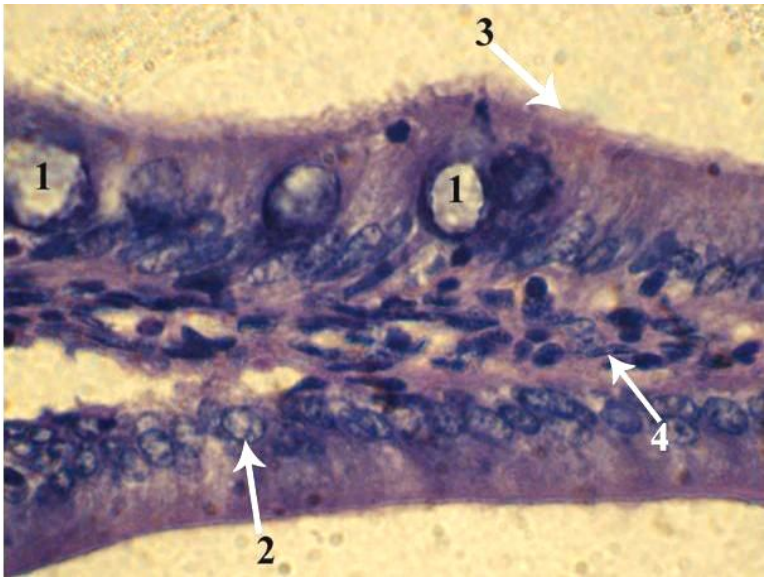

Fig. 6: (1): Goblet cells, (2): Nucleus of the epithelium cell, (3): Microvilli, (4): Smooth muscle cells (H and E, ×1000).

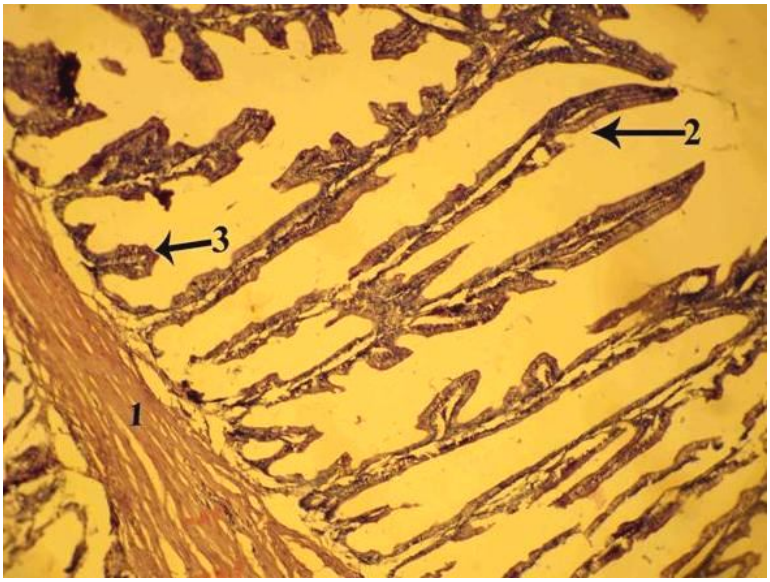

Fig. 7: (1): Trabeculae, (2): Long villi, (3): Short villi (H and E, ×400).

Mean values of capsule thickness, muscular layer thickness beneath trabecula, thickness of connective tissue of trabecula and diameter of lobule and length, width of villous of the pyloric caeca were measured (Table 2).

Table 2

Mean values of capsule thickness, thickness of connective tissue beneath villi, thickness of connective tissue of trabecula, diameter of lobule, length and width of villous in 2 years old *H. huso*

Parameter (µm)/ H. huso	*Diameter of Lobule*	*Width of villous*	*Length of villous*	*Thickness of connective tissue beneath villi*	*Thickness of trabecula*	*Thickness of Capsule*
Male	2866.91±83.14	50.33±1.46	745.86±21.63	20.17±0.92	101±2.93	230.58±6.69
Female	2847.81±82.59	50.00±1.45	751±21.79	17.45±0.84	100.55±2.92	228.00±6.61

Discussion. In most of Osteichthyes pyloric caeca are composed of several blind-end pouches attached to the begging of duodenum. The pyloric caeca in some fishes have one process and in some not present at all (Hideo et al 2003). In the present study it is demonstrated that pyloric caeca in *H. huso* were present like other Ostiechthies. In contrary, it is not in the form of several processes so that the process was attached together and formed a large mass between stomach and duodenum. In many teleosts, pancreas tissue is diffused throughout the adipose tissue that surrounds the pyloric caeca (Yasutake & Wales 1983, Oliveira Ribeiro & Fanta 2000). Our study showed that pancrease tissue like teleosts surrounds the pyloric caeca in *H. huso*. In fishes with several pyloric caeca there are numerous ducts that open into duodenum (Hideo et al 2003, Buddington & Diamond 1986). However in *H. huso* there was only one duct that opened into the beginning of duodenum. Pyloric caeca in piranha (*Pygocentrus nattereri* Kner, 1858) also have a weak positive reaction to Periodic acid Schiff (PAS) but not to Alcian Blue (AB). The pyloric caeca in piranha formed blind, fingerlike projections off the proximal intestine. They serve to increase the effective absorptive surface area of the proximal intestine without increasing intestinal length or thickness, however, pyloric caeca tend to be better developed in carnivores than herbivores, especially in carnivores with short guts (Raji & Norouzi 2010). Walls of pyloric caeca in green sunfish (*Lepomis cyanellus* Rafinesque, 1819) (Centrarchidae) were histologically very similar, consisting a mucosa (epithelial layer), submucosa (lamina propria and stratum compactum), muscularis (circular and longitudinal layers) and the serosa. Cellular constituents of the mucosal layer include absorptive, columnar epithelial cells, mucous-secreting goblet cells,

and various leucocytes, majority of which are lymphocytes (Williams & Nickol 1989). Histological study of pyloric caeca in *Mylossoma acanthogaster* (Valenciennes, 1850), *Mylossoma duriventre* (Cuvier, 1818), *Mylossoma aureum* (Spix & Agassiz, 1829), *Pygocentrus cariba* (Humboldt, 1821) and *Cynopotamus venezuelae* (Schultz, 1944) showed that from the lumen to the most external layer, the mucosa was formed by long and deep folds covered by single cylindrical epithelial tissue with caliciform cells and striated sheets. The submucosa was covered by lax conjunctive tissue. The musculature consisted 3 strata of muscular fibers, except in *M. aureum* which had only 2 strata and the serous formed a simple epithelial plane (Perozo et al 2009). Results of this study showed that in *H. huso,* like other teleosts, the capsule was a rather thick muscular (smooth) layer covering the pyloric caeca and capsule sending trabeculae into its interior. The villi of pyloric caeca was covered by simple columnar epithelium. The mucous secreting cells (goblet cell) were present among the epithelium cells of the villi.

Conclusion. The histological and anatomical studies of the pyloric caeca of this species were similar to that of the majority of teleosts. However, to know physiology of pyloric caeca in Acipenseridae in comparison with teleosts additional studies are necessary. In addition to anatomical studies of pyloric caeca in *H. huso*, the present study revealed that despite short length of pyloric caeca it provides a large absorption area because of numerous villi, special lobulation and mucosal structure. Presence of goblet cells indicates development of the organ toward chemical digestion and mucous secretion that facilitates passage of ingesta in intestine. The thick and strong muscular structure can also play a role in mechanical digestion through contraction.

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