Original article

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Histomorphology of digestive tract in two closely related mountain newts (Salamandridae: *Neurergus kaiseri* and *Neurergus microspilotus*)

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Abstract

Introduction: The digestive system of vertebrates presents various structural and functional adaptations to their diverse feeding habits. **Material and methods:** Anatomical, histological and histochemical examinations were made from digestive tract in two closely related mountain newts (Salamandridae: *Neurergus microspilotus* and *Neurergus kaiseri*). Sections were stained with Hematoxylin and Eosin, Periodic Acid Schiff's and Alcian Blue. **Results:** In both species the pharynx and esophagus are covered by psudostratified ciliated columnar epithelium with goblet cell which is positive with PAS and AB. The stomach in *N. kaiseri* and *N. microspilotus* is a straight, expanded conical tube, laying slightly to the left side of the body cavity, and terminating at the pylorus. The stomach is divided into three distinct parts, the cardia, fundus and pylorus. Although in both species the epithelium of the stomach surface and of the lining of the crypts consists of a single layer of high columnar cell, but the apical portion of the cells in *N.kaiseri* consists of homogeneous acidophilic granules while in *N.microspilotus* is foamy. The duodenum is short and is sharply reflexed along the medial aspect of the stomach. Duodenum in *N. kaiseri* and *N. microspilotus* shows villi which consists of the epithelial covering and a core of connective tissue containing blood and lymph capillaries. The large intestine in both *N. kaiseri* and *N. microspilotus* is located along the median line. The intestine is a coiled tube of a regular diameter, larger than, that of the duodenum. Histologically, these are no villi in large intestine and goblet cells rise to numerous. The epithelium is simple columnar, and the lamina propria and submocosa are strongly reduced. **Conclusion:** The findings of this study demonstrate that the morphological description of the digestive tract of *N. kaiseri* and *N. microspilotus* are very similar and can be extended to the other newts.

Keywords: *Neurergus kaiseri*, *Neurergus microspilotus*, digestive system, histomorohology.

1 Introduction

The digestive system of vertebrates presents various structural and functional adaptations to their diverse feeding habits. The digestive tract also represents a functional link between foraging activity and energy conservation through energy allocation for various activities (HAMMOND, SZEWCZAK and KROL, 2001; SECOR, 2001, 2005; ROMMAO, SANTOS, LIMA et al., 2011). Over the last decades, field observations and experimental laboratory studies have shown that digestive tract anatomy and function of many species are flexible, and can change in response to variation in environmental conditions (PIERSMA and LINDSTROM, 1997; STARCK, 1999; McWILLIAMS and KARASOV, 2000). However, most of these studies have been conducted in birds, mammals and reptiles, while research on histological characteristics in amphibians is scarce. However, attempts have been made to study of the microscopic structure of the amphibians alimentary tract organs. Similarly, Liquoria, Zizzaa, Mastrodonatoa et al. (2005) studied histological and immunohistochemical response of alimentary tract to ATPase mediate acid secretion in gastric glands of *Triturus carnifex* (Amphibia, Caudata). More studies have been carried out on histomorphology of digestive tract in various species of anura (JORQUERA, GARRIDO and PUGIN et al., 1962; FERRI and MEDEIROS, 1975; SENLER and YILDIZ, 2000; SANCAR-BAS, KAPTAN and SENGEZER, 2009).

In Iran, genus Neurergus has a relatively wide geographic distribution, ranging from southern Zagros Mountains to mid-Zagros range and extending into Iraq and southern Turkey (COPE, 1862; BALOUTCH and KAMI, 1995). Sharifi and Assadian (2004) demonstrated that *N. microspilotus* occurs in several highland streams in the mid Zagros Mountains. *N. kaiseri* is endemic to Iran and occurs only in southern parts of Zagros Mountains (SHARIFI, RASTEGAR-PUYANI, AKMALI et al., 2008). *N. microspilotus* is slightly larger than N. kaiseri and live in different climatic regime. Although both species of the genus Neurergus occur in highland first order streams but macro-ecology of these two areas (mid-Zagros and southern Zagros) are distinctively different. In southern Zagros Range where *N. kaiseri* occurs the climate is warm without winter freezing while in western Zagros where *N. microspilotus* occurs the climate is cold with pronounced seasonal variations including a prolonged winter freezing (SHARIFI, RASTEGAR-PUYANI, AKMALI et al., 2008; SHARIFI, PAPENFUSE, RASTEGAR-PUYANI et al., 2009). In both areas the mountain newts are top predators of the diverse benthic macro-invertebrates (SHARIFI and ASSADIAN, 2004).

The objective of this study was to describe the digestive system of two critically endangered mountain newts by means of anatomical and histological inferences. This description should enable better understanding of the digestive processes in these animals, contributing to physiological, pathological and phylogenetic studies and to the management and conservation of amphibian, and to extend their preventive and therapeutic medicine.

2 Materials and Methods

Four adult *N. kaiseri,* two males and two females, were collected from Vejenab in south western Iran (32°56' N, 48°28' E). Four *N. microspilotus,* two males and two females, were collected from Kavat Stream in northern Kermanshah in western Iran (34°53' N, 46°31' E). Permits for collections were issued on the ground of scientific use by regional office of environment in Kermanshah Province for *N. microspilotus* and in Khoramabad Province for *N. kaiseri*. All animals were in resting condition and each with a body length of about 173.91±17.75 mm for *N.kaiseri* and 192.35±10.20 mm for *N.microspilotus*. Specimens from both species, male and female, were dissected. The animals were sacrificed under ether anesthesia and their digestive tracts were quickly removed. The body length was measured as the distance from the tip of the snout to the posterior border of the cloacal opening.

Measurements of the various parts of the digestive tube were taken by digital Vernier Calliper. Body divided into five parts. The specimens were fixed by 10% formaldehyde and dehydrated in a series of ethanol treatments, starting from the 70% storing solution, then were cleared in xylene, embedded in paraffin, and serially sectioned at 7 μm with a rotary microtome. Most sections from each individual were stained with Hematoxylin-Eosin (for general histology), and others sections were treated with Periodic Acid and Schiff's reagent (PAS), for identifying neutral carbohydrates and Alcian Blue (AB) at pH 2.5 to for acidic polysaccharide, according to the protocol of Luna (1968). The control sections for PAS were treated with saliva at 37°C. Sections were observed with an Olympus microscope (Leica Galen III) and were photographed with a digital camera (Leica with Dinocapture 2.) mounted to the microscope.

3 Result

The digestive tract of the *Neurergus kaiseri* and *Neurergus microspilotus* are relatively simple extends from the mouth to cloaca, consist of mouth cavity, pharynx, esophagus, stomach, duodenum, large intestine, rectum (Figure 1).

Pharynx leads directly from the mouth as a wide tube. In both species the pharynx is covered by psudostratified ciliated columnar epithelium with goblet cell. Lamina propria submucosa is loose connective tissue with large blood vessels. A single layer of smooth muscle fibers is presented in tunica muscularis (Figure 2).

Esophagus in both species a wide esophagus follows an almost straight course from the pharynx without any sharp demarcation line. It is situated slightly to the left of the median line, passing dorsal to the heart as it approaches the stomach and narrowing posteriorly (Figure 1). The external walls of both esophagus and stomach, and in fact of the whole gut also, are smooth, but the internal walls are variously corrugated, each section of the gut having its own particular pattern. The length of esophagus in *N.kaiseri* and *N.microspilotus* is 4.5±1.3mm and 7.1±2.68mm respectively and their girth is 1.90±0.70 mm and 2.35±0.65 mm respectively. Histologically the esophagus in *N. kaiseri* and *N. microspilotus* is covered with psudostratified ciliated columnar epithelium with scattered

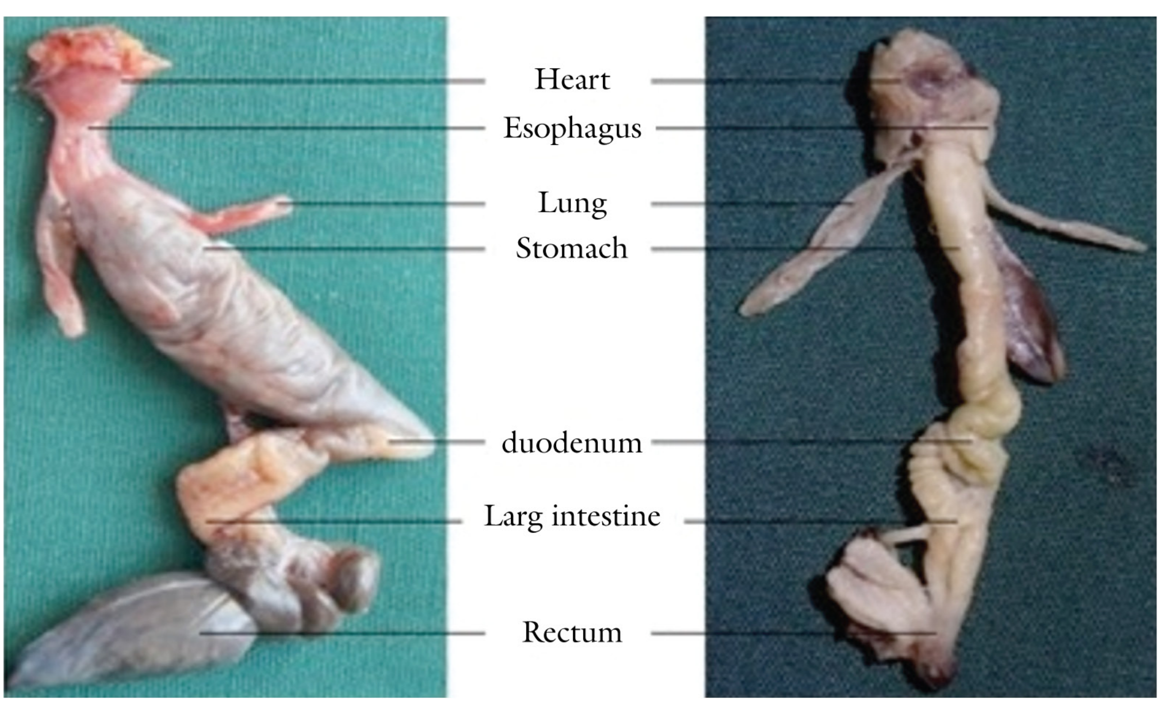

Figure 1. General view of digestive tract in *N. microspilotus* (left) and *N. kaiseri* (right).

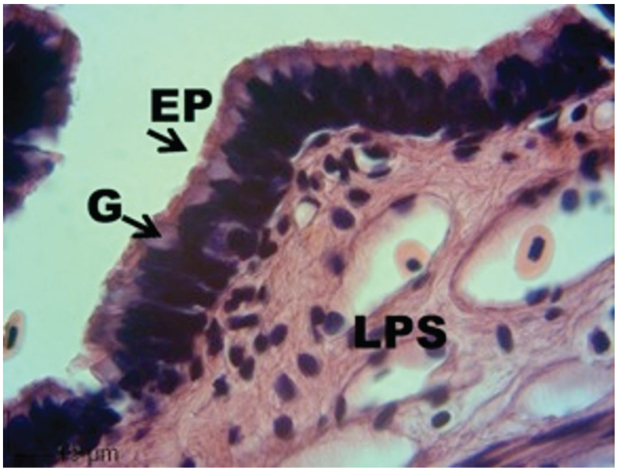

Figure 2. The pharynx is lined with psudostratified ciliated epithelium (EP) (H & E). Unicellular gablet cells (G) are abundant and scattered among the ordinary epithelial cells. The dense connective tissue of the lamina propria submucosa (LPS) is thick and comprises homogeneous collagen fibers and some fibrocytes (H & E).

goblet cells, which release large amount of the mucus to lubricate the epithelium. The mucosa is thrown to longitudinal mucosal folds. These are much serous, mucus and mixed glands in lamina propria and a sparse longitudinally oriented muscularis mucosa is present. The muscular coat consists of inner circular and outer longitudinal layer. The serosa is covered by simple squamus mesothelium. Positive reaction of goblet cells to PAS and AB indicate the presence of neutral and acidic carbohydrate (Figure 3).

Stomach in *N. kaiseri* and *N. microspilotus* is a straight, expanded conical tube, laying slightly to the left side of the body cavity, and terminating at the pylorus. The stomach lies almost longitudinally along the left side of the body. It narrows considerably at its posterior end, and there is always a well-defined constriction between the stomach and the duodenum. The constriction is especially noticeable when the gut is full, and a slight thickening of the wall is discernible in histological cross section (Figure 1). The length of stomach in *N.kaiseri* and *N.microspilotus*is15.65±3.1 mm and 20.25±1.55 mm respectively and their girth is 3.8±0.65mm and 5.65±0.95 mm respectively. In the stomach from the

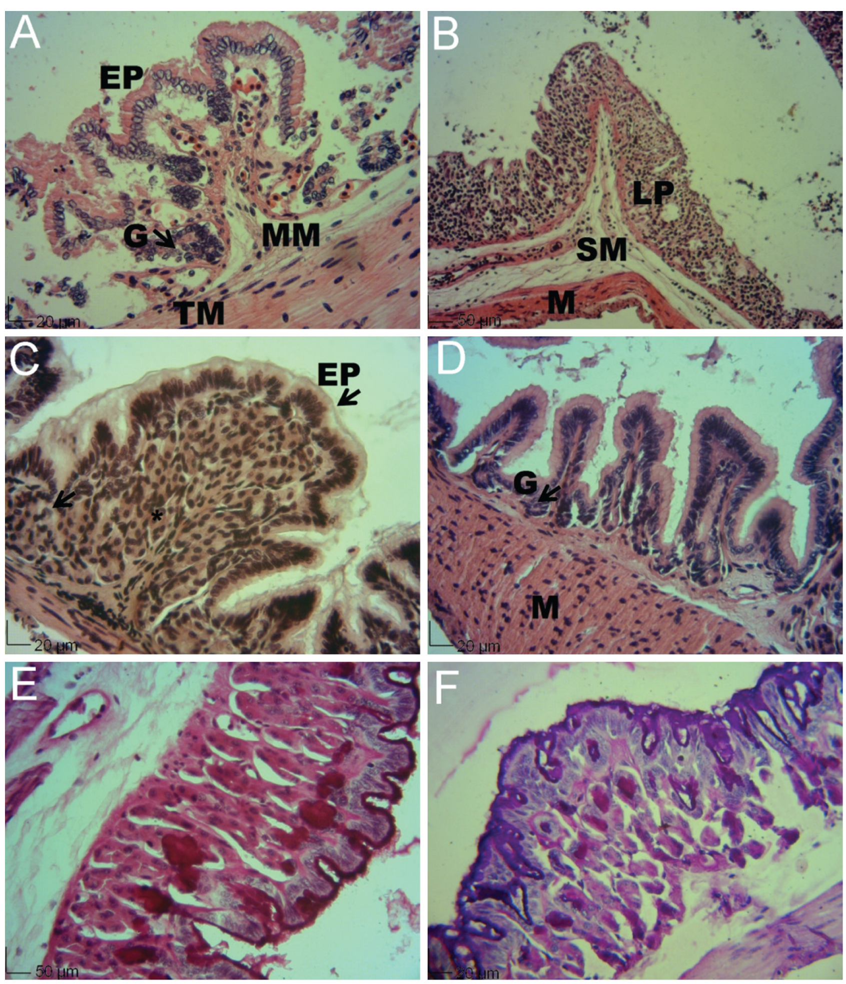

Figure 3. Section of the esophagus (A, B in *N.microspilotus* and B, C in *N. kaiseri*). In this general view, the mucosal epithelial folds are seen . Detail of the esophageal wall. The lumen (L) of the esophagus is lined by a psudostratified ciliated columnar epithelium (EP) containing a huge amount of goblet cells (G) (mucous cells). The lamina propria (LPS) is filled with serous (SG), mucous and mixed glands (MG) (H & E), C: (H & E), Oesophageal goblet cells react with Alcian blue. C, Cilia; M, Tunica muscularis; S, Tunica serosa. A and B (H & E), C (AB).

surface of the epithelium, numerous gastric pits (Crypts) sink down into the mucosa with the gastric gland opening at their bottom in fundus and pylorus in both species. The stomach in *N. kaiseri* and *N. microspilotus* is divided into 3 distinct parts, the cardia, fundus and pylorus. The epithelium of the stomach surface and of the lining of the crypts consists of a single layer of high columnar cell and the apical portion of the cell in *N. kaiseri* acidophil and homogeneous while in *N. microspilotus* is foamy with H-E staining. In the cardia tubular glands empty their secretion into the bottom of the gastric pit. These glands are lined with a single type of secretory cell and extend throughout the connective tissue of lamina propria. The fundus branched tubular glands possess 2 types of cells. Most of the cells are acidophilic which produces both pepsin and hydrochloric acid (Oxyntopeptic cell) and a few mucus cells are situated in the neck of the glands (Mucous neck cell) which secrete a protecting mucous, these cells are fewer in *N. microspilotus.* The pyloric glands are separated more widely from one another and consist of the shorter but less frequently branched tubules. A muscularis mucosa is found and consists of smooth muscle cells disposed longitudinally. The submucosa contains nerves, blood vessels and lymphatics. The muscular coat is composed of the inner circular and outer longitudinal smooth muscle cells and in the pylorus; additional oblique layer makes the sphincter between stomach and intestine. Mucines were observed in the apical portion of columnar cell and mucosa-neck cells, and these parts gave strong PAS and AB reaction. (Figure 4).

Duodenum is definite and readily observable pyloric sphincter occurs consistently in *N. kaiseri* and *N. microspilotus.* The portion of the small intestine between the pylorus and the entrance of the bile duct comprises the duodenum. The duodenum is short and is sharply reflexed along the medial aspect of the stomach. It is indistinguishable from the rest of the intestine except that it receives the pancreatic and bile ducts (Figure 1).The dorsal pancreatic duct enters the proximal end of the duodenum some 2-3 mm or so from the pylorus. The length of duodenum in *N. kaiseri* and *N. microspilotus* is 14.55±0.55 mm and 17.70±1.20 mm respectively and their girth is2.1±0.10mm and 2.7±1.25mm respectively. Duodenum in *N. kaiseri* and *N. microspilotus* shows villi. A villous is a finger-like process of the mucosa which consists of the epithelial covering and a core of connective tissue containing blood and lymph capillaries. The duodenal epithelium of simple columnar is made up of enterocytes possessing a well marked striated border (microvilli) and goblet (mucus-secreting) cells. The simple tubular invagination from the surface is lacking in these newts species, thus no true glands of the Liberkuhn are

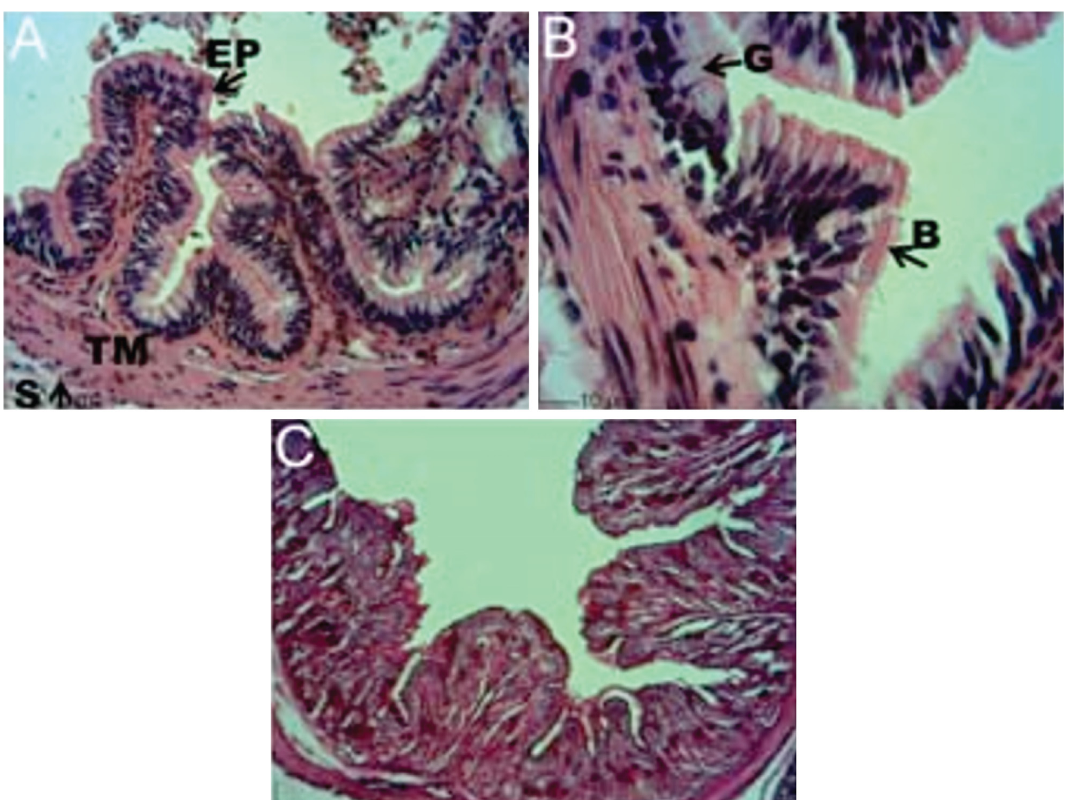

Figure 4. Stomach in *N.kaiseri* (A and D) and *N.microspilotus* (B and C). A. Cardia is lining with simple columnar epithelium (EP). Invagination of tunica mucosa from gastric pits, in which multiple tubular glands (G) drain. The muscularis mucosa (MM) is obvious in this image (H & E). B, C. In the fundus the lining epithelium is simple columnar (EP) and the fundic glands occupy the lamina propria (LP). Glands (*) are constructed mostly of oxynticopeptic cells, which secret both hydrochloric acid and pepsinogen. A few mucosa neck cells (arrow) also present B: (H & E), C: (H & E). D. Note the deep gastric pits in pylorus. The tubular glands (G) in this region are noting convoluted and very few in number. Tunica muscularis (M) become thick to from the pyloroduodenal sphingter (H & E). E, F. Columnar cell and mucus neck cell showed strong affinity for PAS and AB. SM, Tunica submucosa (PAS), F: (AB).

present in lamina propria. The lamina propria and submocusa contain large numbers of wandering eosinophilic granular cells. The muscularis mucosa consists of a thin layer of smooth muscle cells, longitudinal in direction. The muscular coat here well developed to insure peristaltic activity. Mucose secreted by goblet cells was strongly PAS positive. No staining was observed with AB. The brush border corresponding to glycocalyx also reacted with PAS (Figure 5).

Large intestine in both *N. kaiseri* and *N. microspilotus*is located along the median line, except when filled by an abundance of food, or by eggs in gravid female, and it extends straight to the cloaca. The intestine is a coiled tube of a regular diameter, larger than, that of the duodenum (Figure 1). The rectum is an expanded straight flask shaped structure arising quite suddenly from the posterior end of the intestine. It is much thinner walled than the rest of the gut (Figure 1). The length of large intestine in *N. kaiseri* and *N. microspilotus* is 36.30±4.7 mm and 47.55±3.45 mm respectively and their girth is 2.3±0.20 mm and 2.4±0.70 mm respectively. Histologically, these are no villi in large intestine and goblet cells rise to numerous. The epithelium is simple columnar, and the lamina propria and submocosa is strongly reduced. The muscular coat is developed for peristalsis activity. A single layer of mesothelium is covered the outside of the intestine. Goblet cells contain large secretory vesicles that were intensely PAS positive and reacted with AB pH 2.5 (Figure 6). In the rectum, like the large intestine, the epithelium is simple columnar but the number of goblet cells increased. Lamina propria submucosa becomes a very thin layer (Figure 7).

4 Discussion

The pharynx of the *N.kaiseri* and *N.microspilotus* similar to other salamanders is richly vascular and unmarked by any Eustachian tubes and passes undetectably into a wide esophagus. The pharynx leads into the stomach without any sharp line of demarcation (FRANCIS, 1934). Studies conducted by Wonderly (1936) on digestive systems in several species of North American salamanders showed that in *Siren lacertian*, *Notophthalmus v. viridescens*, *Desmognathus o. ochrophaeus*, *Plethodon g. glutinosus*, *P. c. cinereus*, *P. r. richmondi* and *Gyrinophilus p. porphyriticus*, there is a definite, external point of demarcation between the esophagus and stomach. But this is absent in *Cryptobranchus a. alleganiensis*, *Necturus maculosus*, *Amphiuma tridactylum*, *Desmognathus qudramaculatus*, and the genus *Ambystoma* (except for a moderately definite constriction in one specimen of *A. jeffersonianum*). In our study there is no clear demarcation line between esophagus and stomach in *N. kaiseri* and *N. microspilotus.* In a histological study conducted on *Triturus carnifex* (LIQUORI, ZIZZA, MASTRODONATO et al., 2005) it was found that the esophagus was lined by a columnar ciliated epithelium with widespread mucous goblet cells. The mucosa appeared to be folded and no esophageal glands were observed. Goblet cells contained large secretory vesicles that were PAS-positive and reacted with AB

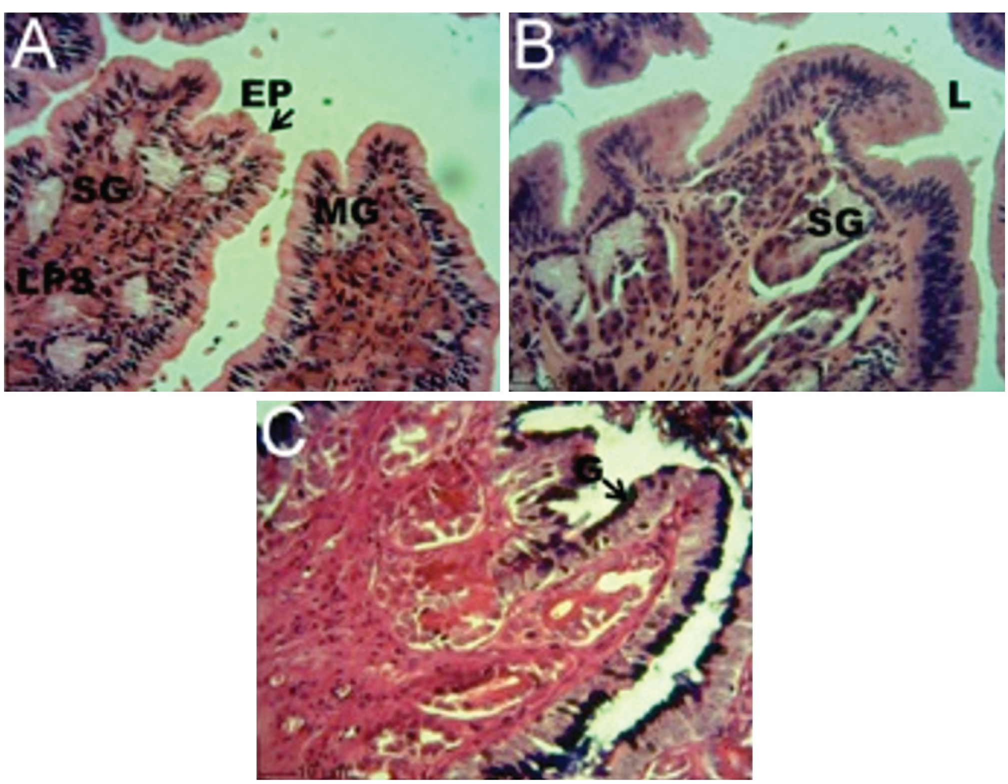

Figure 5. Duodenum in *N. kaiseri* (A) and *N. microspilotus* (B), these micrographs displays elongated villi, deep finger-like processes of the intestinal mucosa extending in the duodenal lumen. These expansions are lined by a simple columnar epithelium (EP) and mucus secreting or goblet cells (G). The brush border (B) of the enterocytes is visible in B. The villous core is filled with connective tissue of the lamina propria (LP) containing blood and lymph capillaries. Tunica muscularis (TM) is covered by the serosa (S). C. PAS positive goblet cells and striated border (H & E), B: (H & E), C: (PAS).

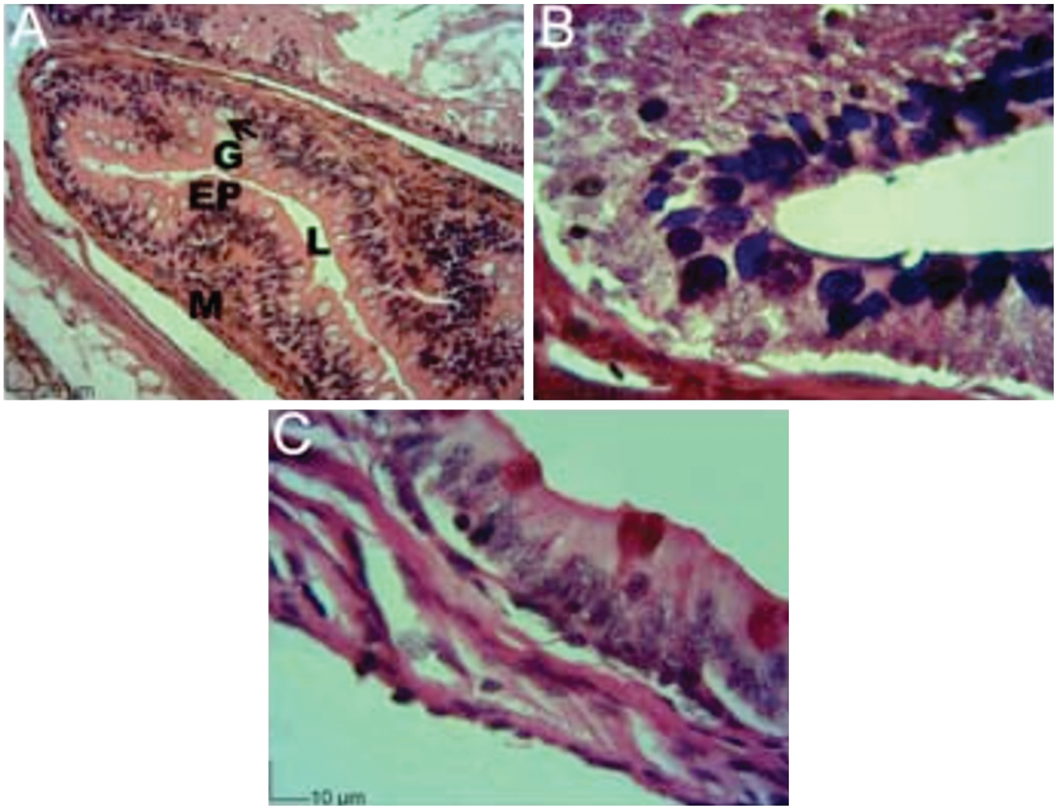

Figure 6. A. Large intestine in *N. microspilotus.* The mucosa presents a flat surface and villi are absent. Numerous goblet cells (G) in the lining epithelium (EP) are characteristic of this part. L: Lumen, M: Tunica muscularis (H & E). B, C. Intestinal goblet cells were PAS positive and react with AB pH. 2.5 (AB), C: (PAS).

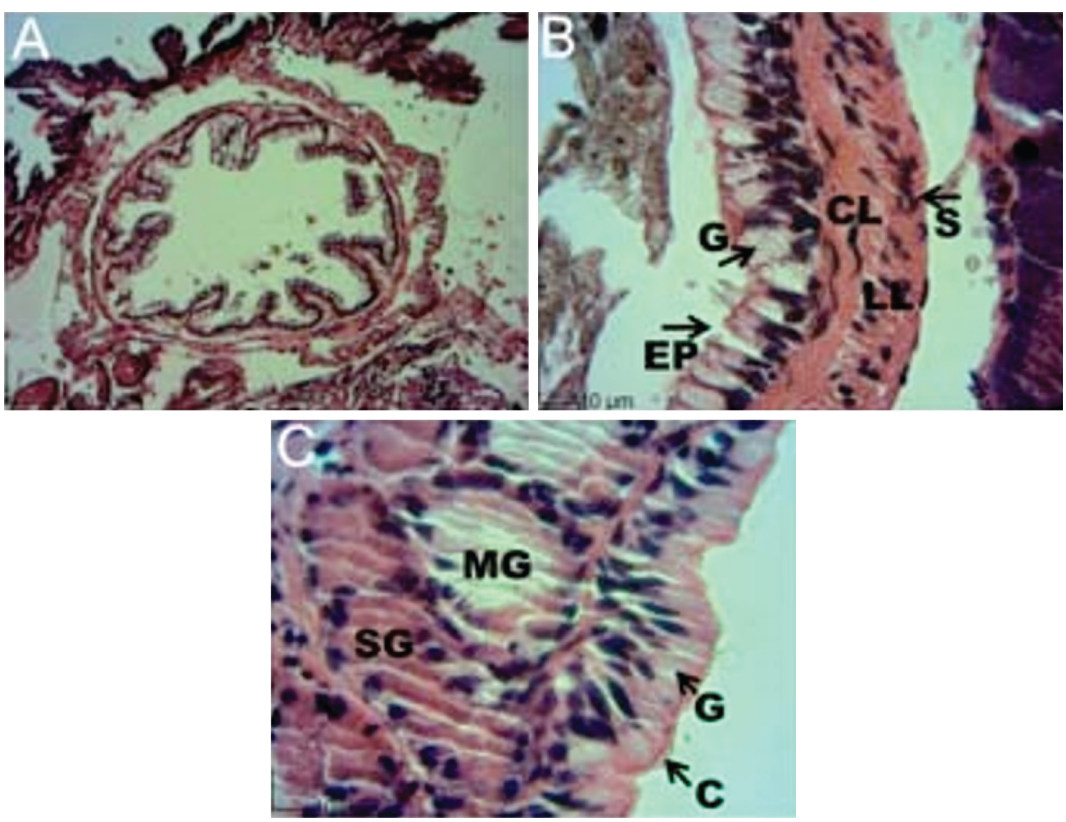

Figure 7. The rectum is lined with a simple columnar epithelium (EP) housing numerous side by side goblet cells (G). The mucosal folds become more flat and the number of goblet cells increases considerably. Lamina propria submucosa is much reduced. Tunica muscularis consists of usual inner circular (CL) and outer longitudinal layer (LL) of smooth muscle fibers lined by the serosa (S) (H & E).

at pH 2.5 (LIQUORI, ZIZZA, MASTRODONATO et al., 2005). But in *N.kaiseri* and *N.microspilotus* the internal covering of esophagus is psudostratified ciliated columnar with large amount of goblet cells.

In *N. kaiseri* and *N .microspilotus* the stomach is divided into 3 parts, the cardia, the fundus and the pylorus and each of them has its own microscopic characteristics. Also, the epithelium of the stomach surface and of the lining of the crypts consists of a single layer of high columnar cell. However, the apical portion of the cell in *N. kaiseri* is acidophil and homogeneous while in *N.microspilotus* is foamy. Similar results have been obtained in other species of Salamandra. The stomach of *Triturus carnifex* (LIQUORI, ZIZZ, MASTRODONATO et al., 2005), *Bufo viridis* (LIQUORI, ZIZZ, MASTRODONATO et al., 2005) and *Bombina variegata* (BANI and CECCHI, 1992) appeared to be histologically subdivided into a corpus, fundus and a wide pars pylorica. In these species the stomach lumen waslined with a single layer of mucus-secreting cells. The mucosa was arranged in a few longitudinal folds. The mucous neck cells were observed only in the oral fundus and were PAS-positive, but they did not react with AB at pH 2.5 (LIQUORI, ZIZZ, MASTRODONATO et al., 2005). A definite and readily observable pyloric sphincter occurs consistently in *Cryptobranchus a. alleganiensis*, *Necturus maculosus*, *Siren lacertina*, and *Amphiuma tridactylum* which is the same of the present study. But in the remaining species studied, very little pyloric constriction is evident, and no appreciable thickening of the wall of the digestive tube at the junction of the stomach and duodenum can be observed (WONDERLY, 1936).

In *N. kaiseri* and *N. microspilotus* similar to many salamanders (FRANCIS, 1934), the duodenum is short and is sharply reflexed along the medial aspect of the stomach, but follows a more transverse direction relative to the body than does that organ. It is indistinguishable from the rest of the intestine except that it receives the pancreatic and bile ducts. The dorsal pancreatic duct or rather the duct of the dorsal pancreas, enters the proximal end of the duodenum some 2 mm or so from the pylorus in both species. The ventral pancreatic ducts, which belong to the two ventral pancrease, discharge into the common bile duct which, in turn, enters the distal end of the duodenum, some 4- 5 mm from the dorsal pancreatic duct. In genus Salamandrina, the intestine is a coiled tube of a regular diameter, approximately equal to, or slightly smaller than, that of the duodenum. Its length is about one-half that of the whole gut, measured from the pharynx to the cloaca (FRANCIS, 1934). The pattern of the internal relief of the duodenum and intestine, considered as a whole, consists of a series of sinuous longitudinal ridges. The ridges are thick proximally that is, at the duodenal end but tend to become thinner and straighter towards the hinder end of the gut, until they are almost knife-like. If the mucous epithelium is scraped or brushed off it is seen that the underlying vascular network is also raised into ridges, and that the capillary loops follow the same wavy outline. The network is much richer at the anterior end of the gut (FRANCIS, 1934). In both *N. kaiseri* and *N. microspilotus* the diameter of large intestine is larger that of the small intestine and occupies approximately one third of the gut.

The findings of this study demonstrate that the morphological description of the digestive tract of *N. kaiseri* and *N. microspilotus* are very similar and can be extended to the other newts. Results obtained from current study are important for understanding the digestive processes, underpinning not only physiological, pathological and Phylogenetic studies but also the management and conservation, and the preventive and therapeutic medicine of these animals.

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