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The Structure of Stomach and Intestine of *Triturus karelinii* (Strauch, 1870) and *Mertensiella luschani* (Steindachner, 1891) (Amphibia: Urodela): Histological and Histometrical Study

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Abstract. In this study, the stomach and small intestine structures of *M. luschani* and *T. karelinii* were evaluated in terms of histology and histometry. The stomach and small intestine tissues of *M. luschani* and *T. karelinii* have similar characteristics. Mucosa consists of lamina propria/submucosa, tunica muscularis and tunica serosa layers. Histometrical results show that *M. luschani'*s fundus ($t=0,003$; $p<0,05$) and pylorus ($t=0,000$; $p<0,05$) epithelial thickness and mean lumen area ($t=0,009$; $p<0,05$) are larger than *T. karelinii'*s fundus-pylorus epithelial thickness and mean lumen area. In intestine, *M. luschani'*s lamina epithelialis mean thickness ($t=0,003$; $p<0,05$) and mean lumen area ($t=0,009$; $p<0,05$) are also larger than *T. karelinii'*s epithelial thickness and mean lumen area.

Keywords: Histology, histometry, stomach, intestine, *T. karelinii, M. luschani.*

1. INTRODUCTION

The vertebrate alimentary canal is a highly specialized structure that brings food into an organism, digests the food, absorbs nutrients, and expels waste products [1]. Digestive tract morphology can affect digestive efficiency and is closely related to food habits. Feeding habits and ecological conditions lead to changes in the basic metabolic rate, which result in variation in dimensions of the alimentary canal [2-4]. Within different groups of animals, the metabolic rate is connected with the type of a diet, and hence with the structure and function of the alimentary canal [5,6].

Differences in food habits among mammals are often reflected in the structure of the alimentary tract [7]. Vorontsov [8] has observed differences in digestive tract morphology to evolutionary adaptations for a herbivorous diet. The transition from a high-energy, high-protein, and high-lipid diet of seeds and small invertebrate, to a low-energy, high-cellulose diet of vegetative parts of plants was hypothesized to have resulted in several evolutionary modifications in the digestive tracts of muroid rodents.

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Studies on small vertebrates, including reptiles [9,10], birds [11] and small mammals [4, 12,13] showed that organs responsible for digestion and distribution of food are associated with the rate of metabolism in animals. The stable rate and threshold of metabolism, which are limiting factors while foraging, and the efficiency of reproduction are associated with adaptations of the digestive system to food ingestion [14]. Factors such as morphology and physiology of the alimentary canal can define both the efficiency of absorption and the rate of assimilation of food and energy [3]. In consequence the size of organs of the alimentary canal often reflects direct metabolic requirements of animals [15].

Numerous studies proved that the increase of energy requirements or decrease of food quality (considering its nutritional value) result in an increase of the intestine length and its capacity [16-18]. It is easy to guess that similar changes in the alimentary canal may have a seasonal course [19-21] and often reflect the physiological state of an organism [22,23]. The small intestine is the main site for enzymatic nutrient digestion and absorption. Formation of crypts and villi enlarges the surface of the mucus membrane in the small intestine. In humans for example, the villi lead to a 5-6 fold enlargement of the absorptive surface of the small intestine [24].

Many authors measured a decrease in villus height with an increasing distance to the stomach [25-27]. Others did not find any regularity regarding villus height [28-30]. This also applies to the depth of the crypt: some authors found a negative correlation between the depth of the crypt and their distance to the stomach [26, 31, 32]. However, Dunsford et al. [22], Makinde et al. [28] and Pluske et al. [29] were not able to confirm this regularity. In contrast, there is a great consensus on a close correlation between the depth of the crypt and the proliferation rate of epithelial cells in both the small and the large intestine of growing pigs [31,33] and also in the small intestine of chickens [34] and rats [35]. Wiese et al. [36] reported that the villi generally were shaped irregularly. However, the villi of the semisynthetic fed piglets were tendency-reflecting, more homogenous and less variable than those of the cereal fed pigs.

Many studies have been carried out on stomachs and the evolution of bowels of some species of amphibia, reptiles and mammals such as mice and rats until the present, but there is no research studying histometric analysis of digestive tract (stomach and intestine) of *M. luschani* and *T. karelinii*. Our study aims to contribute to the literature in this area and make a histometric analysis of stomach and intestine structures of *M. luschani* and *T. karelinii* by measuring tunica mucosa (epithelium, muscularis mucosa), tunica submucosa, tunica muscularis thickness, lumen area and lumen perimeter of these species.

2. MATERIAL AND METHODS

2.1. Histological methods

T. karelinii (n=10) were collected from Osmancalı/Manisa in 2002 and also *M. luschani* (n=10) were collected from Fethiye/Muğla in 2007. Tissue samples that belong to both species have been obtained from animals which were used in our previous studies [37-39]. For this reason a new field work wasn't necessary and animals weren't collected again. Necessary ethics committee approval regarding this study was received (HADYEK-FEF 2014/073).

For histological examination, the stomach and small intestines tissues were dissected and the tissue samples were fixed in Saint-Marie's solution and buffered neutral formalin fixative for 48 h, processed by using a graded ethanol series, and embedded in paraffin. The paraffin sections were cut into 5 μm in a systematic uniform randomized way, producing 15 sections of organs each of the individuals and stained with Hematoxylin-eosin, Gomori trichrome and Periodic acid Shiff (PAS) [40]. The sections were viewed and photographed by using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached photograph machine (Olympus E-330, Olympus Optical Co. Ltd., Japan).

2.2. Histometrical methods

The histologic sections were used to measure the thicknesses of the tissues forming the stomach and small intestine wall. Tunica mucosa (epithelium and muscularis mucosa), crypt length, tunica submucosa, tunica muscularis thickness of fundus and pylorus were measured. Lumen area, villus length, lamina epithelialis (epithelium), perimeter of lumen and tunica muscularis (longitudinal and circular layers) thickness of small intestine were measured. Leica IM50 programme was used for measurements and SPSS 17 packet programme was used to make statistical analysis and draw graphics of the findings.

3. RESULTS

3.1. Histological Results

The stomach consists of two part (fundus and pylorus) in *M. luschani* and *T. karelinii*. The wall of the stomach consists of four layers: tunica mucosa (built from three laminas-lamina epithelialis, lamina propria and lamina muscularis), submucosa, tunica muscularis and tunica serosa (Figs. 1A-D, 2A,B, 3A-C, 4A-C). The mucous membrane is covered with simple columnar epithelium (Figs.1D, F, 2D-E, 3D,F, 4D,E). The glands of fundus are mostly of a simple tubular type (Figs. 1B-D, 3A-C). However, pyloric glands are usually branched tubuler

type (Figs. 2A-C, 4A-B). Both fundus and pylorus mucous neck cells and surface mucous cells are PAS positive (Figs. 1F, 2E, 4E). Also, in the basis of pyloric glands eosinophilic parietal cells have been observed (Figs. 1E, 3E). Submucosa consist of loose connective tissue including collagen fibers and blood vessels (Figs. 1A-D, 2A-C, 3A-C, F, 4A-C). Muscular membrane is the thickest layer building the stomach wall which is built from two muscle layers: internal (circular) and external (longitudinal) (Figs. 1C,D, 2A-C, 3A,B, 4A-C). Tunica serosa is the outermost layer (Figs. 1A, 2A,B, 3A,B, 4A-C).

The small intestine of *M. luschani* and *T. karelinii* consists of tunica mucosa (lamina epithelialis, lamina propria and lamina muscularis), tunica submucosa, tunica muscularis and tunica serosa layers (Figs. 5A,B, 6A,B). Small intestine contains large longitudinal folds called villi (Figs. 5A,B,E,F, 6A-C,E). The mucous membrane of intestine is covered with pseudostratified ciliated epithelium and contains a great number of goblet cells (Figs. 5B-D,F, 6C-F). The glands of the small intestine of both species are of branched tubular type (Figs. 5A,E, 6A,B). There is no muscularis mucosa in intestine of these species (Figs. 5B, E, F, 6A-C). Lamina propria/submucosa layer is rich in collagen fibers and blood vessels. The lamina propria/submucosa layer of *M. luschani* contains lymph follicles (Fig. 5E). Tunica muscularis is very thin and arranged from circular (internal) and longitudinal (external) muscle fibers (Figs. 5B, F, 6A-C). The serosa in the outermost layer is formed from a thin epithelial tissue (Figs. 5A,B, 6A,B).

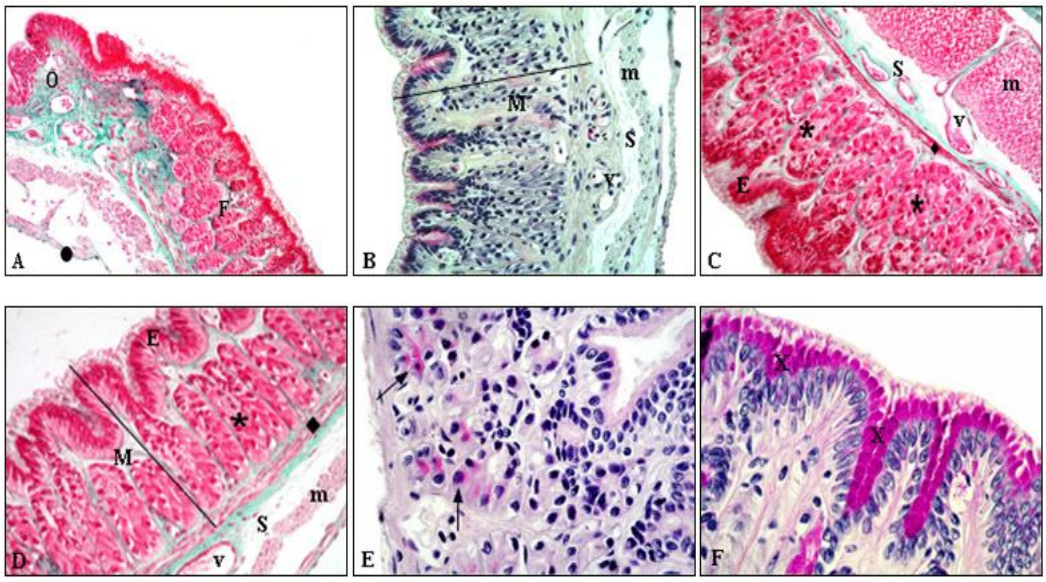

Fig. 1. Histological sections of fundus region of the stomach of *M. luschani*. Oesophagus (O), fundus region of the stomach (F), epithelium (E), simple tubular fundus glands (*), muscularis mucosa (♦), mucosa (M), submucosa (S), vessels (v), muscular layer (m) and serosa of fundus region of the stomach (●), Parietal cell **(→),** mucus cell (x). ***Staining:*** **A-D;** Gomori trichrome, **E;** H&E, **F:** PAS-H. ***Magnification:*** **A;** 10x; **B-D;** 20x; **E, F;** 40x.

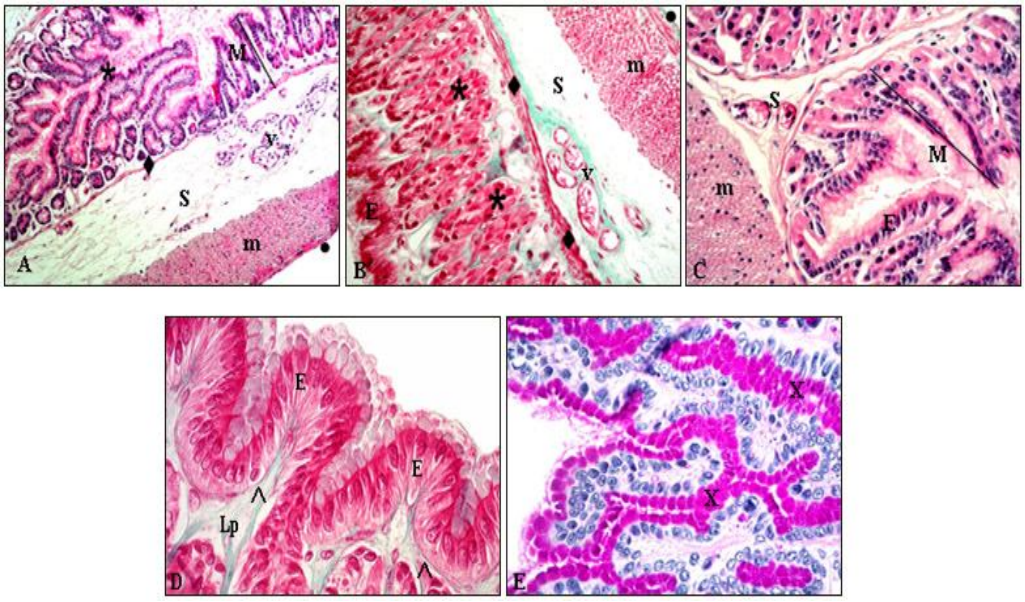

Fig. 2. Pylorus region of the stomach of *Mertensiella luschani*. Epithelium (E), basal lamina (^), branched tubular pyloric glands (*), lamina propria (Lp), muscularis mucosa (♦), mucosa (M), submucosa (S), vessels (v), muscular layer (m) and serosa (●), mucus cell (x). ***Staining:*** **A;** H&E, **B, D;** Gomori trichrome, **C;** H&E, **E;** PAS-H. ***Magnification:*** **A;** 10x; **B, C;** 20x, **D, E;** 40x.

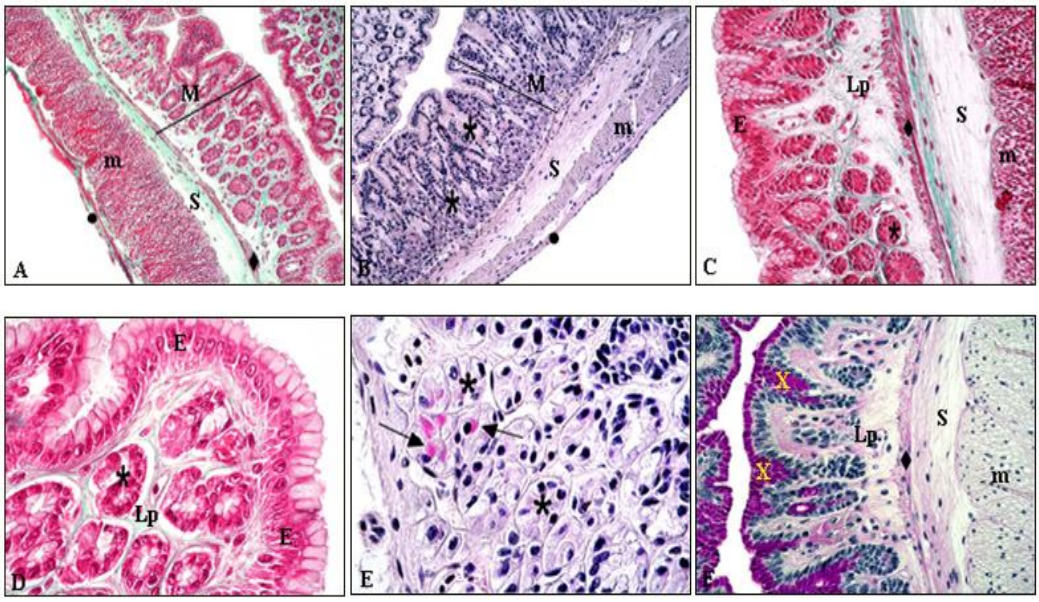

Fig. 3. Fundus region of the stomach of *Triturus karelinii.* Epithelium (E), simple tubular fundus glands (*), lamina propria (Lp), muscularis mucosa (♦), mucosa (M), submucosa (S), vessels (v), muscular layer (m), serosa (●), Parietal cell **(→),** mucus cell (x). ***Staining:*** **A, C, D:** Gomori trichrome, **B, E;** H&E, **F;** PAS-H. ***Magnification:*** **A, B;**10x; **C, F;** 20x, **D**, **E;** 40x.

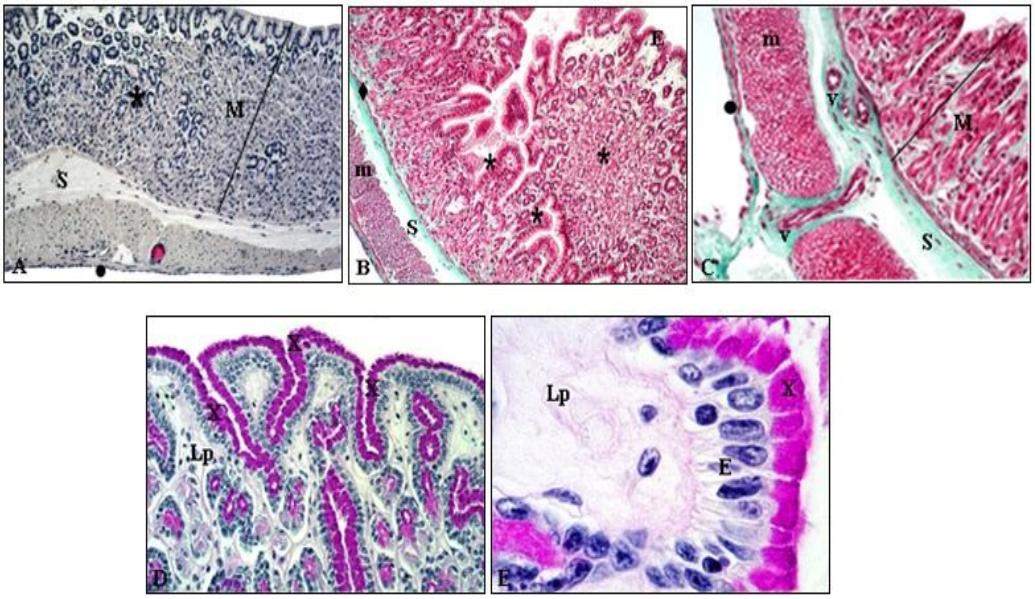

Fig. 4. Pylorus region of the stomach of *Triturus karelinii.* Columnar epithelium (E), branced tubular type pylor glands (*), muscularis mucosa (♦), mucosa (M), submucosa (S), vessels (v), muscular layer (m), serosa (●), lamina propria (Lp), mucus cell (x). ***Staining:*** **A;** H&E, **B, C;** Gomori trichrome, **D-E;** PAS-H. ***Magnification:*** **A, B;**10x, **C, D:** 20x, **E;** 100x.

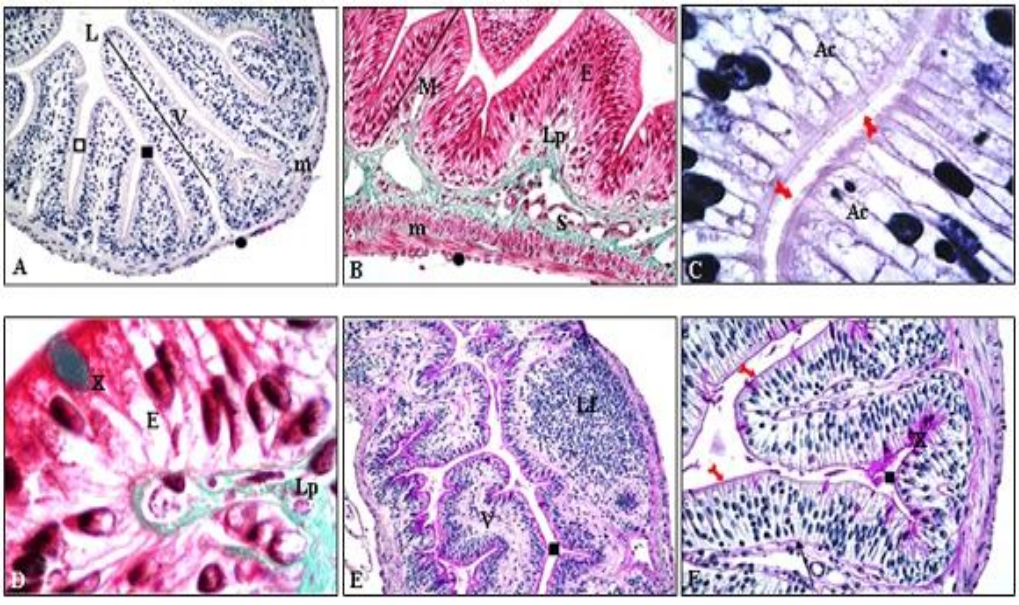

Fig. 5. Small intestine of *Mertensiella luschani*. Lumen (L), mucosa (M), pseudostratified columnar cell (E), lamina propria/submucosa (Lp/S), tubular (□) and branched tubular gland (■), tunica muscularis (m), serosa (●), absortive cells (Ac), cilia (➥), mucus cell (x), lymph follicules (Lf), basal lamina (→). ***Staining:*** **A,C;** H&E, **B, D;** Gomori trichrome, **E, F;** PAS-H. ***Magnification:*** **A, B, F;** 20x, **C, D;** 100x, **E;**10x.

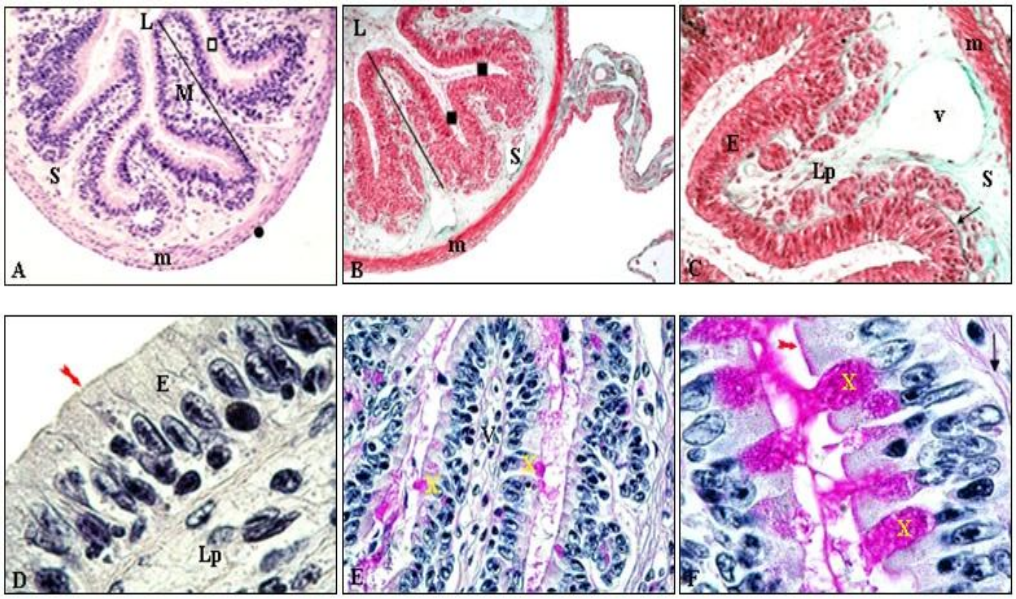

Fig. 6. Small of intestine *Triturus karelinii.* Lumen (L), mucosa (M), pseudostratified columnar cell (E), lamina propria/submucosa (Lp/S), tubular (◘) and branched tubular gland (■), muscular layer (m), serosa (●), cilia (➳), lamina propria (Lp), mucus cell (x), basal lamina (↓). ***Staining:*** **A, D;** H&E, **B, C:** Gomori trichrome, **E, F;** PAS-H. ***Magnification:*** **A, B;** 10x, **C, E;** 20x, **D, F**; 100x.

3.2. Histometrical Results

The epithelial thickness mean of *M. luschani* fundus (t=0,003; p<0,05) and pylorus (t=0,000; p<0,05) were statistically greater than *T. karelinii's* fundus and pylorus. There was no statistically significant difference between the layers of stomach except the epithelial of fundus and pylorus in both species (Fig.7). In intestine, the lamina epithelialis thickness mean (t=0,003; p<0,05) and lumen area mean (t=0,009; p<0,05) of *M. luschani* were larger than *T. karelinii's* lamina epithelialis thickness and lumen area mean. There was no significant difference between all intestine layers of these two species except lamina epithelialis and lumen area mean (Fig. 8).

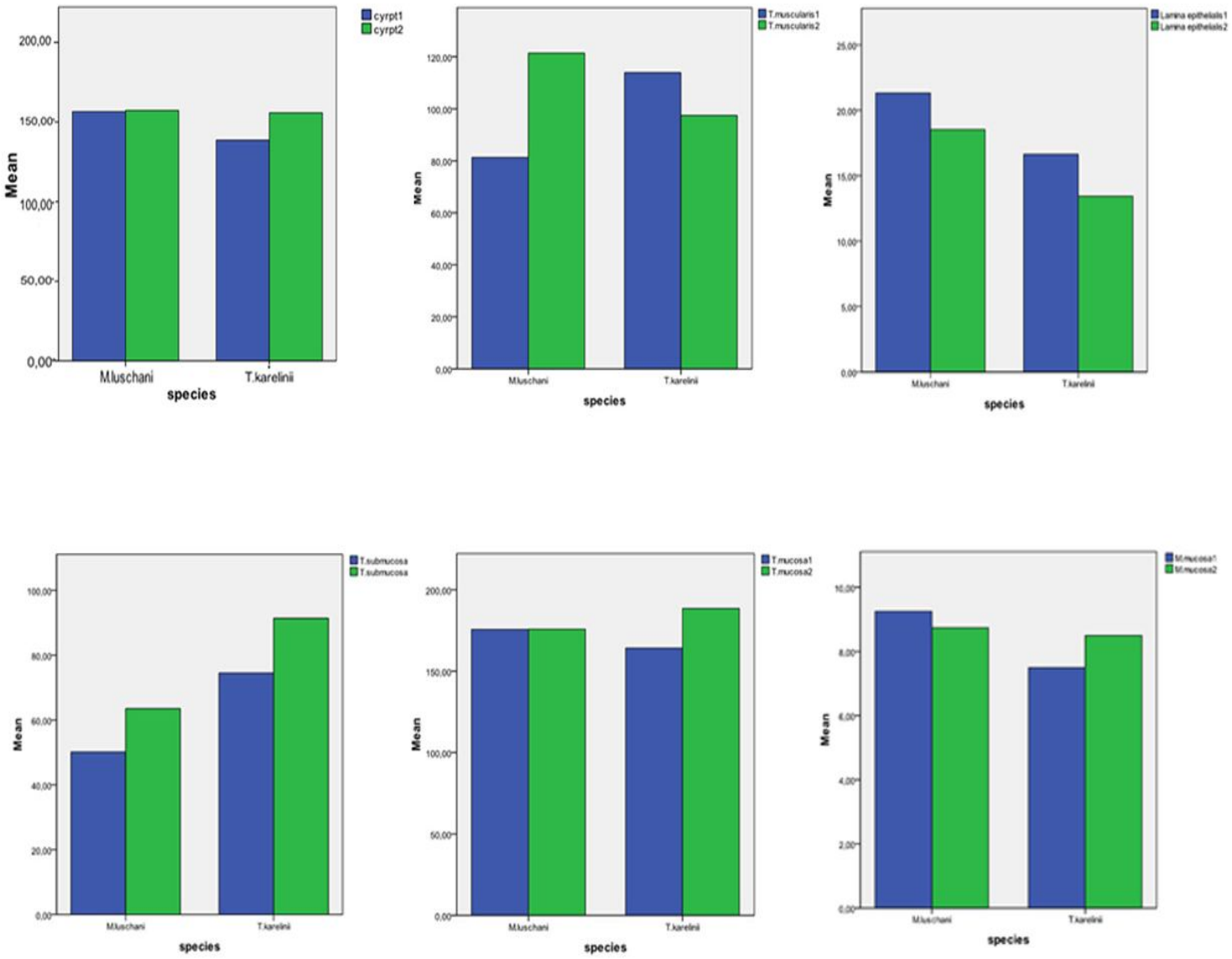

Fig. 7. The averages of crypt, tunica muscularis, lamina epithelialis (epithelium), tunica submucosa, tunica mucosa and muscularis mucosa thickness of fundus (blue bar) and pylorus (green bar) areas of *M. luschani* and *T. karelinii*.

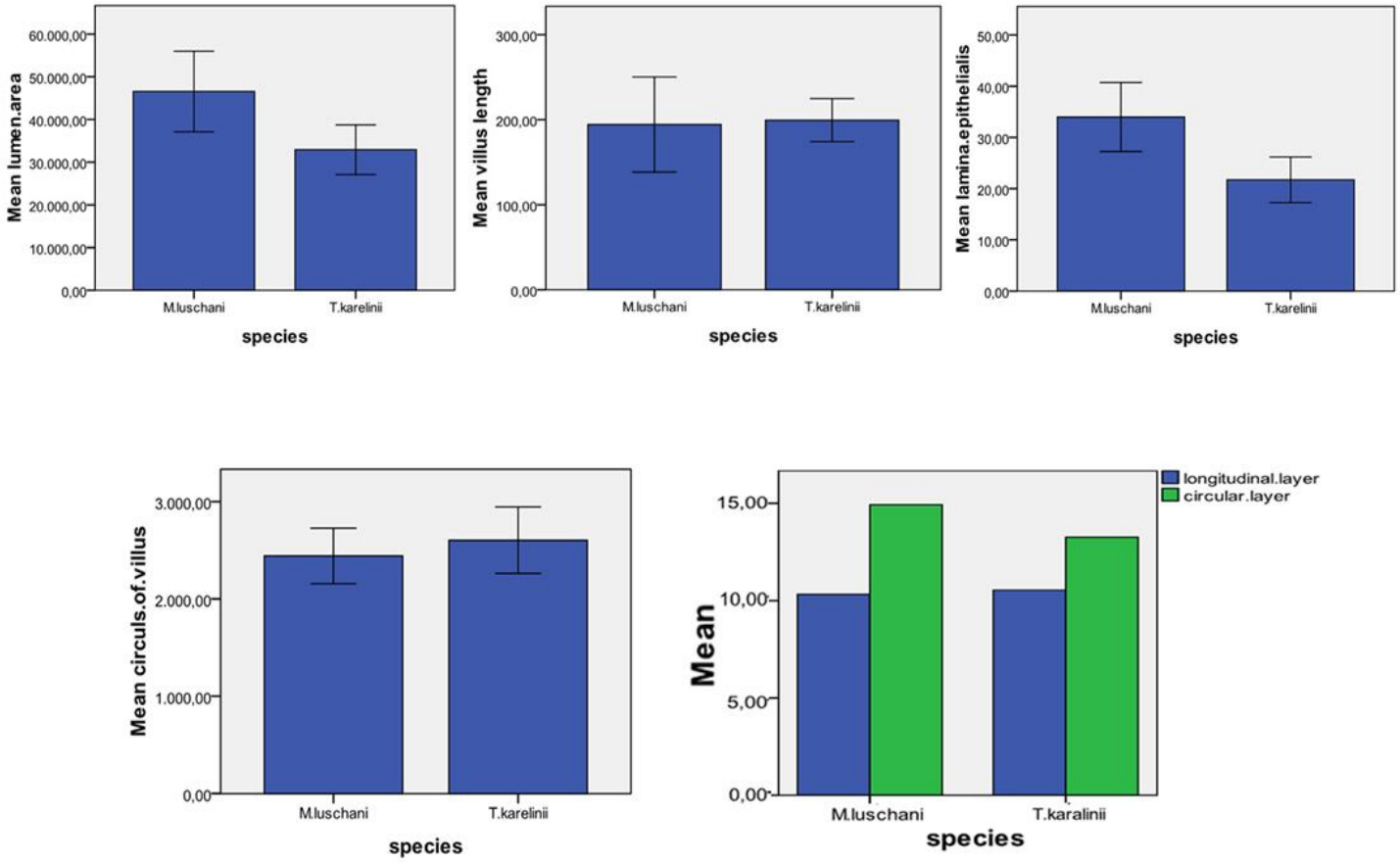

Fig. 8. The averages of lumen area, villus length, lamina epithelialis (epithelium), perimeter of lumen and tunica muscularis (longitudinal and circular layers) thickness of intestine layers of *M. luschani* and *T. karelinii*.

4. DISCUSSION

In this study, histological structure and histometric analyses on sections of the stomachs and intestines in *M. luschani* and *T. karelinii* were investigated. It has been noted that the alimentary canal morphological structures of vertebrates are generally similar but there may be differences in their cell type number and regional distribution [41,42]. Although most of the variation in stomach morphology among different species can be correlated with their diverse diets, the evolutionary origin of the vertebrae stomach is unknown [43]. Stomach can be divided into two sections in most vertebrates. The anterior portion of the stomach is called the fundus, which is characterized by gastric glands that secrete pepsinogen and hydrochloric acid. The posterior, or pyloric, portion of the stomach also features specialized glands, which secrete mucus into the lumen of the stomach [44]. In this study, the stomachs of *M. luschani* and *T. karelinii*

were divided into two sections, called the fundus and pylorus. The wall of the stomach was composed of tunica mucosa (lamina epithelialis, lamina propria and muscularis mucosa), tunica submucosa, tunica muscularis and tunica serosa (Figs. 1A-F, 2A-C).

The structure of the stomach, in particular its glandular layer, is of special interest. In the fundus and pyloric glands develop outstandingly strongly. This is associated with a high-protein diet of these animals [45]. The participation of the glandular layer in the stomach wall in immature males of *S. araneus* can reach even 73.0% of the total thickness of the wall [46]. The fundus and pyloric glands of both species in this study were embedded into the lamina propria and is well-developed. Pyloric glands were branched out and deep when compared to fundus (Figs. 3A-C, 4A-B). There was no significant difference in mucosa layer where gastric glands are situated (Fig.7, Table 1). Mucus secreted by gastric glands forms a mucosal layer on the surface of epithelialis and creates a barrier to pathogens. Pepsinogen and HCl secretion in the amphibian stomach is performed by a single cell type, the oxynticopeptic cell. The distribution of pepsinogen in gastric mucosa of *Bufo marinus* is heterogeneous and higher concentrations are located in the fundus. Both secretions respond to the same secretagogues [47]. The parietal cells are the most conspicuous cells of the gastric mucosa. They produce hydrochloric acid and gastric intrinsic factor. Chief cells (or peptic cell, or gastric zymogenic cell) are short columnar, cuboidal or polyhedral granular cells whose bases lie on the basement membrane, while apices face the lumen of the gland [48].

Like other animals, the secretion of stomach parietal cells presumably plays an important role in hydrolysis of the proteins in *M. luschani* and *T. karelinii.* In *M. luschani* and *T. karelinii*, the two-layer muscular lamina of mucosa (muscularis mucosa) is present in stomach and intestine. The presence of the lamina adapts the stomach and intestines to stretching and its function is to prevent deformation of the glandular layer. Morever, lamina muscularis mucosa may be related to absorbsion of easily digested substrates, such as disacharides and short chain fatty acids [49].

According to Vorontsov [8], diet and alimentary tract morphology are reflected in the life histories of animals. Species that eat high-energy foods and display digestive tract adaptations to a high quality diet will possess life-history traits similar to those of relatively opportunistic species such as high motional ability, high fecundity and the ability to exploit temporarily ideal but relatively unstable habitats, whereas those with adaptations to a herbivorous diet should possess the converse life history traits.

The intestine of *M. luschani* and *T. karelini* is histologically simply organized when compared to that of mammals and resembles that described in fish and other amphibians [50-53]. The typical histology of the gut tube is comprised of four layers. The innermost layer is the

mucosa including the muscularis mucosa, submucosa, muscularis layer and serosal layer which is the outermost [54]. The histological structure of intestine of the species we examined in our study are similar to the above studies. We have found numerous mucus cells in stomach and intestine mucosa (Figs. 1E, 2E, 3F, 4D-E). Although the quantity and compositon of the mucus substance produced in the amphibia by both the cell of the stomach and intestine epithelium and the cells of the glands could be mainly related to the diet and to the environmental functional significance in the stomach and intestine of *M. luschani* and *T. karelinii*.

Many authors determined the depth of the crypts by histometrical examination of the small intestine [28-30]. In order to substantiate these measurments the crypt's function should be focused on. Many authors found a close correlation between the depth of the crypt and the proliferation rate of epithelial cells [31,33]. The crypts are obviously deeper than the width of the lamina propria mucosae. Moreover, as branched crypts have more than one base per opening and previous calculations are based on morphological findings in the murine small intestine with straight and unbranched crypts the number of stem cells has to be reviewed or recalculated [55]. Some studies showed that the increased energy requirements result in an increase of the length and volume of the small intestine [18,56] and food habit may be closely related to gut morphology and structure [5,6]. Schieck and Millar [57] compared the digestive tract morphology for 35 species of rodents and also found that the masses and lengths were greater in herbivores than in granivores, and stated that small intestine lengths in small mammals did not reflect the amount of fiber in the diet of each species. But significant differences were not found in measurements of the small intestine in white-footed mouse (*Peromyscus leucopus*) - an omnivorous species, and meadow vole (*Microtus pennsylvanicus*) - a herbivorous species, the hind gut measurements revealed diet-specific anatomical differences [23].

In our study, *M. luschani'*s lamina epithelialis thickness mean ($t=0,003$; $p<0,05$) and lumen area mean ($t=0,009$; $p<0,05$) in intestine were larger than *T. karelinii'*s intestine epithelialis thickness and lumen area mean. There was no significant statistical difference between both species mean of bowel layers except lamina epithelialis and lumen area (Table 2, Fig. 8). This difference between the lamina epithelialis thickness mean and lumen area mean of these two species may be due to their gender and age gap or nutrition habit and their need to energy. The difference in lamina epithelialis thickness may be derived from the secretion capacity of the cells depending on hunger or fullness of the animals when they are taken from their environment for the study.

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