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Some histomorphological and histochemical characteristics of the digestive tract of the snake-eyed lizard, *Ophisops elegans* Menetries, 1832 (Squamata: Lacertidae)

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Abstract. The current study was designed to evaluate some histomorphological and histochemical characteristics of the digestive tract of *Ophisops elegans*, the most common lizard species in Turkey. The digestive tract was mainly composed of esophagus, stomach, small and large intestine. Each of these consisted of mucosa, submucosa, tunica muscularis and serosa, as in higher vertebrates. The folded esophageal mucosa had ciliated columnar epithelium with mucous secreting goblet cells which stained positive with PAS (Periodic Acid Schiff) and AB (Alcian Blue) procedures. The surface of the columnar cells of the gastric mucosa and gastric glands of stomach were strongly stained with PAS, but did not show any reaction with AB. The mucosa of small intestine was composed of columnar epithelium with goblet cells that exhibited a strong positive reaction to both PAS and AB. Despite the fact that the mucous secreting cells of the large intestine displayed a strong positive reactivity with PAS, they exhibited a weak reaction with AB. In addition, some statistical differences in AB /PAS staining cellular area and epithelial cell/nuclear area among the parts of the digestive tract were noted. The area of PAS positive material in goblet cells was much greater than the area of AB positive mucosubstances (GAGs) in the large intestine.

Key words: *Ophisops elegans,* lizard, digestive tract, goblet cell, histochemistry, statistics.

Introduction

Reptiles include nearly 7500 different species; alligators, turtles, tortoises, lizards and snakes are the most well-known taxa (Elliot 2007). Despite this incredibly large variety of reptile species, information on the reptilian digestive system is based on only a few studies. Besides, reptiles have similar responses to feeding when compared to other commonly used experimental mammals such as mice, rats, rabbits and pigs. Therefore, they are suitable models for studying the physiological regulation of digestive process (Secor & Diamond 1998). On the other hand, it has been reported that some reptilian species, especially lizards, feed on insects which are harmful to many agricultural plants (Kumlutaş 1993).

Ophisops elegans, the snake-eyed lizard or field lizard, is very abundant in Turkey and is mainly distributed in Anatolia, the southern parts of the Balkans, South-Western Asia, and the Aegean islands (Budak & Göçmen 2008). Although it is widely distributed, no studies on the digestive tract of *O. elegans* have been conducted. From this point of view, we aimed to determine the histological and histochemical features of the digestive tract. Some histological and histochemical characteristics were also evaluated statistically.

Material and Methods

Adult individuals of *Ophisops elegans* were caught around Özdere, İzmir-Turkey (N 38° 03' and E 27° 02'). Eight adult lizards (4 males/4 females) were used in this study. The lizards were euthanized by decapitation with a guillotine under ether anaesthesia. This study was approved by Ministry of Forestry and Water Affairs (date: 19 April 2011, number: 34392) and by the animal ethical committee of Ege University, Faculty of Medicine (2011-048). Digestive tracts of lizards were quickly removed and fixed in Bouin's fixative for 24 hours, dehydrated in ethanol and put into xylol for clearing, before being embedded in paraffin. Serial sections of 5 µm were stained with Harris Haematoxylin-Eosin (HE). In order to identify neutral and acidic mucosubstances (GAGs); Periodic acid Schiff (PAS) and Alcian-Blue (AB) pH 2.5 techniques were also used. The slides were examined and photographed with Olympus CX31-Altra 20 Soft Imaging System.

Morphometric analyses were evaluated by measuring the total area of the epithelial cells, the area of the nucleus of the epithelial cells, and the amount of material stained positive with AB and PAS in all parts of the digestive tract in each lizard. One hundred cells per animal were examined and categorized as AB positive staining cellular area, PAS positive staining cellular area, epithelial cell area and its nucleus area. Data are presented as means with standard deviation (SD). The differences were compared for statistical significance by one-way ANOVA and 2-tailed t-tests using SPSS 16.0, with a significance level of $p \leq 0.05$.

Results

All parts of the digestive tract of *O. elegans* are mainly composed of mucosa, submucosa, tunica muscularis and serosa layers. The esophagus was a simple, short tube. The lower border of the epithelium was irregular due to the presence of transitory folds of the lamina propria. The epithelial layer of the esophageal mucosa was formed of ciliated columnar and goblet cells. (Fig. 1a). Mucous-secreting goblet cells exhibited a strong positive reaction to PAS (Fig. 1b) and AB (Fig. 1c).

The stomach was saccular in shape and lined with mucous secreting columnar epithelium that displayed numerous invaginations, the gastric pits, which gave rise to gastric glands (Fig. 2a). Both the gastric surface epithelium and gastric glands exhibited a positive reaction with PAS, but not with AB (Fig. 2b).

The villi, fingerlike projections of the mucosa

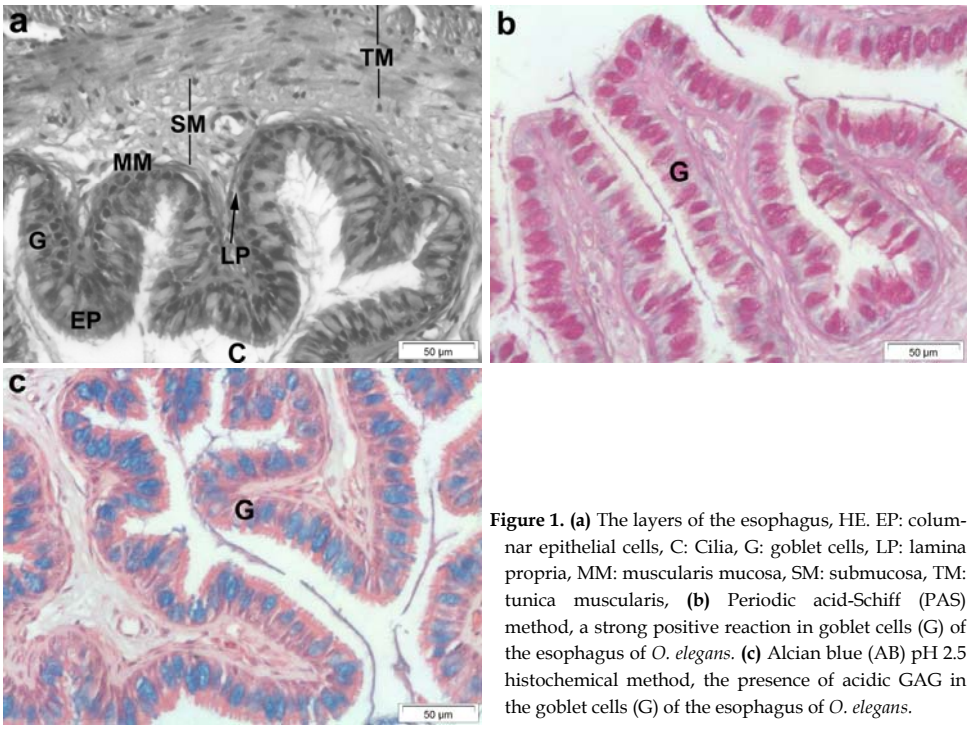

Figure 1. (a) The layers of the esophagus, HE. EP: columnar epithelial cells, C: Cilia, G: goblet cells, LP: lamina propria, MM: muscularis mucosa, SM: submucosa, TM: tunica muscularis, **(b)** Periodic acid-Schiff (PAS) method, a strong positive reaction in goblet cells (G) of the esophagus of *O. elegans*. **(c)** Alcian blue (AB) pH 2.5 histochemical method, the presence of acidic GAG in the goblet cells (G) of the esophagus of *O. elegans*.

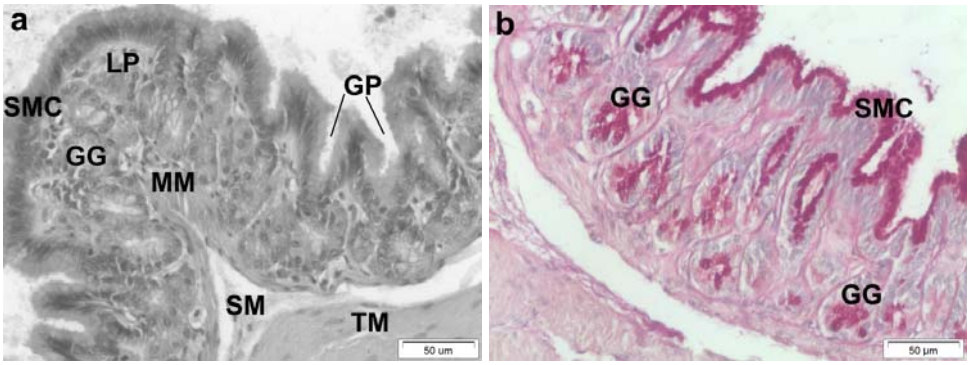

Figure 2. (a) The layers of the stomach, HE. SMC: Surface mucous cells, GP: gastric pit, GG: gastric glands, LP: lamina propria, MM: muscularis mucosa, SM: submucosa, TM: tunica muscularis, **(b)** Detailed view of surface of mucous cells (SMC) and gastric glands (GG) staining with PAS.

of small intestine, were distinguished easily (Fig. 3a). Epithelial layers of mucosa also contained columnar absorptive and goblet cells. The microvilli, thousands of microscopic extensions of the villus were located at the apical surface of absorptive cells (Fig. 3b). The goblet cells that secrete both types of GAGs were stained with both PAS (Fig. 3c) and AB (Fig. 3d).

The epithelial layer of the large intestine was composed of columnar epithelial cells arranged as a single layer and formed as small folds. The goblet cells were widely scattered among the epithelial cells (Fig. 4a). While the PAS reaction of the goblet cells were strongly positive (Fig. 4b), they had a weak staining reaction with AB (Fig. 4c).

As seen in Table 1, some statistical differentiations were noted for all of the digestive parts in AB positive staining cellular area. On the other hand, the values calculated for the esophagus were statistically different from the stomach, small and large intestine in the terms of PAS positive staining cellular area. Similarly, the values of the stomach were also statistically different from the esophagus, and small and large intestines; while no statistical differences were recorded between small and large intestines (Table 1).

In terms of epithelial cell area, the values calculated for the esophagus were statistically different from the small and large intestines. On the other hand, the epithelial area of stomach was discriminated from large intestine statistically. The epithelial area of small intestine also significantly differed from the esophagus and large intestine. Because the values of the large intestine were statistically different from all of the other parts, it can be concluded that it were markedly larger (Table 2).

In terms of the nuclear area of the epithelial cells, the esophagus exhibited a statistical difference when compared to the small and large intestines, while was not different from the stomach. Although the small and large parts of the intestine showed no significant differences, a significant difference was noted between the values of the stomach and intestine (Table 2).

Based on the data given in Table 3, it is concluded that the area of PAS positive materials of goblet cells is much greater than the area of AB positive GAGs in the large intestine. However, the esophagus and small intestine did not show important differences in terms of acidic and neutral GAGs.

Discussion

The structural organization of the digestive system of reptiles is similar to higher vertebrates. Among different reptiles, some adaptive modifications could be seen in esophagus, for instance, the epithelial layers in turtles are keratinized in order to protect the mucosa from abrasive diets. In some reptiles, the stratified and squamous epithelial layers of the esophagus are similar to mammals, however, some typical modifications were also noted for different species (Elliott 2007) such as *Varanus niloticus* which had columnar epithelia (Ahmed et al. 2009). Our results are in accordance with this report. Esophageal glands have been observed in some lizards such as *Tachysaurus rugosus* and *Tiliqua nigrolutea* (Wright & Trethewie 1956), *Acanthodactylus boskianus* (Dehlawi & Zaher 1985). On the other hand, esophagus of crocodilian *Caiman latirostris* does not have submucosal glands in submucosa, but only intraepithelial glands (Machado-Santos et al. 2011). Although the esophageal mucosa of *Lacerta agilis* only consisted of goblet cells (Przystalski 1980), the epithelia is usually composed of ciliated columnar and goblet cells which was also determined in *O. elegans*. Because their secretions have neutral and acidic characteristics, the goblet cells exhibited a positive reaction with PAS and AB in our study. This was not only observed in *O. elegans* but also in *Mabuya brevicollis* (Dehlawi & Zaher 1989), in crocodiles, *Alligator mississippiensis* (Uriona et al. 2005); some other reptiles (Elliott 2007), some fish species such as *Umrio cirrosa* (Parillo et al. 2004), *Claris batrachus, Tilapia spilurus* and *Mylio cuvieri* (Abdulahadi 2005), *Silurus glanis* (Kozaric et al. 2008), *Serrasalmus nattereri* (Raji & Norouzi 2010), and in birds, *Numida meleagris* (Selvan et al. 2008). In *Uromastyx aegyptiaca,* the goblet cells of the esophagus are rich in acid mucopolysaccharides when compared to the small and large intestines (Zaher et al. 2012). This conclusion is compatible with our findings. In addition, in *O. elegans* acidic GAGs of the esophagus are statistically much more numerous than in the small and large intestines. The goblet cells of esophagus showed different affinities for the histochemical techniques in the crocodilian *Caiman latirostris.* These cells were strongly stained by the PAS and after digestion in a-amylase showed slightly less intensity than the PAS reaction. AB at pH 0.4 and 2.5 stained the goblet cells lightly and after double staining with AB-PAS, a small proportion of cells exhibited blue staining, while most

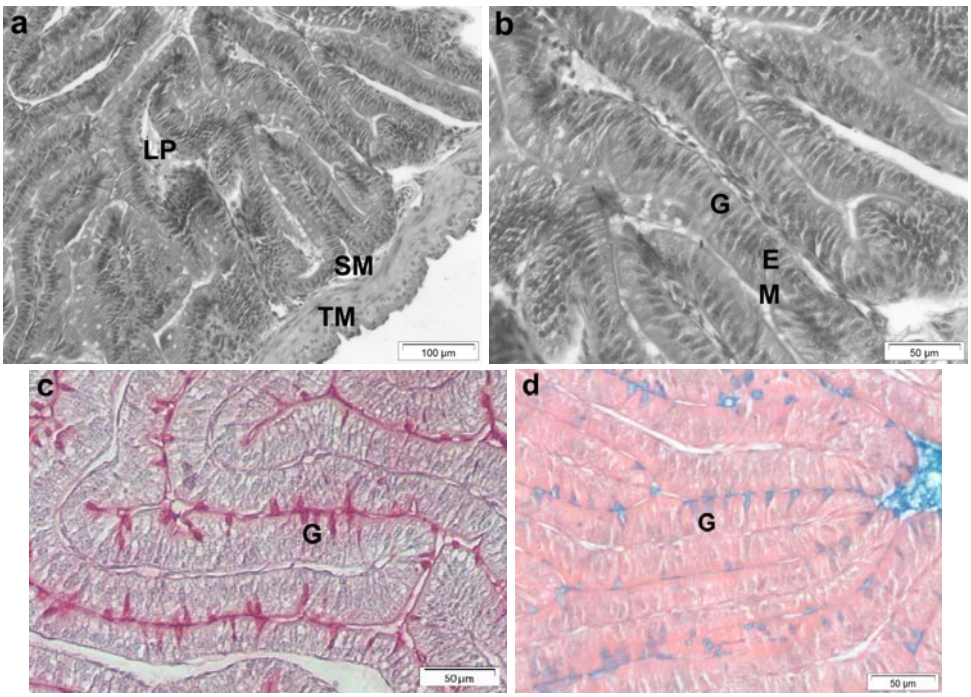

Figure 3. (a) The layers of the small intestine, HE. LP: lamina propria, SM: submucosa, TM: tunica muscularis, **(b)** Absorptive epithelial cells (EP) with microvilli (MV) and goblet cells (G). HE. **(c)** Goblet cells (G) in villi giving a positive reaction with PAS for neutral GAG, **(d)** Goblet cells (G) containing acidic GAG. AB

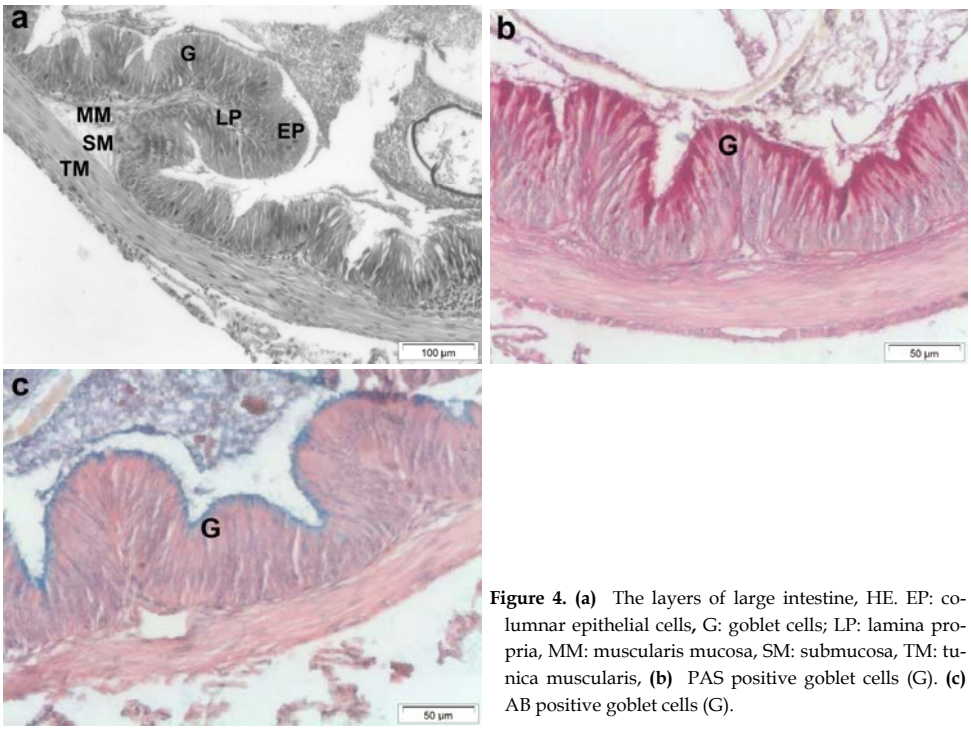

Figure 4. (a) The layers of large intestine, HE. EP: columnar epithelial cells**,** G: goblet cells; LP: lamina propria, MM: muscularis mucosa, SM: submucosa, TM: tunica muscularis, **(b)** PAS positive goblet cells (G). **(c)** AB positive goblet cells (G).

Table 1. AB positive staining cell area (µm²) and PAS positive staining cell area (µm²) in different parts of digestive system. Values are given as mean ± standard deviation. Each part was compared with other parts. * Statistically significant differences are accepted as $p \leq 0.05$

	AB positive staining cellular area		PAS positive staining cellular area	
Esophagus	Stomach	0 *	Stomach	43.54 ± 10.16 *
	Small intestine	90.36 ±42.56 *	Small intestine	93.66 ± 37.98 *
	Large intestine	48.57 ± 17.98 *	Large intestine	94.6 ± 32.91 *
Stomach	Esophagus	205.88 ± 50.04 *	Esophagus	237.46 ± 44.21 *
	Small intestine	90.36 ±42.56 *	Small intestine	93.66 ± 37.98 *
	Large intestine	48.57 ± 17.98 *	Large intestine	94.6 ± 32.91 *
Small intestine	Esophagus	205.88 ± 50.04 *	Esophagus	237.46 ± 44.21 *
	Stomach	0 *	Stomach	43.54 ± 10.16 *
	Large intestine	48.57 ± 17.98 *	Large intestine	94.6 ± 32.91
Large intestine	Esophagus	205.88 ± 50.04*	Esophagus	237.46 ± 44.21 *
	Stomach	0 *	Stomach	43.54 ± 10.16 *
	Small intestine	90.36 ±42.56 *	Small intestine	93.66 ± 37.98

Table 2. Epithelial cell area (µm²) and Nuclear area (µm²) of epithelial cell in different parts of digestive system. Values are given as mean ± standard deviation. Each part was compared with other parts. * Statistically significant differences are accepted as $p \leq 0.05$

	Epithelial cell area		Nuclear area of epithelial cell	
Esophagus	Stomach	181.34 ± 39.48	Stomach	31.48 ± 4.19
	Small intestine	221.24 ± 19.7 *	Small intestine	51.19 ± 14.05 *
	Large intestine	418.72 ± 108.45 *	Large intestine	50.26 ± 9.31 *
Stomach	Esophagus	130.01 ± 35.36	Esophagus	30.27 ± 5.79
	Small intestine	221.24 ± 19.7	Small intestine	51.19 ± 14.05 *
	Large intestine	418.72 ± 108.45 *	Large intestine	50.26 ± 9.31 *
Small intestine	Esophagus	130.01 ± 35.36 *	Esophagus	30.27 ± 5.79 *
	Stomach	181.34 ± 39.48	Stomach	31.48 ± 4.19 *
	Large intestine	418.72 ± 108.45 *	Large intestine	50.26 ± 9.31
Large intestine	Esophagus	130.01 ± 35.36 *	Esophagus	30.27 ± 5.79 *
	Stomach	181.34 ± 39.48 *	Stomach	31.48 ± 4.19 *
	Small intestine	221.24 ± 19.7*	Small intestine	51.19 ± 14.05

Table 3 Mean, standard deviation (S.D), t-value and significance of the measured staining area (µm²). * Statistically significant differences are accepted as $p \leq 0.05$.

PAS-AB Staining	Mean	SD	t-value	Sig. (2-tailed)
Esophagus (PAS)	237.46	44.21	1.5	0.152
Esophagus (AB)	205.86	50.04	1.5	0.152
Small intestine (PAS)	93.66	37.98	0.183	0.857
Small intestine (AB)	90.36	42.56	0.183	0.857
Large intestine (PAS)	94.6	32.91	3.882	0.001 *
Large intestine (AB)	48.57	17.98	3.882	0.002 *

cells exhibited red or intense purple staining. The goblet cells stained with Aldehyde Fuchsin-AB at pH 2.5 appeared purple and blue (Machado-Santos et al. 2011).

The mucous, a viscous fluid composed primarily of highly glycosylated proteins, serves many functions, including lubrication of mucosa and food particles, protection against physical and chemical damage originating from abrasive food (Ahmed et al. 2009), and the trapping and elimination of particulate matter and microorganisms. It has been also related to ionic absorption in fishes (Grau et al. 1992, Albrecht et al. 2001).

In this study, the surface epithelium of the stomach was noted as PAS positive while AB negative. This layer is probably responsible for secretion of neutral GAGs, and that is the case in other reptilian species. Raji and Norouzi (2010) revealed that mucosa of the stomach of walking catfish, *Claris batrachus* and piranha, *Serrasalmus nattereri* were positively stained with PAS, but did not react with AB. In *Natrix natrix*, the secretions

of the fundic mucosa were positively stained by both PAS and AB, pH 2.5 (Scillitani et al. 2012). Neutral glycoproteins have a buffering effect on the acidity of the stomach content (Scocco et al. 1996). Albrecht et al. (2001) reported that the apical portions of columnar epithelial cells, arranged as a single layer, stained positive with PAS in two Neotropical fishes. Citing a study performed by Mousa et al.1956, Dehlawi and Zaher (1989) stated that the stomach of the lizard *A. boskianus* had given a strong reaction with AB, and that the gastric glands of this lizard might be the source of acid mucopolysaccharides. Such a conclusion is in contrast with our data which is largely comparable to other studies. On the other hand, the stomach of *Mabuya brevicollis* gave a positive reaction only to PAS (Dehlawi & Zaher 1989), similar to that of the stomach of *O. elegans.*

In the small intestine of *O. elegans*, the epithelial layer was formed into a large number of villi. In contrast, only a few villi were recorded in *Varanus niloticus* (Ahmed et al. 2009). Despite this difference, both of *O. elegans* and *V. niloticus* had two types of cells in the intestinal epithelium: columnar absorptive cells and goblet cells, which secrete both types of the GAGs that reacted positively to PAS and AB. In the small intestine of *Mabuya quinquetaeniata* and *Chalcides ocellatus*, more absorptive cells were observed (Anwar & Mahmoud 1975). In *Mabuya brevicollis*, the histochemical studies displayed the presence of more goblet cells in the ileum than in the large intestine (Dehlawi & Zaher 1989). In contrast, more goblet cells were observed in the large intestine than in the small intestine of *O. elegans*. Increased populations of goblet cells in the distal parts of intestine was also noted for flower fish, *Pseudophoxinus antalyae* (Çınar & Şenol 2006); may indicate a need for more mucosal protection and lubrication of faeces (Murray et al. 1994). A large quantity of goblet cells contained more neutral than acidic GAGs revealed by giving a strong reaction to PAS. Epler et al (2009) stated that in *Cyprinus carpio* between enterocytes, along the entire length of the intestine, there were mucous cells producing acid (carboxylated and sulphated) mucins. Leknes (2011) pointed out that, when stained with AB followed by PAS, the goblet cells often expressed various colors between blue and purple–magenta; while they revealed blue and red–brown when stained AB followed by neutral red. The author suggested that a true cellular heterogenity was reflected their various functions in lubrication, immunological defence, digestion and absorption.

Citing a study performed by Amer & Ismail 1975, Dehlawi & Zaher (1989) stated that Lieberkuhn glands were not present in *M. quinquetaeniata*. These glands were also absent in *Acanthodactylus boskianus* (Dehlawi & Zaher 1985). Although Lieberkuhn glands were noted in *Uromastyx aegyptia* (El-Toubi & Bishoui 1959), they were not observed in *O. elegans*.

The present study describes some histomorphological and histochemical properties of the digestive tract and statistical evaluations of its mucous content in *Ophisops elegans.* Therefore, it is expected to exhibit a new point of view for further investigations on the physiology of digestive systems of these lizards.

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