Anatomical and Morphometrical Study of the Alimentary Canal of the Lizard Scincus scincus and the snake Natrix tessellata

¹Ahlam M. El- Bakry, ²Ahmed M. Abdeen and ¹Rasha E. Abo- Eleneen

¹Department of Zoology- Faculty of Science, Beni-Suef University ²Department of Zoology- Faculty of Science- Mansoura University amalbakry2@yahoo.com

Abstract: The gastrointestinal tract (GIT) is one of the most interesting systems of reptiles. The present study aimed to study the anatomical, histological and morphometrical features of the gastrointestinal tract (GIT) of the lizard *Scincus scincus* and the snake *Natrix tessellata*. The wall of the different regions of the alimentary tract is built up of four layers, from outside inwards: serosa, muscularis, submucosa and mucosa. The oesophageal mucosa is formed of simple columnar epithelium in *Scincus scincus*, while in *Natrix tessellata* it consists of simple columnar ciliated epithelium with goblet cells. In the studied species, the mucosa of the stomach displayed numerous gastric glands which opened with gastric pits while, the mucosa of the intestine is consisted of simple columnar epithelium containing goblet cells. The intestinal glands are completely absent in the two species. There are great differences between the oesophagus, stomach and intestine histochemically in both species. Generally, the length of the whole gastrointestinal organs is sex-dependent. No significant difference was found in between the organ lengths in both sexes of the studied species. In conclusion, variation in the structure of the GIT appears to be related to the difference in their habitats or their vital activities.

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1. Introduction

Reptiles are always a matter of great interest in many fields of biological sciences. Recently, efforts of the present authors have been directed towards the reptilian gastrointestinal tract (GIT) in order to merge specific morphological merits that would be utilized as an identification key. The gut of various reptiles has been the subject for anatomical, morphometrical, histological and histochemical studies (Zaher *et al.*, **1987 a, b, c & 1989 a; Abo-Taira** *et al.*, **1989; Afifi** *et al.*, **1989 and Abdeen** *et al.*, **1992).** Among lower vertebrates, reptiles always stimulate much interest when compared with the higher ones.

The gastrointestinal tract (GIT) is one of the most interesting systems of reptiles and its morphology, anatomy and histology have been studied extensively on lizards; Scincus officinalis and Scincus scincus (Family: Scincidae), El- Toubi, 1936 and Biomy, 2010 respectively, Uromastyx aegyptia (family: Agamidae, El- Toubi and Bishai, 1958) and Chamaeleon vulgaris (family: Chamaeleontidae, Bishai, 1960). The previous authors showed how the morphological, anatomical and histological patterns of the alimentary tract organs are well adapted to perform their vital functions. Valuable informations were later on added to the subject through the study of other reptilian species; the amphisbaenian Diplometopon zarudnyi (Al- Nassar, 1976), the gecko Gehyra mutilate (Chou, 1977), the scincid lizard *Chalcides levitoni* (El- Taib *et al.*, 1982) and the lacertid lizard *Acanthodactylus ophedureus* (Zaher *et al.*, 1987 a).

Furthermore, combined anatomical and histological studies were carried out on the different species of lizards and snakes, as well as morphometrical investigations were done on the reptilian tract organs of some species in order to cast more light on the importance of the morphometric analysis as a valuable parameter for the identification of different species. In this respect different species of family Lacertidae lizard were studied as Acanthodactylus boskianus (Abo- Taira et al., 1988), family Geckonidae as Tarentola annularis (Abo-Taira et al., 1990) and Chalcides sepsoides (Zaher et al., 1990 b & c). Stimultaneously, great attention was directed to the anatomy and histology of snakes, family Colubridae as Natrix natrix (Przystalski, 1980, Kozaric et al. 2011).

Abdeen et al. (1992) studied the distribution of carbohydrates, proteins and nucleic acids in the oesophageal and gastric mucosal epithelium of three colubrid snakes; *Malpolon monspessulanus*, *Coluber florulentus* and *Tarbophis obtusus* (Family: Colubridae). Several studies are carried out on the histochemical configurations of the reptilian gut mucosa (Zaher et al., 1991 a & b). Mahmoud (1975) resolved the distribution of carbohydrates and proteins in the mucosa of the ileum and rectum of Mabuva quinquetaeniata and Chalcides ocellatus. Recently, in an attempt to creat a novel criterion for classification of reptiles, a regression analysis was applied relating to the snout vent with that of the alimentary canal in the gecko Tarentola annularis et al., 1990) and Mabuya Taira (Aboquinquetaeniata (Zaher et al., 1990 a). These previous works have attracted our attention to design the present investigation which deals with the study of the anatomical, histological and morphometrical aspects of the alimentary tract of the two studied species; the insectivore lizard Scincus scincus (Family: Scincidae) and the water snake Natrix tessellata (Family: Colubridae). This investigation was done to cast more light on the correlation between the anatomical, histological as well as morphometrical configurations of the alimentary canal of Scincus scincus and Natrix tessellata in relation to their habitats. Also, to elucidate if such parameters could be considered with great importance in the field of taxonomy of reptiles.

2. Material and Methods

1- Animals:-

Animal	Family	Habitat
Scincus scincus (Fig. 1)	Scincidae	Restricted to sandy desert areas where it lives mostly under the sand but surfaces occasionally to run on the sand surface at night. It feeds on a variety of insects and captured from Sinai.
Natrix tessellata (Fig. 4)	Colubridae	Never found far from water, often resting or crawling on the bottom of streams or irrigation canals. When distributed it invariably tends to escape by diving into the water. In suitable habitats, large populations can be found in a relatively small area. It feeds on fish and amphibian and collected from

- The animals used were 10 adults of each sex of *Scincus scincus* and *Natrix tessellata*.

2- Gross Anatomy:-

All animals were sacrificed to remove the gut portions (oesophagus, stomach and intestine). These organs were anatomically examined and measured.

3- Morphometry:-

All animals were measured from the snout to the cloacal opening (snout-vent). Lengths of the gastrointestinal tract (GIT) of males and females were expressed as indices and percentages of the total tract length.

4- Statistic Analysis

Statistical significance of female values versus male ones was determined by the application of

student test (Van der Waerden and Nievergelt, 1956). T. test was also applied between the whole gut length and its organs as well as the snout-vent of both sexes of the two species studied.

5- Histological and Histochemical Studies

For histological study of the alimentary tract, parts from different regions of the gut were taken, cleaned with saline and fixed in 10% neutral formalin, then washed and dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections were then cut serially at 5 μ m thickness and stained with Ehrich's haematoxylin and eosin (Mallory, 1944) and Masson trichrome stain (Drury *et al.*, 1976). For histochemical study, the identification of polysaccharides (McManus, 1946), acid and neutral mucins (Mowry, 1956) and total proteins (Mazia *et al.*, 1953) were adopted. The stained sections were examined and photographed using Leitz microscope (Germany).

3. Results

Anatomical Features (Figs. 2, 3, 5 &6):

In *Scincus scincus* (Figs. 2 & 3), the alimentary tract starts with a transverse terminal mouth which surrounds a buccal cavity leading to a short pharynx. The oesophagus is a narrow elongated funnel tube opening directed into the stomach without any external constriction. The stomach is left- curved and slightly wider and longer than the oesophagus. The stomach is terminated by a narrow portion which opens into the intestine.

In Natrix tessellata (Figs. 5 & 6), the oesophagus is subdivided into an anterior wide, funnel- shaped portion and a posterior narrower and extremely longer cylindrical one. The latter enters the visceral cavity dorsal to the pericardioperitoneal septum. The border between the oesophagus and the stomach is only indicated by the nature of the mucous membrane, i.e., there is no anatomical distinction between the oesophagus and the stomach. The stomach has a larger left convex and a smaller right concave curvatures. The stomach leads to the intestine. The intestine is distinguished into the small and large intestine. The large intestine is nearly straight and can be subdivided into two sections: The colon and the rectum. The latter opens into the cloaca. Morphometric observations (Tables 1 – 6 and Figs. 49 - 52):

The application of t- test on the morphometrical data collected from the alimentary canal of the two studied species *Scincus scincus* and *Natrix tessellata* showed that in *Scincus scincus*, there is a significant difference observed between the snout-vent of female (8.4 ± 0.28) and males (9.4 ± 0.15) . The gastrointestinal tract (GIT) of females (12.7 ± 1.72) is always longer than that of males (11.7 ± 0.79) and there is no significant difference is detected in

between them (P > 0.05 level, student t- test) Table (1). In the studied lizard, the female oesophagus length (2.1 ± 0.29) is always longer than the male's oesophagus (1.6 ± 0.37) . No significant difference is detected in between the stomach and intestinal lengths in both species. In both sexes, the longest and shortest organs are the intestine and the oesophagus, respectively. The length of the GIT and its organs, in females and males are diagrammatically represented in (Fig. 49).

There is no significant difference is detected among the index values of the GIT organs in females and males except for the oesophagus (Table 2). The percentage ratios of the GIT organs of female do not significantly differ from those of males in stomach and intestine. However, there is a significant difference in the oesophagus (Table 3). These ratios increase progressively in both sexes through the oesophagus, stomach and intestine. Index and percentage values of the GIT organs in both sexes of Scincus scincus are diagrammatically represented in (Fig. 50), these values are resulted in two closely related curves for the index values, for females and males, and other opposing two for the percentage ratios. These two couples of curves enclose a single genometrically tetragonal area.

The morphometric finding of the alimentary tract organs in both sexes of Natrix tessellata disclosed that there is a significant difference between the snout-vent length of females (85.3 ± 3.34) and males (74.8 ± 9.75) . The GIT length of the females (80.3 ± 3.34) is always longer than that of the males (69.8 ± 9.75) , and there is a significant difference is detected between them (P < 0.005 level, student ttest) Table (4). In both sexes, the longest and shortest organs are the intestine and the stomach respectively. There is a high significant difference between the length of the females and males stomach (P < 0.001level) and a significant difference appears also between females and males intestine (P < 0.05 level). No significant difference is detected between the length of the oesophagus in the two sexes (Table 4 and Fig. 51).

The morphometric data of the alimentary tract organs in both sexes of *Natrix tessellata* showed that there is no significant difference is detected between the index values of stomach and small intestine in females and males but there is a significant difference appears between the oesophagus and large intestine (Table 5).

The percentage ratios increase progressively in both sexes of *Natrix tessellata*, through the stomach, oesophagus and intestine. There is a significant difference between the oesophagus and small intestine (P < 0.005 level, student t- test), but there is no significant difference appears between the stomach and large intestine in both sexes (P > 0.05 level, student t- test) Table (6).

Index and percentage values of GIT organs in both sexes of *Natrix tessellata* are diagrammatically represent in (Fig. 52). The diagrammatical designation of the index and percentage values of the GIT organs in both sexes of the *Natrix tessellta* disclosed two closely related curves inclosing between the two tetragons of unequal areas.

Histological and histochemical studies (Figs. 7-48):

The alimentary canal of *Scincus scincus* and *Natrix tessellata* consists of the usual known regions which are the oesophagus, stomach and intestine. The oesophagus, stomach and intestine of the two studied species are formed of the mucosa, submucosa, muscularis and serosa. **The serosa** represents the outermost layer of the wall in all GIT organs and consists of simple squamous epithelium. This layer is followed by **the muscularis** which consists of smooth muscles and is differentiated into an outer longitudinal and an inner circular muscle layer. In the oesophagus, both layers of muscularis appear, nearly, in the same thickness. However, in all other GIT organs, the outer longitudinal muscle layer is always less developed than the inner circular one.

The muscularis mucosa is well represented in the oesophagus, as well as in the stomach. The oesophageal muscularis mucosa is built up of an outer longitudinal muscle layer which is arranged in several rows and an inner circular muscle layer which appears as a thin sheet. The muscularis mucosa is arranged in the form of a well-developed outer longitudinal muscle layer and well-developed inner circular one.

The submucosa, is well developed throughout all the alimentary canal organs. It consists of an areolar connective tissue and is involved in the structure of the oesophageal, gastric and intestinal folds. The submucosa contains also blood vessels and capillaries in all gut organs. Although the previous layers are nearly similar, some differences are found in the mucosal coat of the oesophagus, the stomach and the intestine of *Scincus scincus* and *Natrix tessellata* which may be referred to their mode of feeding.

1- The oesophagus

Histologically, the oesophageal mucosa of *Scincus scincus* (Figs. 7 & 9), consists of a thin simple columnar epithelium and contains oesophageal glands, while it is thrown into many folds with variable lengths and wavy appearance in *Natrix tessellata* (Figs. 8 & 10). The mucosal epithelium of *Natrix tessellata*, is simple and formed of two types of cells, the elongated ciliated epithelial cells and the goblet cells. The goblet cells are a unicellular glands

and markedly numerous at the bottom of the oesophageal folds. The ciliated epithelial cells of the oesophageal mucosa scattered between the goblet cells.

In *Scincus scincus* (Figs. 11 & 13), the collagenous fibres are scarcely distributed through the mucosa, while in *Natrix tessellata* (Figs. 12 & 14), the collagenous fibres are widely distributed through the mucosa layer.

Histochemically, high contents of polysaccharides which represented by PAS- positive materials are found in the mucosa of both species. The submucosa is moderately stained in Scincus scincus (Fig. 15) while, in Natrix tessellata (Fig. 16), the submucosa is weakly stained. A mixture of neutral polysaccharides (PAS- positive) and acidic ones (Alcian blue positive) are strongly stained in the mucosa of both species in magenta colour (Figs. 17 & 18). Using bromophenol blue stain the oesophageal mucosa of the two studied species shows high content of protein (Figs. 19 & 20). However, low protein content is found in the oesophageal glands of S. scincus (Fig. 19).

2- The stomach

Histologically, in both species, the mucosa of the stomach is mostly consisted of simple columnar cells with oval basally located nuclei. The gastric mucosal coat appeared thick due to the presence of the gastric glands. These gastric glands are embedded in the lamina propria and open into the gastric folds through the gastric pits. The glands consist mainly of a secretory portion, gland neck and gastric pit which open into the lumen (Figs. 21 - 28).

Histochemically, the stomach mucosa of Scincus scincus is strongly stained with PAS indicating high content of neutral polysaccharides (Fig. 29), while the stomach mucosa of Natrix tessellata is moderately stained with PAS indicating the presence of moderate content of neutral polysaccharides (Fig. 30). On the other hand, the muscularis and the submucosa of the two species are moderately loaded with neutral polysaccharides. PAS- Alcian blue (pH 2.5), showed weak reaction in the mucosa, the muscularis and the gastric glands of Scincus scincus (Fig. 31), while PAS- Alcian blue (pH 2.5), showed strong reaction in the mucosa and the gastric glands of Natrix tessellata (Fig. 32). This reaction appeared magenta indicating high content of mixed neutral (purple) and acidic (blue) polysaccharides present in the mucosa. In addition, moderate magenta colour (mixed polysaccharides) was observed in the muscularis layer. Bromophenol blue stained materials indicated high protein contents in the mucosa of both species, while the submucosa appeared moderately stained. However, the low protein contents in gastric glands are weakly stained (Figs. 33 & 34).

3- The intestine

Histologically, the intestinal mucosae of both studied species are thrown up into numerous elongated and deep folds lined with simple epithelia containing scattered goblet cells (Figs. 35 - 38). The intestinal glands are absent in the two species. The collagenous fibres are scarcely distributed through the muscularis layer of *Scincus scincus* and *Natrix tessellata* (Figs. 39 & 40), while these fibres are greatly distributed in the mucosa of both species (Figs. 41 & 42).

Histochemically, PAS positive materials are represented in the muscularis and mucosa of *Scincus scincus* indicating moderate content of neutral polysaccharides (Fig. 43). However, strong PAS reaction (high content of neutral mucins) is indicated in the mucosa of *Natrix tessellata* (Fig. 44), while the muscularis is moderately stained with PAS.

PAS- Alcian blue (pH 2.5), showed moderate magenta colour (mixed neutral and acidic mucins) in the mucosa of both species. The goblet cells are stained magenta for mixed mucins and blue for acid mucins in both species (Figs. 45 & 46), while in *Natrix tessellata* acid mucins predominate in the goblet cells (Fig. 46). Application of bromophenol blue, showed high protein contents in the mucosa and the muscularis layer of both species (Figs. 47 & 48). On the other hand, the goblet cells appeared weakly stained for protein in *Scincus scincus* and devoiding proteins in *Natrix tessellata*.

Table (1): Lengths (cm) of the snout vent and alimentary tract organs in both sexes of *Scincus scincus*

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GIT organ	Females	Males	Р
Snout-vent	8.4 ± 0.28	9.4 ± 0.15	**
Alimentary tract	12.7 ± 1.72	11.7 ± 0.79	ns
Oesophagus	2.1 ± 0.29	1.6 ± 0.37	**
Stomach	2.7 ± 0.52	2.4 ± 0.28	ns
Small intestin	0.3 ± 1.2	5.2 ± 1.36	ns
Large intestine	2.6 ± 0.6	2.5 ± 0.6	ns

The values are means \pm standard deviation (n = 10 0f each sex); ns: Non-Significant (student t-test, P > 0.05 level); **: Significant values (student t- test, P < 0.001 level).

Table (2): Index values of gastrointestinal tract (GIT) organs in both sexes of *S. scincus*

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GIT organ	Females	Males	Р
Oesophagus	6.6 ± 1.17	7.3 ± 1.16	*
Stomach	4.4 ± 0.5	4.3 ± 0.61	ns
Small intestine	2.4 ± 0.25	2.5 ± 0.41	ns
Large intestine	5.4 ± 0.96	4.7 ± 0.78	ns

Index is the gastrointestinal tract length / organ length; Values are means \pm standard deviation (n = 10 0f each sex); ns: Non- Significant (student t- test, P > 0.05 level); *: Significant values (student t- test, P < 0.05 level).

Table (3): Percer	hage ratios of	the gastrointes	linai
tract (GIT) organs	in both sexes	s of Scincus scin	cus
GIT organ	Females	Males	Р

Off ofgun	1 enhares	iviares	1
Oesophagus	16.7 ± 2.35	13.6 ± 2.9	*
Stomach	21.4 ± 3.16	20.8 ± 2.54	ns
Small intestine	42 ± 4.42	44.4 ± 5.3	ns
Large intestine	20.4 ± 2.3	21.3 ± 3.2	ns
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The values are means \pm standard deviation (n = 10 0f each sex) Percentage ratio is the organ length \times 100 / gastro-intestinal length; ns: Non- Significant (student t- test, p > 0.05 level); *: Significant values (student t-test, p < 0.05 level).

Table (4): Lengths (cm) of the snout vent and alimentary tract organs in both sexes of *Natrix tessellata*

GIT organ	Females	Males	Р
Snout-vent	85.3 ± 3. 34	74.8 ± 9.75	*
Alimentary tract	80.3 ± 3.34	69.8 ± 9.75	*
Oesophagus	21.6 ± 1.58	20.5 ± 1.96	ns
Stomach	10.4 ± 1.19	8.1 ± 0.74	**
Small intestine	36.6 ± 11.8	30.2 ± 8.31	*
Large intestine	11.7 ± 3.4	11 ± 2.8	*

The values are means \pm standard deviation (n = 10 0f each sex); ns: Non- Significant (student t- test, P > 0.05 level); *: Significant values (student t- test, P < 0.05 level); **: Significant values (student t- test, P < 0.001 level).

Table (5): Index values of gastrointestinal tract (GIT) organs in both sexes of *Natrix tessellata*

GIT organ	Females	Males	Р
Oesophagus	3.5 ± 0.69	3.2 ± 0.23	*
Stomach	7.7 ± 1.39	8.6 ± 1.25	ns
Small intestine	2.3 ± 0.4	2.5±0.19	ns
Large intestine	6.8 ± 1.45	6.6 ± 1.3	*

Index is the gastrointestinal tract length / organ length. Values are means \pm standard deviation (n = 10 0f each sex); ns: Non- Significant(student t- test, p > 0.05 level); *: Significant values (student t- test, p < 0.05 level).

Table (6): Percentage ratios of the gastrointestinal tract (GIT) organs in both sexes of *Natrix tessellata*

GIT organ	Females	Males	Р
Oesophagus	26.9 ± 2.1	29.6 ± 2.84	*
Stomach	12.9 ± 1.63	11.6 ± 1.13	ns
Small intestine	45.5 ± 7.2	43.2 ± 3.8	*
Large intestine	$14.5 \pm 2.$	16 ± 2.6	ns

The values are means \pm standard deviation (n = 10 0f each sex); Percentage ratio is the organ length \times 100 / gastro-intestinal length; ns: Non- Significant (student t- test, P > 0.05 level); *: Significant values (student t- test, P < 0.05 level).



- Fig. 1: Photograph of the lizard (*Scincus scincus*) in a lateral view.
- Fig. 2: Dissected specimen of male *Scincus scincus* exposing the general viscera.Oesophagus (OES), Stomach (ST), Intestine (I), Testis(T), Rectum (R), Coloaca (C).
- Fig. 3: Dissected specimen of female *Scincus scincus* exposing the general viscera. Oesophagus (OES), Stomach (ST), Intestine (I), Ovary(O), Rectum (R), Coloaca (C).



- Fig. 4: Photograph of the snake (*Natrix tessellata*) in a lateral view.
- Fig. 5: Dissected specimen of male *Natrix tessellata* exposing the general viscera. Oesophagus (OES), Stomach (ST), Gall Bladder (GB), Intestine (I), Right Testis(RT), Left Testis(LT).
- Fig. 6: Dissected specimen of female *Natrix tessellata* exposing the general viscera. Oesophagus (OES), Stomach (ST), Gall Bladder (GB), Intestine (I), Ovary (O).



- **Fig. 7:** T.S. of the oesophagus of *Scincus scincus* showing the structure of the mucosa (MU) and the submucosa (SM) of loose connective tissue. H.E., X 100.
- Fig. 8: T.S. of the oesophagus of *Natrix tessellata* displaying the columnar epithelial structure of the mucosa (MU). The submucosa (SM) is formed of loose connective tissue and thick muscularis layer (M) of striated muscles covered with serosa (S). H.E., X 100.
- **Fig. 9:** T.S. of the oesophagus of *Scincus scincus* showing the structure of the mucosa (MU), the esophageal glands (OG) and the submucosa (SM). H.E., X 200.
- **Fig. 10:** T.S. of the oesophagus of *Natrix tessellata* displaying the ciliated epithelial cell (C) and the goblet cell (GC). H.E., X 200.
- **Fig. 11:** T.S. of the oesophagus of *Scincus scincus* showing the columnar epithelial structure of the mucosa (MU) and the collagen fibres (CF) scarcely distributed in the submucosa (SM). Masson's trichrome stain., X 100.
- **Fig. 12:** T.S. of the oesophagus of *Natrix tessellata* showing the condensation of the collagen fibres (CF) in the muscularis (M) and the mucosa (MU). Masson's trichrome stain., X 100.
- **Fig. 13:** T.S. of the oesophagus of *Scincus scincus* showing the collagen fibres (CF) in the muscularis (M), the submucosa (SM) and the mucosa (MU). Masson's trichrome stain., X 200.
- **Fig. 14:** T.S. of the oesophagus of *Natrix tessellata* showing the collagen fibres (CF) are distributed in the mucosa (MU). Masson's trichrome stain., X 200.



- Fig. 15: T.S. of the oesophagus of *Scincus scincus* denoting high polysaccharide content in the mucosa (MU) and the esophageal glands (OG). PAS, X 100.
- **Fig. 16:** T.S. of the oesophagus of *Natrix tessellata* showing accumulation of high polysaccharide content in the mucosa (MU). PAS., X 100.
- Fig. 17: T.S. of the oesophagus of *Scincus scincus* displaying high content of neutral and acidic mucins in the mucosa (MU) and showing high content of acidic mucins in the esophageal glands (OG) (in blue). PAS-Alcian., X 100.
- Fig. 18: T.S of the oesophagus of *Natrix tessellata* denoting high content of acidic mucins in the mucosa (MU). The muscularis (M) are moderately stained magenta. PAS-Alcian., X 100.
- Fig. 19: T.S. of the oesophagus of *Scincus scincus* showing high protein content in the mucosa (MU), the muscularis (M) and the serosa (S). The esophageal glands (OG) are moderately stained. Bromophenol blue., X 100.
- Fig. 20: T.S. of the oesophagus of *Natrix tessellata* displaying high protein content in the mucosa (MU). Bromophenol blue., X 100.
- Fig. 21: T.S. of the stomach of *Scincus scincus* disclosing the serosa (S), the muscularis (M), the submucosa (SM) and the mucosa (MU). H.E., X 100.
- Fig. 22: T.S. of the stomach of *Natrix tessellata* displaying the serosa (S), the muscularis (M), the submucosa (SM) and the mucosa (MU). H.E., X 100.



- **Fig. 23:** T.S. of the stomach of *Scincus scincus* showing the mucosa (MU), the gastric pits (GP) and the gastric glands (GG). H.E., X 200.
- **Fig. 24:** T.S. of the stomach of *Natrix tessellata* displaying the mucosa (MU), the gastric pits (GP) and the gastric glands (GG). H.E., X 200.
- Fig. 25: T.S. of the stomach of *Scincus scincus* showing the collagen fibres (CF) in the serosa (S) and the mucosa (MU). Masson's trichrome stain., X 100.
- **Fig. 26:** T.S. of the stomach of *Natrix tessellata* showing the collagen fibres (CF) in the serosa (S) and the mucosa (MU). Masson's trichrome stain., X 100.
- **Fig. 27:** T.S. of the stomach of *Scincus scincus* showing the collagen fibres (CF) in the mucosa (MU). Masson's trichrome stain., X 200.
- **Fig. 28:** T.S. of the stomach of *Natrix tessellata* showing the collagen fibres (CF) in the mucosa (MU). Masson's trichrome stain., X 200.
- Fig. 29: T.S. of the stomach of *Scincus scincus* showing high polysaccharide contents in the mucosa (MU). The muscularis (M) is weakly stained. PAS., X 100.
- **Fig. 30:** T.S. of the stomach of *Natrix tessellata* showing moderate content of polysaccharides in the mucosa (MU). The muscularis (M) is lightly stained. PAS., X 100.



- Fig. 31: T.S. of the stomach of *Scincus scincus* displaying weak reaction of PAS-Alcian blue in the mucosa (MU) and the muscularis (M). PAS-Alcian blue., X 100.
- Fig. 32: T.S. of the stomach of *Natrix tessellata* showing high content of neutral and acidic-mucins in the mucosa (MU). The muscularis (M) appear moderately stained magenta. PAS-Alcian., X 100.
- **Fig. 33:** T.S. of the stomach of *Scincus scincus* displaying high protein content in the mucosa (MU). The muscularis (M) is moderately stained while the gastric glands (GG) are weakly stained. Bromophenol blue., X 100.
- Fig. 34: T.S. of the stomach of *Natrix tessellata* displaying high protein content in the mucosa (MU) and the muscularis (M). The gastric glands (GG) are weakly stained. Bromophenol blue., X 100.
- Fig. 35: T.S. of the intestine of *Scincus scincus* showing the serosa (S), the muscularis (M) and the mucosa (MU). H.E., X 100.
- Fig. 36: T.S. of the intestine of *Natrix tessellata* displaying the serosa (S), the muscularis (M) and the mucosa (MU). H.E., X 100.
- Fig. 37: T.S. of the intestine of *Scincus scincus* showing the mucosa (MU) and numerous goblet cells (GC). H.E., X 200.
- Fig. 38: T.S. of the intestine of *Natrix tessellata* showing the mucosa (MU) and the goblet cells (GC). H.E., X 200.



- Fig. 39: T.S. of the intestine of *Scincus scincus* showing the collagen fibres (CF) scarcely distributed in the muscularis (M). Masson's trichrome stain., X 100.
- Fig. 40: T.S. of the intestine of *Natrix tessellata* disclosing the collagen fibres (CF) in the muscularis (M). Masson's trichrome stain., X 100.
- Fig. 41: T.S. of the intestine of *Scincus scincus* showing the condensation of collagen fibres (CF) in the mucosa (MU). Masson's trichrome stain., X 200.
- Fig. 42: T.S. of the intestine of *Natrix tessellata* displaying the condensation of collagen fibres (CF) in the mucosa (MU). Masson's trichrome stain., X 200.
- Fig. 43: T.S. of the intestine of *Scincus scincus* disclosing moderate polysaccharide content in the muscularis (M) and the mucosa (MU). The goblet cells (GC) appear unstained. PAS., X 100.
- Fig. 44: T.S. of the intestine of *Natrix tessellata* displaying high content of polysaccharide in the mucosa (MU). The muscularis (M) is moderately unstained. PAS., X 100.
- Fig. 45: T.S. of the intestine of *Scincus scincus* showing moderate PAS-Alcian blue positive materials in magenta colour (neutral and acidic mucins) in the mucosa (MU) and the muscularis (M). The goblet cells (GC) are strongly stained with a mixture of Alcian blue (acid mucins) and PAS (neutral mucins). PAS-Alcian blue., X 100.
- Fig. 46: T.S. of the intestine of *Natrix tessellata* showing mixed neutral (red) and acidic (blue) polysaccharides (magenta) in the mucosa (MU). Acid mucins predominate in the goblet cells (GC). PAS-Alcian blue., X 100.
- Fig. 47: T.S. of the intestine of *Scincus scincus* showing high content of protein in the muscularis (M) and the mucosa (MU). The goblet cells (GC) are devoid of protein. Bromophenol blue., X 100.
- Fig. 48: T.S. of the intestine of *Natrix tessellata* disclosing high protein content in the muscularis (M) and the mucosa (MU). The goblet cells (GC) are negatively stained for protein. Bromophenol blue., X 100.



Fig. 49: Histogram illustrating the snout-vent, mean length of the alimentary tract and its organs in both sexes of *Scincus scincus*.



Fig. 50: Indices and percentage ratios of organ length relative to the length of the whole alimentary tract in both sexes of *Scincus scincus*. Note the tetragonal areas in between the two curves.







Fig. 52: Indices and percentage ratios of organ lengths in relation to the total tract length in both sexes of *Natrix tessellata*. Note the two, more or less, similar tetragonal areas in between the curves.

4. Discussion

Anatomically, in the two examined species, there is a relatively long and narrow oesophagus. It becomes gradually wider towards the stomach. The stomach is formed of an elongated straight sac. Stated previously, the insectivorous species Scincus scincus has no delimitation between the oesophagus and the stomach. This is a relevant adaptation to permit the passing of insect prev without any obstruction, similar to that found in Scincus officinalis (El-Toubi, 1936), Mabuya brevicollis (Zaher et al., 1989 b) and Scincus scincus (Biomy, 2010) displayed also the absence of such a constriction. The stomach is highly elongated and extremely narrow in the present lizard. The differentiation of the intestine of Scincus scincus is closely similar to that described in other members of scincidae (El-Toubi, 1936; Zaher et al., 1989 b and Biomy, 2010,). Also, the present finding indicates the absence of the oesophageal stomach demarcation in Natrix tessellata. This is also observed in other snakes, Coluber florulentus (Dehlawi and Zaher, 1989 and Abdeen et al., 1992). The disappearance of such anatomical limit in snakes is believed to be the major reason for facilitating the passage of prey which is swallowed occasionly from the oesophagus to stomach. As observed in several lizards and snakes, the stomach of both studied species displays a large left convex curvature and a small right curvature. Therefore, this gastric appearance is most likely a characteristic feature of order squamata. A similar observation was recorded in lacertilian and ophidian species (Bishai, 1959; Zaher et al., 1990 a & b and Afifi et al., 1990, Kozaric et al. 2011).

The morphometric analysis:

The statistical analysis of the morphometric variabilities has got a special attention in the last few years. Good results were obtained for evaluation of sexual dimorphism in reptiles (Kminiak and Kaluz, 1983) and for determination of some indices (Estrada and Rodriguez, 1984). Moreover, extensive studies were carried out by icthyologists using regression analysis and morphometric indices in the field of fish taxonomy (Botros *et al.*, 1970; Lachner and Jenkins, 1971; Ezzat *et al.*, 1979; Mahmoud, 1980, Kozaric *et al.* 2011).

As demonstrated morphometrically, there is no statistical significant difference in between the gastrointestinal organs of the males and females of the two studied species. However, in *Natrix tessellata*, the small intestine of females was found to be longer than that of males.

The present morphometric demonstration divulged that no differences were observed between males and females of *S. scincus* in all studied

measurements. Except for the stomach of M. brevicollis which is longer in males than females all other morphometric aspects have no sex differences (Zaher et al., 1989 b). This may suggest that the sex relationships which inferred from the present data are not correlated with sex dimorphism in family scincidae. It is not surprising that the small intestine is the longest gut organ in S. scincus. This elongation is most probably to compensate the limited number of intestinal villi observed microscopically and consequently to increase the surface of absorption. In contrast, which in the present Scincus scincus, the oesophagus appears as the shortest organ. Since the mucosal folds of the oesophagus are highly convoluted, the shortening of this organ does not interrupt the oesophageal function. The present morphometric data disclose that in S. scincus the snout- vent represents 70 % of the alimentary tract length. This means that, when the snout-vent as an external element of an animal is measured, the length of the GIT and its organs, as an internal system can be estimated without the need for animal sacrifice. Furthermore, the illustrations of both index and percentage ratio of all tract regions of an animal always gives rise to a specific geometrical outline. This specific illustration may support the predicted species identification. In the present species (S. scincus), the resulted geometrical outline is a tetragonal area which is dissimilar to the hexagonal one obtained in the skink lizard *M. brevicollis* (Zaher et al., 1989 b). The present tetragonal outline is similar to that described in the lizards (Chalcides sepoides; Zaher et al., 1990 b & c and Acanthodactylus boskianus; Abo- Taira et al., 1988) and in the snake (Echis carinatus; Afifi et al., 1990).

The morphometric analysis of *N. tessellata* revealed that the female GIT is more elongated than that of the male. In addition, the length of each alimentary canal organ except the large intestine is significantly higher in females than in males. Similar observations have been reported in other reptilian species (**Przystalski, 1980 and Abo- Taira** *et al.*, **1988).** However, it seems likely that the correlation between the female sex and the length of GIT is a remarkable character of snakes. This sex- dependency is perhaps connected with the production of eggs in females (**Przystalski, 1980).**

In the present species, both the stomach and oesophagus are shortest and longest GIT organs, respectively. Similar results were obtained by **Zaher** *et al.* (1991a) in *Echis carinatus*.

By considering the index and percentage of all GIT regions of *N. tessellata* none of the organs exhibits any significant difference between both sexes except for the index of the female large intestine which is slightly higher than that in males. Therefore, both parameters (index and percentage) cannot be accepted as a base for differentiating the sex. In addition, these values are different from those reported in *Chalcides sepoides* (Zaher *et al.*, 1990 b & c). However, such measurements are most probably related to the zone where the animal lives and where the nature and amount of food as well as ecological conditions differ from one zone to another.

The combined representation of both index and percentage values in *N. tessellata* discloses a double geometrical design. Each of the resulting two areas is a tetragon and most likely a specific rule for snakes, since the geometrical description reported previously was a single hexagon in *Mabuya brevicollis* (Zaher et al., 1989 b) and also a single tetragon in *Stenodactylus slevini* (Zaher et al., 1989 a).

Histologically, the oesophageal mucosa of Scincus scincus is formed of simple columnar epithelium and consists mostly of oesophageal glands, while the oesophageal mucosa of Natrix tessellata is simple and formed of two types of cells, the elongated thin ciliated epithelial cells and the goblet cells. Such a condition was also recorded in other reptilian species such as Chalcides levitoni and Mauremyes caspica (El- Taib et al., 1982 and El-Taib and Jarrar, 1983, Kozaric et al. 2011). The oesophageal mucosa of Scincus scincus, is nearly similar to that of Uromastyx aegyptius (Abo-Eleneen, 2010). In Natrix tessellata, such a structure is greatly similar to that described in Varanus niloticus (Abo-Eleneen, **2010**) and in Acanthodactvlus boskianus (Dehlawi and Zaher, 1985a). The presence of the oesophageal glands in Scincus scincus is nearly similar to that of several reptiles such as C. vulgaris (Bishai, 1960) and turtles (Gabe, 1973). The oesophageal glands are mucous in nature and secrete mucin to help the oesophagus to convey food from the buccal cavity to the stomach.

On the other hand, in Natrix tessellata, the oesophageal glands are completely absent. This observation was also recorded in C. levitoni (El- Taib et al., 1982), T. vermicularis (Heyder, 1974) and A. boskianus and P. rupestris (Dehlawy and Zaher, 1985a & b). The presence of the goblet cells in the oesophageal mucosa of Natrix tessellata as well as of other reptiles, such as Ablephorous pannonicus (Greschik, 1917), Chamaeleon vulgaris (Bishai, 1960) and U. philbyi (Farag, 1982), agrees with the basic task of the oesophagus which conveys food from the buccal cavity to the stomach. This function is facilitated by the mucous secretion of the goblet cells, which causes the inner surface of the oesophageal lumen to be viscous and thus facilitates the swallowing of the prey and its gliding through the oesophageal lumen to reach the stomach.

The collagenous fibres of *Scincus scincus* are scarcely distributed through the mucosa while, the collagenous fibres of *Natrix tessellata* are widely distributed through the mucosa. This collagenous fibre gives the oesophagus its strength.

Histochemically, the present study revealed the presence of exaggerated amounts of carbohydrates in the mucosal epithelium of both species. This finding is in agreement with that observed by Abo- Eleneen (2010) in Uromastyx aegyptius and Varanus niloticus. The oesophageal mucosa of Scincus scincus and Natrix tessellata showed accumulation of PAS and Alcian blue positive materials indicating the presence of mixed neutral and acidic mucins which help in producing mucous necessary for lubricating food. In S. scincus and N. tessellata, the oesophagus is richly supplied with protein particularly in the mucosa and muscularis. This indicates the importance of protein materials in constituting the contracting muscles. However, low protein content is found in the oesophageal glands of S. scincus showing that these glands are more responsible for the production of mucus which is mostly acidic in nature.

The stomch mucosa of Scincus scincus and Natrix tessellata is consisted of gastric glands and gastric pits. In S. scincus (insectivorous), the gastric glands are represented with the oxyntic type while. these glands are represented with peptic cells in Natrix tessellata (carnivorous). A review to the published works of the history of the alimentary tract of reptiles shows a considerable controversy on the nature of the granular cells forming the glandular bodies of the gastric glands of the reptilian stomach. Smit (1962), Gabe and Saint Girons (1964) and Skoczylas (1970) reported that these cells play a role in the secretion of pepsin and hydrochloric acid. They termed them oxyntico-peptic cells. However, in U. aegyptia, which is purely herbivorous (El- Tobui and Bishai, 1958), termed these cells oxyntic cells. On the other hand, Bishai (1959) in Varanus griseus, which is purely carnivorous, termed these cells peptic cells. The same author identified such in the insectivorous C. vulgaris as oxyntic cells. In both species studied, the collagenous fibres are scarcely distributed through the serosa and the mucosa.

Histochemically, the gastric mucosa is heavily loaded with mixed neutral and acidic mucins in *Natrix tessellata* while, the gastric mucosa is weak reaction with PAS- Alcian blue. The positive response of the gastric secretory portion cells of PAS-Alcian blue stain denotes the mucous nature of their secretions that facilitate the passage of digested food from the stomach to the intestine. Meanwhile, the presences of polysaccharides which play an important role in the production of energy are greatly represented in the mucosa. Moreover, the gastric mucosa of both species shows heavy protein contents. The same results were recorded in *Uromastyx aegyptius* and *Varanus niloticus* (Abo- Eleneen, 2010, Zaher *et al.*, 1991c).

The absence of the intestinal glands observed in the present work was similar to that found in *Mabuya quinquetaentiata* (Amer and Ismail, 1975), *Acanthodactylus boskianus* (Dehlawi and Zaher, 1985 a), in *Typhlops vermicularis* and *Echis carinatus* (Heyder, 1974 and Afifi *et al.*, 1990) respectively and in *Uromastyx aegyptius* and *Varanus niloticus* (Abo- Eleneen, 2010).

As outlined microscopically, the intestinal mucosa of *Scincus scincus* and *Natrix tessellata* lacks granular crypts. This outcome is closely similar to that recorded in both lacertilian and ophidian species (Greschik, 1917). Moreover, the response of the mucous membrane cells and goblet cells in the intestinal wall indicates the abundance of mucous secretion. This may be considered as an adaptation for the intestine to perform well its role in water absorption (Bishai, 1960). The collagenous fibres are greatly distributed in the mucosa of both species studied. These fibres give the intestinal mucosa its strength and compactness which are associated with the mechanical properties.

Histochemically, the intestinal goblet cells of the two species studied are strongly stained with a mixture of neutral and acidic mucins. The secretion of these cells is probably responsible for mucus secretion. However, acidic mucins are predominated in the goblet cells of *Natrix tessellata*. Meanwhile, the goblet cells of both species are weakly loaded with proteins, whereas high content of protein is found in the mucosa. Similar observations were reported in reptiles (Abo- Eleneen, 2010) and in mammals (Abdeen *et al.*, 2008).

In conclusion, it is obvious that the anatomy and morphometry as well as the histology of the alimentary tract in *S. scincus* and *N. tessellata* demonstrate certain specific characteristics of functional adaptation.

Corresponding author

Ahlam M. El- Bakry

Department of Zoology- Faculty of Science- Beni-Suef University amalbakry2@yahoo.com

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