

Ultrastructural Study of the Alimentary Tract of Two Reptilian Species the Lizard *Scincus scincus* and the Snake *Natrix tessellata*

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Abstract

The ultrastructural difference of the alimentary tract of the insectivore, *Scincus scincus* and the carnivore, *Natrix tessellata* was described by using scanning and transmission electron microscopy. The oesophagus of *S. scincus* with many prominent longitudinal folds while in *N. tessellata* it has many primary longitudinal folds and secondary ramification. The oesophageal epithelium of both species has numerous goblet cells. The mucosal epithelial cells in both species contain large nucleus, endoplasmic reticulum, lysosomes and some vacuoles. Stomach had a meshwork of primary folds in *S. scincus* and it forms various folds forming many discrete, irregular pockets in *N. tessellata*. The mucosal cells in the stomach of *S. scincus* have many secretory granules while in *N. tessellata* it provided with numerous mucinogen granules in the gastric columnar cells. In *S. scincus*, the intestinal mucosa with many primary folds but it appeared as longitudinal secondary mucosal folds in Zig-Zag pattern enclosing a number of concavities in *N. tessellata*. The intestinal mucosal cells in both species consisted of regularly formed microvilli. There were numerous goblet cells in the intestine of both species. In conclusion, structural variation of the alimentary tract in the two species examined was related to difference in feeding habits.

Keywords: Reptiles; Alimentary tract; SEM; TEM

Introduction

The digestive system is as important as food is to survival in animals. The anatomical characteristics of this system are dependent upon the food, habitat and nutritional status of the organism [1]. In reptiles, the digestive system contains all the structures present in other higher vertebrates, from the oral cavity to the cloaca. The oral cavity is lined by mucous membrane made by non-ketatinized stratified squamous epithelium with salivary glands distributed in the submucosa [2].

In reptiles, the first vertebrates to adapt completely to the terrestrial life, the oesophagus shows adaptive modifications from group to group, the stomach varies in shape and the intestine may be highly convoluted as in turtles or relatively straight as in snakes to facilitate absorption, secretion and excretion [3-6].

The ultrastructural studies of the Gastrointestinal tract have been widely used to provide a detailed anatomical description that betters to understanding the operation of this system as in Typhlopidae Snake *Rhamphotyphlops Braminus* and *Scincus scincus* [7,8]. Carrassón & Matallanas [9] used SEM (Scanning electron microscope) to study the digestive tract in *Dentex dentex*; while Imtiyaz & Ashok [10] used the same technique to examine the intestinal tract of *Schizothorax curifrons*.

The present work aims to study the ultrastructural features of the digestive tract of two reptilian species *Scincus scincus* and *Natrix tessellata*, inhabiting different locations and with different mode of feeding, in order to increase current knowledge in basic structures of the reptilian alimentary tract.

Materials and Methods

Sample collection

5 specimens of both species, *Scincus scincus* and *Natrix tessellata* were used. The first species was captured from sandy desert areas of Sinai and it feeds on a variety of insects while the second one is collected from irrigated canals of Faiyum. It feeds on fish and amphibian. The two species were dissected and oesophagus, stomach and intestine were

removed. The animals were anaesthetized and decapitated according to the international protocol for biomedical investigation with Human Being and Animals.

Scanning electron microscopy

The dissected oesophagus, stomach and intestine were cut into pieces and fixed in glutaraldehyde in 0.1M cacodylate buffer. The specimen were post-fixed in a solution of 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for one hour, and washed several times in the 0.1 M sodium cacodylate buffer. The tissues were dehydrated through either acetone or alcohol series, critical point drying and platinum-palladium ion-sputtering were applied. The specimens were then investigated by scanning electron microscopy (JSM -5400LV; JEOL).

Transmission Electron Microscope (TEM)

Small fragments of the oesophagus, stomach and intestine were fixed in fresh 3 % glutaraldehyde in 0.1 M cacodylate buffer (Ph 7.2) for 4 h. Afterwards, the specimens were post-osmified in 0.1 M cacodylate buffer (Ph 7.2) for 2 h. They were then washed again in the buffer, dehydrated, and embedded in epoxy-resin araldite mixture. Semithin sections were cut by ultramicrotome, stained with toluidine blue, then examined. Ultrathin sections were cut using a diamond knife and stained using uranyl acetate and lead citrate, then examined by CX100 transmission electron microscope (JEOL, operated at an accelerating voltage of 60 Kv).

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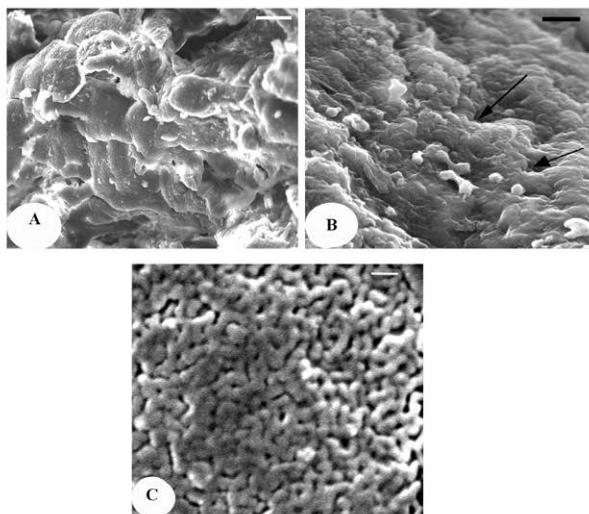


Figure 1: SEM micrograph of the oesophagus of *Scincus scincus*, showing (a) many prominent longitudinal folds. (Scale bar, 50 µm). (b) many goblet cells (arrows) Scale bar, 50 µm. (c) Surface wavy microridge (Scale bar, 1 µm).

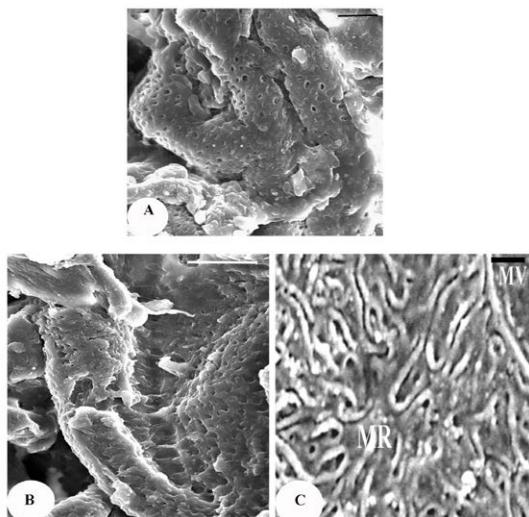


Figure 2: SEM micrograph of the oesophagus of *Natrix tessellata*, showing (a) primary and secondary folds. (Scale bar, 50 µm). (b) many goblet cells (Scale bar, 20 µm). (c) apical fingerprint-like microridge(MR) alternates with microvilli (MV) in some regions (Scale bar, 1 µm).

Results

SEM observation showed that, the oesophageal mucosal surface in *S. scincus* was provided with many prominent longitudinal folds leaving long furrows in between them. Many circular opening of goblet cells and well-defined wavy microridges were also detected (Figure 1a-c). While in *N. tessellata*, the oesophageal surface has many primary longitudinal folds of different sizes and secondary ramification (Figure 2a). Some pocket marks representing the luminal surface of goblet cells were found in between the cell junctions (Figure 2b). The superficial oesophageal epithelium provided with fingerprint-like microridge alternates in some regions with apical microvilli (Figure 2c).

By TEM, The oesophageal mucosal cells of *S. scincus*, containing oval-shaped nucleus oriented parallel to the epithelial surface (Figure 3a). Some mitochondria, vacuoles, endoplasmic reticulum and lysosomes were observed (Figures 3a and 3b). Numerous goblet cells are found in the mucosa of stratified epithelium occupying the whole length of the oesophagus (Figure 3c). On the other hand, the mucosal cells in the oesophagus of *N. tessellata*, with an oval nucleus (Figure 4a). Lysosomes, vacuoles and numerous mitochondria were found scattering in the cytoplasm of the epithelial cells (Figure 4a and 4b). Goblet cells and microvilli were also observed (Figure 4c).

In *S. scincus*, stomach had a meshwork of primary folds by SEM

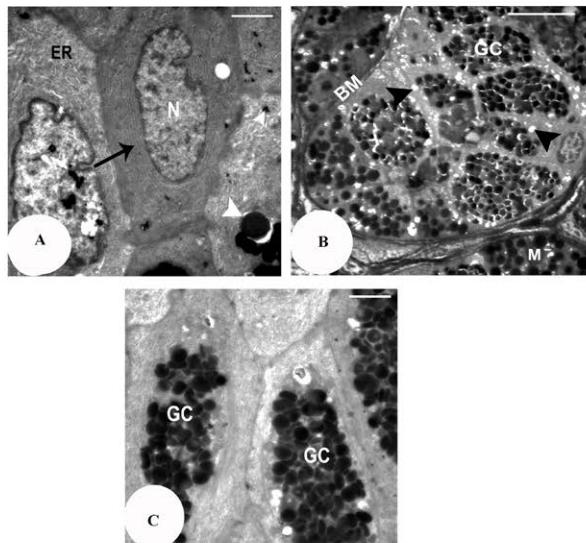


Figure 3: TEM micrograph of the oesophagus mucosal cells of *Scincus scincus*, showing (a) large nucleus (N) (arrow), endoplasmic reticulum (ER) and lysosomes (arrowhead). (Scale bar, 2 µm). (b) the basal membrane (BM), mitochondria (M) and vacuoles (arrowhead). (Scale bar, 10 µm). (c) numerous goblet cells (GC). (Scale bar, 2 µm).

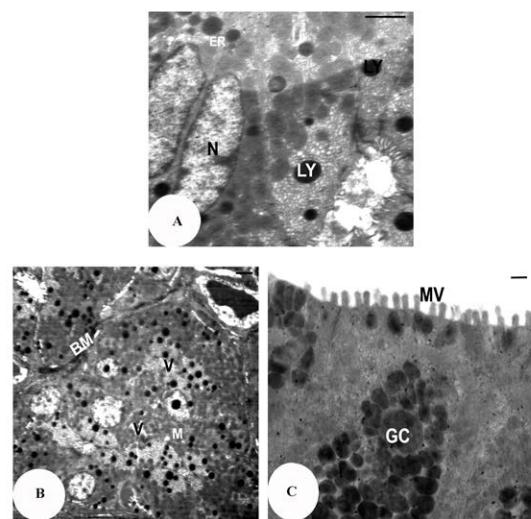


Figure 4: TEM micrograph of the oesophagus mucosal cells of *Natrix tessellata*, note, (a) nucleus (N) and lysosomes(LY). (Scale bar, 2 µm). (b) the basal lamina (BL), mitochondria (M) and vacuoles (V). (Scale bar, 500 nm). (c) microvilli (MV) and the goblet cells (GC). (Scale bar, 500 nm).

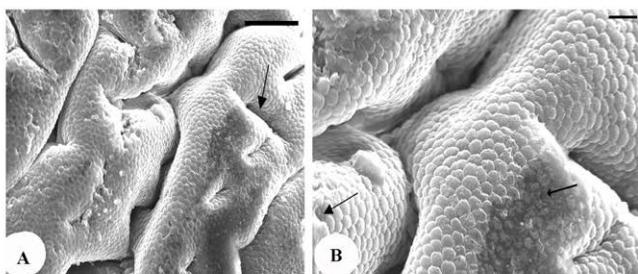


Figure 5: SEM micrograph of the stomach of *Scincus scincus*, showing (a) primary longitudinal folds and the luminal surface of epithelial cell represented by pentagonal elevations, Note many gastric crypts appeared (arrow) (Scale bar, 50 µm). (b) the presence of gastric pit (arrow). (Scale bar, 10 µm).

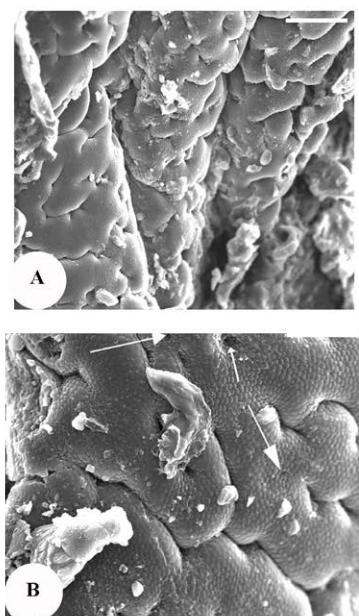


Figure 6: SEM micrograph of the stomach of *Natrix tessellata* showing (a) various folds and the luminal surface of epithelial cells with pentagonal and hexagonal elevations (Scale bar, 100 µm). (b) gastric pit (arrows) and crypts of the gastric glands. (Scale bar, 50 µm).

observation, and the luminal surface of columnar cells was pentagonal in shape and the gastric glands open into gastric crypts in the stomach (Figure 5a). Many gastric pits were also observed (Figure 5b).

In *N. tessellata*, under low magnifications of SEM, the mucosal surface of stomach was seen to form various folds forming many discrete but irregular pockets (Figure 6a). Under higher magnifications, it was found to be divided into pentagons and hexagons (Figure 6a and 6b). These correspond to the luminal surface of the epithelial cells. Occasional presence of prominent gastric pits surrounded by the epithelial cells was detected in this region (Figure 6b). Many gastric crypts were appeared in which gastric gland open (Figure 6b).

The mucosal cells of stomach of *S. scincus*, containing spherical secretory Granules that filled with homogenous material (Figure 7a). A euchromatinic nucleus, endoplasmic reticulum, vacuoles, lysosomes and many small mitochondria were also observed (Figure 7b and c). While in *N. tessellata*, the epithelium of the stomach has numerous

mucinogen granules (Figure 8a). The epithelial cells appeared with oval euchromatinic and irregular nucleus with a large nucleolus, vacuoles, numerous mitochondria and lysosomes were also seen (Figure 8b and 8c).

In *S. scincus*, Under scanning electron microscope, there was many primary folds of intestinal mucosa extends along the entire length of the intestinal tract and it observed projecting into the lumen without

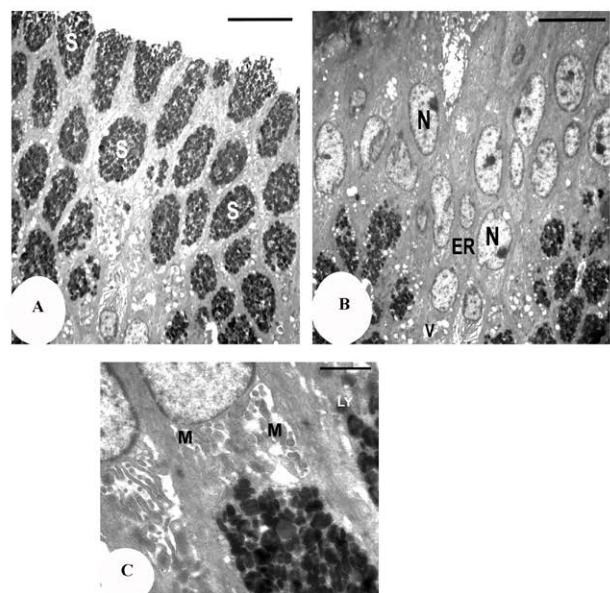


Figure 7: TEM micrograph of the stomach mucosal cells of *Scincus scincus*, showing (a) numerous electron dense secretory granules (S). (Scale bar, 10µm). (b) endoplasmic reticulum (ER), nucleus (N) and vacuoles (V). (Scale bar, 10 µm). (c) Golgi apparatus (GA), mitochondria (M) and lysosomes (LY). (Scale bar, 2 µm).

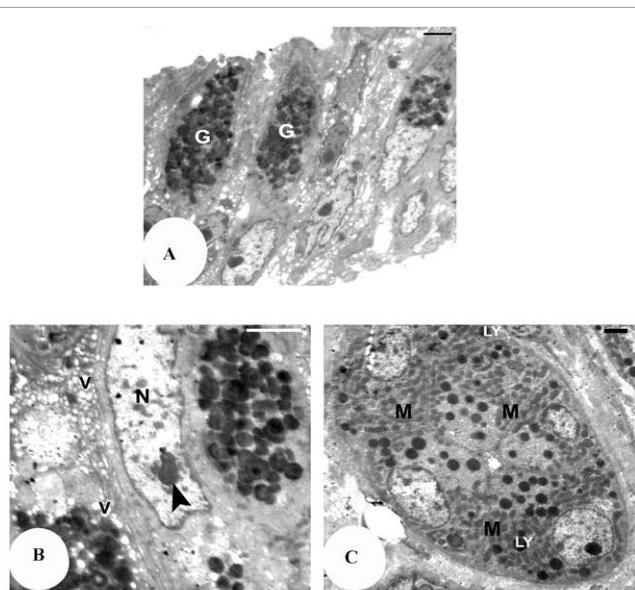


Figure 8: TEM micrograph of the stomach mucosal cells of *Natrix tessellata*, showing (a) numerous granules (G). (Scale bar, 2 µm). (b) vacuoles (V) and the basal position of the nucleus (N) with a prominent nucleolus (arrowhead). (Scale bar, 2 µm). (c) numerous mitochondria (M) and lysosomes (LY). (Scale bar, 2 µm).

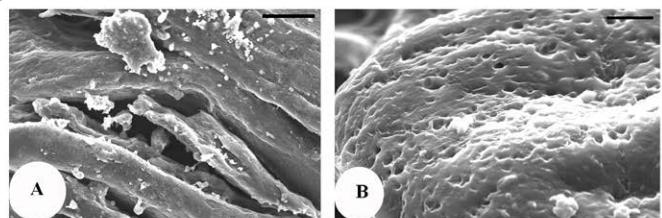


Figure 9: SEM micrograph of the intestine of *Scincus scincus*, showing (a) primary folds. (Scale bar, 100 μ m). (b) scattered goblet cells (Scale bar, 20 μ m).

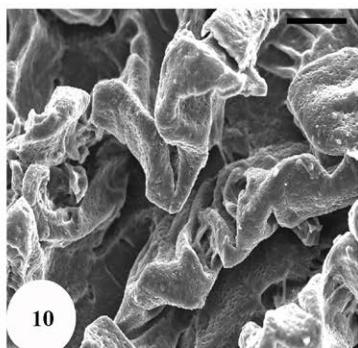


Figure 10: SEM micrograph of the intestine of *Natrix tessellata*, Note wavy longitudinal secondary mucosal folds. (Scale bar, 100 μ m).

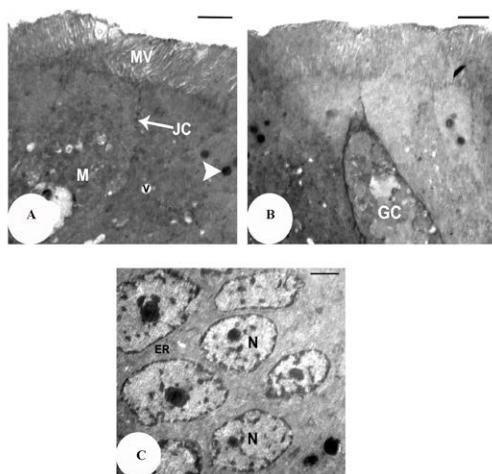


Figure 11: TEM micrograph of the intestine mucosal cells of *Scincus scincus*, showing (a) microvilli (MV), mitochondria (M) and lysosomes (arrow head). Note also, the junctional complexes (JC) situated just below the free surface. (Scale bar, 2 μ m). (b) goblet cell (GC). (Scale bar, 2 μ m). (c) Euchromatic nucleus (N). (Scale bar, 2 μ m).

following a definite pattern in their orientation and many goblet cells observed (Figure 9a and 9b). While the most characteristic feature of the intestine in *N. tessellata* is the presence of irregular, wavy longitudinal secondary mucosal folds in the fashion of Zig-Zag pattern enclosing a number of concavities in between them (Figure 10). Each species have many scattered goblet cells and lacks granular crypts.

At ultrastructural level, the columnar cells of the intestine of *S. scincus* have many mitochondria and microvilli toward the lumen and are joined together at the apical surface by junctional complexes. Lysosomes, vacuoles and mitochondria are scattered in the supranuclear cytoplasm of the enterocyte (Figure 11a). Goblet cells are seen near the luminal surface of intestinal epithelium (Figure 11b). A basal nucleus with a large nucleolus and rough endoplasmic reticulum are seen in the enterocytes (Figure 11c). The mucosal cells in the intestinal epithelium of *N. tessellata*, are consisted of columnar cells bearing microvilli and joined at the apical surface by the junctional complex, including the evident desmosomes, their cytoplasm contains numerous mitochondria and lysosomal bodies (Figure 12a). Goblet cells appeared elongated (Figure 12b). The nucleus and vacuoles are seen in the enterocytes (Figure 12c).

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 alimentary tract, for instance, the epithelial layers in the esophagus of turtles are keratinized in order to protect the mucosa from abrasive diets [5,11].

The results of the present work revealed some differences in the structure of the oesophagus of both species related to type of food and feeding habits of both species. The oesophageal mucosal surface in *S. scincus* was provided with many prominent longitudinal folds while in *N. tessellata*, the oesophageal surfaces have primary longitudinal folds of different sizes and many secondary ramification. This result agree with that observed in *Uromastyx aegyptius* and *Varanus niloticus*

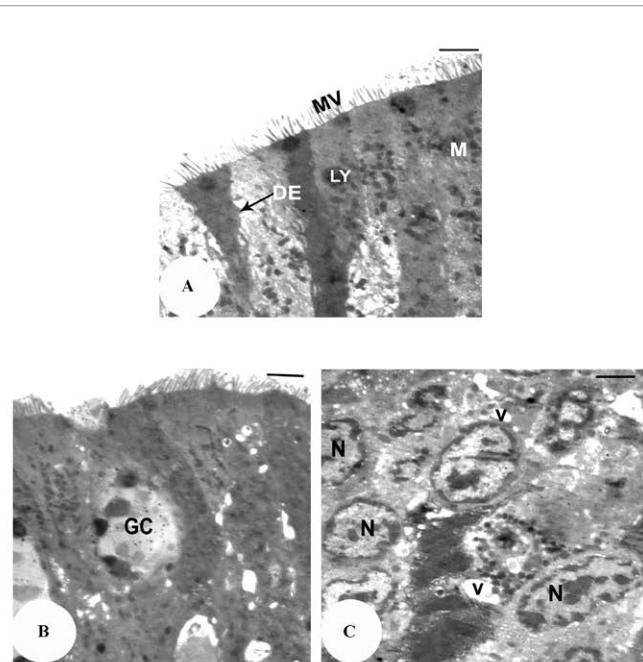


Figure 12: TEM micrograph of the intestine mucosal cells of *Natrix tessellata*, containing (a) microvilli (MV) covering the surface of the intestine in a regular manner, lysosomes (LY), desmosomes (DE) and abundant mitochondria (M). (Scale bar, 2 μ m). (b) goblet cell (GC). (Scale bar, 2 μ m). (c) vacuoles (V) and irregular shape of nucleus (N). (Scale bar, 2 μ m). (arrow head). Note also, the junctional complexes (JC) situated just below the free surface. (Scale bar, 2 μ m). (b) goblet cell (GC). (Scale bar, 2 μ m). (c) Euchromatic nucleus (N). (Scale bar, 2 μ m).

[6,12]. This feature is common for the reptiles, *Uromastyx philibyi* [13] and *Pristurus rupestris* [14]. The esophagus of the crocodile *C. crocodilus yacare* [15] and *C. latirostris* [16] has longitudinal folds in the esophageal mucosa.

The esophagus lumen of the cranial portion in the Japanese lizard has no fold. However, the middle and caudal portions are formed of very complicated folds [17]. These folds are totally distensible, to enable expansion of the esophagus, allowing it to store food that is ingested [15].

In catfish, the anterior part of the oesophagus was characterized by the presence of numerous mucosal folds that may allow maximal distension for prey and broken down food, the oesophagus of carnivorous fish acted as a constitutive adaptation that protected the oesophagus against live prey damages [18].

Our results revealed that the oesophagus of both species possessed a high density of goblet cells. The presence of the goblet cells in the oesophageal mucosa of *Natrix tessellata* as well as of other reptiles, such as *Ablephorus pannonicus* [19], *Chamaeleon vulgaris* [20] and *U. philibyi* [13], 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. In *Uromastyx aegyptica*, the goblet cells of the esophagus are rich in acid mucopolysaccharides when compared to the small and large intestines [12].

The increased number of goblet cells in the oesophagus of all fish species in general was probably due to the absence of salivary glands, as the mucin excreted in the oesophagus and buccal cavity compensates the absence of salivary glands in fish [21].

In the present species *S. scincus*, The oesophageal mucosal epithelial surface contain many well-defined wavy microridges while in *N. tessellata* it provided with fingerprint-like microridge alternates in some region with microvilli. Microridges by virtue of their nature would seem to spread and hold mucus film secreted by the adjacent goblet cells [22]. Microridges have also been reported to be present on various epithelial surfaces, such as skin [23], gills [24], which are subject to mechanical stress. This would appear to be an advantage in having a scupled surface for absorbing impacts [22]. The superficial cell layer the oesophageal epithelium in catfish, provided with microvilli that indicates an adaptation to rapid ion absorption [25] while the presence of fingerprint-like microridges may represent a mechanical adaptation that would withstand the trauma resulting from ingesting bulky materials [26]. The fingerprint-like microridges observed on the esophageal surface cells in *N. tessellata* are similar to those reported in *Solea senegalensis* [27], in *Dentex dentex* [9], in *Seriola dumerilli* [28], in *Rita rita* [29], and in several other seawater and freshwater fishes [25]. These ridges have been thought to play an important role of protecting the buccal-oesophageal surface from trauma and providing an anchor to the mucus secreted from the goblet cells [30].

The oesophageal mucosal cells of the present *S. scincus*, showed the presence of oval-shaped nucleus, the perinuclear cytoplasm contained mitochondria, few rough endoplasmic reticulum, lysosomes, few electron light vesicles and numerous goblet cells are found in the mucosa of stratified epithelium occupying the whole length of the oesophagus. These observations are similarly met with in *Micropogonias furnieri* [31], in *Uromastyx aegyptius* [6] and in *Scincus scincus* [8]. While, the mucosal cells of oesophagus in *N. tessellata*, are columnar cells with

basal microvilli. The surface epithelial cells showed large nucleus, vacuoles, endoplasmic reticula, lysosomes and numerous mitochondria scattering in their cytoplasm. These cells are probably adapted for ingestion of small fishes and other small aquatic animals. The goblet cells which arranged in the oesophageal epithelia are important for rapid and consistent lubrication of food particles during swallowing [6,32]. 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 [5] such as *Varanus niloticus* which had columnar epithelia [33]. In lizards, it is formed of folds lined by ciliated columnar epithelium with goblet cells. Some snakes have mucous glands along their submucosa [5]. The esophageal mucosa of *Lacerta agilis* and *Ophisops elegans* is composed of ciliated columnar and goblet cells [34,11].

In some lizards such as *Tachysaurus rugosus*, *Tiliqua nigrolutea* [35] and *Acanthodactylus boskianus* [14] many esophageal glands have been observed although the esophagus of crocodilian *Caiman latirostris* does not have submucosal glands in submucosa, but only intraepithelial glands [16].

In the present study, as examined by SEM, the stomach of *S. scincus* had a meshwork of primary folds. The luminal surface of columnar cells was pentagonal and with many gastric pits. While in *N. tessellata*, the mucosal surface of stomach was form various folds forming many discrete but irregular pockets and a prominent gastric pits appeared. This observation agrees with that done on *Salmo gairdneri* [36], in *Mystus aor* [22], and in *Rhamdia quelen* [37]. In *Lates niloticus*, the complex foldings of stomach may probably provide for the extension of the stomach capacity during ingestion and increase the surface area during digestion [38]. The complex nature of folding in stomach wall would probably allow for stretching during food consumption and also increase surface area for digestive activity [22]. Grau et al. [28] found that the stomach of *Seriola dumerilli* displayed a large number of primary longitudinal folds which contained secondary folds.

But the cardiac surface of *O. angorae* is a meshwork of primary folds; the fundic and pyloric surfaces have many deep regular longitudinal folds with transverse secondary folds [39].

Our result in the present species, *N. tessellata*, showed that the gastric glands open into gastric crypts in the stomach. This result as that observed in many fishes that are bottom feeders [40], and they were also related to digestion time, and to carnivorous and voracious feeding [41]. The secretions of the gastric glands probably protect the mucosa from the acidic contents of the stomach as in *O. angorae* [39]. According to Martin and Blaber [42], gastric glands in *Ambassidae* were packed beneath the epithelial layer, but the epithelium did not have goblet cells.

The fine structure of gastric cells of *S. scincus*, showed that the apical part of cytoplasm contained variable numbers of secretory granules, vacuoles, lysosomes and many small mitochondria. Similar observations were recorded by Abo-Eleene, Biomy [6,8]. While the mucosal cells in the stomach of *N. tessellata*, contain mucinogen granules. These mucinogen granules are discharged from the columnar cells to the surface of the mucosa; they form a layer of adherent membrane protecting the mucosa which is similar to that found in the soft-shelled turtles that have similar food interest as *Varanus niloticus* [43]. The mucinogen granules of *Natrix tessellata*, were found also in fishes, amphibians and birds [44]. In the present study, the endoplasmic reticula, lysosomes and vacuoles of the epithelial columnar cells are nearly similar to those of some carnivorous fishes such as *Channa argus*, *Parasilurus asotus*, *Peltobagrus fulvidraco* [45] and in *Varanus niloticus* [6].

In the present study, SEM observation showed that the intestinal mucosa of *S. scincus* has primary folds along the entire length of the intestinal tract. While in *N. tessellata* it appeared as wavy longitudinal secondary mucosal folds in the form of Zig-Zag pattern. Similar observations were reported by Jacobshagen, (1937) [46] on studying intestine of crocodiles and snakes. Zigzag longitudinal folds of the intestinal surface of the present study the same as reported by Clarke and Witcomb [47]. A great amount of folds were observed in loop I of the intestine of *Trichomycterus brasiliensis*, increasing the surface area, and enhancing the absorptive activity, typical for carnivorous species [48]. In *Schizothorax curvifrons*, the large intestine of is thrown into irregularly arranged mucosal folds, minor mucosal folds and the concavity being totally absent [49]. The intestinal mucosa of the present species lacks granular crypts. This outcome is closely similar to that recorded in both lacertilian and ophidian species [19].

The columnar cells of the intestine of *S. scincus*, have many mitochondria and microvilli located toward the lumen and are joined together at the apical surface by junctional complexes. Lysosomes, vacuoles, endoplasmic reticulum and mitochondria were scattered in the supra-nuclear cytoplasm. These ultrastructural observations confirm the earlier findings of Goro-Takahata [50] in *Oryzias latipes*, Banan khojasteh et al. [51] in *Oncorhynchus mykiss*, Dai et al. [52] in *Monopterus albus* and Abo-Eleneen [6] in *Uromastyx aegyptius*.

The intestine of *S. scincus* contained goblet cells. These cells are similar to those described in fishes, amphibia, reptiles and mammals [53-55] and other reptiles [56,57]. The goblet cells contain mucusubstance mainly formed by carboxylated glycoconjugates [58].

At the ultrastructural level, the enterocytes of the epithelium of *N. tessellata*, showed regular microvilli to increase the surface area. The presence of microvilli and lysosomes in the apical region of the intestinal columnar epithelial cells play part in collection of the nutritive substances from the lumen and their intracellular digestion. The enterocytes are joined laterally near the free surface by junctional complexes. Similar observations were recorded by Caceci and Hrubec [59]. The goblet cells of the intestine of *N. tessellata* contained many mucusubstances and secrete more mucilage for efficient digestion. This finding is similar to that found in the rice field eel *Monopterus albus* [52], in other fish species [28,60-63] and in *Varanus niloticus* [6] and in Typhlopis Snake *Rhamphophlops Braminus* [7].

The presence of many mitochondria suggests that a high amount of energy is required for active transport. Similar observations were recorded by Arellano et al. and Alexandre et al. [64,65].

We can conclude from the present study that the ultrastructural differences in the alimentary tract among the two reptilian species examined exhibited important specific features reflecting the mode of life and feeding habits of these animals.

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