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ORIGINAL ARTICLE

COMPARATIVE HISTOLOGICAL STUDY OF THE ESOPHAGEAL STRUCTURE OF SQUIRREL (*SCIURUS ANOMALUS*) AND MONGOOSE (*HERPESTES EDWARDSII*)

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ABSTRACT : This study presents a histological comparison of the esophagus between squirrels and mongooses, illustrating herbivorous and carnivorous dietary adaptations, respectively. Histological sections were examined from both species to compare the tunica mucosa, submucosa, muscularis, and adventitia. Esophageal samples were collected from adult specimens that were stained with hematoxylin and eosin (H&E) and Masson's trichrome, then examined microscopically. Results revealed that squirrels exhibited a thick wall with non-keratinized epithelium and fully striated muscularis, reflecting a plant-based diet. In contrast, mongooses displayed regional variation in wall thickness and thinner mucosa in the upper third and a transition from striated to smooth muscle along the esophagus that suited their diet. Vascularization in the submucosa also differed significantly between species. These findings enhance the understanding of the relationship between esophageal histology and feeding ecology in these mammals and contribute to broader knowledge of the evolutionary patterns of the digestive system in response to dietary specialization.

Key words : Histological comparison, squirrels, mongooses, esophagus, non-keratinization, epithelium.

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INTRODUCTION

The mammalian esophagus plays a vital role in digestion, functioning as a conduit that coordinate the movement of ingested material from the mouth to the gastric chamber (Hyder, Reddy and Mukherjee, 2023; Stanforth *et al*, 2021). Its histological structure varies among species, reflecting evolutionary adaptations to different diets and feeding behaviors (Cai *et al*, 2022). Squirrels and mongooses serve as ideal models for studying such adaptations due to their contrasting diets and lifestyles. Squirrels are primarily herbivorous, feeding on pine cones or cedar cones and consuming abrasive plant materials such as nuts, seeds, and fibrous tissues (Menéndez *et al*, 2023; Honda and Saito, 2023; Kaur, Singla and Kalra, 2022; Tamura, Ito and Hayashi, 2021). In contrast, mongooses are carnivorous, feeding on a diverse array of prey including insects and small vertebrates (Hinton and Dunn, 2023; Khatoon *et al*, 2022; Hays, 2019). Comparative histological and histomorphometric analyses of squirrels have previously been conducted to investigate cellular and tissue-level differences between species. For example, a study by Kadhim and Khaleel (2021) compared chromaffin cells between the Squirrel and Hamster, highlighting species-specific histological features. This supports the relevance of interspecies comparisons in understanding adaptive morphological traits. Structural features of the mammalian esophagus exhibit marked interspecies variations, involving differences in the mucosal epithelial lining, the organization pattern of the muscularis mucosae and the structural configuration of the tunica muscularis. The differences are influenced by the chemical and physical characteristics of ingested materials and their physiological effects on digestive system. A similar pattern was noted in the European beaver, where (Martyniuk *et al*, 2023) described the histological layers of the esophagus. A comparison was offered of the esophagus in both species, centering on the tunica mucosa, submucosa, muscularis and adventitia.

By connecting these histological traits to feeding behavior, it aims to expand the understanding of structure-function relationships in the mammalian digestive system and contribute to comparative histological knowledge of dietary adaptations (Berentsen, Pitt and Sugihara, 2018).

MATERIALS AND METHODS

Sample collection and preparation

Adult specimens of squirrels (n = 5) and mongooses (n = 5) were ethically obtained from the Al-Gazal market in Baghdad, following the ethical protocols set by the institutional Ethics Committee for Scientific Research at the College of Science (Approval No. CSEC/0125/0003). Chloroform inhalation was employed to anesthetize both specimens prior to dissection that maintained tissue integrity for histological analysis. The esophagus was immediately dissected and divided into three regions: upper (proximal), middle and lower (distal) thirds for comprehensive analysis of regional variations (Mnati, Ahmed and Mutlak, 2022; Al-Nakeeb *et al*, 2019).

Histological processing

Tissue samples were preserved in 10% neutral-buffered formalin for a duration of 24 hours. Samples underwent progressive dehydration using graded ethanol concentrations (70%, 80%, 90%, 95% and 100%), followed by xylene clearing and paraffin embedding. Consecutive sections were prepared with a rotary microtome and placed on glass slides. For histological examination were stained with hematoxylin and eosin (H&E) and Masson's Trichrome.

Histomorphometric analysis

Microscopic examination was performed using a light microscope equipped with digital imaging capabilities.

Protocol established by (Mnati, Ahmed and Mutlak, 2022; El-Nahass, 2023). For each specimen, five random fields per section were analyzed at different magnifications (4×, 10× and 40×) to assess the following parameters:

1. Epithelial thickness (µm) including measurement of individual layers (basal, spinous, granular and lucent layers)
2. Muscularis mucosae thickness (µm) and organization
3. Submucosal thickness (µm) and vascular density (vessels per mm²)
4. Tunica muscularis measurements including:
   - Outer circular layer thickness (µm)
   - Outer longitudinal layer thickness (µm)
   - The ratio between circular and longitudinal layers
5. Tunica adventitia thickness (µm) and composition

All measurements were performed using calibrated digital image analysis software (ImageJ, National Institutes of Health, USA) following methodology described by Maidana *et al* (2020).

Table 1 : Comparison between Squirrel and Mongoose in the Tunics of Esophagus.

Type/ animal	Mean ±SE of Esophagus / um					
	Epithelial height 40x	Lamina propria 10x	Muscularis mucosa 10x	Submucosa 10x	Muscularis 4x	Serosa 10x
Squirrel	72.31 ±1.86 a	11.03 ±0.54 d	11.65 ±0.54 a	34.07 ±1.24 c	148.82 ±2.38 b	13.45 ±0.60 b
Mongoose	51.76 ±1.72 a	47.71 ±2.74 c	16.73 ±0.71 ab	127.92 ±0.95 a	338.05 ±10.68 a	33.25 ±0.71 b
T-test	6.49 **	5.902 **	3.27 **	16.75 **	22.51 **	5.66 **

Means having with the different letters in same row differed significantly. * ($P \leq 0.05$), ** ($P \leq 0.01$).
Averages that carry different letters differ significantly from each other, and averages that carry common letters do not differ significantly from each other. The highest average takes a, and so on. If you find an average that takes two letters, such as ab, this does not differ from a or b.

Statistical analysis

The Statistical Packages of Social Sciences -SPSS (SPSS, 2019) program was utilized to assess the influence of variable factors (type and anatomical region) on the studied parameters. T-test and Least significant difference-LSD was used to significant compare between means in this study.

RESULTS

Findings revealed that the esophagus of the squirrel exhibited a robust tubular wall, comprising the standard four tunicae: tunica mucosa, tunica submucosa, tunica muscularis, and tunica adventitia (Fig. 1). The The mucosal tunic was organized into three distinct layers: epithelial lining, lamina propria and the muscularis mucosa. The epithelium consists of a thick layer of non-keratinized stratified squamous cells consisting of a basal cell layer, a spinous cell layer, granulosum cell layer, and a leucidum cell layer, while the lamina propria contained sparse loose connective tissue populated with fibroblasts, and the muscularis mucosa composed of a dual-layered arrangement of smooth muscle fibers (Figs. 2, 3 and 4). The tunica submucosa was formed of a thin layer of loose

Fig. 1 : Histological section of the esophagus (squirrel) showing the mucosa (black arrow), submucosa (red arrow), muscularis (green arrow), and tunica adventitia (green arrow). Hematoxylin and eosin stain (H&E). 4.X

Fig. 2 : Histological section of the esophageal wall (squirrel) showing the lining cells of the tunica mucosa (black arrow), the muscularis mucosa (red arrow), the loose connective tissue of the submucosa (green arrow), the inner circular fibers of the tunica muscularis (I), the outer longitudinal fibers of the tunica muscularis (O), and the tunica adventitia (blue arrow). (H&E). 10x

connective tissue consisting of several fibroblasts (Fig. 4). Tunica muscularis was composed of two layers of skeletal muscle, the first a thick, circular, inner layer and the second a thin, longitudinal, outer layer (Fig. 4). The results showed that the tunica adventitia of the esophagus was a thick layer of loose connective tissue rich in blood vessels and nerves (Fig. 5). The mongoose esophagus featured a relatively thinner-walled tubular morphology, comprising the canonical four tunics: tunica mucosa, tunica submucosa, tunica muscularis and tunica adventitia (Fig. 6). The figures of the upper third of esophagus showed that the tunica mucosa consists of three layers: (1) the epithelial layer, (2) the lamina propria and (3) the

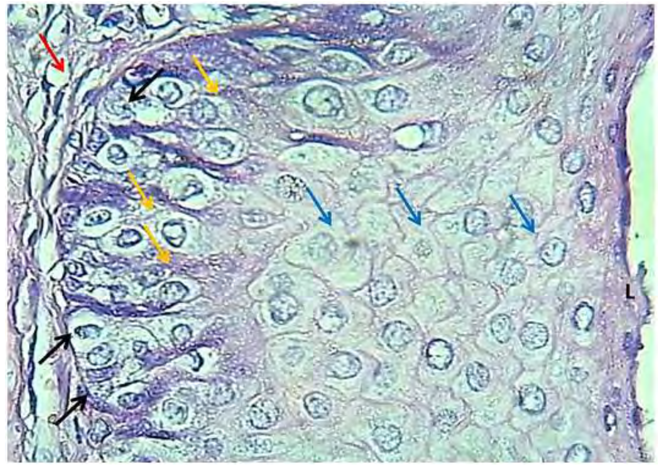

Fig. 3 : Histological section of the esophageal mucosa (squirrel) showing the lining cells of the tunica mucosa composed of a thin layer of basal cells (black arrow), a spinous cell layer (yellow arrow), a granular cell layer (blue arrow), a lucent cell layer (L) and the loose connective tissue of the lamina lata (red arrow). (Blue arrow). Hematoxylin and eosin stain. 40x

muscularis mucosa. The epithelium consisted of a narrow band of stratified squamous epithelium lacking keratinization consisting of a layered into basal, spinous, granular, and lucid components, the lamina propria consisted of a thin layer of dense collagenous connective tissue that rich with fibroblasts, and the muscularis mucosa consists of 2-3 layers of smooth muscle fibers (Fig. 8). The tunica submucosa was composed of thick layer of vascular loose connective tissue (Figs. 7 and 8). The muscular layer contained two distinct striated skeletal muscle bands: an inner robust circular layer and an outer delicate longitudinal one (Figs. 6 and 9).

The figures of the middle and lower thirds of the esophagus showed very thick wall and the tunica mucosa displayed many long simple mucosal folds (Fig. 10), the epithelium consisted of thick layer of non-keratinized stratified squamous cells, the lamina propria consisted of a thick layer of dense collagenous connective tissue that rich with fibroblasts and the muscularis mucosa consists of 6-8 layers of smooth muscle fibers (Fig. 10). Tunica muscularis was very thick that composed of two layers of smooth muscle, the first was very thick circular, inner layer, while the second was thinner outer longitudinal layer of smooth muscle (Fig. 6).

General Esophageal structure

Both squirrels and mongooses exhibited the typical four-layered structure of the mammalian esophagus: tunica mucosa, submucosa, tunica muscularis, and tunica adventitia described by (Uehara, Elmore and Szabo, 2018; Al-Taai, Nsaif and Almayahi, 2021). However, notable differences were observed in the relative development

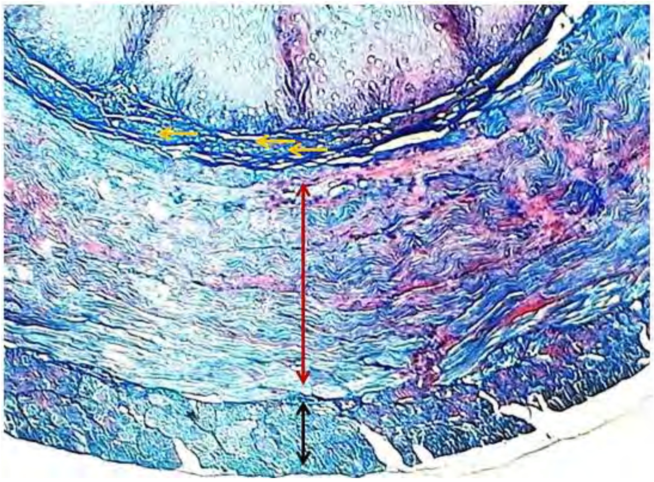

Fig. 4 : Section of esophagus (squirrel) shows collagen fibers of submucosa (yellow arrows), inner circular layer (red double arrow), outer longitudinal layer of tunica muscularis (black double arrow). Masson's trichrome stain. 4x

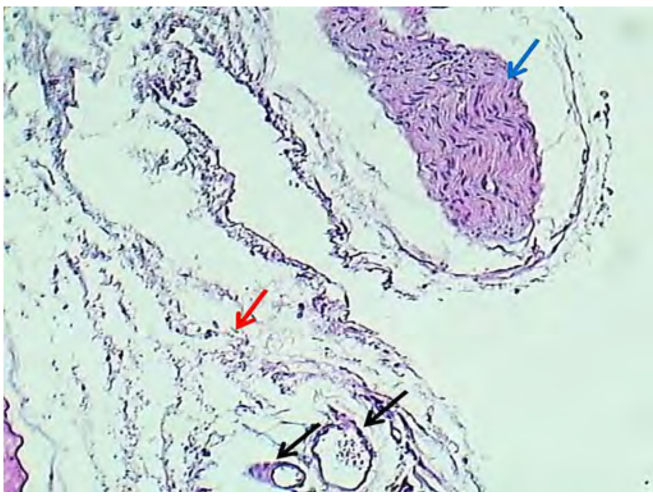

Fig. 5 : Histological section of the esophageal tunica (squirrel) showing the loose connective tissue (red arrow) rich in nerves (blue arrow) and blood vessels (black arrow). Hematoxylin and eosin stain. 10x

and composition of these layers between the two species, reflecting their distinct feeding adaptations.

Tunica Mucosa

The esophageal mucosa of both species was lined with stratified squamous epithelium, but with marked differences in structure. The esophagus of the Squirrel features a non-keratinized stratified squamous epithelium (Goodarzi *et al*, 2019).

which contain a thin basal cell layer, a well-developed spinous layer, a distinct granular layer and a superficial lucent layer (Fig. 3). The total epithelial thickness in squirrels (72.31 ±1.86 a µm) was significantly greater than in mongooses (51.76 ±1.72 a µm; p < 0.01).

In contrast, mongooses exhibited a thinner epithelial layer with less distinct stratification (Fig. 8). And it showed thin non-keratinization layers (The lamina propria in squirrels encompassed loose connective tissue with few elastic fibers. In mongooses, the lamina propria was featured by denser fibrous connective tissue (Fig. 8) with a higher density of elastic fibers, supporting findings by Al-Taai (2022) in the histological features of esophagus. The lamina propria in mongooses was (47.71 ±2.74 c µm), while in squirrels was (11.03 ±0.54 d µm; p < 0.01) (Al-Taai, 2022). The muscularis mucosae in squirrels was noted as a thin, often discontinuous layer of smooth muscle fibers. Unlike the mongooses exhibited a more sturdy muscularis mucosae consisting of 2-3 layers of smooth muscle cells (Figure 8), consistent with observations by Abood *et al* (2023), who stated similar structures in wild mongooses. Muscularis mucosae thickness also differed between species, which mongooses recorded a thicker muscularis mucosae layer (16.73 ± 0.71 a µm) compared to squirrels (11.65 ± 0.54 c µm; p < 0.05). The examined esophagus appears common histological features to those of other mammals, and comparable to those reported in other mammals such as guinea pigs (Al Shreefy and Al Taai, 2024).

Submucosa

The submucosa consisted of loose connective tissue including blood vessels, lymphatics and nerve fibers in both species. However, significant differences were observed in vascular density and organization. Squirrels exhibited a submucosa rich in nerves and blood vessels (Fig. 5) consistent with observations in other mammals such as rabbits (Ranjan and Das, 2018). Submucosal thickness also differed between species, with mongooses showing a thicker submucosal layer (127.92 ±0.95 a µm) compared to squirrels (34.07 ±1.24 c µm; p < 0.01). Additionally, the submucosal connective tissue in mongooses appeared more vascularized throughout all esophageal regions, particularly in the middle portion (Fig. 10), aligning with observations by de Aro *et al* (2019) also noted in other mammalian species such as the rocky cavy (*Kerodon rupestris*).

Tunica Muscularis

The tunica muscularis in both species consisted of inner circular and outer longitudinal smooth muscle layers. In the mongoose, the Muscularis (338.05 ±10.68 a µm) was approximately thicker than in squirrels (148.82 ±2.38 b µm P≤0.01).

Regional variations were also observed in the muscularis organization. In the upper third of the mongoose esophagus, the inner circular fibers were more prominent (Fig. 9), while in the lower third, both layers showed more balanced development.

Similar structural organization of the muscularis

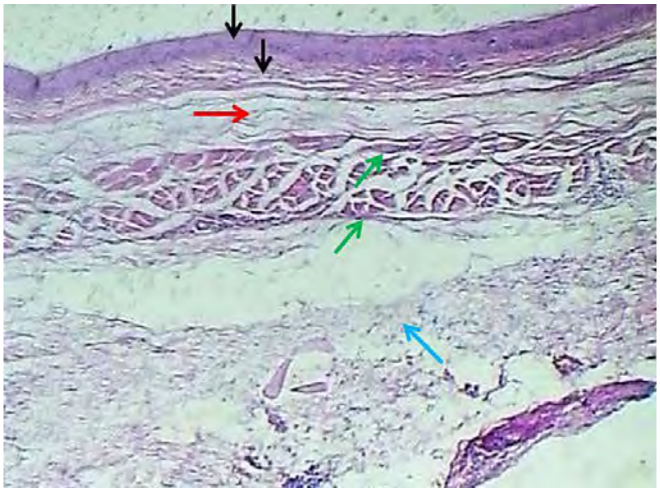

Fig. 6 : Histological section of esophagus-upper third (mongoose) show; tunica mucosa (black arrows), submucosa (Red arrow), muscularis (Green arrow) & adventitia (Blue arrow).4x

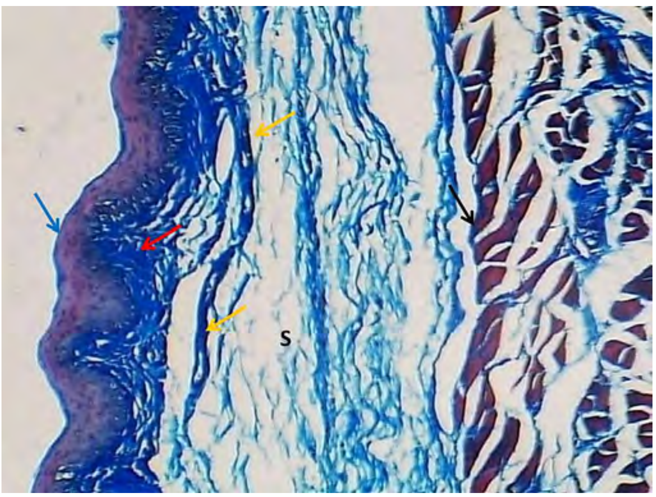

Fig. 7 : Histological section of the esophagus-upper third (mongoose) shows; mucosal lining cells (blue arrow), fibrous tissue of lamina propria (red arrow), muscularis mucosa (yellow arrows), submucosal loose connective tissue (S) and skeletal muscle of muscularis (black arrow). Masson's Trichrome stain. 10x

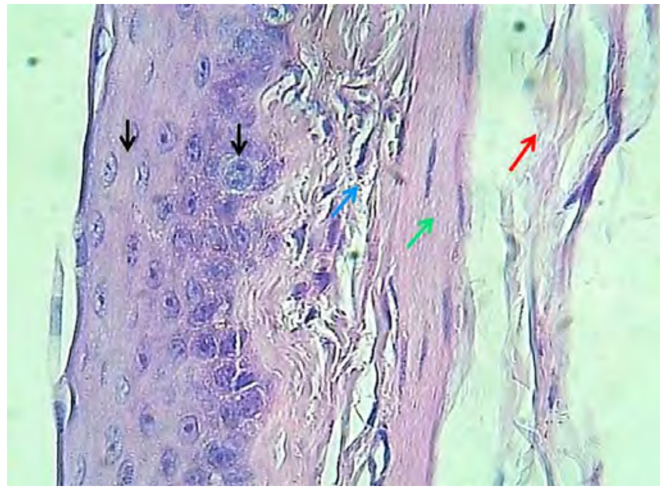

Fig. 8 : Histological section of the esophagus-upper third (mongoose) shows; thin stratified squamous cells (black arrows), dense fibrous tissue of lamina propria (Blue arrow), 2-3 layers of smooth muscle of muscularis mucosa (Green arrow) & submucosal vascular loses connective tissue (Red arrow). 40x

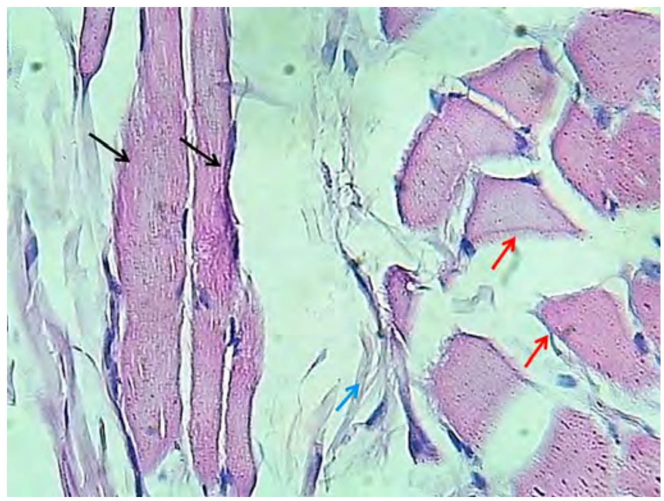

Fig. 9 : Histological section of the esophagus-upper third (mongoose) shows; inner circular fibers (black arrows), outer longitudinal fibers of tunica muscularis (Red arrows) & fibrous tissue of perimysium (Blue arrow). 40x

mucosa in the esophagus has also been described in carnivorous mammals such as dogs (McKnight, Diehl and Bergin, 2024).

The transition between skeletal and smooth muscle fibers in the upper esophagus occurred more proximally in mongooses compared to squirrels. Additionally, the perimysium (connective tissue separating muscle bundles) was more developed in mongooses (Fig. 8), containing more abundant elastic fibers, as in their comparative myology of the mammalian esophagus.

Tunica Adventitia\ serosa

The tunica adventitia in both species comprised of loose connective tissue with variable amounts of elastic fibers, adipose tissue, blood vessels and nerve fibers (Barbero, Teta and Cassini, 2021). Squirrel was characterized by a relatively reduced- thickness adventitial layer (13.45 ±0.60 b µm) comparison with mongooses (33.25 ±0.71 b µm; $p < 0.05$) and the adventitia made up more abundant elastic fibers and seen greater vascularity, in particular the middle and lower regions in esophagus.

DISCUSSION

Epithelial adaptations

The shown variations in epithelial structure between squirrels and mongooses signify their divergent feeding ecologies behavior (Goodarzi *et al*, 2019) indicated that the thicker, non-keratinized epithelium in squirrels which represents an acclimation to a diet containing abrasive plant materials, providing protection against mechanical harm during the passage of food these findings also

Fig. 10 : Histological section of esophagus-middle part (mongoose) show; mucosal folds (black arrows), submucosa (Red arrow), muscularis (Blue arrows) & adventitia (green arrow). 4x

mentioned by (Paksuz and Paksuz, 2021) in their study about the esophagus in bats (*Myotis myotis*), contrary to mongoose the thinner non-keratinization epithelium in mongooses is consistent to their carnivorous diet, which typically involves less coarse food objects (Al-Taai, 2022).

Muscularis Development and Functional implications

The differences in muscularis organization between the two species have important functional implications (Kilarski, 2019). The relatively thicker inner circular layer observed in squirrels may facilitate more controlled propulsion of fibrous plant materials through the esophageal lumen. Conversely, the more developed outer longitudinal layer in mongooses, particularly in the lower esophagus, likely enables more rapid transit of animal prey, consistent with the carnivorous feeding strategy, which has been observed in carnivorous mammals such as dogs (McKnight, Diehl and Bergin, 2024). The more proximal transition from skeletal to smooth muscle in mongoose esophagus suggests adaptation for faster food bolus progression, such muscular adaptations correlate with biomechanical properties that optimize esophageal function for specific dietary requirements. This proximal transition from skeletal to smooth muscle within the esophagus may reflect biomechanical adaptations that enhance the efficiency of bolus propulsion, which is consistent with the findings of Lang *et al* (2023) regarding the role of muscle coordination in the swallowing function. The well-developed muscularis mucosae in mongooses, consisting of 2-3 layers of smooth muscle cells, likely provides enhanced mucosal mobility and potentially contributes to localized contractions that aid in the passage of meat boluses. The thinner, sometimes discontinuous muscularis mucosae in squirrels may reflect less need for such localized control due to the more homogeneous nature of their plant-based diet. The examined esophagus shows great histological similarities to those of other mammals, such as those reported by Kuloðlu, Tuncer and Ýlgün (2024) example in carnivores and Al Shreefy (2024) in herbivores.

Vascularization patterns and Regional specializations

The higher vascular density observed in the mongoose submucosa may be associated with increased metabolic requirements for processing protein-rich diets. Gerussi *et al* (2024 and Freund *et al* (2022) proposed that enhanced vascularity in carnivore digestive tracts facilitates more efficient nutrient absorption and transport. Additionally, the more vascularized submucosa may support more active secretory functions. Regional specializations, particularly the prominent mucosal folds observed in the middle portion of the mongoose esophagus (Fig. 5), likely represent adaptations that increase surface area and enhance functional flexibility during the passage of heterogeneous food materials. Similar regional adaptations were reported by Patole *et al* (2025) in their study.

Evolutionary and Ecological Perspectives

From an evolutionary context, the denoted histological differences between squirrels and mongooses indicate how digestive tract structure is shaped by nutritional ecology. Martyniuk *et al* (2023) proposed that the evolution of histology in esophagus tightly parallels dietary differentiation across mammalian lineages. The epithelial non-keratinization in squirrels was signified by adaptation close to that seen in other herbivorous and omnivorous mammals as Massoud *et al* (2023), Mahdy and Mohammed (2024) also explained in their studies of esophagus, reflecting evolutionary responses to similar selective constraints. Similarly, the musculars aspects in mongooses align with patterns found in other carnivores, implying common functional requirements despite diverse phylogenetic origins (Metz *et al*, 2024). Environmental causes may also influence these adaptations. Wu (2022) stated that small carnivores have evolved digestive systems that can rapidly process protein-rich diets, extracting necessary nutrients from their food with efficiency. Barbero, Teta and Cassini (2021) revealed that dietary changes in response to seasonal resource availability which induce plasticity in digestive system histology. The histological features illustrated in this study represent adaptations that optimize digestive efficiency within the ecological niches occupied by these species. Information about food ecology plays an important role in a wide range of decisions related to biological research and environmental conservation (Llobat *et al*, 2024).

CONCLUSION

This comparative histological analysis reveals slight but remarkable architectural variations between the squirrels and mongooses esophagus, which are attributed distinct structural adaptations that signify their dissimilar dietary habits and environmental niches. Squirrels are adapted for consuming more abrasive plant material and the squirrel esophagus showed a consistently thick-walled morphology with prominent non-keratinized stratified squamous epithelium and an entirely striated muscularis tunica, referring enhanced voluntary control and protect against abrasive plant materials. On the other hand, the mongoose esophagus exhibited regional variety, with a thinner upper wall and gradual thickening towards the distal end, coupled with a transition from striated to smooth muscle fibers an adaptation facilitating rapid propulsion of softer carnivorous. These observations highlight the relationship between diet and tissue structure, and also enhance our knowledge of adaptive changes in the digestive system. The histomorphological observations presented contribute valuable baseline data for future comparative anatomy and evolutionary biology research.

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Data availability : The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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