

Identification of enteroendocrine cells of *Tropidurus torquatus* and *Salvator merianae* (Squamata: Lacertilia)

Fernanda Barbosa Lopes, Maria Luiza Assis Rodrigues, Carlos Henrique de Souza Silva, Sirlene Souza Rodrigues Sartori

Department of Animal Biology, Federal University of Viçosa, Viçosa, 36570-900, 7 Minas Gerais, Brazil.

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ABSTRACT

The Squamata lizards *Tropidurus torquatus* and *Salvator merianae* are omnivores with different diets and foraging strategies, which requires adequate physiological responses of enteroendocrine cells, since they control digestive events, gastrointestinal epithelium renewal, metabolism and food intake. Thus, we aim to describe the distribution and frequency of enteroendocrine cells in the digestive tract of these two lizard's species. Five individuals of each species were collected, euthanized and the digestive organs removed. Histological sections were submitted to the techniques of Grimelius for argyrophilic cells and Masson-Fontana for argentaffin cells. Both endocrine cells were found in the lining and glandular epithelium, with a pyramidal, oval, rounded, or elongated shape. Argyrophilic cells were observed in both species, predominantly in the stomach, but absent in the cranial esophagus. Argentaffin cells were distributed in almost the entire digestive tract but absent in the cranial portion of the esophagus and small intestine in both species, and with high frequency in the *T. torquatus* pyloric region. The absence of argentaffin cells in the cranial portion of the small intestine is intriguing and may reveal greater neural control over certain secretomotor functions involving this segment. We concluded that the inter-specific and inter-regional variations in the distribution and frequency of these cells may reflect the regulatory functions and mechanisms of each organ, taking also into account the oscillations of the food and nutritional status of animals in the wild.

Key Words: Diet; Argentaffin cells; Argyrophilic cells; Histology; Morpho-physiology; Reptiles.

RESUMO

Os lagartos Squamata *Tropidurus torquatus* e *Salvator merianae* são onívoros com diferentes dietas e estratégias de forrageamento, o que requer respostas fisiológicas adequadas de células enteroendócrinas, uma vez que estas controlam os eventos digestivos, a renovação do epitélio gastrointestinal, o metabolismo e a ingestão alimentar. Portanto, pretendemos descrever a distribuição e frequência de células enteroendócrinas no trato digestivo destas duas espécies de lagartos. Foram coletados cinco indivíduos de cada espécie, eutanasiados e os órgãos digestivos removidos. Seções histológicas foram submetidas às técnicas de Grimelius para células argirófilas e Masson-Fontana para células argentafins. Ambas as células endócrinas foram encontradas no epitélio de revestimento e glandular, com formatos piramidal, oval, arredondado ou alongado. Células argirófilas foram observadas em ambas as espécies, predominantemente no estômago, mas ausentes no esôfago craniano. As células argentafins estiveram distribuídas em quase todo o trato digestivo, mas ausentes na porção cranial do esôfago e intestino delgado em ambas as espécies, e com alta frequência na região pilórica de *T. torquatus*. A predominância das células endócrinas no estômago pode estar relacionada, por exemplo, à maior permanência do alimento neste órgão, ao contrário do esôfago. A ausência de células argentafins na porção cranial do intestino delgado é intrigante e pode revelar maior controle neural sobre certas funções secretomotoras envolvendo este segmento. Nós concluímos que as variações inter-específicas e inter-regionais na distribuição e frequência destas células podem refletir as funções e mecanismos regulatórios de cada órgão, levando-se também em conta as oscilações do estado alimentar e nutricional dos animais na natureza.

Palavras-chave: Dieta; Células argentafins; Células argirófilas; Histologia, Morfofisiologia; Répteis.

Introduction

Enteroendocrine cells constitute a small proportion of the digestive tract epithelium in all vertebrates,

however their functions are essential for digestive physiology and organism's homeostasis (Dockray,

2006; Rodrigues *et al.*, 2005). These cells release peptides or amines to regulate the secretion, absorption, motility and proliferation of digestive tract epithelial cells (Rindi *et al.*, 2004; Gribble and Reimann, 2019). In addition, they are involved with the regulation of appetite and consequent food intake, regulation of hepatic and pancreatic secretions, glucose concentrations and energy metabolism (Gutierrez-Aguilar and Woods, 2011; Moran-Ramos *et al.*, 2012). Enteroendocrine cells can be evidenced by histochemical techniques using silver salts, and are classified into argentaffins, which absorb and reduce silver salts; and argyrophilic, which only absorb silver salts but do not reduce it, requiring an exogenous substance with a reducing capacity (Polak *et al.*, 1993). Enteroendocrine cells have already been identified in all vertebrates, from fish, amphibians and reptiles to birds and mammals (Ku *et al.*, 2000; Lee and Ku, 2004; Santos *et al.*, 2013; Lin *et al.*, 2016; Mazzoni *et al.*, 2018), and in invertebrate taxa some gut cells resemble those that produce gastrointestinal peptides in vertebrates, such as in insects, echinoderms and protochordates (Dimaline and Dockray, 1994; Neves *et al.*, 2003; García-Arrarás *et al.*, 2019). In the primitive group cnidaria regulatory peptides are secreted only by neurons, and the peptide production in endocrine cells probably may have derived from these neuropeptides (Dimaline and Dockray, 1994).

Reptiles are an animal model for studies of the gastrointestinal tract because they have intestinal morphological flexibility, ingest large prey, and have exacerbated physiological and structural responses to ingestion, such as increased intestinal mass, metabolic rate, and nutrient transport rates (Holmberg *et al.*, 2003). For this reason, they are useful for studies on the regulation of digestive processes and the evolution of regulatory mechanisms, including those carried out by enteroendocrine cells (Secor and Diamond, 1998; Starck and Beese, 2002).

Squamata are one of the most diverse groups of terrestrial vertebrates, including lizards, snakes, and amphisbaenians (Pyron *et al.*, 2013). The *Tropidurus torquatus* (Wied-Neuwied, 1820) is an omnivorous, generalist and opportunistic lizard with “sit-and-wait” eating strategies (Bergallo and Rocha, 1994; Carvalho *et al.*, 2007). The tegu *Salvator merianae* (Duméril and Bibron, 1839) is also an omnivorous lizard, but it is an active forager, locating its prey with the aid of its long and bifid tongue (Vitt, 1995). It consumes everything ranging from vertebrates, mollusks, and arthropods to decomposing animals,

in addition to vegetables, being important seed dispersers (Castro and Galetti, 2004; Sazima and D'Angelo, 2013). The objective of this study was to describe and compare the distribution and frequency of argyrophilic and argentaffins enteroendocrine cells in *T. torquatus* and *S. merianae* in order to improve knowledge about the digestive physiology of these species, which are omnivorous but differ in diets and feeding strategies.

Materials and methods

Study area and capture of animals

Five individuals, among males and females, of each species studied *T. torquatus* and *S. merianae*, were captured in the municipalities of Viçosa and Guiricema, which are part of the Zona da Mata in the State of Minas Gerais, Brazil (IBAMA: 10504-1). After a period of fasting, the animals were euthanized with pentobarbital overdose (120 mg/kg) injected intraperitoneally, and then measured with a tape measure and weighed on a digital scale. The peritoneal cavity was exposed by a median longitudinal incision in the ventral region and the digestive tract was removed and measured with a tape measure, collecting fragments of the esophagus (cranial and caudal), esophagus-gastric transition, stomach (fundic and pyloric), gastro-intestinal transition, small intestine (cranial, middle and caudal), transition between intestines, and large intestine (colon and rectum). The study was authorized by the Ethics Committee for the use of animals (CEUA, protocol 27/2016).

Histological and histochemical analyzes

The organs fragments were washed in saline solution and fixed in Carson's formalin for 24 hours (Carson *et al.*, 1973) and dehydrated in an increasing series of ethyl alcohol, embedded in histological paraffin (paraplast) and sectioned with a manual rotating microtome (Olympus America Inc. CUT 4055), obtaining sections 5 µm thick. These sections were submitted to silver impregnation: Grimelius for argyrophilic cells (Grimelius and Wilander, 1980) and Masson-Fontana (Barbosa *et al.*, 1984) for argentaffin cells; as positive control, histological sections of monkey's stomach and duodenum, previously tested, were used.

Morphometric analyzes

Argyrophilic and argentaffin cells were quantified using an optical microscope (Nikon E100 LED)

with 40X objective lens and 10X eyepiece lens. Ten random areas of the mucosa of four histological sections (20 μm spacing between sections), for each segment of the digestive tube of each animal ($n=10 \times 4 \times 5=200$) were analyzed. This quantification was represented with a frequency scale: absent; rare, 1 to 2 cells / area; few, 3 to 4 cells / area; many, 5 to 8 cells / area; and elevated, 9 or more cells / area. The area was calculated using the formula: $A=\pi \times r^2$, with $r=0.25$ mm (given the characteristics of the objective and ocular lenses), obtaining the value of 0.2 mm². The photographic record of the histological sections was performed using an optical microscope (Olympus BX60) coupled with a digital camera (QColor3 DP73 Olympus).

Results

The rostro-cloacal length and the weight of *T. torquatus* and *S. merianae* were 9.7 cm and 110.43 g and 26.8 cm and 441.75 g, respectively. The digestive tube of *T. torquatus* (Fig. 1) measured an average of 9.6 cm in length, representing 99% of its body length; and that of *S. merianae* (Fig. 2) measured 30.8 cm in average length, which represents 115% of its body length.

The digestive tract was composed of the four typical layers: mucosa with lining epithelium,

lamina propria and muscularis mucosae; submucosa (without glands); muscular with its circular and longitudinal sublayers; and serosa. The lining epithelium was varied, prismatic pseudo-stratified, with ciliary cells, goblet cells and basal cells in the esophagus; simple prismatic in the stomach; simple prismatic with goblet cells and striated border cells in the small and large intestines. Mucosa's glands were registered in the caudal esophagus and in different gastric regions, even though they were absent in the small intestine and were scarce in the large intestine. Enteroendocrine cells, when present, were located diffusely through the lining and / or glandular epithelium, with a rounded or oval shape, characteristic of closed type cells, and pyramidal or spindle shape, characteristic of closed type cells; in addition to an uncolored nucleus and cytoplasm marked in brown or black, especially around or below the nucleus (Figs. 3 to 6). The frequency of argyrophilic and argentaffin enteroendocrine cells in the different 159 regions analyzed, for both species, is shown in Figs. 7 and 8.

Although argyrophilic cells were not found in the *T. torquatus* esophagus, few were present in their esophageal-gastric transition (Figs. 3a and 7a), and rare were found in the esophageal glands of *S. merianae* (Figs. 3b and 7b). Rare argentaffin cells have been identified in the caudal esophageal epithelium

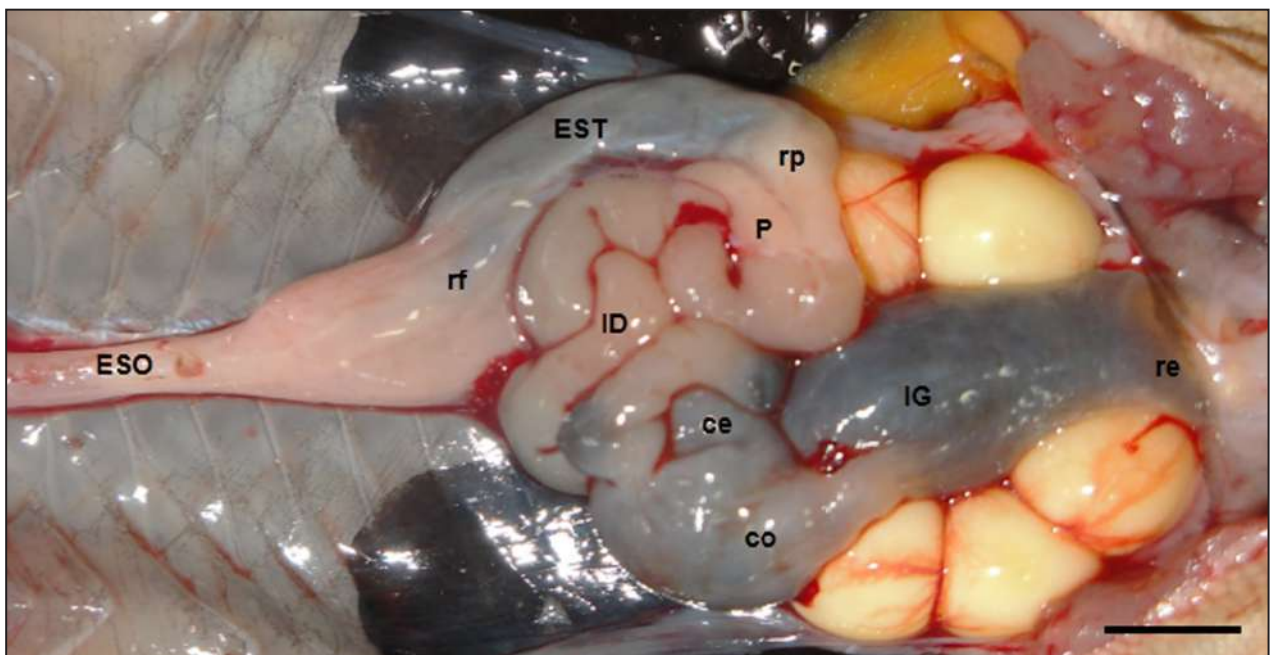


Figure 1. Digestive tube of *Tropidurus torquatus* with indication of its segments: esophagus (ESO), stomach (EST) with the fundic (rf) and pyloric (rp) regions, small intestine (ID), large intestine (IG) with cecum (ce), colon (co) and rectum (re), and pancreas (P). Bar=5 mm.

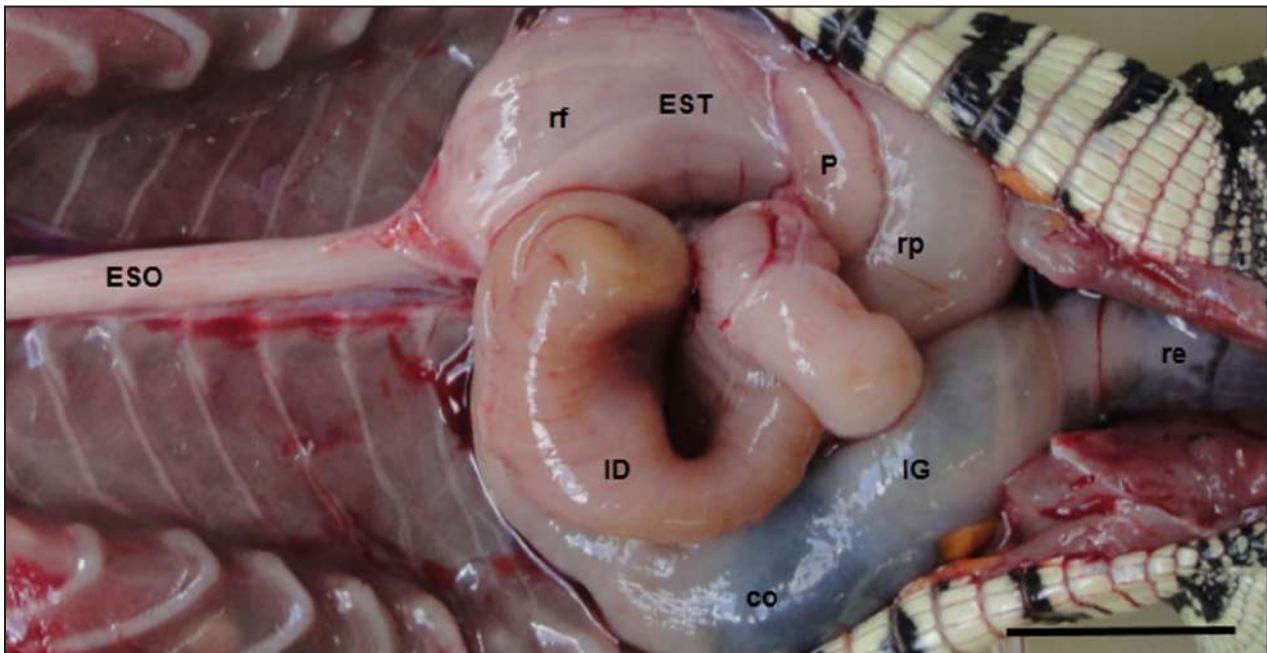


Figure 2. Digestive tube of *Salvator merianae* with indication of its segments: esophagus (ESO), stomach (EST) with the fundic (rf) and pyloric (rp) regions, small intestine (ID), large intestine (IG) with colon (co) and rectum (re), and pancreas (P). Bar=20 mm.

of both species (Figs. 4c and d, 8a and b), however many were seen in the esophageal-gastric transition in *T. torquatus* (Fig. 8a).

Argyrophilic cells were observed in the different regions of the stomach, on the surface, in the pits and especially in the glands of both species (Fig. 4a to d), with variable frequency: in *T. torquatus* there were many in the entire stomach (Fig. 7a); in *S. merianae*, many were found only in the pyloric region, being rare in the fundic region (Fig. 7b). Likewise, argentaffin cells were identified in the lining and glandular epithelium of both species (Fig. 4e to h) with variable frequency: in *T. torquatus* they were few in the fundic region and elevated in the pyloric (Fig. 8a); while in *S. merianae* they were few in the fundic and rare in the pyloric region (Fig. 8b). In the gastro-intestinal transition there were few argyrophilic cells and rare argentaffin cells in both species (Figs. 5a and b, 8a and b).

Rare argyrophilic cells were found in the small intestine of *T. torquatus* and *S. merianae* (Figs. 5c and d, 7a and b). Rare argentaffin cells were also observed in the small intestine of these species, except for the cranial segment in which they were absent (Figs. 5e and f, 8a and b). In the transition between the intestines, argyrophilic cells were many in *T. torquatus* and few in *S. merianae*, although rare in the large intestine of these species (Figs. 6a and b, 7a and b). Argentaffin cells showed the same pattern in the

transition and in the large intestine, they were rare in *T. torquatus* and few in *S. merianae* (Figs. 6c to f, 8a and b), and some had an inverted morphological configuration, with granules concentrated in the supra-nuclear region (Fig. 6c).

Discussion

The enteroendocrine cells found in the digestive tract of *Tropidurus torquatus* and *Salvator merianae* presented different formats, which allows categorizing them in “closed type” or “open type”. The first one, with a rounded to oval shape, does not communicate with the lumen; and the second one, pyramidal to spindle shape, presents an apical extension communicating with the lumen, although this extension cannot always be seen in histological sections (Latorre *et al.*, 2016). “Closed type” cells are believed to respond to mechanical distension or humoral stimulation, while “open type” cells detect changes in luminal pH or content composition (Ceccarelli *et al.*, 1995; Solcia *et al.*, 2000; Gribble and Reimann, 2016).

The *T. torquatus* and *S. merianae* enteroendocrine cells were argyrophilic and / or argentaffins, with variable distribution and frequency in the different organs of the digestive tract. According to Grimelius and Wilander (1980), all enteroendocrine cells are argyrophilic, except for cholecystokinin

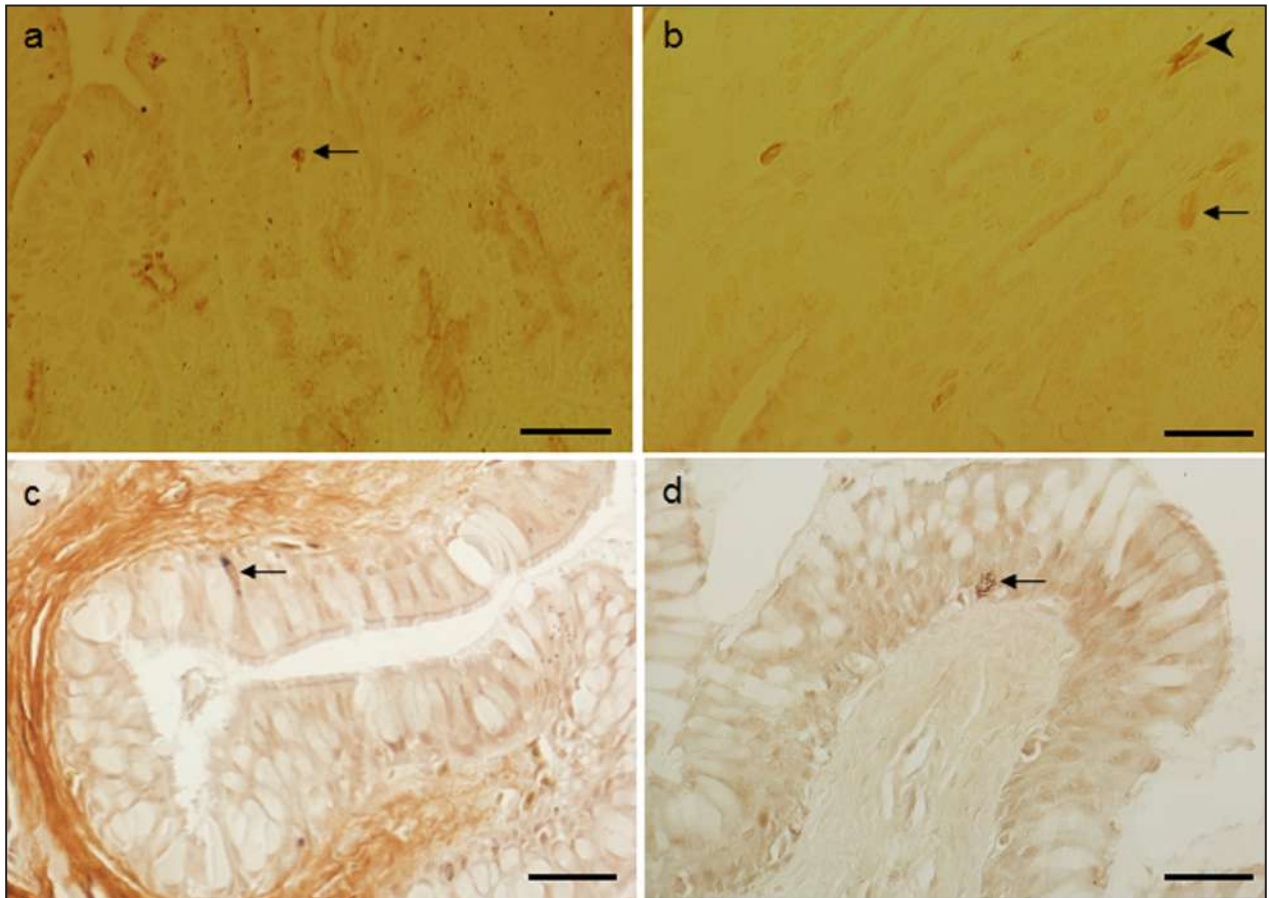


Figure 3. Histological sections of the esophagus of *Tropidurus Torquatus* and *Salvator merianae*, with arrows indicating endocrine cells: argyrophilic (a and b; Grimelius technique) and argentaffin (c and d; Masson-Fontana technique). Esophagus-gastric transition (a) and caudal esophagus (c) of *T. torquatus*. Caudal esophagus of *S. merianae* (b, d). Arrowhead: red blood cell. Bar= 20 μ m.

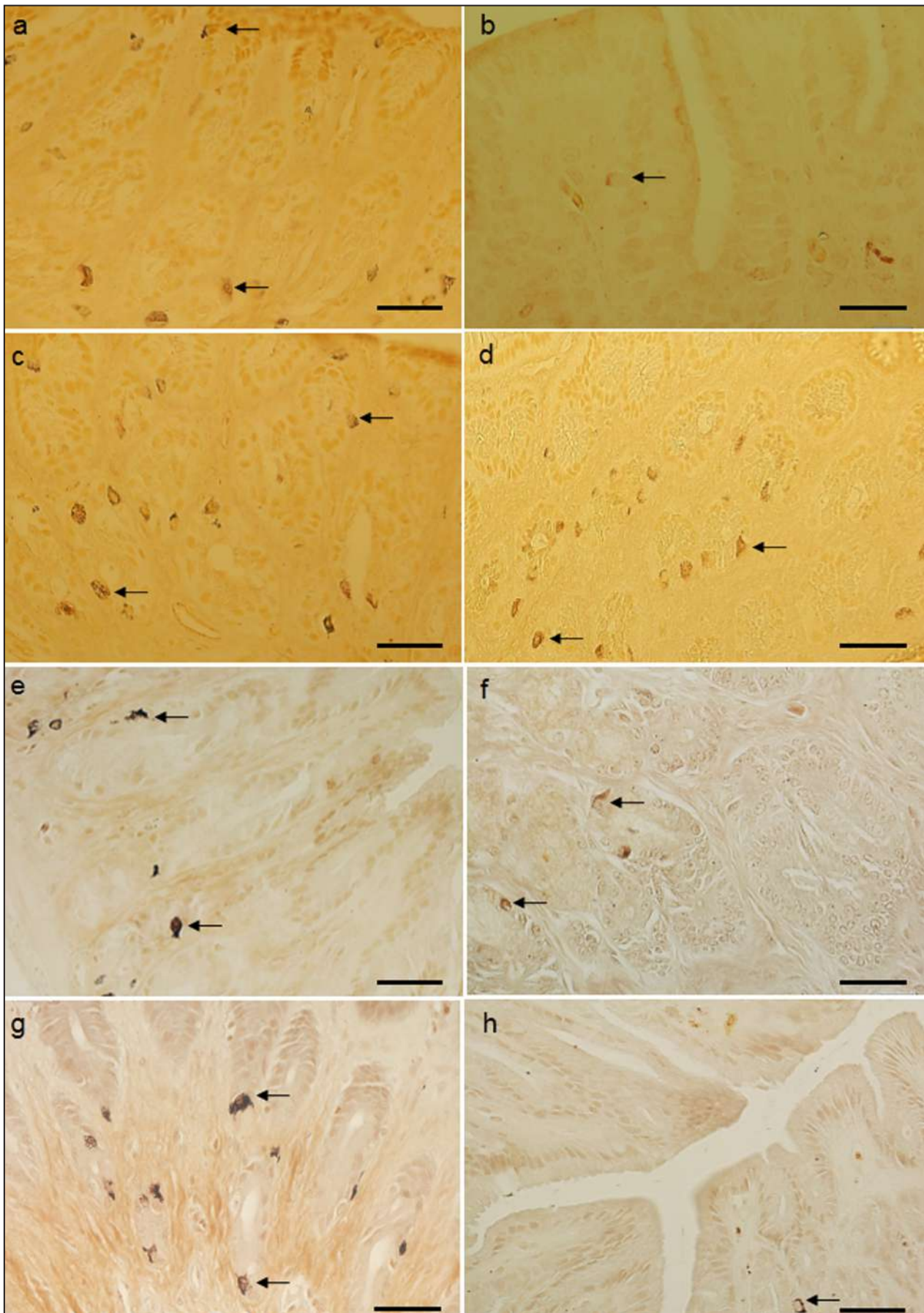
and somatostatin producing cells, while argentaffin cells are serotonin-producing cells, called type I (serotonin and substance P) or type II (serotonin and motilin) enterochromaffin cells.

In our study, we observed enteroendocrine cells in the caudal esophagus of *T. torquatus* and *S. merianae*, especially in the glands that appear in this esophageal region, allowing us to infer that they are involved with the control of glandular secretion. According to several authors, who worked with different reptilian species (Zamith, 1952; Ferri *et al.*, 1976; El-Salhy and Grimelius, 1981; Madrid *et al.*, 1989; Zug, 1993; Pereira *et al.*, 2005; Rodrigues-Sartori *et al.*, 2015), these glands secrete mucus for protection and lubrication of the esophagus, which has a storage function in addition to conduction of food, especially in reptiles that swallow whole prey. Thus, in *T. torquatus* and *S. merianae* the existence of endocrine cells in the caudal esophageal portion may also be associated with the permanence of food in this location, which presents itself as an extension of the stomach for the storage of larger prey. Endocrine

cells may also be involved in controlling esophageal motility in reptiles. Serotonin released by argentaffin cells is a paracrine mediator known to stimulate the contraction of smooth muscle in the digestive tract, and which may also be involved in the regulatory mechanism of ciliary movement in the reptilian esophagus (Perez -Tomas *et al.*, 1989).

Among the organs analyzed, the stomach was the one with the largest number of enteroendocrine cells, certainly because it is a place of greater permanence of the food content, being important in the control of gastric secretion and motility, unlike the esophagus. Argyrophilic cells were numerous in the pyloric region of both species studied, as well as argentaffin cells in *T. torquatus*. The argyrophilic and argentaffin cells were present in the stomach of different reptiles, with a predominance in the pyloric glands (Deng and Chu, 2006; Ahmed *et al.*, 2009; Rodrigues-Sartori *et al.*, 2011; Pereira *et al.*, 2015).

Argyrophilic and argentaffin cells were identified in the intestines of both species, except in the cranial portion of the small intestine, in which there



were no argentaffin cells. According to Furness *et al.* (1999), the intrinsic sensory neurons that detect luminal changes can do it directly or indirectly, through the release of chemical mediators by enteroendocrine cells, with serotonin being one of

the possible intermediary in this mucosal signal transduction. Since the argentaffin cells release serotonin and other mediators related to motility, it is assumed that certain motor functions involving the cranial intestine are performed directly by the

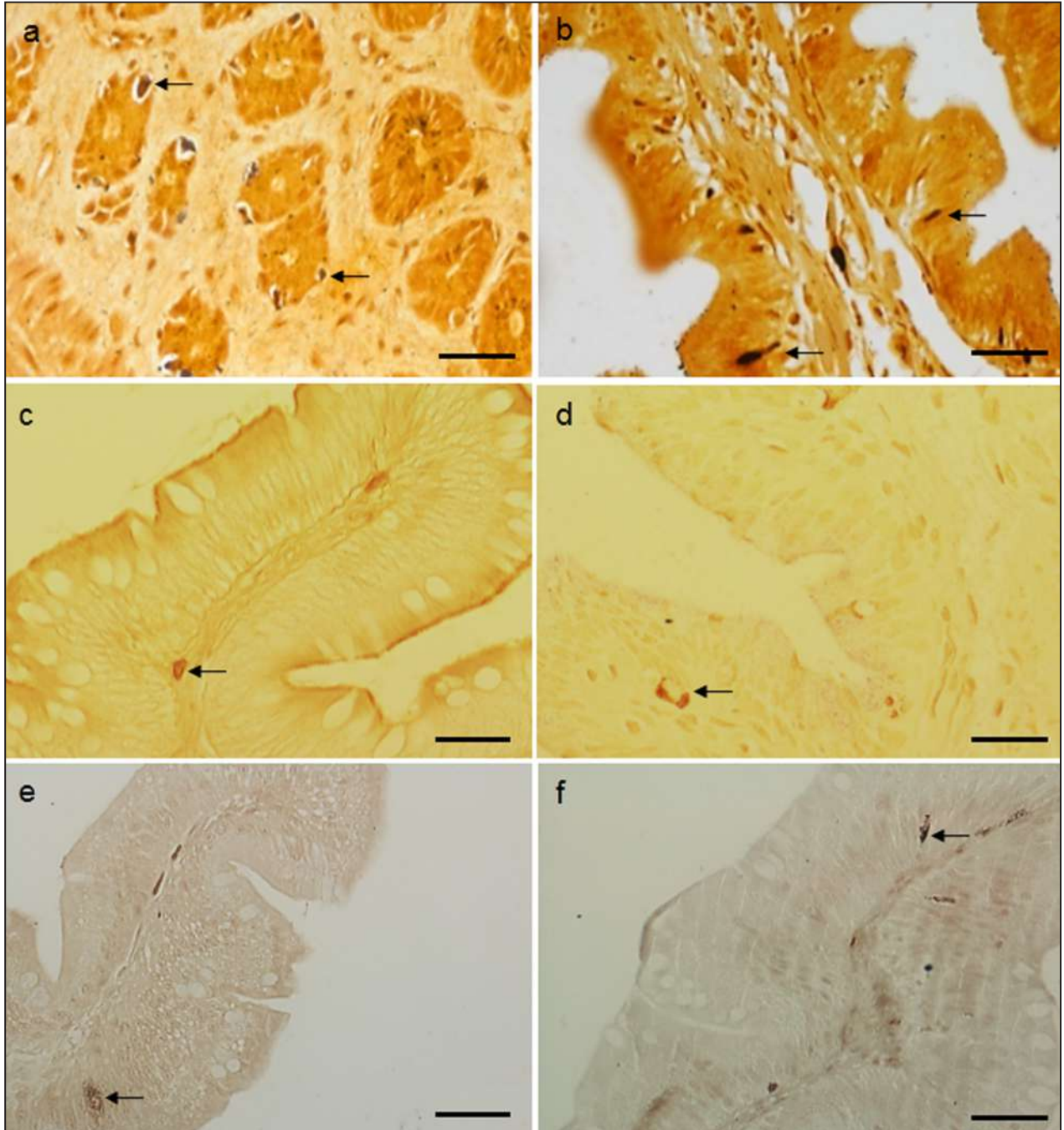


Figure 5. Histological sections of the small intestine of *Tropidurus torquatus* and *Salvator merianae*, with arrows indicating endocrine cells: argyrophilic (a to d; Grimelius technique) and argentaffin (e, f; Masson-Fontana technique). Gastro-intestinal transition (a), and cranial (c) and medium (e) small intestine of *T. torquatus*. Gastro-intestinal transition (b), and caudal (d) and medium (f) small intestine of *S. merianae*. Bar= 20 μ m.

Figure 4. Histological sections of the stomach of *Tropidurus torquatus* and *Salvator merianae*, with arrows indicating endocrine cells: argyrophilic (a to d; Grimelius technique) and argentaffin (e to h; Masson-Fontana technique). Fundic region (a, e) and pyloric region (c, g) of *T. torquatus*. Fundic region (b, f) and pyloric region (d, h) of *S. merianae* (b, f). Bar= 20 μ m.

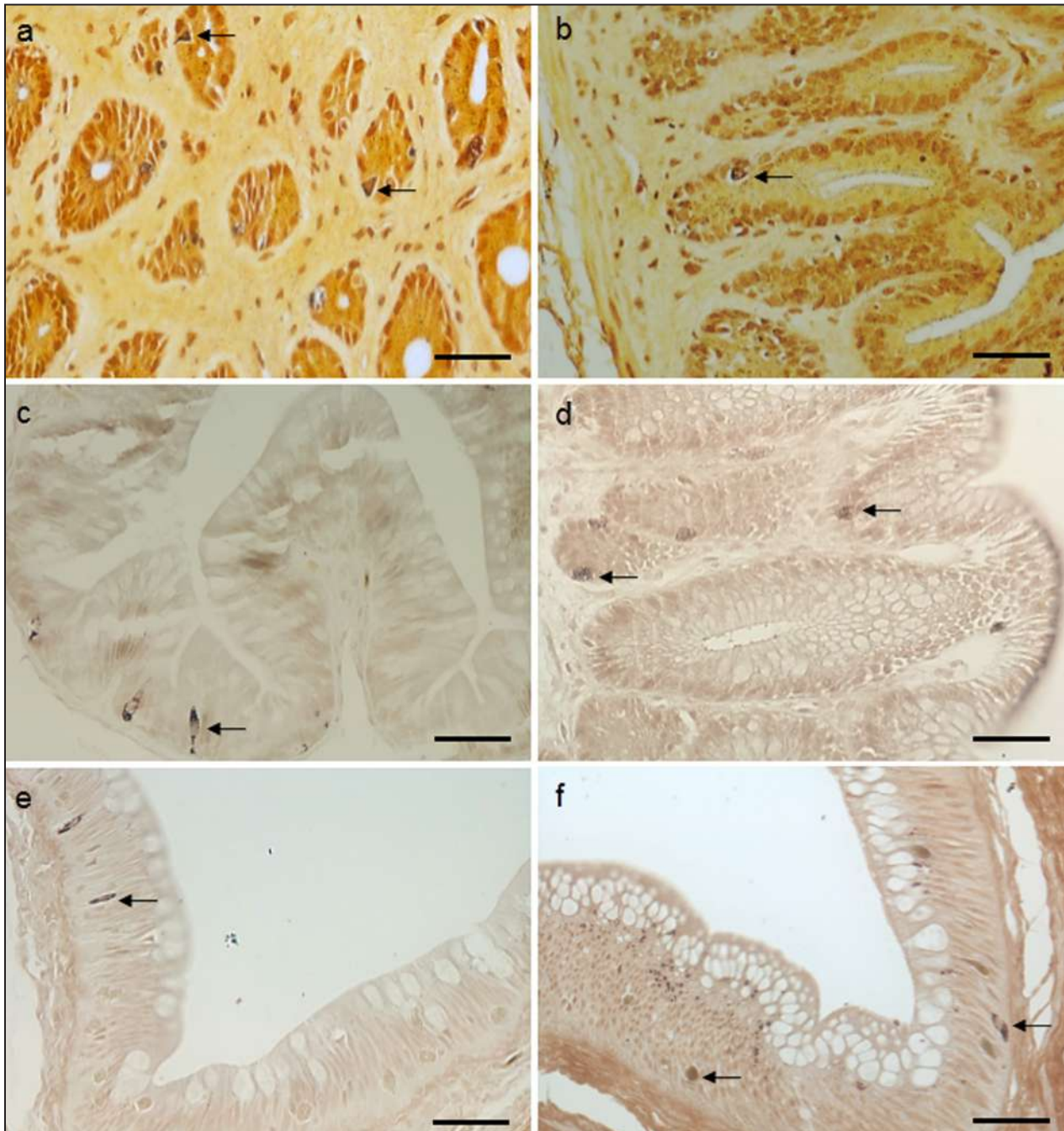


Figure 6. Histological sections of the large intestine of *Tropidurus torquatus* and *Salvator merianae*, with arrows indicating endocrine cells: argyrophilic (a, b; Grimelius technique) and argentaffin (c - f; Masson-Fontana technique). Transition between small and large intestines (a), colon (c) and rectum (e) of *T. torquatus*. Transition between small and large intestines (b), colon (d) and rectum (f) of *S. merianae*. Bar= 20 μ m.

nervous system.

The low number of enteroendocrine cells in the intestines of the studied reptiles is certainly related to the relatively short intestinal length and the absence or scarcity of glands, that is, it is less complex organ when compared to those of mammals. In the intestines of mammals, enteroendocrine cells are located mainly in the glands, however in many reptiles the

intestinal glands or crypts are non-existent (Perez-Tomas *et al.*, 1989; George *et al.*, 1998; Hamdi *et al.*, 2014; Rodrigues-Sartori *et al.*, 2014), or exist only in the large intestine, as in *T. torquatus* and *S. merianae*, and other reptilian species (Luppa, 1977). Thus, argyrophilic and / or argentaffin cells have also been located in the intestines of several reptiles, generally sparse (Ferri *et al.*, 1976; Martin-Lacave *et al.*, 1982;

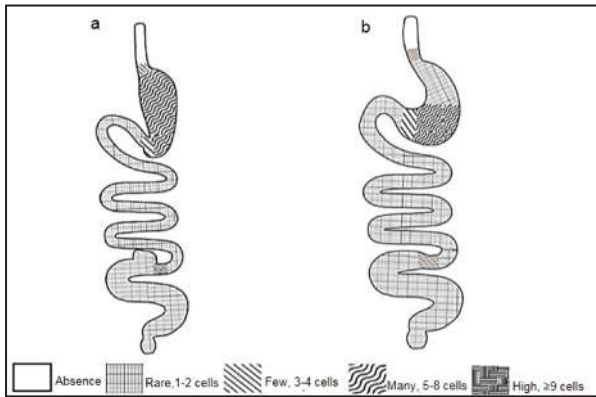


Figure 7. Schematic drawing showing the frequency of argyrophilic endocrine cells (number of cells / 0.2 mm²) in the digestive tract of *Tropidurus torquatus* (a) and *Salvator merianae* (b).

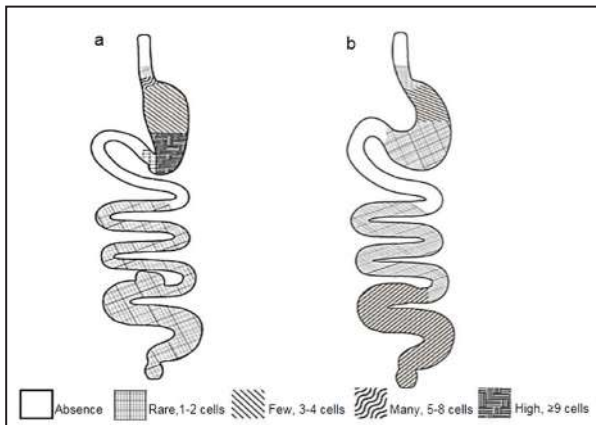


Figure 8. Schematic drawing showing the frequency of argentaffin endocrine cells (number of cells / 0.2 mm²) in the digestive tract of *Tropidurus torquatus* (a) and *Salvator merianae* (b).

D'Este *et al.*, 1993; Deng and Chu, 2006; Rodrigues-Sartori *et al.*, 2014). In addition, serotonin immunoreactive cells have been identified in the epithelium of the surface and glands in the large intestine of reptiles, suggesting that this mediator has a trophic action on the intestinal epithelium (Perez-Tomas *et al.*, 1989; Tarakçi *et al.*, 2005; Rodrigues-Sartori *et al.*, 2014). Comparing the two species studied, the intestinal crypts are more prominent in *S. merianae*, and perhaps that is why it presented a slightly larger number of argentaffin endocrine cells. On the other hand, many argyrophilic cells were found in *T. torquatus*, in the transition between the intestines, which may be related to the existence of the cecum in this species.

From the general analysis of the species studied, we can see how they differ in the number of argyrophilic and argentaffin cells when compared

with mammal species (Shimizu and Nozaki, 1994; Fonseca *et al.*, 2002; Bressan *et al.*, 2004; Machado-Santos *et al.*, 2009; Basile *et al.*, 2012; Freitas-Ribeiro *et al.*, 2012; Rodrigues-Sartori *et al.*, 2018). The lower density of these cells can be compensated by a greater direct neural participation in the control of digestive functions, which we understand as a primitive characteristic given that the nervous system arose before the endocrine in the evolution of homeostatic control systems (Falkmer, 1993). Thus, the discrepancies between reptiles and mammals can be attributed to evolutionary aspects, while the differences between reptilian species can be due not only to phylogenetic but also ecological issues, such as the animal's diet and food status, in view of the flexibility of the digestive tract.

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