

Morphological Description of the Green Turtle Tongue (*Chelonia mydas*)

Luana Félix de Melo¹; Marisol León Cabrera¹; Ana Clara Bastos Rodrigues¹;
Anaelise de Oliveira Macedo Turqueti¹; Edris Queiroz Lopes²; Rose Eli
Grassi Rici¹

¹University of São Paulo, Faculty of Veterinary Medicine and Animal Science - FMVZ / USP, São Paulo, Brasil.

²Institute of Marine Biology and Environment - IBIMM. Peruibe - SP, Brazil.

Corresponding author: Luana Félix de Melo

University of São Paulo, Faculty of Veterinary Medicine and Animal Science - FMVZ / USP, São Paulo, Brasil.

Abstract – In chelonians, the gastrointestinal tract is anatomically diverse among the large variety of reptiles, and this fact requires further studies to understand their particularities. For that, four green turtles were used to find beached dead, for macroscopic and microscopic analysis using light and scanning microscopy in search of the description of their morphology. The tongue presented as a non-protuberant organ, with a rigid and highly keratinized aspect. Concluding that the morphological structure of the turtle's tongue is adapted according to its abrasive feeding during capture and swallowing of the food.

Keywords— *microscopy, morphology, chelonians, keratin.*

I. INTRODUCTION

Sea turtles are reptiles belonging to the Reptilia class of the order Chelonia and suborder Cryptodira (1), and there are only representatives of the families Dermochelyidae (1) and Cheloniidae (3). Physiological, anatomical and behavioral adaptations allowed sea turtles to inhabit both marine and estuarine environments (4).

The green turtle (*Chelonia mydas*) belongs to the Cheloniidae family and inhabits the tropical and subtropical oceans (5). Using estuaries of rivers and lakes (6), they make use of the Brazilian coast for feeding and spawning (7). Its name is due to the greenish coloration of its fat, but that is not related to the external appearance (8)(6).

The diet of *C. mydas* varies throughout its life. In their early years they have omnivorous eating habits, with a carnivorous tendency, guaranteeing a fast growth, allowing them to avoid predators (9) (10). When reaching the juvenile stage, from 25 to 35 cm of Curvilinear Length of Carapace (CCC), the green turtle becomes preferentially herbivorous, the only species of marine turtle to present

this type of diet (2)(11)(12)(13). At this stage, *C. mydas* generally uses shallow areas to feed, while preferring deeper areas to rest (14). The dietary habits of *C. mydas* are largely associated with on-site food availability, turtle selectivity and /or habitat type (13)(15)(16)(17).

The green turtle is the only turtle that feeds on sea grass, being its largest consumer in tropical and subtropical waters (15). Marine grasses are angiosperms that grow only in marine environments (18)(19). These angiosperms play a key role in coastal ecosystems: they have high primary production, capture carbon from the atmosphere, participate in nutrient cycling, feed on coastal food webs and serve as habitat for microbes, invertebrates and vertebrates. Currently marine grasses are threatened by factors directly related to human practices, such as fishing, aquaculture, coastal area constructions, as well as indirect human impacts, such as changes in global temperatures and sea level rise (19). causing these animals to seek other means of feeding.

The juvenile green turtles also have a diet composed of animal material, and it may be occasional with the animal adhering to seaweed and sea grass (20). Invertebrate consumption can be considered to occur due to the opportunities present at the feeding site when they are abundant, and invertebrates in their diet are supplemented by their diet to obtain vitamins, minerals and essential amino acids (20)(21)(22). Live turtles and jellyfish are also widely consumed by green turtles (20)(23), and since the movement of these items is similar to plastic debris in the water, green turtles can often accidentally ingest such waste and confuse it with their usual food (24)(25).

In chelonians, the gastrointestinal tract is anatomically diverse among the large variety of reptiles, and this fact requires further studies to understand their anatomical specificities (26). Traumatic lesions in the oral

cavity, tongue and esophagus, may progress to secondary bacterial infection.

Thus, as described above, the green turtle's diet is highly diversified and abrasive, making it necessary to perform work with a description of its gastrointestinal tract to assist in filling in and updating existing gaps in the literature, providing subsidies for new work involving their macro and microscopic anatomy, morphological changes during a pathological process, as well as help in the attempt to trace new mitigating strategies

II. Material and Methods

Four marine turtle specimens were used, found dead on Guaraú beach, Peruibe - SP and obtained with authorization and licenses approved by ICMBio / SISBio: 50132-1 and CEUA-IBIMM: 005/18. For histological analysis, samples of the tongue were collected and fixed in 10% formaldehyde for 48 hours, in the sequence dehydrated in increasing series of ethanols (70 to 100%) and diaphanized in xylol, with subsequent inclusion in paraffin. 5 µm thick cuts were performed on the microtome (Leika and German 1988) and stained with hematoxylin -

eosin, and Mallory staining. The images were obtained through the Nikon Eclipse E-800 light microscope.

Part of the material was prepared for scanning electron microscopy. After 10% formaldehyde fixation were dehydrated in increasing series of alcohols in concentrations of 70%, 80%, 90% and 100%, dried in a LEICA EM CPD 300 critical point apparatus, glued with carbon paste in metallic aluminum bases (stub) and silver (sputtering) in the EMITECH K550 metallizer, and analyzed and photodocumented in LEO 435VP scanning electron microscope (SEM).

III. Results and Discussion

Macroscopically, the tongue of the green turtle is attached to the oral floor and not projectable, thick and wider in breadth than length, with red-whitish color and rigid appearance and rough surface. The glottis is located in the final portion of the tongue, as an opening opening function to close the airways. The esophagus begins at the basal portion of the tongue, presenting itself as a muscular tube through which food passes to the stomach (Figure 1).

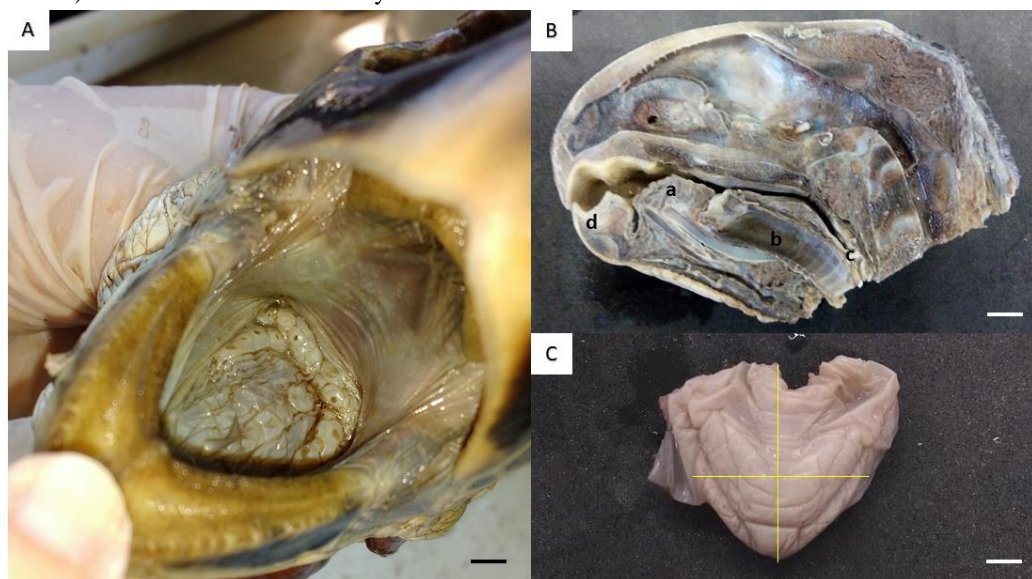


Fig.1: A: oral cavity of the green turtle (*Chelonia mydas*). B: sagittal cranial cut of *C. mydas*, demonstrating the organization of the organs arranged in the oral cavity, serigraphy (d), non-projectile tongue, trachea (b), esophagus (c). C: Language removed from the turtle. Bar 1cm.

According to Ovalle and Nahimey (27), in mammals language participates in the chewing, tasting and swallowing processes. It is covered by stratified squamous epithelium, and the dorsal surface, which is in contact with the hard palate in swallowing, in speech and at rest, is keratinized. The upper face of the tongue is irregular, due to protrusions of the epithelium and underlying loose connective tissue: the papillae (28)(29).

Despite being a group with growing interest in the study, the gastrointestinal tract of the chelonians is anatomically diversified, due to its great variety of species,

adaptations and food customs, and still little described in the literature. According to Wynken (30) the tongue of the tortoise is fixed to the floor of the mouth and not protuberant, corroborating with our findings. However, in his studies, it is described that the glottis occurs in the medial portion of the tongue, whereas in our specimens we describe that the glottis is present in the final portion of the tongue, next to the portion of the frenulum of the tongue where the muscular tube begins. esophagus.

In the microscopic analyzes of light and scanning electron, the tongue of the green turtle is covered by a

squamous keratinized stratified squamous epithelium from its apical to basal portion, presenting germinative extract with presence of keratinocytes, stratum spinosum, dense

and loose connective tissue and a large layer of skeletal striated muscle. The epithelium is covered by small short projections and flattened throughout its length (Figure 2).

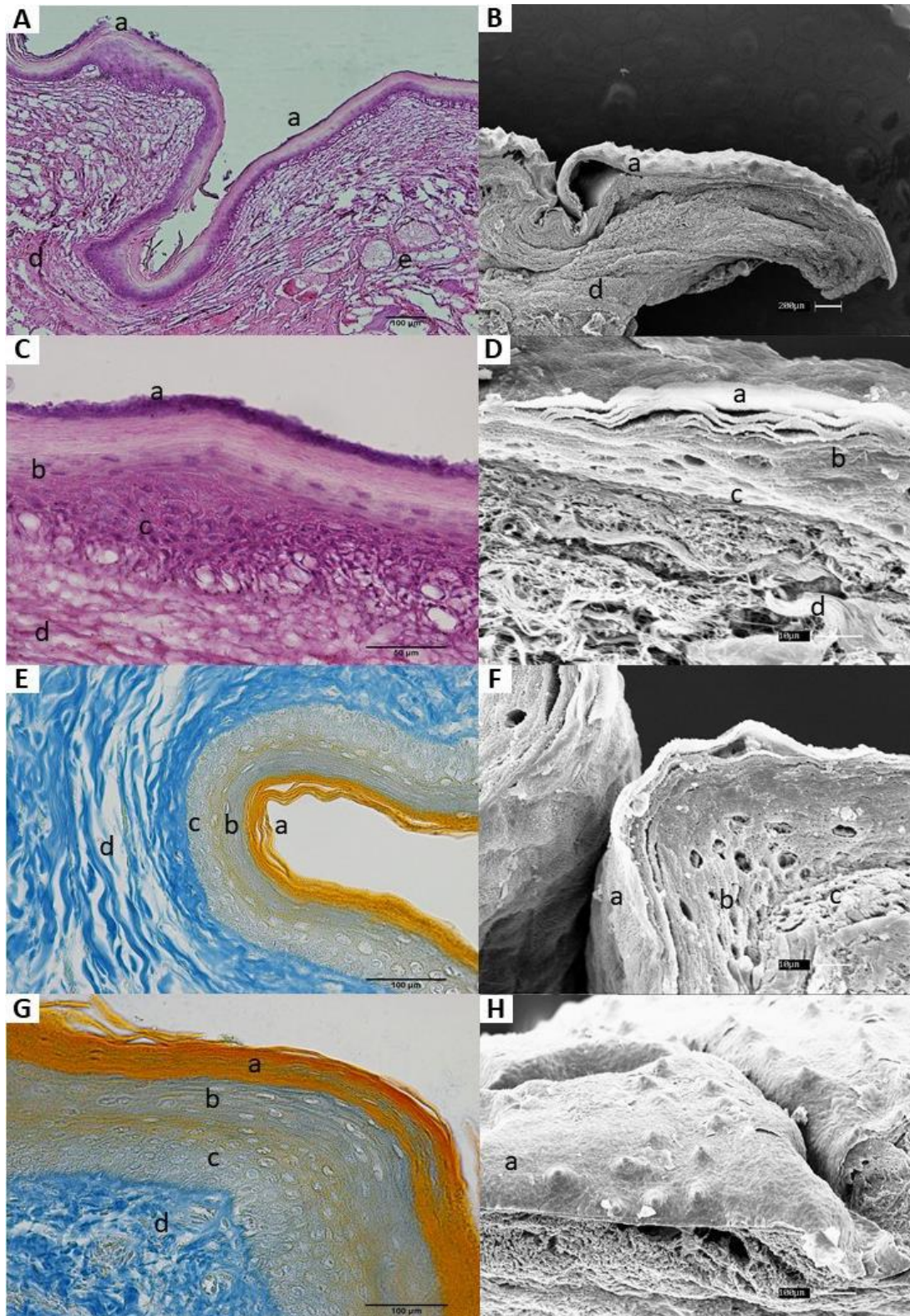


Fig.2: Green-tongue photomicroscopy (*Chelonia mydas*), by light and scanning microscopy, showing squamous keratinized stratified squamous epithelium, divided into a layer of keratin (a), stratum spinosus (b), germ stratum (c) where keratinocytes are matured. Below muscle tissue (d) richly vascularized by small venules (e) with presence of nucleated erythrocytes. A and C: light microscopy with HE staining. E and G: Mallory coloring, blushing keratin in orange. B: Scanning electron microscopy, sagittal cut of the tongue of the tortoise, with a scaly layer of keratin with small protuberances of keratin. D and

F: scanning image of the keratinized pavement epithelial tissue. H: scanning image of the rough surface of the turtle's tongue, evidencing the thick layer of keratin with varied short and flattened keratin projections.

In the work of Silveira (31) where they studied the oral cavity of *Podocnemis expansa*, the Amazonian turtle, although they are different species with different eating habits and environments, there are some similarities in their characteristics.

Silveira (31) describes the tongue as a muscular structure, with rhombus shape with different colorations in its regions, with papillae scattered on the surface of the epithelium, with the presence of taste buds attached to the coating epithelium. Regularly covered by cylindrical pseudo-lamellar epithelium, with non-keratinized goblet cells and with considerable presence of mucous cells. In our study with *C. mydas*, the tongue also presented a rhombus shape, but with only one coloration throughout its length. Width larger than length, its surface is covered by projections of the epithelium being formed only by keratin, without any presence of papillae or buds attached to the palate. Its epithelium is pavement keratinized and without presence of mucous cells. The entire floor is covered by a thick scaly layer of keratin with projections of keratin for all its extension only with mechanical function, its morphology indicates adaptation to the abrasive feeding and protection of the oral cavity.

Junqueira and Carneiro (32) describe filiform papillae with a pointed and keratinized shape that have mechanical paper helping to scrape the food increasing the friction in the mastication (27)(28)(29)(33) that despite a different shape from that in the *C. mydas* described in our results, they are also formed from keratin, thus attributing the same mechanical function aiding in the rubbing and scraping of the food during swallowing of the turtle.

IV. CONCLUSION

The morphological structure of the turtle's tongue is adapted according to its abrasive feeding during food capture and swallowing, using the thick layer of keratin to protect its oral cavity and also serving as the basis for new studies to identify pathological alterations advising with conservation.

V. ACKNOWLEDGEMENTS

Thanks to CAPES financial support. To the Advanced Center in Diagnosis by Image - CADI-FMVZ / USP.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

REFERENCES

- [1] Márquez M. R. Sea turtles of the world : an annotated and illustrated catalogue of sea turtle species known to date [Internet]. Food and Agriculture Organization of the United Nations; 1990 [cited 2018 Oct 24]. 81 p. Available from: <http://www.fao.org/docrep/009/t0244e/t0244e00.HTM>

- [2] Vandelli, D. Epistola de holothurio, et testudine coriacea ad celeberrimum Carolum Linnaeum equitem naturae curiosum Dioscoridem II. 1761. *Conzatti, Padua*.
- [3] Linnaeus, *Chelonia mydas*. Testudines, Cheloniidae. 1758.
- [4] Musick J.A., Limpus C. Habitat utilization and migration in juvenile sea turtles. In: The biology of sea turtles. 1997, CRC Press, Boca Raton, FL, p 137–164.
- [5] Ernest, C.H.; Barbour, R.W. Turtle of the world. 1ed., Washington: Smithsonian Institution Press, 1989. 313p.
- [6] Hirth, H.F. Synopsis of the biological data on the green turtle, *Chelonia mydas* (Linnaeus 1758). Washington: United States Fish and Wildlife Service Biological Report, 1997. 120p.
- [7] Moreira L., Baptisttpte C., Scalfone J., Thomé J.C., Almeida A.P. Ocorrência de *Chelonia mydas* on the Island of Trindade Brazil. Mar Turtle News. 1995;70:2.
- [8] Pritchard, P.C.H.; Trebbau, P. The Turtles of Venezuela. Athens: Society for the Study of Amphibians and Reptiles, 1984. 403p.
- [9] Chevalier J. & Lartiges A. Les Tortues Marines des Antilles. Ed. Office. National de La Chasse et de la Faune Sauvage. CNERA Fauned'Outre Mer, 2001. Paris. 59p.
- [10] Fidelis S.V., Ballabio T.A. & Guebert F.M. Análise da relação corporal do trato gastrointestinal da *Chelonia mydas* (tartaruga verde) juvenil do litoral do Paraná. 2005. Anais II.
- [11] Bjorndal K.A. Foraging ecology and nutrition of sea turtle, The Biology of Sea Turtle. Marine Science Series. In: Lutz P.L. & Musick J.A. (Eds). 1997 New York p.199-231.
- [12] Mortimer J. Feeding ecology of sea turtles. In: Bjorndal KA (ed) Biology and conservation of sea turtles. Smithsonian Institution Press, 1982. Washington, DC, p 103–109.
- [13] Ross J.P. Biology of the Green Turtle, *Chelonia mydas*, on an Arabian Feeding Ground. J Herpetol [Internet]. Society for the Study of Amphibians and Reptiles; 1985 Dec [cited 2018 Oct 24];19(4):459. Available from:

- <https://www.jstor.org/stable/1564198?origin=crossref>.
- [14] Reisser J., Proietti M., Sazima I., Kinan P., Horta P., Secchi E. Feeding ecology of the green turtle (*Chelonia mydas*) at rocky reefs in western South Atlantic. Mar Biol [Internet]. Springer Berlin Heidelberg; 2013 Dec 13 [cited 2018 Oct 25];160(12):3169–79. Available from: <http://link.springer.com/10.1007/s00227-013-2304-7>.
- [15] Bjorndal K.A. Nutrition and grazing behaviour of the green turtle *Chelonia mydas*. Mar Biol 1980, 56:147–154.
- [16] Garnett S.T., Pirce I.R., Scott F.J. The diet of the green turtle, *Chelonia mydas* (L.), in Torres Strait. 1985. Wildl Res 12: 103–112.
- [17] Brand-Gardner J.M.L., Limpus C. Diet selection by immature green turtles, *Chelonia mydas*, in subtropical Moreton Bay, south-east Queensland. Aust J Zool 1999 47: 181–191.
- [18] Charpy Roubaud C., Sournia A. The comparative estimation of phytoplanktonic, microphytobenthic and macrophytobenthic primary production in the oceans. Mar Microb Food Webs [Internet]. 1990 [cited 2018 Oct 25];4(1):31–57. Available from: <http://www.documentation.ird.fr/hor/fdi:34226>.
- [19] Duarte CM. The future of seagrass meadows. Environ Conserv [Internet]. 2002 Jun 21 [cited 2018 Oct 25];29(02). Available from: http://www.journals.cambridge.org/abstract_S0376892902000127.
- [20] Castell, E.D. Hábitos alimentares de juveniles de tortuga verde (*Chelonia mydas*) en Cerro Verde, Rocha. 2005. 56 f. Relatório de estágio (especialização em etologia) – Sección Etología, Facultad de Ciencias, Universidad de la República, Montevideo.
- [21] Bjorndal K.A., Bolten A.B. Digestive Processing in a Herbivorous Freshwater Turtle: Consequences of Small-Intestine Fermentation. Physiol Zool [Internet]. University of Chicago Press ; 1990 Nov 30 [cited 2018 Oct 25];63(6):1232–47. Available from: <https://www.journals.uchicago.edu/doi/10.1086/physzool.63.6.30152642>.
- [22] Hirth, H. F., M. Huber, T. Frohm, and T. Mala. A natural assemblage of immature green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) turtles on the fringing reef of Wuvulu Island, Papua New Guinea. Micronesica 1992. 25:145–153.
- [23] Bustard, R. Australian sea turtles, their natural history and conservation. 1972. London, Collins, 220p.
- [24] Bugoni L, Krause L, Virgínia Petry M. Marine Debris and Human Impacts on Sea Turtles in Southern Brazil. Mar Pollut Bull [Internet]. Pergamon; 2001 Dec 1 [cited 2018 Oct 25];42(12):1330–4. Available from: <https://www.sciencedirect.com/science/article/pii/S0025326X01001473>.
- [25] Melo C.M.F, Santos, Amora, Oliveira. Estudo do impacto fisiológico do lixo na tartaruga verde através da análise do aparelho digestivo [Internet]. [cited 2018 Oct 25]. Available from: <http://www.tamar.org.br>.
- [26] Costa, M.F.; Ivar do Sul, J.A.; Silva-Cavalcanti, J.S.; Araújo, M.C.B.; Spengler, A.; Tourinho, P.S. On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. Environmental Monitoring and Assessment 2009. v.168, p.299-304. Congr. Bras. Oceanografia, Vitória, p.1-3.
- [27] Ovalle, William K.; Nahirney, Patrick C.; Netter, Frank Henry. Netter bases da histologia. 2. ed. 2008. Rio de Janeiro: Elsevier.
- [28] Lowe, J. S.; Anderson, P. G. Stevens & Lowe's. Human Histology. 4.ed. Philadelphia: Elsevier, Mosby, 2015. pp. 186-188, 197.
- [29] Ross, M. H.; Pawlina, W. Histologia: texto e atlas em correlação com a biologia celular e molecular. 6. ed. 2012. Rio de Janeiro: Guanabara Koogan.
- [30] Wyneken (PDF) The Anatomy of Sea Turtles The Anatomy of Sea Turtles [Internet]. 2001. Available from: https://www.researchgate.net/publication/265924061_The_Anatomy_of_Sea_Turtles_The_Anatomy_of_Sea_Turtles.
- [31] Silveira T.B., Agostinho E.S., Santos F.G.A., Oliveira A.C.P., Medeiros L.S., Carvalho Y.K., et al. Avaliação da cavidade orofaríngea da tartaruga da Amazônia, *Podocnemis expansa* (Schweigger, 1812). Pesqui Veterinária Bras [Internet]. 2015 Dec [cited 2018 Oct 25];35(12):1002–8. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-736X2015001201002&lng=pt&tlng=pt.
- [32] Junqueira L.C.U, Carneiro J. Basic histology : text & atlas [Internet]. Lange Medical Books, McGraw-Hill, Medical Pub. Division; 2003 [cited 2018 Oct 25]. 515 p. Available from: https://books.google.com.br/books/about/Basic_Histology.html?id=y7RqAAAAMAAJ&redir_esc=y.

- [33] Gartner, L. P. & Hiatt, J. L. Tratado de Histologia em Cores. 2 ed. 2003. Rio de Janeiro: Guanabara Koogan.