Journal of Morphological Sciences
J Morphol Sci
Brazilian Society of Anatomy

Original article
J Morphol Sci 2021;38:27-37

Morphological Aspects of the *Monodelphis Domestica* Dentition

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Disclose and conflicts of interest: none to be declared by all authors

ABSTRACT

Introduction: the description of tooth morphology and dental tissues of laboratory opossum may contribute to a better understanding of this animal's behavior, including mammalian evolution and how different morphologies can result from tooth development.

Methods: the dental morphology of *Monodelphis domestica* was stereomicroscopically identified and its tissues microscopically analyzed in demineralized and abraded sections of teeth.

Results: tooth crown morphology is complex and has a low crown-root length ratio. Inside the crown, the dentine tubules are narrow, branched and more densely arranged than in the root. The dentinal tubules in the root are narrower and slightly tortuous, with more widespread ramifications. Cementum is predominantly cellular, presenting a very stratified layer and being abruptly initiated, giving a shoulder-like appearance to the cervical limit and a stick-like shape to root apex. Enamel had tubules and was identified at the furcation. Width variation in the periodontal ligament of the same tooth was observed, which tended to become narrower with age. Epithelium was also observed at the furcation.

Conclusion: some peculiarities of the *Monodelphis domestica* dentition have been first described, such as cementum thickness, presence of enamel and epithelium at the furcation, narrowing of periodontal ligament width related to aging and low crown-root length ratio.

Keywords: *Monodelphis domestica*; Tooth morphology; Dental tissues; Mammalian tooth morphology; Didelphidae tooth morphology; Short-tailed opossum.

Introduction

Monodelphis domestica is a small animal belonging to the *Didelphidae* family (Van de Berg & Robison, 1997; Macrini, 2004) which inhabits South America (Macrini, 2004) and has been suitable to different areas of research (Sasagawa & Ferguson, 1991; Van de Berg & Robison, 1997; Wang *et al*., 2009; Moustakas *et al*., 2011; and Gasse *et al*., 2015). This animal has a diversified dentition with numerous teeth, that is, a deciduous third premolar and fifty teeth in the permanent dentition. Its dental formula is I (5/4), C (1/1) P (3/3), M (4/4), thus making this model more relevant in studies on evolution of mammals (Van de Berg & Robison, 1997; and Sasagawa & Ferguson, 1991). The numerous altricial offspring in this non-pouched mammal, which occurs at 14 days of gestation, allows the pups to be manipulated when odontogenesis begins (Van Nievelt & Smith, 2005). These characteristics make the short-tailed opossum a suitable prototype for studies on tooth development as it has an obvious advantage over murine models, which are nevertheless widely used besides their reduced dental formula, i.e. I (1,1), M (3,3), and continuously growing incisors.

The dental tissues of the laboratory opossum have not yet been fully described, despite their importance for understanding the evolutionary development and changes in mammals. The description of tooth morphology and dental tissues of laboratory opossum may contribute to a better understanding of this animal's behavior, including mammalian evolution and how different morphologies can result from tooth development. This study aims to describe the morphological characteristics of the teeth and dental tissues of *Monodelphis domestica* by means of stereomicroscopy and light microscopy.

Material and methods

This research was approved by the animal research ethics committee of the Federal University of Mato Grosso do Sul (CEUA/UFMS) according to protocol number 487/2012.

Table I shows the distribution of the animals according to age and methodology used. The animals were dismissed from the UFMS vivarium because of aging or genetic refinement. Euthanasia was performed in a CO_2 chamber (Sharp & Lawson, 2006) before decapitation and removal of soft tissues, and the surgical pieces were immersed in Morse's solution (Morse, 1945) for a period ranging from 10 days to four weeks to obtain 5-µm sections of the demineralized material. Next, analysis of the dentin, cementum and periodontal ligament was performed.

DOI: https://doi.org/ 10.51929/jms.38.6.2021
ISSN 2177-0298

Table 1. Distribution of the animals by age and methodology in which they were used.

Age	2 mo old	3 mo old	5 mo old	8 mo old	1 yr 3 mo old	1 yr 5 mo old	2 yr10 mo old	3 yr old
Quantity	1	1	1	1	1	1	1	3
Methods	S/LM-dem	S	S	S	S/LM-dem	LM-dem	S/LM-dem	LM-abr

S: Stereomicroscopic analysis; LM-dem: Light microscopic analysis of demineralized section; LM-abr: Light microscopic analysis of abraded sections.

The skulls which were selected for stereomicroscopic analysis were fixed in formaldehyde 10%. Prior to jaw disarticulation, the occlusion pattern was observed in lateral view at magnifications of 0.8, 1, 2, 3, 4 and 5 times to identify the morphology of the dental crown. Illustrative images were obtained with an iPhone-7® camera attached to the eyepiece of the stereomicroscope, and proximity was obtained manually by positioning the specimens under the objective lens and varying the magnification to allow better focus.

The heads, which provided abraded sections, were first immersed in 2.5% sodium hypochlorite solution until denaturation of organic tissues. The extracted teeth were embedded in epoxy resin (Redelease®, São Paulo, Brazil) to obtain small rectangular blocks for sectioning with a diamond knife (Isomet Buehler, Lake Bluff, IL, USA), which resulted in sections of 0.4-0.6 mm thick. The sections were manually abraded with wet sandpaper of decreasing roughness (i.e. 150, 400, 500 and 600 grits, Norton, Guarulhos, Brazil) until reaching a thickness of 0.2-0.05 mm, which was measured by a digital micrometer (Digimatic, Chapecó, SC, Brazil) and microscopically. After being rinsed for 24 hours under running water, the sections; which were inside individual cocoons made of gauze; were allowed to dry at room temperature for 48 hours before being mounted with Canada balsam. These sections allowed enamel, dentin and cementum to be analyzed. Photomicrographs were obtained with a camera (Leica DM 5500B, Heerbrugg, Switzerland) to illustrate the findings.

A skull and a mandible were sagittally sectioned with a carborundum disc mounted on a low-speed handpiece under stereomicroscopy and then X-rayed by using a digital X-ray sensor (Kodak RVG 6100®, Carestrem Health, Rochester, USA) with a 0.15-second exposure. Next, the radiographs were edited for light/dark contrast by using the software TDO Professional® (TDO, San Diego, USA), to analyze number, size and direction of the dental roots. To obtain the root length and crown-root length ratio, the extracted teeth were placed on a graph paper (A4 paper size = 210 mm x 297 mm) and measurements were obtained based on this scale.

The dental notation used in this investigation identifies maxillary teeth with upper case letters followed by Arabic numerals; e.g. I1 - first maxillary incisor; and mandibular teeth with lower case letters followed by Arabic numerals; e.g. i1 - first mandible incisor; (Swindler, 2002; Van Nievelt & Smith, 2005). This well-accepted dental notation (Swindler, 2002; Van Nievelt & Smith, 2005; Moustakas *et al.*, 2011) is widely used to register the dentition of *Monodelphis domestica*, which has five maxillary incisors and four mandibular ones. If the modified Triadan system (Floyd, 1991) was used, for instance, inaccuracy might have affected the identification of the following teeth of the arches. The terminology used for morphological description was that presented by Swindler *et al.* (2002).

Results

Stereomicroscopic Analysis

The morphological characteristics of the 8-month-old animals' teeth were described. The dentition of animals at other ages was also examined to identify morphological variations, including tooth wear and loss. The overall morphological aspects of the dental crowns are listed in Tables 2 and 3.

Diastemas were present in both arches. The incisors were very much alike each other, except the central maxillary one, which is cylindrical and very tiny. The mandibular incisors had a hook-shaped projection in their lingual surface, which differs from the cingulum. The latter one is present and gives convexity to other vertical teeth's surfaces (Figures 1A and 2A). Maxillary and mandibular canines were very similar, but the former were smaller than the latter (Figures 1B and 2B). The premolars had similarities (Figures 2A and 2B) as well, but with a tiny projection close to the cervical limit of the mesial edge in mandibular ones, which were also smaller than the maxillary teeth (Figure 2B). In the maxilla, the tribosphenic molars had very sharp projections and edges. The forth molar was the smallest and had a flattened aspect. The mesial surface was more convergent to the occlusal surface than the distal one and the occlusal projections were aligned from the mesial-buccal to the distal-lingual region (Figure 1D). Mandibular molars had similar morphology among themselves. The forth molar had a volume equivalent to the third one, but with the talonid basin presenting only one projection separated from the trigonid basin by a hill-shaped surface (Figure 2D).

The roots of almost all teeth were at least three times larger than their crown (Figure 3). The canine crown and root had approximately the same length, whereas the maxillary central incisor root was two times the crown dimension (Table 2). The crown-root length ratio was similar in both arches, with mandibular teeth being larger (Tables 2 and 3). The teeth were predominantly in perpendicular position, except the anterior ones, which had inclination of their long axes towards the lingual region. The cervical limit was highlighted and the furcation accompanied the end of the anatomical crown.

As for presence/maintenance of teeth and its relationship with chronological age, incisors absence was observed in animals with one year and three months old or more and m1 absence was observed in an exemplar of one year and three months. Tooth wear was observed in older animals, being more evident in molars and increasing with age, but no pattern could be identified.

Light Microscopic Analysis

The aspects observed in both abraded and demineralized sections are described together, including findings exclusively provided by the two methodologies.

Enamel exhibited surface irregularities and variable thickness, being thicker in the buccal surface of maxillary incisors as well as in the lingual surface

Table 2. Morphology of the crowns and roots of the *Monodelphis domestica* teeth – Maxilla.

Teeth	Crown					Root	
	Morphology	Volume in the group/ arch	Flattening direction		Mean tooth size (mm)	Quantity	Mean root size (mm)
			MD	BP			
I1	Cylindrical, slightly tapered	Smaller than the other incisors			1.8	1	1.2
I2, I3, I4, I5	Spoon aspect; flattened-spear shaped	Decreasing order of volume		X	3.0	1	2.5
C	Tapered, with pointed incisal border	Highest crown, projected beyond the occlusal line		X	6.0	1	3.5
P1, P2, P3	Three projections aligned mesio-distally: paracone, protocone (more bulky and designed) and metacone	Crescent order of volume		X	P1– 3.0 P2 – 3.7 P3 – 4.1	2	P1 – 1.7 P2 – 2.2 P3 – 2.5
M1, M2, M3, M4	Three occlusal projections: two buccal (mesial: paracone and distal: metacone) and one lingual (protocone). M4's paracone and metacone are smaller, aligned with protocone in a compression aspect	Increasing order of volume from the first to the third. M4 is the smallest of the group.	X		3.5	3	2.5

MD: mesio-distally; BP: buccolingual

Table 3. Morphology of the crowns and roots of the *Monodelphis domestica* teeth – Mandible.

Teeth	Crown					Root	
	Morphology	Volume in the group/arch	Flattening direction		Mean tooth size (mm)	Quantity	Mean root size (mm)
			MD	BP			
i1, i2, i3, i4	Paddle shape	Decreasing order of volume	X		3.2	1	2.8
c	Tapered, with pointed incisal border	Highest crown, projected beyond the occlusal plane		X	6.7	1	3,8
p1, p2, p3	Pointed occlusal border, with three mesio-distally aligned projections: paraconid, protoconid (more bulky and designed) and metaconid	Increasing volume order		X	p6 – 3.1 p7– 4.0 p8 – 4.5	2	p6 – 1.7 p7 – 2.5 p8– 3.0
m1, m2, m3, m4	Five occlusal projections in two bases: trigonid, with protoconid, paraconid and metaconid projections. Talonid (distal), with hypoconid and entoconid projections. m4 has only one projection in the talonid basin	m3 has the largest volume. m4 is the smallest in the group		X	4.2	3	3.0

MD: mesio-distally; BP: buccolingual

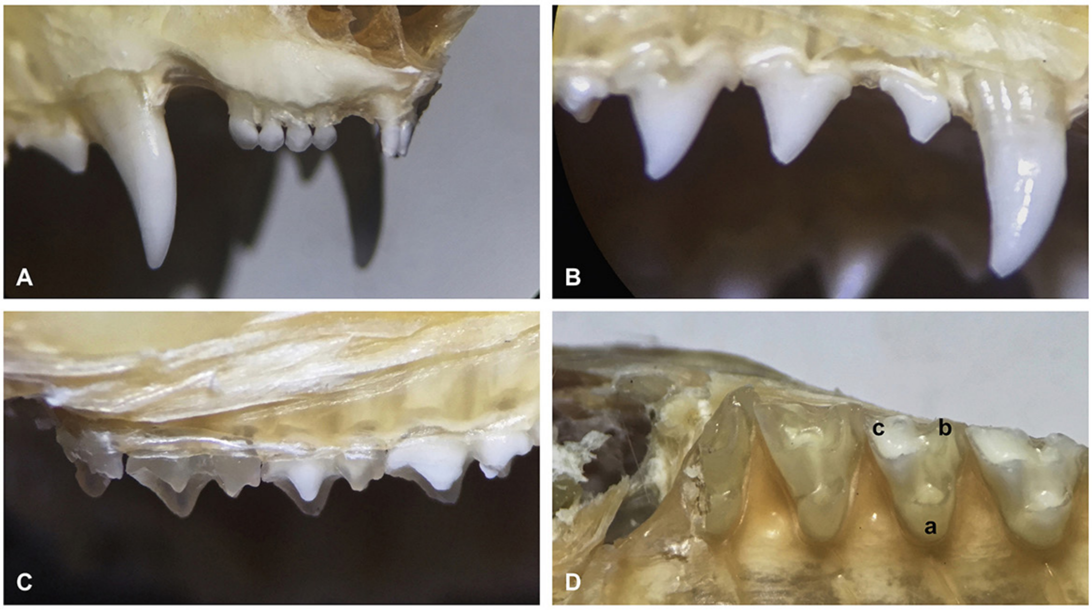

Figure 1. Photograph of maxillary teeth of *Monodelphis domestica* observed by a stereomicroscope. A. Buccal surface of incisors; B. Buccal surface of canines and premolars; C. Buccal surface of molars; D. Occlusal surface of molars. a: protocone; b: paracone; c: metacone.

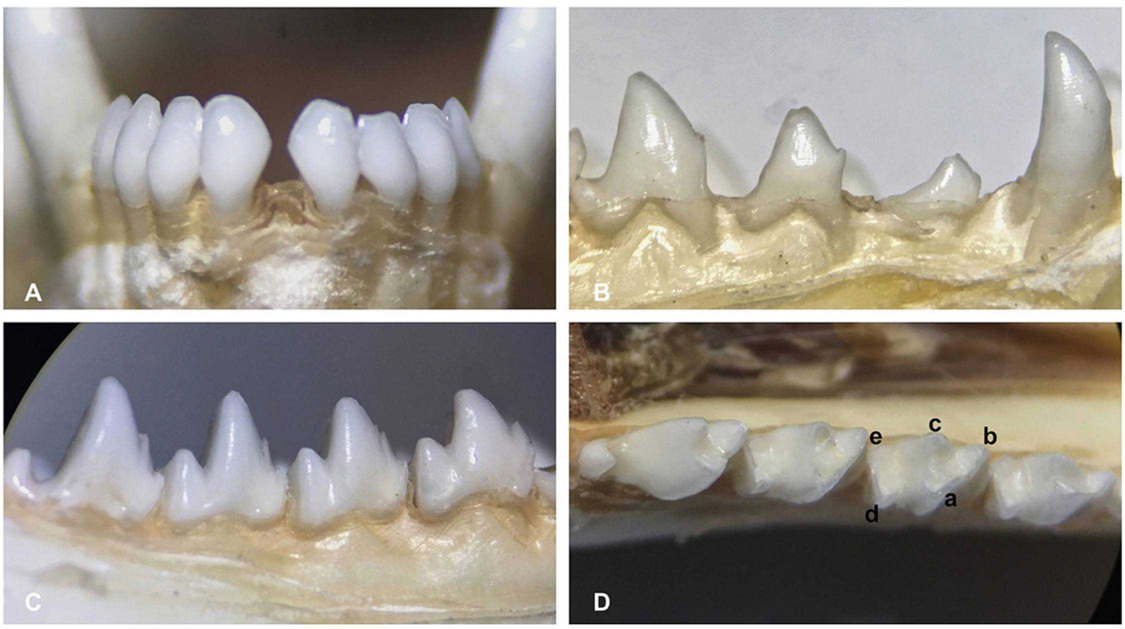

Figure 2. Photograph of mandibular teeth of *Monodelphis domestica* observed by a stereomicroscope. A. Buccal surface of incisors. B. Buccal surface of canines and premolars. C. Buccal surface of molars. D. Occlusal surface of molars. Projections of the trigonid basin, a: protoconid; b: paraconid; c: metaconid. Projections of the talonid basin; d: entoconid; e: hypoconid.

of maxillary canines and ranging from thinner to thicker in the occlusal surface of molars (Figure 4A). Its presence was identified at the furcation region (Figure 4B). Longitudinal and transverse striations were identified (Figure 4D). Areas compatible with lamellae were more frequently observed in the enamel of anterior teeth and casually in the continuity with dentin (Figure 5B).

Enamel deposition at the enamel-cement junction varied in the same tooth, sometimes determining a gap between both tissues rather than exposing the dentin (Figures 6A). A butt-welding shape (Figure 4C) and even an overlapping of these two tissues were observed as well. Enamel tubules were not frequently identified in the cervical third region of the crown, even being absent in incisors. On the other hand, these tubules were observed in the middle third of the enamel layer, being densely present in the occlusal area and even more densely near the enamel-dentin junction, where this limit is uniform (Figure 5B).

Tubules in the crown dentin were high-density organized and exhibited ramifications through the whole tissue, being more scattered near the dental pulp chamber and more intensely ramified near the enamel-dentin junction (Figure 5B). The direction of the tubules varied in diameter and trajectory, which was predominantly straight (Figure 5B), with constant diameter after the initial ramification which decreased near the enamel-dentin junction. The ramifications spread and reached the mantle dentin, maintaining their trajectory and penetrating into the enamel (Figure 5B). In the cervical third of the crown, some tubules were straighter (or not identified) than those observed in other areas and were in continuity with the mantle dentin. Areas resembling *tratus mortus* (Figure 5A) and tubules with segmented aspect were identified in the crown (Figure 5B) next to the incisal edge and just beneath the occlusal projections.

In the root, the density of dentin tubules decreased towards the apex, exhibiting a perpendicular orientation and being notoriously tortuous in the apex area. Numerous ramifications were already present near the root canal and the diameter of the tubules was wider than those of the crown, remaining constant towards the cementum-dentin junction, where they branched profusely (Figures 5C and D).

Cellular cement was predominantly stratified, frequently with an abrupt start, giving a shoulder appearance to the cervical area and a stick-like shape to the roots (Figure 6A), mainly in the incisors. In the canines, a greater thickness was observed in the lingual surface as well as in the distal surface of premolars (Figure 6A). The surface of cementum presented an irregular and wavy appearance, with layer lines being better observed in the demineralized sections (Figure 6B).

The cementum thickness was wider and the surface irregularity was more pronounced in older animals (Figure 7B). In some sections, cementum was absent in the root surface next to the furcation, where periodontal ligament fibers were directly attached to the dentin. The cementum-dentin junction was regular in the cervical and middle thirds (Figure 7A). This limit became irregular and was not well defined in the apex area and in those related to root developmental groove (Figures 6C and D) and furcation.

Periodontal ligament consisted of extensively vascularized connective tissue, whereas the blood vessels were wider in the apex area. Horizontal disposition of the fibers was more frequently found, but oblique orientation and haphazard arrangement were also randomly observed, with the latter in the apex and furcation regions. In the cervical root third, the arrangement of the periodontal ligament fibers was varied, extending from the cementum to the mucosa or from the cementum to the bone. Continuous periodontal fibers extending from the cervical area of a mandible incisor to the contiguous one were identified with no bone septum between them.

The width of periodontal ligament also varied broadly from narrow to wide in the same specimen, with intermediate widths being more frequently observed in the cervical and middle thirds and wider ones in the furcation and apical regions. In the canines, the periodontal ligament was narrower in the buccal surface.

Irregularity in bone and cementum surfaces was identified in animals of all ages and could be related to aging. In older animals, narrowing of the periodontal ligament and remarkable increase of cementum layers were observed (Figure 7B). In some areas, it was difficult to precise the limit between bone and cementum. Hyaline areas and ossification points were observed in the periodontal ligament, which is suggestive of ossification process.

Demineralized sections allowed for identifying epithelium in an adjacent position to that of the enamel, that is, in the furcation area (Figures 7B and C).

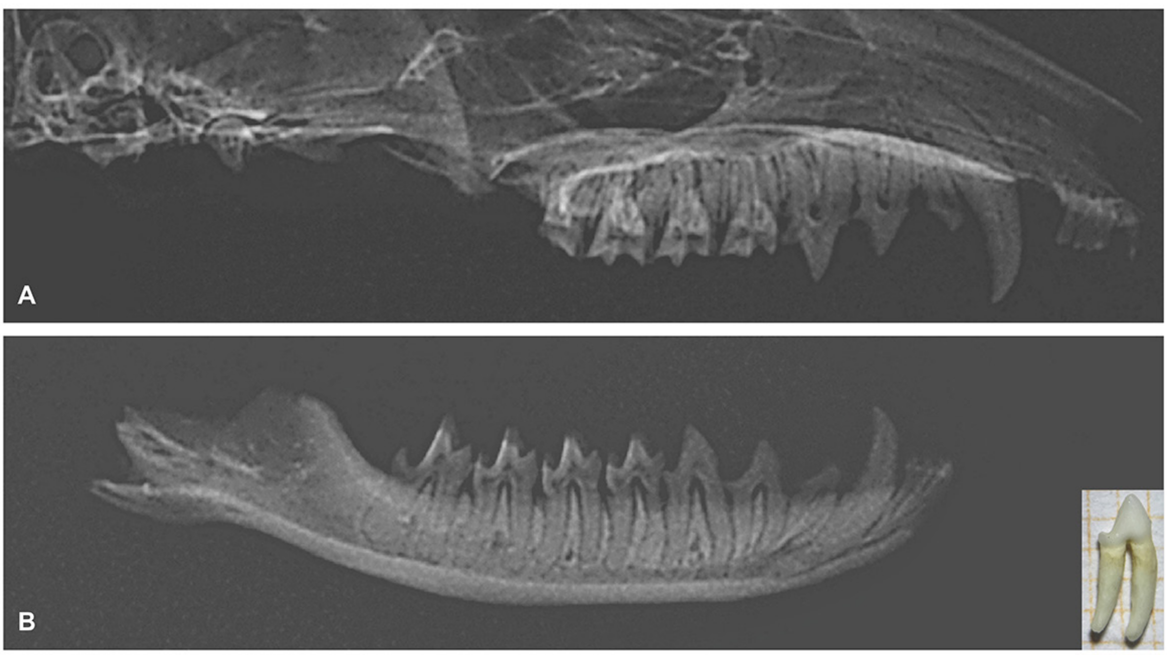

Figure 3. X-ray of the *Monodelphis domestica*'s teeth. A. Maxillary hemi-arch; B. Mandibular hemi-arch; C. p3 positioned over graph paper to measure crown-root length ratio.

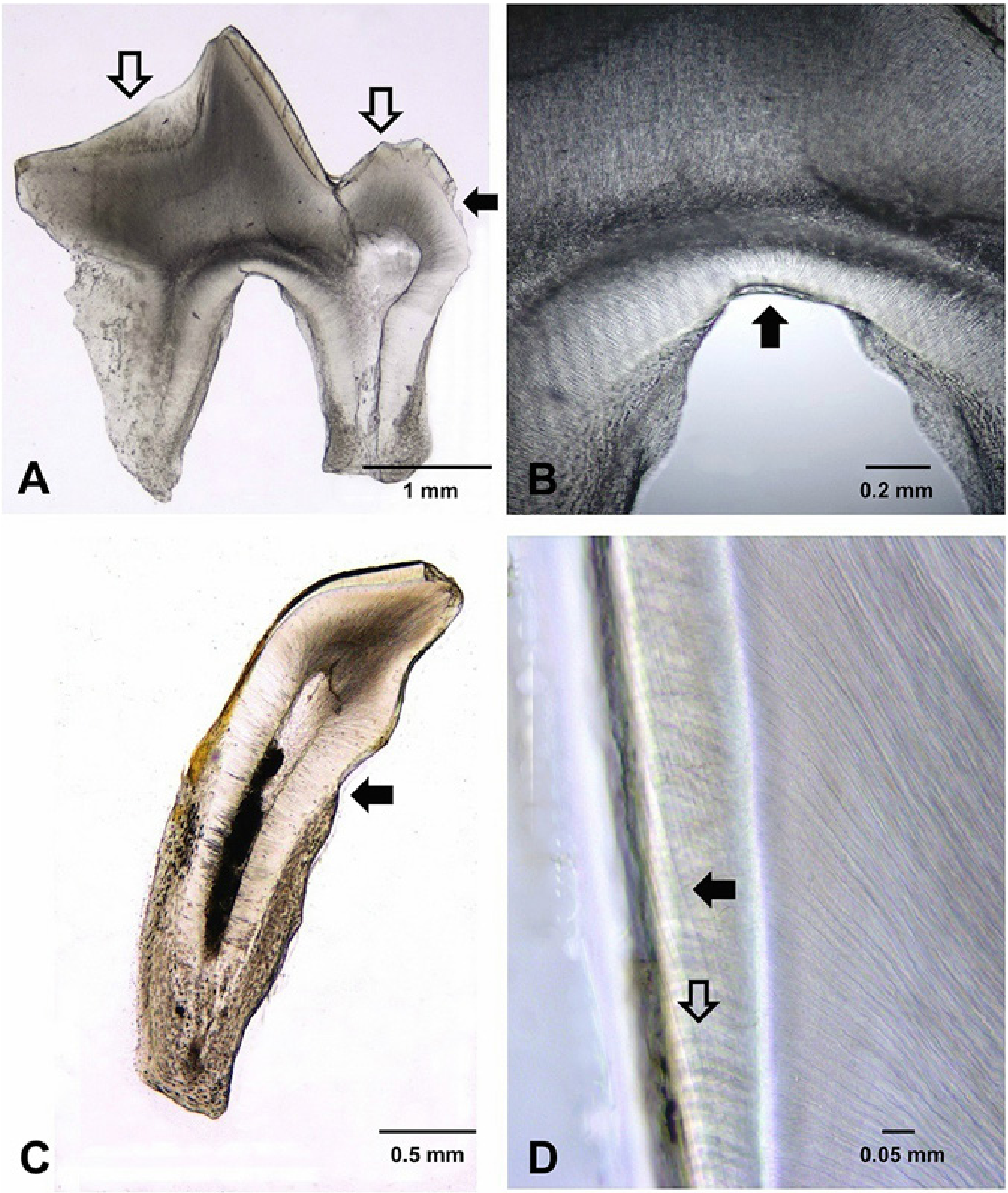

Figure 4. Photomicrograph of the enamel aspects of the *Monodelphis domestica's* teeth. A. Difference in enamel thickness in the occlusal surface (empty arrow) and loss of enamel caused by fracture of m2 (full arrow); B. Presence of enamel at the furcation of m2 (full arrow); C. Enamel-cement junction in I5, showing initiation of enamel and cementum with an edge-to-edge relationship between them (full arrow); D. Longitudinal (full arrow) and transversal striations (empty arrow).

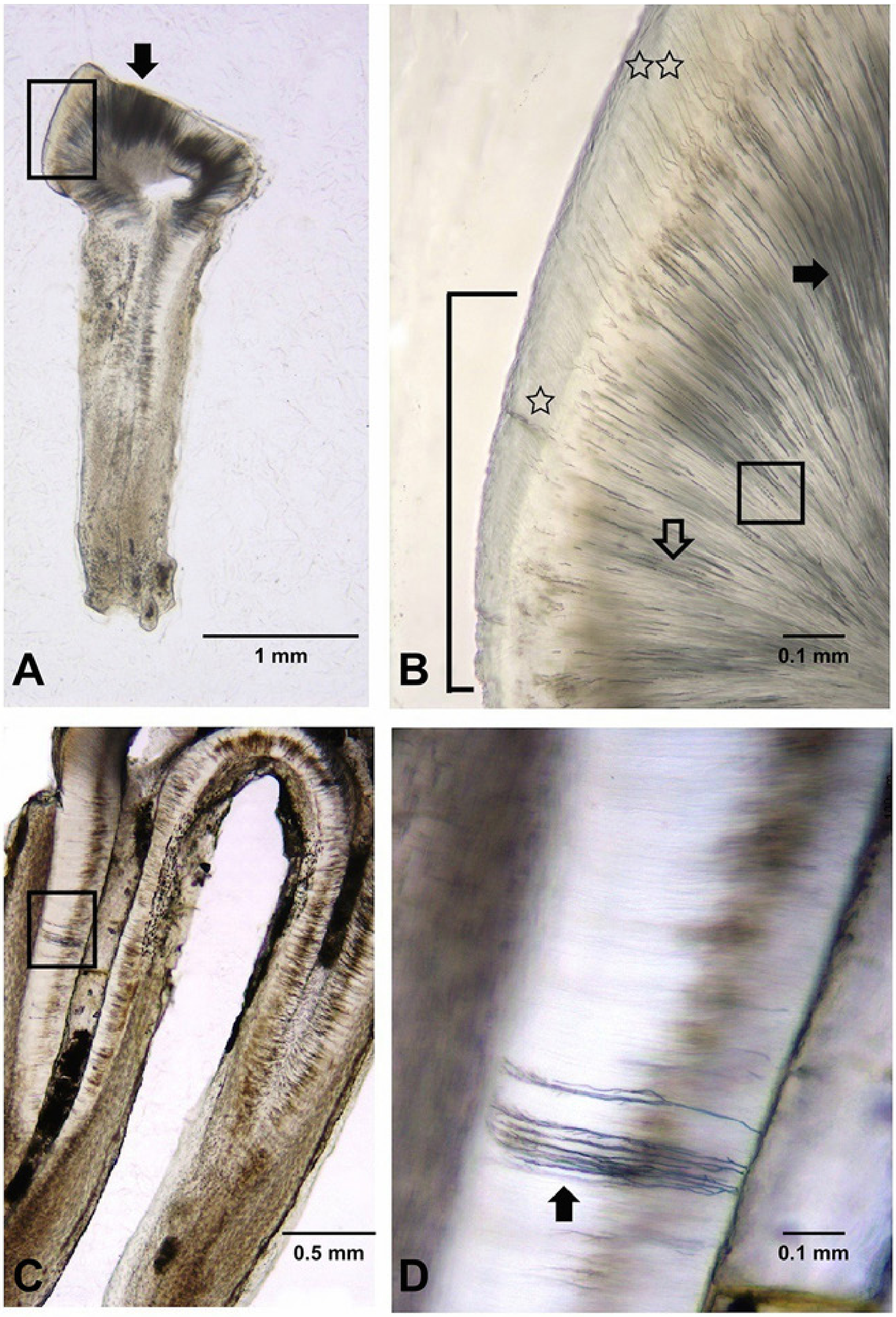

Figure 5. Photomicrograph of enamel and dentin in the crown and root of the *Monodelphis domestica's* teeth. A. Featured area of M2 (empty square) showing tratus mortus in the dentin beneath the vertex occlusal projection (full arrow); B. Lamellae in the enamel (star), ramification of dentinal tubules near the enamel-dentin junction, continuity of tubules through the enamel-dentin junction (two stars), absence of tubular ramification in the cervical third (brackets) and tubules with linear and longitudinal directions, considering the tooth's long (full arrow) and transversal (empty arrow) axes. Tubules presenting a segmented aspect (empty square); C. Featured area showing ramification of dentinal tubules in m1 (empty square); D. Greater emphasis to tubular ramification near the root canal in the middle third and near the cementum-dentin junction (full arrow).

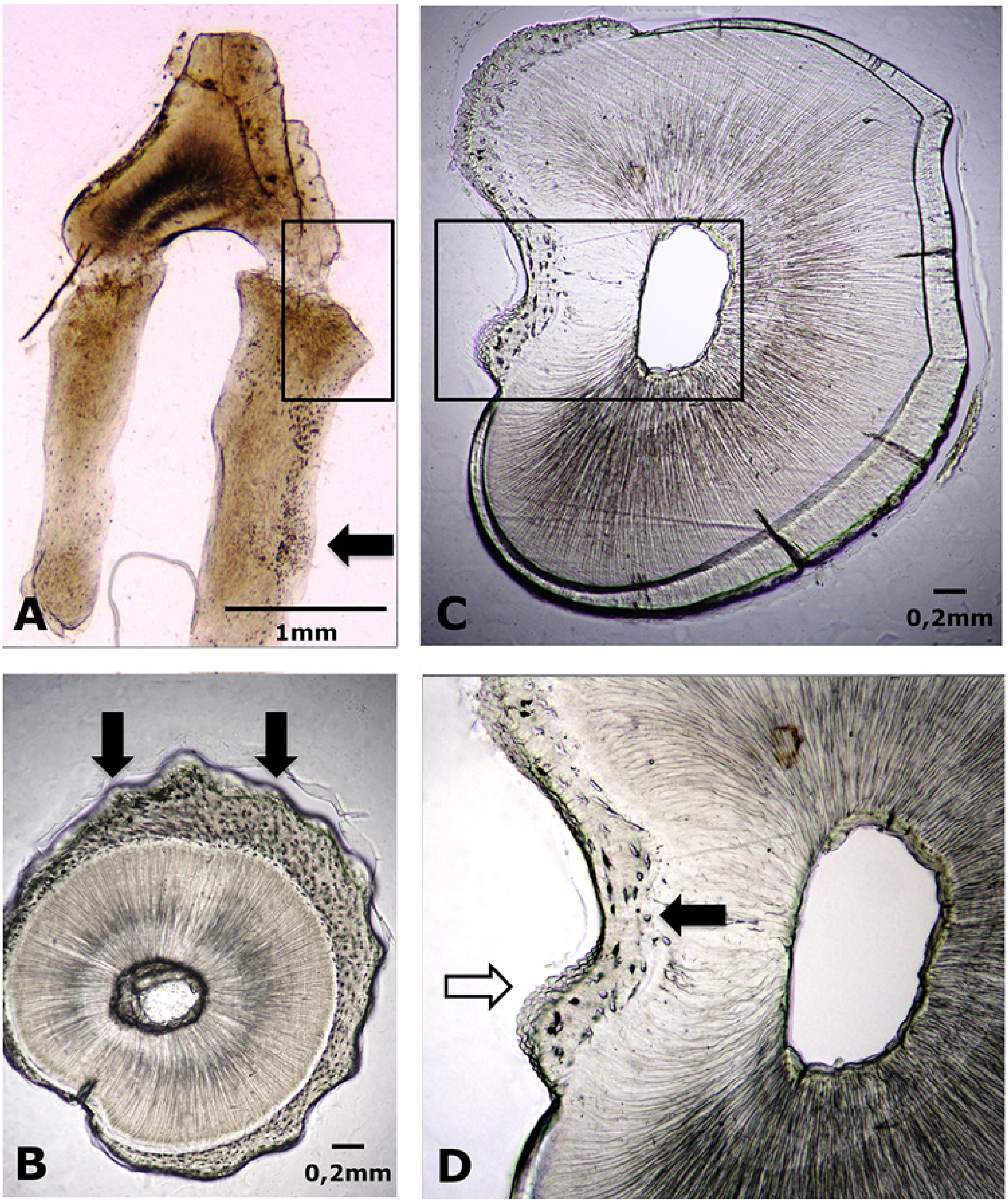

Figure 6. Photomicrograph of the cementum aspects of the *Monodelphis domestica's* teeth. A. Emphasis to the shoulder-like deposition of cervical cementum in P2 (empty square) and stick-like shape of the roots (full arrow); B. Irregularities in the cellular cementum surface (full arrow) in the middle third of the root of m2; C. Emphasis to root developmental groove (empty square) in the cervical third of the root of p3; D. Irregularity of cementum-dentin junction in the root development groove (full arrow) and presence of surface striations (empty arrow).

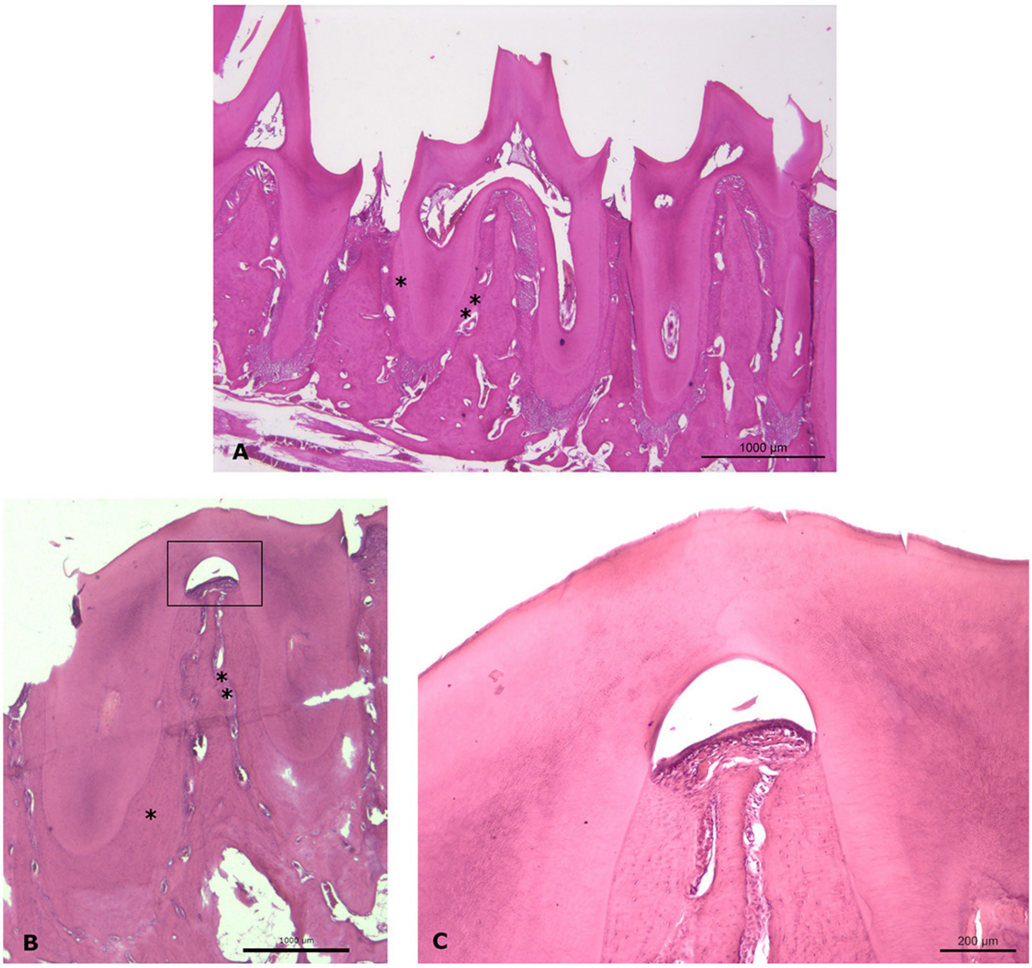

Figure 7. Photomicrograph of the aspects of cementum and periodontal ligament of the *Monodelphis domestica*'s teeth, stained with hematoxylin and eosin (HE). A. Thinner layer of cementum (*) and wider periodontal ligament (**) in posterior teeth of a young adult animal; B. Thicker layer of cementum (*) and narrower periodontal ligament (**) in posterior teeth of an older animal and area of absence of cementum and presence of epithelium at the furcation region (empty square); C. Greater emphasis to epithelium at the furcation region.

Discussion

Monodelphis domestica dentition suggests an evolution in mammalians (Casella, 2011) because it is morphologically diversified and has numerous teeth, which indicates that this omnivorous species relies on different dietary resources (Cifelli, 2016). This is considered an important factor in the diversity of species, as is the case of the tribosphenic molar, a homoplastic acquisition in the modern mammalians (Stern, 1989; Luo, 2007; Schultz & Martin, 2014).

The laboratory opossum has extremely small teeth, presenting a remarkable morphology. The extremely steep occlusal projections or/and irregularities resulting from fractures to enamel, as those observed in the histological sections in the present study, seems to be suitable for a diet (Stern *et al*., 1989, Luo, 2007, Casella, 2011) which ranges from small insects to carcass of small rodents (Casella, 2011). Boyde & Lester (1967) identified enamel tubules inside prisms in well-mineralized areas, which evidences that these tubules cannot be related to hypo mineralization as their presence might make the enamel more susceptible to fractures instead. Tubules were not frequently observed or even absent in the cervical third of the enamel (proximal surfaces), which are not grinding areas supposed to break and create irregularities. This animal's aggressive behavior and mating ritual might explain the fractures to enamel and teeth due to grinding (Macrini, 2004). The crown-root length ratio and lack of long axis inclination seem to be adequate for anchoring this animal's teeth as its diet/behavior demands/results in highly stressful occlusal forces. Teeth with reduced root size and/or inclined long axes; e.g. incisors; were the most frequently lost ones, especially with aging. Intense tooth wear was observed in older animals and, sometimes, in relatively young ones.

The thickness of enamel varied from a thinner layer; e.g. in shearing-crushing areas; to a thicker one; e.g. in shared outer surfaces; as previously described (Stern, 1989). The tubules continuity from dentin to enamel, passing through the enamel-dentin junction, strongly suggests that tubules in the enamel are the continuation of those from the dentin. Presence and distribution of tubules in the enamel showed a similar pattern to that observed in marsupial teeth, as described by Tomes (1849). Odontoblastic processes, ameloblastic projections (Sasagawa & Ferguson, 1991) and Tomes' processes (Luo, 2007) were previously described inside the enamel tubules in marsupials.

In this study, dark oblique lines in the enamel were observed in abraded longitudinal sections, extending from the enamel-dentin junction towards the tooth surface. These features are observed in human enamel and are related to the enamel prism arrangement (Nanci, 2018), which prevents the enamel from cracking (Stern, 1989).

The ramifications of dentine tubules were found to be profuse, beginning in the mantle dentin, especially in the root, and continuing through the enamel, with exception of the cervical third area. A previous study investigating *Didelphis virginiana* identified numerous ramifications of dentin tubules on their course towards enamel and break up into several branches nearby it (Tomes, 1949). Below the vertex occlusal projections, one could identify *tratus mortus*, a morphological feature related to the presence of air in empty tubules as a result of shrinkage of odontoblastic processes or of cell death (Nanci, 2018), which seemed to be related to the animal aging.

A peculiar deposition of cellular stratified cementum was identified, with an abrupt beginning and giving a shoulder appearance to the cervical area and a stick-like shape to the roots. It is different from human teeth, where cellular cement is mostly confined to the apical third (Nanci, 2018) and root developmental groove (Ennes & Lara, 2004), becoming thicker with age (Nanci, 2018; Bosshardt & Selvig, 2000) but without altering the conic shape of the root. Increase in the cementum lines or annuli (Swindler, 2002) and presence of thickness could also be related to aging.

Periodontal ligament of *Monodelphis domestica* showed width variation in the same tooth, and narrowing related to aging. The width variation can also be observed in human teeth (Nanci 2018), but not the narrowing can be related to aging.

The dentition of *Monodelphis domestica* has a striking and complex morphology, especially in posterior teeth, where there are numerous steep projections on the occlusal surface circled by smaller ones. The enamel has variable thickness and irregular surfaces due to fractures. There is a remarkable low crown-root length ratio in these teeth. All these features seem to be linked to the evolution of a dentition for dietary diversification.

Dental tissues present peculiar aspects, such as presence of enamel and epithelium in the furcation area, presence of tubules through the whole extension of the enamel, profusely ramified dentinal tubules, predominantly cellular cementum with abrupt deposition and a shoulder-like shape in the cervical area, being markedly stratified and thus giving a stick-like form to the roots. The periodontal ligament width becomes narrower with aging. Some of these aspects justify a renewed interest in further investigations.

Conclusions

Some peculiarities of the *Monodelphis domestica* dentition have been first described, such as cementum thickness, presence of enamel and epithelium at the furcation, narrowing of periodontal ligament width related to aging and low crown-root length ratio.

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Received: November 10, 2018
Accepted: May 7, 2019

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