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Evolutionary tinkering of the mandibular canal linked to convergent regression of teeth in placental mammals

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SUMMARY

Loss or reduction of teeth has occurred independently in all major clades of mammals [1]. This process is associated with specialized diets, such as myrmecophagy and filter feeding [2,3], and led to an extensive rearrangement of the mandibular anatomy. The mandibular canal enables lower jaw innervation through the passage of the inferior alveolar nerve (IAN) [4,5]. In order to innervate teeth, the IAN projects ascending branches directly through tooth roots [6,5], bone trabeculae [6], or bone canaliculi (i.e. dorsal canaliculi) [7]. Here, we used micro-computed tomography (µ-CT) scans of mandibles, from eight myrmecophagous species with reduced dentition and 21 non-myrmecophages, to investigate the evolutionary fate of dental innervation structures following convergent tooth regression in mammals. Our observations provide strong evidence for a link between the presence of tooth loci and the development of dorsal canaliculi. Interestingly, toothless anteaters present dorsal canaliculi and preserve intact tooth innervation while equally toothless pangolins do not. We show that the internal mandibular morphology of anteaters has a closer resemblance to that of baleen whales [7] than to pangolins. This is despite masticatory apparatus resemblances that have made anteaters and pangolins a textbook example of convergent evolution. Our results suggest that early tooth loci innervation [8] is required for maintaining the dorsal innervation of the mandible and underlines the dorsal canaliculi sensorial role in the context of mediolateral mandibular movements. This study presents a unique example of convergent redeployment of the tooth developmental pathway to a strictly sensorial function following tooth regression in anteaters and baleen whales.

Keywords

Mandibular canal; edentulous; Convergence; Mammals; Homology.
Results

Evolution of dorsal canaliculi after tooth regression

Three-dimensional (3D) models of the mandibles, teeth, and mandibular canals of 26 species were investigated (Figure 1, S1-2). Detailed anatomical descriptions of the mandibular canal in each myrmecophagous species and sister taxa are provided as Supplemental Information (Figures S1, Data S1). A summary description of additional species used to reconstruct the ancestral condition of placental mammals is provided as Supplemental Information (Figure S2, Data S1). For cetacean comparisons, we used a dataset that was recently published by Peredo et al. [7]. All toothed mammals get teeth innervated and vascularized, but this innervation/vascularization only occasionally happens through dorsal canaliculi. These canaliculi correspond to narrow tubular channels that connect the mandibular canal to tooth alveoli (Figure 2E and F). The teeth of the giant otter shrew (Potamogale velox), the aardwolf (Proteles cristatus) and the dog (Canis lupus) are rooted in close contact with the mandibular canal, with alveoli often surrounded by trabecular bone (Figures 1, S1,I,J and N). The investigated additional species revealed similar patterns with either deeply rooted teeth or trabeculae surrounding the alveoli (or both; See SI; Figure S2). These species all lack dorsal canaliculi, even for dorsally implanted teeth (e.g., aardwolf molars) (Figure S1I). Dorsal canaliculi were present in three (Pilosa, Cingulata and Tubulidentata) out of the 18 mammals orders sampled (including one marsupial). Ancestral reconstruction unambiguously showed that the absence of dorsal canaliculi likely represents the ancestral condition in placental mammals where alveolar branches (IAN and inferior alveolar artery, IAA) pass through the trabecular structure of the bone or directly through tooth roots. In contrast, armadillos (Dasypus novemcinctus, D. pilosus, and Priodontes maximus), sloths (Bradypus tridactylus and Choloepus hoffmanni), and the aardvark (Orycteropus afer), present dorsal canaliculi (Figures 1, 2E,F, 3C,D, S1, S4), the so-called “dorsal branches” as previously described in baleen whales [7], whose dorsoventral length...
increases as the tooth is implanted further from the mandibular canal (Figure 2E and F). Most of these dorsal canaliculi are located in the anterior part of the mandible where teeth are reduced or simply missing. In the nine-banded armadillo, we observed one anterior dorsal canaliculus that divides to open in four dorsal foramina (Figures 1, S1F), while in the hairy long-nosed armadillo we observed a dorsal canaliculus that divides in a plexus of three branches (Figures 1, S1G). In the aardvark, three free dorsal canaliculi split and open in six dorsal foramina (Figures 1, S1M). Intraspecific variation was detected and described (Figure S4; see SI), but the presence and pattern of dorsal canaliculi is always consistent among specimens. Ontogenetic variation was studied in two species of sloths in which fetuses display dorsal canaliculi associated with vestigial teeth loci (Figure 3A,B). These dorsal canaliculi are still present in adults of both species despite the resorption of vestigial teeth (Figure 3C,D).

Surprisingly, toothless species (anteaters and pangolins) display contrasted mandibular canal morphologies (Figures 1, 2). All three investigated anteater species present dorsal canaliculi that open in small foramina (Figures 3A-C, S1A-C). These foramina are placed along the anterior flattened dorsal margin of the mandible, which defines the dental pad, with only limited intraspecific and bilateral variations among specimens of the three species (Figure S4). Ontogenetic variation is equally limited, with similar patterns of dorsal canaliculi being observed in both adults and juvenile giant anteaters (M. tridactyla, Figure S4L, M and N) and nine-banded armadillos (D. novemcinctus, Figure S4A-F). Conversely, pangolins lack dorsal canaliculi (Figure 3D), with only several minute canaliculi that are parallel to the mandibular canal, both dorsally and ventrally, but rarely connected to it (Figure 1). Foramina associated to these parallel canaliculi are scarce, invisible to the naked eye, and only occasionally open dorsally to the mandibular canal.

Histological evidence for the passage of nerves and blood vessels in dorsal canaliculi
Three-dimensional analyses were complemented by histological series, which enabled to identify internal soft structures associated to the dorsal canaliculi. The LFB (see Method details in STAR methods) stained histological slices of the collared anteater (*T. tetradactyla*) mandible enabled us to describe the soft tissues encapsulated in the mandibular canal (Figure 4). We observed dorsal canaliculi that allow for the passage of an ascending branch of the inferior alveolar nerve (IANab; Figure 4C,C’), of the inferior alveolar artery (IAAab; Figure 4C), and of the inferior alveolar vein (IAVab; Figure 4B’). In *T. tetradactyla*, a keratinous dental pad (pa) covers the dorsal part of the mandible (Figure 4C). Ventrally, the epidermis (ep) consists of a small layer (Figure 4C), which lies dorsally to a thick dermis layer (de; Figure 4C). In addition to connective tissue, this dermis layer presents small blood vessels and nerve branches.

Histological slices of bowhead whale [9] (Figure S3) show a similar structuration of soft tissues, with accessory branches and large IAN and IAA that most likely connect the mandibular canal to the vestigial tooth alveoli through dorsal canaliculi. The histological section [9] suggests a pronounced anterior projection of the dorsal canaliculi, similar to the pattern for the IAN ascending branches in odontocetes [4].

**Discussion**

**Shadows of regressed tooth buds in dorsal canaliculi**

Enamelless sloths, armadillos, and aardvarks all present dorsal canaliculi that are associated with either tooth alveoli or vestigial tooth loci in the anterior part of the mandible. The corresponding nervous and vascular ascending branches should then be considered homologous to alveolar branches. If the number and shape of alveolar branches can vary [6,10], especially in terminal bifurcations, each main alveolar branch usually corresponds to a single tooth root [6]. Establishing the direct homology between ascending and alveolar branches in anteaters is hindered by the absence of teeth. However, their closest relatives, sloths and armadillos, as well as the aardvark, also display some anterior dorsal canaliculi with no apparent
connection to tooth alveoli (Figures 1, 2, S1, S2). Long-nosed armadillos present dorsal
canalculi in the anterior part of the mandible and the anterior most alveoli, while sloths present
minute dorsal canalculi in cheek teeth. This suggests that dorsal canalculi likely evolved
concomitantly with tooth simplification in cingulatans and pilosans. All these species have been
shown to display vestigial tooth buds in the anterior part of the mandible during pre-natal
development [11–13]. The observed pattern of anterior dorsal canalculi in long-nosed armadillos
(Dasypus) and the aardvark roughly matches the distribution of previously described vestigial
teeth [12,14–16]. In nine-banded armadillos, we found dorsal canalculi that consistently split into
three to six distinct dorsal foramina in the anterior part of the mandibles (Figure 1, S1F,S4A-F),
while Martin [12] identified five to six vestigial incisors. This difference is not surprising given the
frequent dental formula variation observed in nine-banded armadillos [17]. The observed
variation in dorsal canalculi count of anteaters and nine-banded armadillos (Figures 1, S4) was
to be expected as the number and shape of alveolar branches vary in humans [6,10], both at the
intraspecific and bilateral levels. This variation also matches the variation observed in tooth
count of placentals showing a reduced dentition such as the aardvark [13], long-nosed
armadillos [17] and the giant armadillo [18]. Such bilateral variation in tooth number might result
from the lack of stabilizing selection due to an absence of strict occlusion, as hypothesized for
mysticetes [9]. In the aardvark [15], the lower milk dentition is normally composed of eight to ten
teeth, with the second generation of teeth varying from five to eight in number depending on the
presence of vestigial anterior premolars and canines [15,16]. We found dorsal canalculi that
could correspond to two-four anterior premolars, one canine and three incisors (Figure S1M).
This number coincides with the deciduous dental formula of the aardvark [13]. Our observations
therefore provide convincing evidence for a link between the presence of teeth, vestigial or not,
and the development of dorsal canalculi.

This developmental link was corroborated by the study of ontogenetic series of both extant
sloth genera. Comparisons between sloth pre- and post-natal stages allowed us to directly
associate one long anterior dorsal canaliculus to a vestigial tooth locus (Figure 3), which is resorbed during development and is absent in adults. Based on histological sections (Figure 4), we showed that ascending branches of the IAN and IAA pass through dorsal canaliculi in the collared anteater (*T. tetradactyla*). Teeth innervation was likely retained after tooth resorption in all pilosans, and this could extend to all xenarthrans if we consider the anterior dorsal canaliculi of armadillos as representative of vestigial tooth loci. Wadu et al. [6] showed that human tooth nerve bundles can also be retained – although slightly reduced – after tooth loss induced by senescence. Our observations of histological sections of toothless whale fetuses (see [9] and SI, Figure S3) suggest that vestigial tooth loci are associated to IAN and IAA ascending branches during development, a situation that mirrors the condition observed in toothed cetaceans [4,5].

Ridgway et al. [4] described an elongation of the ascending branches of the inferior alveolar nerve in dolphins, while an anterodorsal inclination was also reported for the dorsal canaliculi of mysticetes [7]. However, the homology between alveolar branches and ascending branches carried by dorsal canaliculi was recently challenged for whales [7]. Instead, Peredo et al. [7] proposed that dorsal canaliculi and associated foramina constitute a derived character of crown Mysticeti since no internal evidence of such a structure was visible in other edentulous taxa [7].

Our results clearly contradict this assertion, as we showed that anteaters display both foramina and dorsal canaliculi. In our view, the ascending branches of IAN and IAA should be considered as homologous to alveolar branches since they include identical structures and are linked to the development of teeth. This implies that dorsal canaliculi evolved convergently in xenarthrans, aardvarks, and baleen whales following tooth reduction.

Unlike the other toothless species investigated, pangolins do not present dorsal canaliculi in their mandibles. The small parallel canaliculi (Figure S1D,E) present a distinct shape and topology hindering a hypothetical homology with the dorsal canaliculi. Tims [19] reported the presence of 13 to 14 tooth rudiments in the mandible of *M. javanica*, which he also compared to hair follicles. However, the observed number of up to four tooth vestiges per coronal slice [19]
seems inconsistent with the position of the teeth along an anteroposterior axis. Unless *M. javanica* presents four tooth generations, no more than two tooth buds should be expected for each coronal slice [9,20]. In this context, the 13-14 teeth reported by Tims (1908) should be considered with caution and might correspond to distinct structures. The lack of tooth buds [21] might therefore explain the absence of dorsal canaliculi. Although an early dental lamina may be present in pangolin embryos [14,19], its development appears to be drastically reduced when compared to anteaters [21]. On the other hand, the lack of these structures in pangolins could be explained by phylogenetic constraints. Since dorsal canaliculi are also absent in carnivorans (Figures 1, S1I,J), their absence might represent the ancestral state for Ferae (Pholidota + Carnivora). Additionally, complete tooth loss probably happened much earlier in pangolins than in anteaters, since the almost certainly toothed most recent common ancestor (MRCA) of Pilosa (~58 Mya, [22]) is much more recent than the MRCA of Ferae (~80 Mya; [23]). With the oldest fossil pangolin (~45 Mya,[24]) being already toothless, the absence of dorsal canaliculi in pangolins might simply reflect a more ancient tooth loss. Importantly, our study shows that the external resemblances of the mandibles in anteaters and pangolins, which made them a textbook example of convergent evolution, have overshadowed the complex evolution of their internal morphology.

**Functional role of dorsal canaliculi in toothless species**

Previous studies proposed that mammalian teeth might play a sensorial role in detecting a wide array of external stimuli including pressure, proprioception, and percussion [25] in addition to their role for food intake. Our results suggest that the development of dorsal canaliculi might be linked to the presence of tooth loci/vestigial teeth in both anteaters and baleen whales. One puzzling fact however is that the development of vestigial teeth remained preserved for so long during the evolutionary history of these taxa. In sloths, Hautier et al. [11] showed that the mineralization and resorption of the vestigial teeth is an integral part of prenatal dental
development. Given the conservatism of sloth dental formula in the fossil record [26], they proposed that these vestiges were kept for at least 30 million years [22,26], which implies that there is still a strong selective pressure for developing these structures. Such a complex and energetically costly developmental pathway might be the consequence of a strong developmental constraint in preserving the associated innervation and vascularization of the mandible. A similar developmental constraint was proposed for the initial development of a normal eye in blind cavefish [27]. These fish present normal eye development before showing sign of later degeneration. Given that there is no separation between cells giving rise to the retina and to the forebrain at the early stage of the nervous system (neural plate stage), the development of a viable embryo with a well-formed forebrain implies the early development of the eyes [27]. Tooth innervation develops synchronously with tooth development, being controlled by local molecular signals [8]. We therefore argue that tooth development, even in initiation stages, might be required for maintaining the dorsal innervation of the mandible. As a matter of fact, embryos of pygmy anteaters present early tooth development (dental lamina-tooth buds) [21,28].

Histological slices allowed revealing the presence of dorsal projections of the IAN and IAA passing through the dorsal canaliculi in a mandible of the collared anteater. We propose that these structures respectively innervate and vascularize the mandibular keratinous pad that covers most of the dorsal margin of the horizontal ramus (Figure 4C). The oral sensory receptors that project via the IAN (a branch of the trigeminal nerve) may confer a somatosensory role to the mandibular keratinous pad. In fact, the pattern of innervation of this keratinous pad resembles that of bird beak [29]. Both structures display a superficial keratinous layer followed dorsally by epidermis and a large dermis with blood vessels and free nerve endings [29] (Figure 4). Early tooth development (bud stage) was previously reported in anteaters [21]. In birds, the keratinous ramphoteca was proposed to be responsible for the early interruption of odontogenesis at the lamina stage [30]. An early keratinization might be the triggering event for
subsequent odontogenesis disruption during the development of both anteater keratinous pad and bird beak. Interestingly, toothless whales also present keratinous structures (baleens) that develop after odontogenesis interruption [9]. Thewissen et al. [9] argued that tooth development is a *sine qua non* condition to the development of baleens. This is in line with both fossil and molecular evidence suggesting a step-wise transition between teeth and baleen in mysticetes [3]. Ekdale et al. [31] also recently suggested that foramina for baleen vascularization in the upper jaw of toothless whales are likely homologous to tooth alveoli. Similarly, odontogenesis and associated dorsal canaliculi might be a prerequisite for mandibular keratinization in anteaters. Accordingly, the lack of keratinous pad and vestigial teeth might explain the absence of dorsal canaliculi in pangolins.

In rorqual whales, the peculiar distribution of dorsal canaliculi along the mandible was proposed to be related to movement coordination of lower jaws and alignment with the baleen plates during filter feeding [7]. Pyenson et al. [32] argued that nerves passing through the most anterior canaliculus connected the brain to a symphyseal organ/vibrissae system responsible for mandibular motor coordination and prey detection, respectively. Given the absence of external vibrissae in anteaters and the fact that they mainly use olfaction to detect their prey [33], dorsal canaliculi and respective extensions of the IAN are unlikely related to prey detection. However, several studies [34 and references therein] described the synchronization between tongue protrusion and mandible closing during feeding in anteaters (i.e. *Myrmecophaga* and *Tamandua*) as well as in ant-eating echidnas. In these groups, the mandibles rotate medio-laterally, with the oral cavity widening when the tongue is retracted and narrowing when it is protruded, forming a tube-like mouth to serve as physical support [34,35]. This type of integrated movement would require a tactile feedback originating from the dorsal margin of the mandible, which is in contact with the protruding tongue and the upper jaw. A similar hypothesis has been proposed in whales [7] for the coordination between mandibles and baleens during gulping. Tooth pulp stimulation was shown to trigger a response of the digastric muscle in cats [36]. Dong et al. [37] also
showed that cat teeth stimulation results in discharge signatures for different textures (e.g., rough or smooth). Furthermore, teeth are known to respond to non-painful stimuli in humans [38]. We propose that the ascending branches of the IAN might be part of the somatosensory system involved in mechanoreception, which could explain the convergent evolution of dorsal canaliculi in the toothless mandibles of baleen whales and anteaters. In contrast, no medio-lateral rotation of the mandibles was reported in pangolins [35], while the presence of a fused symphysis likely helps maintaining the tube-like shape of the oral cavity while feeding [39]. Compared to anteaters and baleen whales, such a relatively reduced mandibular mobility could potentially explain the absence of dorsal canaliculi in pangolins.

Our results support the hypothesis of convergent exaptation of the dorsal canaliculi in anteaters and baleen whales following tooth regression. We unequivocally showed that the IAN as well as blood vessels branch through the dorsal canaliculi, and argue that keratinous structures and vestigial teeth have a crucial role in mandibular innervation, maintaining the sensorial function associated to the presence of teeth while strong selective pressures induced their loss. Despite the superficial resemblance of the masticatory apparatus between anteaters and pangolins, convergent tooth loss resulted in divergent structures in the internal morphology of their mandible. We propose that these differences likely reflect divergent phylogenetic histories and/or divergent functional constraints. Rewiring of the mandibular canal in anteaters and baleen whales provides a striking example of evolutionary tinkering linked to the regression of teeth.

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**Author contributions**

S.F.-C., L.H., F.D. collected the data, conceived the study, designed the study. S.F.-C., L.H., F.D. contributed to discussion, drafted and edited the manuscript. L.H., F.D. coordinated the study. All authors gave final approval for publication.

**Declaration of Interests**

The authors declare no competing interest.
References


Figure legends

Figure 1. Evolution of dorsal canaliculi linked to tooth regression in 28 placentals and 1
marsupial. Circular timetree (according to Kumar et al. [40]) with corresponding 3D reconstructions of the internal mandibular morphology with dorsal canaliculi (orange), mandibular canal (cyan), mental branches (pink), and teeth (dark blue). Tree branches are colored in orange (presence of dorsal canaliculi) and black (absence of dorsal canaliculi). Animal silhouettes are colored in black (sampled species) and gray (species from [7]).

**Figure 2. Evolution of dorsal canaliculi after tooth regression.** (A) Cyclopes didactylus; (B) Tamandua tetradactyla; (C) Myrmecophaga tridactyla; (D) Manis javanica; (E) Orycteropus afer; (F) Priodontes maximus. Bone is transparent. Dorsal canaliculi – orange; mental branches – purple; mandibular canal – cyan; teeth – dark blue. Scale in mm.

**Figure 3. Ontogenetic evidence of the association of dorsal canaliculi to vestigial tooth alveoli.** 3D models of the mandibles of sloth fetuses (A) Bradypus tridactylus and (B) Choloepus didactylus) and adults (C) B. tridactylus and (D) Choloepus hoffmanni in medial (A, C and D) and lateral (B) views. Bone is transparent. B is a mirrored lateral view for a better perspective of the dorsal canaliculus. Dorsal canaliculi – orange; mental branches – purple; mandibular canal – cyan; teeth – dark blue. Zoomed details of the dorsal canaliculi associated with the vestigial teeth are shown. Scale in mm.

**Figure 4. Histological evidence for the passage of blood vessels and nerves in dorsal canaliculi.** (A) 3D-model of a mandible with a purple dashed rectangle indicating where the sagittal cut was performed; black dashed rectangles indicate the limits of the coronal cut. (B) Coronal cut showing the mandibular canal and a dorsal canaliculus; soft tissues are present dorsally to the mandibular canal, the dorsal canaliculus and the external dorsal, and dorso-
lateral surfaces of the mandible; the red square represents the area of interest to be zoomed in
(B'); the purple dashed line represents the sagittal section performed on the cut portion of the
mandible (A). (C) Sagittal section showing the mandibular canal, two dorsal canaliculi and
associated soft tissues; soft tissues on the dorsal surface of the mandible are identified; the red
square delimits the area zoomed in (C'). (C') An ascending branch of the IAN is present in the
dorsal canaliculus. Abbreviations: bo bone; de dermis; ep epidermis; IAA inferior alveolar artery;
IAAab inferior alveolar artery ascending branch; IAN inferior alveolar nerve; IANa inferior
alveolar nerve accessory branch; IANab inferior alveolar nerve ascending branch; IAV inferior
alveolar vein; IAVab inferior alveolar vein ascending branch; pa keratinous dentary pad.

**Supplemental figure legends**

**Figure S1.** 3D models of fourteen mandibles (five toothless and nine toothed) in lateral
view. Related to Figure 1. (A) Cyclopes didactylus (MNHN 1986-1115); (B) Tamandua
tetradactyla (BMNH 34.9.2.196); (C) Myrmecophaga tridactyla (ISEM – 065 V); (D) Manis
crassicaudata (BMNH_67.4.12.298); (E) Manis javanica (BMNH 9.1.5.858); (F) Dasypus
novemcinctus (USNM 033867); (G) Dasypus pilosus (ZMB 19240); (H) Priodontes maximus
(ZMB 47528); (I) Proteles cristatus (BMNH 34.11.1.5); (J) Canis lupus (LAMC 23010); (K)
Bradygus tridactylus (MNHN 1999-1065); (L) Choloepus hoffmanni (Hautier pers. Coll.); (M)
Orycteropus afer (BMNH 27.2.11.113); (N) Potamogale velox (ZMB 71587). Bone is transparent.
Dorsal canaliculi (numbered) – orange; mental branches (mb) – purple; mandibular canal –
cyan; parallel canaliculi – yellow. Scale in mm.

**Figure S2.** 3D models of 12 toothed mandibles in lateral view. Related to Figure 1. (A)
Dasyurus hallucatus (TMM M-6921); (B) Lemur catta (DPC-O92); (C) Cynocephalus volans
Figure S3. Histological slice of coronal sections of the mandibles of a bowhead whale and a collared anteater. Related to Figure 4. Coronal sections of the mandibles of (A) Balaena mysticetus [9] and (B) a formalin preserved Tamandua tetradactyla (ISEM 778N; LFB stained). For B bone tissue is colored in dark blue and soft tissues stained in different tones of blue and purple. The dark blue colored myelins layers can be observed. Coronal cuts showing the mandibular canal and dorsal canaliculi; soft tissues are present on mandibular canals and dorsal canaliculi. bo bone; IAA inferior alveolar artery; IAAa inferior alveolar artery accessory branch; IAAab inferior alveolar artery ascending branch; IAN inferior alveolar nerve; IANa inferior alveolar nerve accessory branch; IANab inferior alveolar nerve ascending branch; IAV inferior alveolar vein; IAVab inferior alveolar vein ascending branch; vt vestigial tooth (1st generation).

Figure S4. 3D models showing intraspecific variation of dorsal canaliculi. Related to Figures 1 and 2. (A-F) Dasypus novemcinctus (USNM 033867, BMNH 11.10.27.3, LSUMZ 8538, LSUMZ 29160, ZMB 84-357, USNM 020920); (G-H) Cyclopes didactylus (MNHN 1986-1115, BMNH 24.12.4.68); (I-K) Tamandua tetradactyla (BMNH 34.9.2.196, MVZ 153482, ISEM 778N); (L-N) Myrmecophaga tridactyla (ISEM 065V, MVZ 185238, ISEM 071N). Teeth are segmented in (A) only. Bone is transparent. Dorsal canaliculi – orange; mental branches – purple; mandibular canal – cyan; teeth – dark blue. Scale in mm.

Figure S5. Ancestral state reconstruction of the presence/absence of dorsal canaliculi in
28 placental and one marsupial species. Related to Figure 1. Posterior probabilities (%) for the presence of dorsal canaliculi are presented for each node. Presence of dorsal canaliculi – orange; absence of dorsal canaliculi – cyan.

STAR * methods

- Key resources table
- Contact for resource sharing
- Method details
- Data and software availability

Contact for resource sharing

Further information and requests for resources should be directed to, and will be fulfilled by the Lead author Frédéric Delsuc (frederic.delsuc@umontpellier.fr).

Method details

Comparative anatomy
We present an anatomical description of the mandibles and mandibular canals of 26 species (Figure 1) representing the following taxa: Vermilingua (anteaters) – *Cyclopes didactylus*, *Tamandua tetradactyla*, *Myrmecophaga tridactyla* (juvenile + adult); Cingulata (armadillos) – *Dasypus novemcinctus*, *Dasypus pilosus*, *Priodontes maximus*; Folivora (sloths) – *Bradypus tridactylus* (fetus + adult); *Choloepus* spp. (fetus + adult); Tubulidentata (aardvarks) – *Orycteropus afer*; Afrosoricida (tenrecs) – *Potamogale velox*, *Tenrec ecaudatus*; Macroscelidea (elephant shrews) – *Rhynchocyon petersi*; Hyracoidea (hyraxes) – *Procavia capensis*; Pholidota (pangolins) – *Manis crassicaudata*, *Manis javanica*; Carnivora (carnivores) – *Proteles cristatus* and *Canis lupus*; Perissodactyla (odd-toed ungulates) – *Tapirus indicus*; Chiroptera (bats) – *Molossus molossus*; Eulipotyphla (moles and shrews) – *Talpa europaea*; Lagomorpha (hares and rabbits) – *Lepus europaeus*; Rodentia (rodents) – *Rattus norvegicus*; Primates (primates) – *Lemur catta*, Dermoptera (colugs) – *Cynocephalus volans*; Scandentia (tree shrews) – *Tupaia montana*; Dasyuromorphia (carnivorous marsupials) – *Dasyurus hallucatus*. Using this dataset, we were able to compare the morphology of the mandibular canal and dorsal canaliculi of closely related toothed and toothless taxa, as well as a wide range of species belonging to 18 mammalian orders. We additionally compared our results to the previously published research on cetaceans and even-toed ungulates (Cetartiodactyla) by Peredo et al. [7]. We segmented a total of five *D. novemcinctus*, three *M. tridactyla* (two adults + one juvenile), three *T. tetradactyla*, and two *Cy. didactylus* in order to account for intraspecific variation. We also segmented left and right hemi-mandibles of the anteater specimens to assess the level of bilateral variation in dorsal canaliculi count. Both fetus and adult specimens of *B. tridactylus* and *Choloepus* spp. were studied (Figure 3) in order to seek the presence of a dorsal canaliculus linked to the development of vestigial teeth [11] and to confirm that there was no major variation in the number of dorsal canaliculi through ontogeny. Mandibles were chosen to study dental innervation patterns because they are composed by a single bone (dentary) enclosing the IAN and the IAA. The mandibular canal soft tissues enter in a continuous canal posterior to the tooth.
row and project anteriorly to its anterior end. Compared to the cranium, all branching patterns of
the mandibular canal are therefore more easily traceable. The mandible is, therefore, a simpler
model to study innervation patterns with the techniques used for this manuscript.

Acquisition of data

The studied specimens belong to the following collections: Natural History Museum, London
(BMNH); Muséum National d'Histoire Naturelle, Paris (MNHN); Museum für Naturkunde, Berlin
(ZMB); University Museum of Zoology, Cambridge (UMZ); Institut des Sciences de l'Évolution,
Montpellier (ISEM); Museum of Vertebrate Zoology, Berkeley (MVZ); Louisianna State University
Museum of Zoology (LSUMZ); United States National Museum (USNM); L.H. personal
collection. High-resolution microtomography (µCT) was performed at Montpellier Rio Imaging
(MRI; Microtomograph RX EasyTom 150, X-ray source 40-150 kV) platform, Imaging Analysis
Center (BMNH; Nikon Metrology HMX ST 225, X-ray source 225 kV), and the Helmholtz
Zentrum Berlin (HZB, Hamamatsu L8121-3, X-ray source 40-150 kV). The scan resolution
differed according to the size of specimens (Table S1). Eight specimens were obtained from
MorphoSource (www.morphosource.org), one from Digimorph (www.digimorph.org), and one
from the Digital Morphology Museum (www.dmm.pri.kyoto-u.ac.jp). The left hemi-mandibles (the
right one was used if the left one was missing or broken) were reconstructed, with respective
mandibular canals and teeth. Avizo 9.4.0 (Visualization Sciences Group) was used to perform
the 3D reconstructions. When teeth were absent, the mental branches were distinguished from
the dorsal canaliculi by their larger diameter and their lateral projection to the mental foramina on
the lateral aspect of the mandible [5,7]. Dorsal canaliculi were defined as dorsal projections of
the mandibular canal opening on the dorsal surface of the mandible.

Histology
We also dissected the head of an adult specimen of *T. tetradactyla* (ISEM 778N) fixed in formalin. The right mandible was extracted and its associated musculature removed. The mandible was then subjected to decalcification during forty five days, following standard protocols. A portion of the mandible was then cut (as shown in Figure 4A) with the use of a scalpel, and fixed in paraffin. Serial slicing was performed to produce 3µm thick coronal and sagittal sections of the mandible. Luxol Fast Blue (LFB) was used to stain the slices in order to allow for the blue staining of the myeline sheaths insulating nerve cell axons. The LFB staining protocol was composed of several phases (1-11) in which acetic acid (10%), Luxol Fast Blue (0.1%), lithium carbonate (0.05%), and cresyl violet (0.1%) were used. (1) Histological sections were placed in a sealed container with the Luxol Fast Blue solution and were left overnight inside an oven at 58°C; (2) Sections were rinsed with ethanol (96%); (3) Sections were rinsed with distilled water; (4) Sections were differentiated in a lithium carbonate solution during 30 seconds; (5) Sections were placed in a rack with ethanol (70%) and were then stirred manually twice during periods of 15 seconds; (6) Sections were placed in a rack with distilled water and were then stirred manually during one minute; (7) A counterstain was performed by placing the sections in a cresyl violet solution during ten minutes; (8) Samples were quickly rinsed twice with ethanol (96%); (9) Sections were placed on ethanol (100%) for two periods of two minutes each; (10) Sections were placed on xylene (dimethylbenzene) for the same duration as in step (9); (11) Sections were mounted with a resinous medium (Pertex). A histological slice of a coronal section of the mandible of *Balaena mysticetus* (provided by J.G.M. Thewissen [22]) was used for anatomical comparisons (Figure S3A).

**Supplemental item titles**
**Data S1.** Supplemental Text for anatomical descriptions, histological comparison of the anteater and the bowhead whale, intraspecific variation, Vermilingua ancestral tooth pattern, and ancestral state reconstruction of the internal anatomy of the mandible. Related to Results and Discussion.

**Table S1.** Resolution of the performed μ-CT scans and scanning platform information. Related to STAR methods.
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**Software and Algorithms**

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Evolutionary tinkering of the mandibular canal linked to convergent regression of teeth in placental mammals

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Author list footnotes

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Supplemental information:
- 1. Anatomical descriptions
- 2. Homology of dorsal canaliculi between anteaters and baleen whales
- 3. Intraspecific and ontogenetic variation of dorsal canaliculi number
- 4. Insights into the ancestral number of teeth in anteaters
- 5. Ancestral state estimation of the tree internal nodes
1. Anatomical descriptions of myrmecophagous mammals and sister taxa

Pygmy anteater (Cyclopes didactylus)

The mandible of *Cyclopes didactylus* is low and slender. In lateral view, the horizontal ramus is thin, elongated and is highly curved with a ventral concavity, which makes it distinctive from all other anteaters. The ascending ramus is extremely inclined laterally, with the hook-like coronoid process projecting laterally. The mandible presents seven mental foramina (Figure S1A). We here designate the flattened part of the horizontal ramus of the mandible as the dentary pad. This dentary pad extends from the most anterior tip of the mandible to the posterior end of the coronoid process. This pad displays several dorsal foramina with foramina for dorsal canaliculi 9 to 15 forming a continuous groove. Nineteen foramina are present on the left dentary pad, 20 on the right mandible.

The mandibular canal displays a straight trajectory until it reaches the dentary pad, from where it curves ventrally, accompanying the shape of the mandible. The mandible has an elliptic-like section, and the major axis rotates, in anterior direction, from an almost transversal plane parallel position to a coronal one. The mandibular canal is positioned dorsally. The size of the mandibular canal remains constant along the horizontal plane, and decreases towards the symphysis. The mandibular canal presents several dorsal canaliculi (dc), which connect dorsomedially with the opening foramina. In total, there are fifteen dorsal (eighteen on the right mandible) and seven mental branches. Some of the dorsal canaliculi bifurcate and vary in diameter, with dc14 and dc15 being the largest (Figure S1A). The first four dorsal canaliculi (dc1 to dc4) are
anterodorsally oriented, as well as dc12, dc13 and the anterior projections of the bifurcating canaliculi. The mental branches also vary in diameter, with the most anterior one (mb1) being, by far, the largest, but no specific trend observed.

Collared anteater (*Tamandua tetradactyla*)

In *Tamandua tetradactyla* the edentulous horizontal ramus is long, slightly curved ventrally, and gets gradually shallower postero-anteriorly. The dentary pad is more anterior, narrower in its most posterior part, and not as medially inclined as in *C. didactylus* (*Figure S1B*); it is perforated by 17 foramina (*Figure S1B, dc 1-15*); a small and shallow groove connects the most posterior ones. Only one anterior mental foramen is present (*Figure S1B, mb1*). The ascending ramus is rather shallow and presents incipient coronoid and angular processes; the condyloid process is posteriorly projected.

The mandibular foramen (mb1, *Figure S1B*) is parallel to the mandibular canal. The mandibular canal opens on the medial surface of the ascending ramus and projects anteriorly with a slight ventral inclination. It projects dorsally from the posterior edge of the dentary pad, as is typical of toothed mammals after tooth loss [S1, S2]. Similarly to *C. didactylus*, dorsal canaliculi are present. Each quadrant presents 15 dorsal canaliculi; only dc1 and dc15 are bifurcated (*Figure S1B*). The canals of the dorsal canaliculi are relatively narrower than those observed in *C. didactylus*. Although the most anterior canals (dc1 to dc6) are slightly anteriorly oriented, this trend is not present on the most posterior ones (dc7 to dc14), which display a more random inclination. The most posterior canaliculus (dc15) is posteriorly inclined. Dorsal canaliculi dc1 and dc2 are spatially and morphologically distinct from the remaining (*Figure S1B*), and highly anteriorly inclined. Dc1 is extremely elongated and splits into two parts anteriorly.
Giant anteater (*Myrmecophaga tridactyla*)

The mandible of the adult *Myrmecophaga tridactyla* is, in many ways, similar to that of *T. tetradactyla*. It is larger in size but presents the same general morphology, with the body being elongated, transversely compressed and lacking teeth, and the ascending ramus being extremely simplified when compared to toothed xenarthrans. As in *T. tetradactyla*, the anterior part of the mandible is slightly curved, with the anterior tip tapering ventrally.

A dentary pad occupies more than half of the total length of the jaw (Figure S1C), which is narrow posteriorly. Sixteen foramina are present on the dentary pad, and correspond to the dorsal opening of the dorsal canaliculi. Two mental foramina are present in the anterior part of the mandible, with the most anterior one (mb1, Figure S1C) opening laterally at the level of dc4 and the second one (mb2, Figure S1C), much smaller, opening at the level of dc5.

The mandibular canal opens in the medial surface of the ascending ramus, just dorsally to the position of the coronoid process, with an aperture with a long antero-posterior axis. A groove runs anteriorly along the canal. The latter gradually descends along the lengthy horizontal ramus, until it reaches the level of the posterior margin of the dentary pad (Figure S1C). It then projects dorsally to reach the level of dc5, from where it maintains its position in the mandible until it opens to the mental foramen (mb1), anteriorly. Along this path, thirteen main dorsal canaliculi open dorsally in the dentary pad. Dc13, the longest and most posterior dorsal canaliculus projects postero-dorsally. Dc12 to dc6 project dorsally, with some dorsal canaliculi displaying a simple column shaped, while others bifurcate dorsally. Dc5 is anteriorly inclined and has a much reduced diameter. Close to the symphysis, the first five dorsal canaliculi (dc1-5) form a distinct complex of long and anteriorly inclined canaliculi, similar to *T. tetradactyla*. In summary, the morphology of the mandibular canal of *M. tridactyla* is similar to *T.*
tetradactyla, except for the dorsoposteriorly oriented dorsal canaliculus (i.e. 13), and for
the larger number of dorsal canaliculi on the anterior complex.

A juvenile specimen (ISEM - 071 N) was also analysed to check on the number
of dorsal canaliculi. There are 16 dorsal canaliculi on the left side and 18 dorsal
canaliculi on the right hemi-mandible. The posterior part of the mandibular canal is not
well ossified and the division between branches is less clear. The posteriormost dorsal
canaliculus is long and posteriorly projecting and does not reach the top of the mandible.

Indian pangolin (Manis crassicaudata)

The mandible of Manis crassicaudata is greatly compressed transversely and shallow.
The ascending branch lack a true coronoid process as consists of a slightly dorsally
inclined posterior knob-like projection. Three mental foramina (Figure S1D, mb1-3) are
present on the anterior part of the horizontal ramus.

The mandibular foramen is antero-posteriorly elongated and has an anterior
position in the ascending ramus. The mandibular canal projects anteriorly in a straight
trajectory, near the dorsal margin of the mandible. From about the middle of the
horizontal ramus the mandibular canal trajectory accompanies the dorsal edge
mandible. This contrasts with anteaters, in which the mandibular canal position is ventral
until it drifts dorsally at the posterior part of the dentary pad. The transversal section of
the canal varies between a circular and an oval shape. From its most posterior part, the
canal displays several small canaliculi that depart from the main mandibular canal,
parallel to it. These parallel canaliculi are present around the mandibular canal, along its
entire length, both dorsally and ventrally. Their density is higher on the anterior half of
the horizontal ramus and the posterior part of the ascending ramus. On the anterior part
of the mandible, the dorso-laterally projecting pseudo-tooth displays a small foramen for
a parallel canaliculus and the very anterior tip of the horizontal ramus presents two of
these canaliculi. Most parallel canaliculi show no connection to the mandibular canal and only a few open in microscopic foramina (Figure S1D). The most posterior mental branch (Mb3) is short and opens in an elongated foramen just before it bifurcates. The remaining two mental foramina (associated to mb2 and mb3) open laterally close to the sharp anterior tip of the mandible. The small mb2 splits posteriorly to the pseudo-tooth while the large and elongated mb1 drifts laterally at the level of this prong.

**Malayan pangolin (Manis javanica)**

The mandible in *Manis javanica* is shallower than in *M. crassicaudata*. It presents five anterior mental foramina (Figure S1E, mb1-5), with the second most anterior (mb2) being clearly larger than the remaining ones. Between the posterior end of the symphysis and the pseudo-tooth, two small circular foramina open medially (Figure S1E).

The mandibular foramen in *M. javanica* differs from *M. crassicaudata*; it displays an elliptical shape with an extremely elongated antero-posterior axis. The dorsal and ventral margins of the foramen fade posteriorly and its internal surface becomes continuous with the medial surface of the ascending ramus.

Similar to its sister taxon, *M. javanica* displays a mandibular canal that presents a rather homogeneous shape and position along its path. Posteriorly, it slightly descends accompanying the inclination of the mandibular foramen and follows the general shape of the dorsal margin of the horizontal ramus until it reaches the mental foramina. The transversal section of the mandibular canal becomes slightly dorso-ventrally compressed at mid-length, but immediately assumes its former shape anteriorly. As described for *M. crassicaudata*, several parallel canals are present along the entire length of the mandible. The density of these small canals is particularly high in the anterior portion of the mandible as well as in the posterior part, at the level of the mandibular foramen.
Their morphology is in all aspects similar to the structures observed in *M. crassicaudata*, including the presence of a couple of vertical canaliculi that project dorsally and open through two small foramina in the pseudo-tooth, as well as the presence of a couple of canaliculi that reach the mandible surface in all directions. The mandible presents five mental branches: the three more posterior ones (Figure S1E, mb3-5) are short and small, and open laterally in small elliptical foramina posterior to the pseudo-tooth; the largest mental branch (Figure S1E, mb2) drifts antero-laterally to form a short branch at the level of the prong, opening in a large diamond shaped foramen; the most anterior mental branch (Figure S1E, mb1) represents the last remaining part of the main mandibular canal that projects anteriorly and opens just ahead of mb2 in an low and antero-posteriorly elongated foramen anterior to the pseudo-tooth.

**Nine-banded armadillo (Dasypus novemcinctus)**

*Dasypus novemcinctus* displays a thin, low and elongated horizontal ramus and a slightly broader ascending ramus characterized by its high and slender coronoid process. Anteriorly, the mandible is extremely low and transversely compressed, and displays a short symphysis. It is higher posteriorly and becomes transversely wider at the level of the tooth row. The dental formula is c 1, p 7, m 1. The anterior quarter of the mandible is completely toothless but an alveolus for a canine [S3,S4] is visible at the level of the first mandibular foramen (Figure S1F, mb1), and is separated from the rest of the tooth row by a small diastema. The molar is not erupted. This specimen displays two mental foramina: the most posterior one (mb2) is located between the second and the third premolars while the anterior one (mb1) opens anteriorly to p 1. Below the anterior edge of the coronoid process, a slightly elongated mandibular foramen opens on the medial surface of the ascending ramus, at roughly the same height of the alveoli. It projects ventrally with an inclination of around 14° until the
The canal describes a shallow dorsal facing concavity contouring the alveoli ventrally and accompanying the curvature of the ventral edge of the horizontal ramus. Immediately anteriorly to the third premolar, a slightly antero-dorsally inclined posterior mental foramen is present on the medial side of the mandible. Anteriorly to the first molar, the main branch of the mandibular canal opens laterally in an antero-posteriorly elongated mental foramen. This foramen is particularly large and elongated. Anteriorly to the first molar, a dorsal canaliculus with a rather large diameter is also oriented antero-dorsally in the direction of the alveolus for the canine. The canaliculus opens in the alveolus and continues anteriorly – with less than half the diameter – to split into two ramifications. The most posterior ramification bifurcates just dorsally to mental foramen 1 and opens into two small foramina (Figure S1F, dc 1c,d) on the dorsal margin of the mandible. The anterior ramification (dc 1a,b) describes a long trajectory antero-ventrally to dc 1c,d, and bifurcates near the anterior tip of the mandible. All bifurcation of the dorsal canaliculus (dc 1a,b,c,d) open into tiny foramina on the dorsal side of the mandible.

In a juvenile specimen (USNM 020920), the canal does not open directly into the first five premolars and the canine. Therefore, the juvenile mandible displays longer dorsal canaliculi for the premolar dentition than the adults. A large dorsal canaliculus is associated to the first molar, which is absent. Anterior to the canine, a dorsal canaliculus projects anterodorsally, splitting in a plexus of three smaller canaliculi that open dorsally in three individual foramina. The dorsal canaliculi pattern is similar to the one observed in adults.

**Hairy long-nosed armadillo (Dasypus pilosus)**

The anterior part of the slender mandible is sharp and its height slightly increases at mid-length of the horizontal ramus. A row of rather spaced peg-like teeth occupies the
second third of the mandible. *D. pilosus* presents a c 1, p 7, m 1 lower dental formula.

The c1 and m1 present a basic peg-like shape. The anterior four mental foramina are positioned between the p 3 and the margin of the most anterior and largest mental foramen (Figure S1G, mb1), at the level of the fused symphysis. The most posterior and smallest of the five mental foramina is located posterior to the m 1 alveolus.

The symphysis is elongated, reaching the level of the anterior margin of mb1. The mandibular canal is ventrally positioned at the level of the alveolar portion of the mandible. The canal opens posteriorly in a high mandibular foramen and extends ventrally towards the centre of the tooth row at the level of the fourth premolar. From this point it projects slightly dorsally, accompanying the shape of the mandible, until the level of mental foramen 3. Anterior to mb3, the mandibular canal presents a straight trajectory. Immediately dorsal to the mandibular canal, the horizontal ramus displays a small canal that originates in the p 6 alveolus and passes through all alveoli until p 2. From this point it projects antero-dorsally, as a dorsal canaliculus, to reach the p 1 alveolus. From this alveolus it projects further antero-dorsally to the c 1 alveolus and extends in the same direction until the level of third mental foramen. This dorsal canaliculus presents a bifurcation anterior to c 1 (Figure S1G). A second dorsal canaliculus arises at the level of mental foramen 3, bifurcating and ending just anteriorly. Neither of these canaliculi reaches the dorsal margin of the mandible. No foramina open in the anterior part of the mandible, close to the symphysis.

**Giant armadillo (*Priodontes maximus*)**

The mandible is long and displays a long tooth row extending for about half its total length. Similarly to *D. novemcinctus* and *D. pilosus*, the mandible is narrow, transversely compressed, although it is slightly more robust. As in anteaters, this mandible presents a medio-lateral rotation anteriorly. The left tooth row is composed of 22 hypselodont teeth,
10 23 on its right counterpart. In lateral view, three mental foramina (Figure S1H, mb 1-3) 
24 can be observed just anterior the tooth row. A fourth small one is present in the same 
25 area, but appears to be an extra branching of mental foramen (mb1) with no symmetrical 
26 branch on the right hemi-mandible. 
27 The transversely compressed mandibular canal of *P. maximus* exhibits some 
28 differences compared to the other genus of armadillo here studied. The mandibular 
29 foramen is dorsoventrally elongated as in *Dasypus* but its position is more dorsal in the 
30 ramus when compared to the alveolar plane. The canal extends ventrally with a weak 
31 inclination (~12.2°). Posterior to mf 22, a large dorsal canaliculus (Figure S1H, dc3), 
32 slightly inclined posteriorly, projects dorsally and opens into several small foramina. 
33 Anteriorly, although alveoli for mf 1 and mf 2 are almost adjacent to the canal, small 
34 dorsal canaliculi are visible. As the canal projects ventrally towards alveolus for mf16, 
35 the dorsal canaliculi become longer. Their morphology varies, being anteriorly oriented 
36 and sometimes bifurcated. These canaliculi vary in diameter and can bifurcate (e.g., 
37 below alveolus for mf 17 and mf 18, Figure S1H), with a branch projecting dorsally and 
38 the other anteriorly, to merge with the immediately anterior dorsal canaliculus. Anteriorly 
39 to alveolus of mf 16 the mandibular canal displays a parallel trajectory to the alveoli row 
40 until alveolus for mf 9. From this point, it projects dorsally until mental foramen 1, being 
41 positioned immediately ventral to the nine most anterior alveoli. Along its path to the 
42 symphyseal portion of the mandible, the mandibular canal displays several minor mental 
43 branches before it splits into three larger mental canals (Figure S1H, mb 1-3) anterior to 
44 the first alveolus. The most posterior and smaller mental branch splits from the main 
45 canal right anteriorly to alveolus for mf 1, projecting anterodorsally and then drifting 
46 laterally. A second larger mental branch (mb2) splits anteriorly, departing from the 
47 dorsolateral part of the mandibular canal and opening in an antero-posteriorly elongated 
48 foramen. The most anterior and largest mental branch (mb1) also opens into an antero-
posteriorly elongated foramen. A more anterior small branch is visible on the lateral side of the mandible, but corresponds to a small ramification of the largest mental branch (mb1). Another canaliculus of the mandibular canal, which splits from the posterior part of the mb1, continues anteriorly toward the antero-dorsal end of the mandible. This small canaliculus (dc1) divides into three smaller ramifications, which open on the medial side of the anterior tip of the mandible.

**Aardwolf (Proteles cristatus)**

*Proteles cristatus* displays a mandible with a relatively high horizontal ramus displaying an anteriorly projected anterior margin. The specimen displays the following dental formula: i 3, c 1, p 2, m 2. This mandible displays three mental foramina (Figure S1I, mb1-3), with the first and second mental foramina displaying oval apertures of similar size. The first mental foramen is located on the anterior margin of the mandible and the second one is anterior to the canine, below the anterior end of the diastema. The third mental foramen is located below the diastema, half way through the height of the mandible.

The mandibular foramen is oval shaped, located in the middle of the ascending ramus, antero-ventral to the dorsal tip of the coronoid process. The mandibular canal has an oval section, being noticeably dorso-ventrally elongated from the anterior end of the coronoid process to the level of the m 1. The mandibular canal projects slightly ventrally until the posterior limit of the diastema. None of the four cheek teeth alveoli is adjacent to the mandibular canal. The first and second molars are dorsally placed relative to the canal; no dorsal canaliculus reaches their alveoli. The inferior alveolar nerve and artery branches reach the teeth through the many hollow cavities of the alveolar portion of the mandible. The trabecular nature of the molar area is particularly evident anteriorly (Figure S1I), dorsally and posteriorly to the premolar alveoli, almost
reaching the dorsal edge of the mandible. Posteriorly, fused trabeculae form a sort of posteriorly projecting canals, which reach the dorsally placed m 1 and m 2 alveoli.

Anterior to the c, below the diastema, three mental branches (Figure S1I, mb1-3) split and project antero-dorsally. Mb3 projects dorsally and opens laterally right ventrally to the midpoint of the diastema. Mb2 projects antero-dorsally and is laterally inclined, opening latero-ventrally to the canine. Mb1 is long and projects antero-dorsally, passing through the middle of i 2 and i 3 and opens in the anterior margin of the mandible.

**Dog (Canis lupus)**

*Canis lupus* presents a mandible with a high ascending ramus, with a relatively broad coronoid process. The mandible presents 11 tooth loci (i 3, c 1, p 3, m 3) and contrasts with *P. cristata* in its complete tooth row (no diastema), its shorter symphysis and rather vertical anterior margin. Four mental foramina are present (Figure S1J, mb1-4). The first mental foramen opens anteriorly, ventral to i 2. The second and largest mental foramen opens laterally between p 1 and c 1. The third mental foramen opens at the level of the anterior root of p 3 and the fourth mental consists of a rather small lateral aperture located between the ventral tips of the anterior and posterior roots of p 4 and p 3, respectively.

The mandibular foramen resembles *P. cristata*, yet the canal projects slightly more ventrally. Just posteriorly to the anterior edge of the coronoid process, the mandibular canal becomes more extended anterodorsally (Figure S1J), displaying a pseudo elliptical cross section. This is a main characteristic of the mandibular canal of the dog. With the exception of m 3, teeth are rooted deeply enough to be in close contact with the dorsal edge of the mandibular canal. The trajectory of the mandibular canal follows the shape of the ventral margin of the mandible from the level of the posterior root of m 1 until p 2, where it branches (Figure S1J). The large m 1 is so deeply
rooted that both its roots protrude ventrally into the mandibular canal. Along its trajectory, the mandibular canal is topped by a complex of trabeculae that surrounds the tooth roots. From about the second m 1 cusp, the mandibular canal gradually decreases in height anteriorly. All but the small fourth mental branch project anterolaterally. Mb4 is a very thin branch that projects posterolaterally below the posterior edge of p 3. Mb3 is comparatively larger and splits from the mandibular canal just ventrally the anterior root of p 3. Mb2 is large in diameter and projects anterolaterally ventrally to p 2 to open in an oval-shaped foramen between p1 and c (Figure S1J). Mb1 is a rather thin and extremely long ramification of the mandibular canal that projects anteriorly to pass dorsally to the c and i 1-3 roots and open in the anterior margin of the horizontal ramus. In its trajectory, mb1 opens to the alveolus of i 3. Dorsally to the branching point of mb1, a relatively large accessory branch of the mandibular canal projects anterodorsally. This branch is medially oriented and almost reaches the symphysis, posterior to which it divides into several trabeculae.

Pale-throated three-fingered sloth (Bradypus tridactylus)

In the adult Bradypus tridactylus, the mandible is characterized by a short but robust horizontal ramus, bearing one caniform (Cf) and three molariform teeth (mf 1-3), and a high ascending ramus. The tooth row almost occupies the entire length of the body of the mandible, with teeth inclination becoming more medial along the antero-posterior axis. All four teeth are tall, lack roots and are deeply rooted in the mandible. The mandible presents two mental foramina with one at the anterior tip of the mandible (Figure S1K, mb 1) and a larger one at the level of the mf 4 (Figure S1K, mb2).

The morphology of the mandibular canal is very distinct from that observed in anteaters and armadillos. The mandibular foramen is elliptical in shape, with the major axis oriented anteroposteriorly with a similar inclination to the condylar process. The
canal projects antero-ventrally from the mandibular foramen until it reaches its lowest position in the mandible, at the level of the mf 3. At the same level, but higher in the body of the mandible, the largest and most posterior mental branch opens on the lateral side of the mandible, after branching midway between the mandibular foramen and the lowest mandibular canal position. The mandibular canal then runs anteriorly and gradually projects dorsally in the mandible until it reaches mental foramen 1. There is one small dorsal canaliculus (dc 1-3) for each of mf 1, mf 2 and mf 3. Dc4 is originates ventrally to mf3 but extends anterodorsally to reach the alveolus on its anterior margin.

The mandibular canal runs medial to the first tooth and divides into dorsal and mental branches. The mental branches follow the same direction and inclination as the main branch and eventually reach the anterior tip of the mandible, lateral to the symphysis. The dorsal canaliculus assumes a steep trajectory (roughly parallel to the anterior margin of the mandible) and exits the mandible dorsally. This dorsal canaliculus likely corresponds to a remnant of the innervation/vascularization of an additional tooth locus identified in a previous work [S5] and opens anteriorly to alveolus for Cf. The juvenile specimen (ZMB 18834) displays a vestigial tooth in the same position; its mandibular canal of the foetus presents a similar general morphology, differing in number of mental and medial branches. The foetal mandible presents four foramina for accessory branches that open in the medial side of the mandible and four mental foramina. This mandible is much less ossified, compared to the adult.

**Two fingered sloth (Choloepus hoffmanni)**

The mandible of *Choloepus hoffmanni* presents shallower horizontal and ascending rami compared to *B. tridactylus*. The anterior tip of the horizontal ramus is shovel-shaped and elongated. The mandible bears three molariform teeth (mf 1-3) and one anterior caniniform tooth (cf). In total, the *C. hoffmanni* mandible presents four mental foramina,
a large one at the level of the root of the coronoid process and three small ones anterior to the caniniform tooth.

Due to the differences in mandible shape, the morphology of the mandibular canal is also different between the two sloth genera. Here again, the dorsal canaliculi of the mandibular canal are present when the tooth alveoli are more dorsal. The canal starts at the level of the dorsal edge of the horizontal ramus and runs anteriorly until it reaches the level of the posterior margin of the tooth row. At this level, the mandibular canal bifurcates, with a mental branch (Figure S1L, mb4) projecting antero-laterally to open in a large mental foramen, just anteroventrally to the root of the coronoid process.

The main branch projects medially from the mandibular foramen, passing marginally ventrolaterally to mf 3. A small dorsal canaliculus of the mandibular canal (Figure S1L, dc 10) projects medially and allows for the innervation and vascularization of mf 3, which is medially oriented. As the mandibular canal runs anteriorly, its position gradually changes to a more medial one. This contrasts with the morphology of the mandible of the adult *B. tridactylus*, in which the mandibular canal is medially positioned along the entire length of the tooth row. Two dorsal canaliculi of the mandibular canal (df8-9) are ventral to the anterior half of mf2. Three dorsal canaliculi (dc5-7) reach the alveolus for mf 1. Dc5 is a small canal that splits at mid-length of the alveolus and joins the main canal just anteriorly to it (Figure S1L). From this canal, three small dorsal projections reach the alveolus. Two smaller additional innervation/vascularization canaliculi are present posteriorly (Figure S1L, dc 6-7). Close to the symphysis, the mandibular canal steadily projects dorsally. The main branch passes medially to the cf and, as in *B. tridactylus*, its position is dorsal to the base of the alveolus. In fact, dorsal canaliculi reach the alveolus of the cf. The more posterior canaliculus (Figure S1L, dc4) bifurcates from the mandibular canal antero-ventrally just anterior to the alveolus for the cf.

Anteriorly, a small canaliculus (dc3) splits from the mandibular canal and projects
laterally into the same alveolus. The third canaliculus that connects to the alveolus of the
cf (dc1) is located just anterior to the mandibular canal steep antero-dorsal projection in
the direction of the anterior tip of the mandible. A long dorsal canaliculus (Figure S3B,
dc2) splits from the mandibular canal in the direction of the rudimentary alveolus of the
vestigial tooth that is observed on earlier ontogenetic stages. The vestigial alveolus,
located just antero-medially to the anterior edge of cf, is also innervated/vascularized,
with the dorsal canaliculus further splitting in two ventrally to its dorsal opening. Three
small mental branches (Figure S1L, mb1-3) are present in the anterior part of the
mandible: the most posterior (mb3) departs from the mandibular canal to open laterally
at the level of mb2; mb2 projects dorsolaterally near the dorsal margin of the mandible;
mb1 splits at about the same length as mb2, but projects anterolaterally. Anteriorly to the
level of the foramen for mb1, a small branch (Figure S1L, ab) reaches the mandible
shovel-shape projection and further divides into two small canals that open dorso-
medially. These canals are not present in *B. tridactylus*.

The mandible of the foetus of *Ch. didactylus* (ZMB 4949) presents a lower level
of ossification and allometric shape differences. Nevertheless, the mandibular canal of
the foetus presents the same branch pattern as observed in the adult.

**Aardvark (*Orycteropus afer*)**

This mandible consists of a transversely compressed long and shallow horizontal ramus
and a comparatively very high ascending ramus. The anterior half of the horizontal
ramus is edentulous, while its posterior half presents three molars and two premolars (i
0, c 0, p 2, m 3; Figure S1M). The molars are large and deeply rooted and display an
oblanceolate shape in dorsal view; the premolars are much smaller, transversely
compressed, and are rooted more dorsally. The mandible of *Orycteropus afer* displays
four major mental foramina (Figure S1M, mb1-4), with six small mental branches
laterally positioned compared to the tooth row (Figure S1M, mb5-10). A total of seven foramina for dorsal canaliculi are present. The anteriormost four foramina are aligned just anteriorly to p 5 (sensu [S6]; dc3). Another small foramen is present anteriorly, at the level of the third mental foramen (dc2). Two slightly larger and longer foramina open between mental foramina 1 and 2 (dc2). The most anterior foramen (dc 1) opens anteriorly in the middle of the anterior apical portion of the mandible. The dc1 foramen resembles the reticular alveolar foramen of whales [S7,S8].

The mandibular foramen is narrow and located well above the tooth row level, from where the canal projects ventrally. Between m 3 and m 1, the trajectory of the mandibular canal changes as it gradually projects dorsally, accompanying the height decrease of the horizontal ramus. The mandibular canal is adjacent to alveoli of m 1-3 and dorsal canaliculi are absent. From m 3 to p 5, only mental branches bifurcate from the mandibular canal (mb 5-10). The p5 and p6 alveoli are placed more dorsally in the mandible and are connected to the mandibular canal by two dorsal canaliculi, which project antero-dorsally. The two canaliculi dorsal to p 5 and p 6 (dc3 and dc4) are connected by a small canal parallel to the mandibular canal. Anterior to p 5, the main dorsal canaliculus (dc3) steadily extends dorsally, and then divides into four small canals linked to foramina dorsally. At the level of mb4, dc3 projects ventrally to merge with the mandibular canal, and a new dorsal canaliculus (dc2, Figure S1M) projects antero-dorsally. Dc 2 is long and presents a small diameter. It bifurcates three times: just anteriorly to its origin, a small ramification projects antero-dorsally to open at the level of mb4; the dorsal canaliculus continues anteriorly and bifurcates again between mb1 and mb2, with one branch projecting antero-dorsally but never opening dorsally; a thin branch of dc2 continues until it splits in several smaller canals near the third mental foramen, with one of them opening dorsally at the level of the second mental foramen. Mb2 and dc1 split near the anterior end of the mandibular canal. Dc1 bifurcates – with
one branch projecting antero-dorsally and the other anteriorly – before it opens in two
foramina. The most posterior branch of dc1 opens at the level of the second mental
foramen. The most anterior canaliculus reaches the anterior tip of the mandible, ending
in a foramen similar in position to the reticular alveolar foramen found in rorquals
[S7,S8].

Giant otter-shrew (*Potamogale velox*)

The mandible of *Potamogale velox* presents a relatively low horizontal ramus with a
rather long and anteriorly inclined symphysis. The ascending ramus of is high due to the
presence of a broad sub-triangular coronoid process. The tooth row is composed of ten
teeth (i 3, c 1, p 3, m 3). Three mental foramina are present in the mandible (Figure S1N,
mb1-3). The first mental foramen is small and opens on the anterior margin of the
mandible. The second and third mental foramina are relatively larger and open laterally
at the level of p1 and the posterior root of m1, respectively.

The mandibular foramen has a pseudo circular shape. Posteriorly, the
mandibular canal presents a slightly descending trajectory until it reaches the m 2. The
trajectory is then practically horizontal until the canine. Small complexes of trabeculae
are present dorsally to the mandibular canal. The trabeculae surround the tooth roots,
which are deeply implanted in the mandible. With the exception of the c and i 1-3, all
alveoli enter in contact of the mandibular canal. The most posterior mental branch
(Figure S1N, mb3) is the largest in diameter and projects laterally at the level of the tip of
m 1 posterior root and opens slight dorsally in an elliptical shaped foramen. At the level
of p 2, the trajectory of the canal drifts slightly dorsally, accompanying the small ventral
concavity of the ventral edge of the horizontal ramus. Two branches split from the
mandibular canal, dorsal to p 1, and project laterally. These branches merge into one
larger mental branch, opening laterally in a single foramen (Figure S1N, mb2). Mb1
consists of a rather long branch, small in diameter, which projects anteriorly (Fig S1N, mb1). This branch passes ventrally to c alveolus and gradually projects dorsally to open anteriorly, between i 2 and i 1. Two accessory branches of the mandibular canal split at the level of p 1 and c. The posterior one projects anterodorsally until it divides into a trabeculae complex at the level of i 3 and i 2 alveoli. The anterior one also projects anterodorsally but it meets the trabeculae briefly anteriorly to its branching point. This trabeculae complex is located medially in the mandible and is associated with the i 2 alveolus and the symphysis.

Anatomical description of additional taxa

A total of 12 species were segmented in order to reconstruct the phylogenetic history of the absence/presence of dorsal canaliculi (Figure 1). One Afrosoricida (Tenrec eucaudatus) and one Macroscelidea (Rhynchocyon petersi) were additionally segmented to increase the number of species with relatively close phylogenetic affinities to Orycteropus afer (Figure 1). Neither of the 12 species described in this section presents dorsal canaliculi. With the exception of the brown rat (Rattus norvegicus) and the velvet free-tailed bat (Molossus molossus), all the species present mandibular canals very similar in shape (Figure S2A-I). The mandibular foramen displays a descending trajectory anteriorly until the third molar (m 3). It projects anteriorly, ventrally to the tooth row, with its dorsal margin that usually opens directly to adjacent post-canine tooth alveoli (Figure S2). In some species (e.g., Dasyurus hallucatus, Lemur catta, Cynocephalus volans, or Procavia capensis; Figure S4A-D) a large amount of trabecular tissue is visible around the teeth, and connects the mandibular canal to the alveoli, only when both structures are not adjacent to each other. The Malayan tapir (Tapirus indicus, Figure S2E) constitutes the most remarkable case of innervation/vascularization via trabeculae, with a mandible characterized by non-adjacent tooth alveoli and mandibular
canal. In almost all species, an incisor branch projects anteriorly at the level of the most anterior mental branch, and usually splits into several trabeculae that wrap both canine and incisor teeth. In the rock hyrax (*Procavia capensis*), however, the incisor branch opens directly into the corresponding alveolus. The tailless tenrec (*Tenrec ecaudatus*, Figure S2F) also presents a slightly different morphology of the incisor branch, with the mandibular canal opening directly into the alveolus of the canine, with the latter being connected to the following alveolus. In the brown rat (*Rattus norvegicus*, Figure S2K) the incisor branch is absent, with the alveolus of the incisor tooth extending back into the ascending ramus of the mandible. The innervation/vascularization of the incisor tooth arrives ventral to the coronoid process, as the mandibular canal partially merges with the alveolus. Although the Malayan tapir exhibits a classical incisor branching of the mandibular canal, the latter differs in from other species by its extremely reduced diameter (Figure S2E). The overall shape of the mandibular canal of the brown rat is peculiar due to the extreme posterodorsal projection of the incisor alveolus (Figure S2K). The mandibular canal merges with the incisor alveolus medially, just anteriorly to the mandibular foramen. It projects anteriorly at the level of the m3 and its trajectory follows the shape of the dorsal margin of the incisor alveolus. The mandibular canal opens directly to the m3 and m2. In the most anterior portion of the alveolar part of the mandible, which corresponds to the alveolus of the m1, the mandibular canal presents a relatively ventral position relative to the first molar alveolus, the tooth being likely innervated/vascularized via trabecular bone. The mandibular canal opens dorsolaterally in a single mental foramen, just anteriorly to the m1. The velvet free-tailed bat also presents a distinct mandibular canal morphology (Figure S2L). Compared to other species, the mandibular canal is unusually high. Here again, all pre-canine tooth alveoli are adjacent to the mandibular canal. However, the mandibular canal seems to extend dorsally, in between tooth roots (Figure S2). This might be a result of the scan resolution
compared to the reduced size of the specimen, for in other segmented placental mammals, those spaces are normally filled with trabeculae (Figure S2). Apart from this abnormal height, the mandibular canal of the velvet free-tailed bat resembles those of other toothed species; it is positioned ventrally to the tooth row and bifurcates anteriorly, with a mental branch that projects laterally while the other branch of the canal opens into a trabecular system wrapping the canine and the incisors. An anterodorsally projecting mental branch is also present and opens ventrally to the incisors, similar to the condition observed in carnivores (Figure S2D, E).

2. Homology of dorsal canaliculi between anteaters and baleen whales

The trigeminal nerve originates in the pons and has two different roots, a sensory and a motor root [S9]. The mandibular branch of the trigeminal is composed of both sensory and motor roots and is designated by inferior alveolar nerve (IAN) [S9]. The nerve enters the medial side of the mandible through the mandibular foramen, and is accompanied by the inferior alveolar artery [S10]. Typically, the inferior alveolar nerve separates into four types of branches [S9,S10]: the mylohyoid branch supplies the hyoid musculature; the dental/inferior dental branches supply molars and premolars, each nerve branch innervating one tooth; the incisor branch supplies canine and incisors. The mental branch supplies the lip integument (the number of mental branches may vary between taxa). Here, we will group dental and incisor branches to follow the nomenclature of previous references for edentulous placentals [S7].

In Tamandua tetradactyla, the large inferior alveolar nerve (Figure 4B’, IAN) is placed dorsally in the mandibular canal. Two accessory branches of the IAN (Figure 4B’, IANa) are present dorsolaterally to the IAN. The inferior alveolar artery (Figure 4B’, IAA) lies dorsally to the IAN. An irregularly shaped branch of the inferior alveolar vein (Figure
4B’, IAV) is placed laterally to the IAA. A third branch of the IAN (Figure 4B’ and S3B, IANa) is visible dorsally to the IAA. Dorsally to it, the ascending branch of the IAV (Figure 3B’ and S3B, IAVab) is present in the dorsal canaliculus of the mandibular canal. A keratinous dental pad covers the dorsal part of the mandible (Figure 4C, pa). Ventrally, the epidermis consists of a small layer (Figure 4C, ep) and, which lies dorsally to the thick dermis layer (Figure 4C, de). In addition to connective tissue, the dermis presents several blood vessels and small nerve branches. The most posterior dorsal canaliculus allows for the passage of an ascending branch of the IAN (Figure 4C, C’, IANab1); the anterior dorsal canaliculus displays a large ascending branch of the IAA (Figure 4C, IAAab) and likely features a second ascending branch of the IAN (Figure 4C, IANab2) that projects anterodorsally.

A histological section (coronal plane) of the mandible of a bowhead whale (Balaena mysticetus) showing the 15th vestigial tooth [S11] shows the mandibular canal and associated soft tissues (inferior alveolar nerve, blood vessels) with two small canals (Figure S3A, red squares) dorsally. The most dorsal small canal is most likely associated to the vestigial teeth observed in the figure, while the most ventral one is associated to the 14th vestigial tooth. Its position between the mandibular canal and the tooth row, plus the presence of branches of the inferior alveolar artery and the inferior alveolar nerve inside these small canals, highly suggest that these are homologous to the dorsal canaliculi observed in the collared anteater (T. tetradactyla). Dorsal canaliculi are anteriorly inclined in whales [S7], thus histological slices produce circular section for each canaliculus.
3. Intraspecific and bilateral variation of dorsal canaliculi

Dorsal canaliculi were present in all segmented anteater (n=8) and long-nosed armadillo (n=6) mandibles (Figure S4). All six long-nosed armadillos displayed an anterior dorsal canaliculi plexus splitting in three to six dorsal foramina (Figure S4A-F), including the juvenile (Figure S4F). A canine, as well as its corresponding dorsal canaliculus, was present in two specimens (Figure S4A, F). The mandibular canal morphology was similar in the six specimens. The two pygmy anteater (C. didactylus, n=2) mandibles presented bilateral variation and showed between 15 and 17 dorsal canaliculi (MNHN 1986-1115, 15L/17R; BMNH 24.12.4.68, 17L/15R; Figure S4G, H). Bilateral variation was observed in two collared anteaters (T. tetradactyla, n=3) and two giant anteaters (M. tridactyla, n=3). The collared anteaters (T. tetradactyla) showed between 14 (MVZ 153482, 14L/14R) to 17 (ISEM – 788N, 17L/16R) dorsal canaliculi (Figure S4I-K). The two adult giant anteaters presented 14 (ISEM – 065 V) and 13/14 (MVZ – 185238) dorsal canaliculi, while the juvenile presented slightly more canaliculi with a slightly larger bilateral variation (16L/18R; Figure S4L-N). Nevertheless, the observed pattern of dorsal canaliculi morphology and distribution along the mandible is similar.

4. Insights into the ancestral number of teeth in anteaters

All known anteater fossil skulls are toothless [S12,S13]. However, our data confirmed that the earliest representatives of the group were toothed, assuming the homology between dorsal canaliculi and alveolar vestiges. There are, however, several limitations in using the number of dorsal canaliculi to estimate tooth number: 1) it is not clear weather bifurcating dorsal canaliculi would correspond to one or more alveoli– although bifurcating dorsal canaliculi mostly appear to correspond to only one tooth in P. maximus.
– which can lead to an overestimation of the number teeth with the present method; 2) a high mandibular canal merges the lower part of the dorsal canaliculi from the posterior part of the mandibular canal of the juvenile *M. tridactyla* makes it difficult to discriminate dorsal canaliculi; 3) intraspecific variation may partly explained the observed number of dorsal canaliculi, especially when comparing the bilateral variation to the interspecific variation observed in other clades within Xenarthra (Cingulata). If we were to reconstruct the dental formula based on the number of dorsal canaliculi regardless of the abovementioned arguments, we would propose that each mandibular quadrant of the earliest Vermilinguans comprised between thirteen and seventeen teeth.

5. Ancestral state estimation of the tree internal nodes

Based on our dataset composed of extant species, we reconstructed the ancestral condition of placental mammals concerning the presence of dorsal canaliculi (Figure S5). We used the re-rooting method of Yang et al. [S14] to perform an ancestral state reconstruction under a continuous time Markov chain. The posterior probabilities for the presence of dorsal canaliculi are presented for each node (Figure S5). We used the same tree as presented in Figure 1, obtained from www.timetree.org [S15]. This tree includes 29 species and 28 internal nodes. Due to the absence of fossil taxa in the analysis, all nodes presented posterior probabilities close to zero or one. The probability of the presence of dorsal canaliculi was high in all nodes within Xenarthra. Excluding this clade, the highest probability for the evolution of dorsal canaliculi was found in the most recent common ancestor (MRCA) of cetaceans (node 12), with 4.86%. We found a 0.37% probability for the evolution of dorsal canaliculi in the MRCA of all placentals (node 2). This suggests that the absence of dorsal canaliculi (0) likely corresponds to the
ancestral condition of placentals. The ancestral state reconstruction was performed with the phytools package [S16] in R [S17].
Supplemental references


Table S1. List of all studied specimens with corresponding collection number and voxel size.

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