

3D models related to the publication: "From teeth to pad: tooth loss and development of keratinous structures in sirenians".

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Abstract

This contribution contains the 3D models described and figured in the following publication: Hautier L, Gomes Rodrigues H, Ferreira-Cardoso S, Emerling CA, Porcher M-L, Asher R, Portela Miguez R, Delsuc F. 2023 From teeth to pad: tooth loss and development of keratinous structures in sirenians. *Proceedings of the Royal Society B*. <https://doi.org/10.1098/rspb.2023.1932>

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INTRODUCTION

We present here the 3D models of the mandibles extant and extinct sirenian species. Sirenians (extant and extinct sea cows) are characterized by a tooth reduction associated with the development of horny pads. These keratinized structures were especially pronounced in the recently extinct Steller's sea cows, which was completely toothless. CT scans of sirenian skulls were used to investigate the morphology of the mandibular alveolar canals as osteological proxies of tooth innervation, as well as to understand the correlation between the evolution of sirenian orofacial anatomy and tooth reduction (Hautier et al., 2023). We used a comparative framework of ontogenetic, paleontological, and genetic evidence to trace the evolutionary history of tooth loss in sirenians. We argue that the dental neurovascular anatomy was co-opted for innervating and supplying blood to the keratinized pads. Such a case of evolutionary tinkering is similar to that observed in other toothless tetrapods, and demonstrates that the tooth developmental pathway has been recycled multiple times to improve the neurovascularisation of disparate neomorphic keratinized structures, from baleen of mysticetes to beaks of non-avian dinosaurs (Hautier et al., 2023).

METHODS

We reconstructed 3D models of the internal mandibular morphology of the four extant species of Sirenia: the dugong (*Dugong dugon*), the West Indian manatee (*T. manatus*), the Amazonian manatee (*Trichechus inunguis*), and the West African manatee (*T. senegalensis*). We also included 3D models for six key extinct taxa: *Prorastomus sirenoides* (lower Lutetian), *Libysiren sickenbergi* (lower Lutetian), *Halitherium taulannense* (Priabonian), *Eosiren libyca* (Priabonian), *Rytiodus capgrandi* (lower Miocene), *Ribodon limbatus* (Late Miocene), and *Hydrodamalis gigas* (Steller's sea cow, extinct since the 18th century). The 27 3D models presented here belong to the following collections: Natural History Museum, London (NHMUK); Muséum National d'Histoire Naturelle, Paris (MNHN); Muséum d'Histoire Naturelle de Toulouse, Toulouse (MHNT); University Museum

of Zoology, Cambridge (UMZC); the Institut Royal des Sciences Naturelles de Belgique, Brussels (IRSNB); Institut des Sciences de l'Évolution, University of Montpellier (UM). High-resolution microtomography (μ CT) was performed at Montpellier Rio Imaging (MRI) platform, the Imaging Analysis Center (NHMUK), the Cambridge Biotomography Centre, and the AST-RX platform (MNHN). Scan resolutions are listed in Table 1. Image segmentations of the mandibles were performed on the μ CT images with Avizo 2019.4 (Thermo Fisher Scientific) software using the segmentation threshold selection tool. The 3D virtual restoration was performed with MorphoDig software (<https://morphomuseum.com/morphodig>). The 3D surface models are provided in .vtk and .ply format, and can therefore be opened with a wide range of freeware.

DESCRIPTION

We here provide detailed anatomical descriptions of the internal anatomy of each extant species, as well as of the recently extinct Steller's sea cows, for which we had access to intraspecific variability. These descriptions could not be included in the original paper. For each of these species, one specimen is described and illustrated in details (Figures 1, 2, 3, 4, and 5) and then compared to other 3D reconstructed specimens of the same species.

Dugong dugon

The mandible NHMUK 1848.8.29.7 (Fig. 1) is short and high; its body is thick, especially at the level of the symphysis. The ascending ramus is high, with a large masseteric fossa and a shallow angular process. The body of the mandible displays three molars. They are large, with high crowns (hypsodonty) and no roots. The two posterior molars (m2 and m3) are longer than the anterior one (m1). The symphysis area has an anteriorly inclined vestigial incisor tooth (di), with a dorso-ventral axis almost perpendicular to the molars. It is thin and long antero-posteriorly. An empty socket is also visible just dorsal to the di. The symphysis is wide and thick, and forms a right angle with

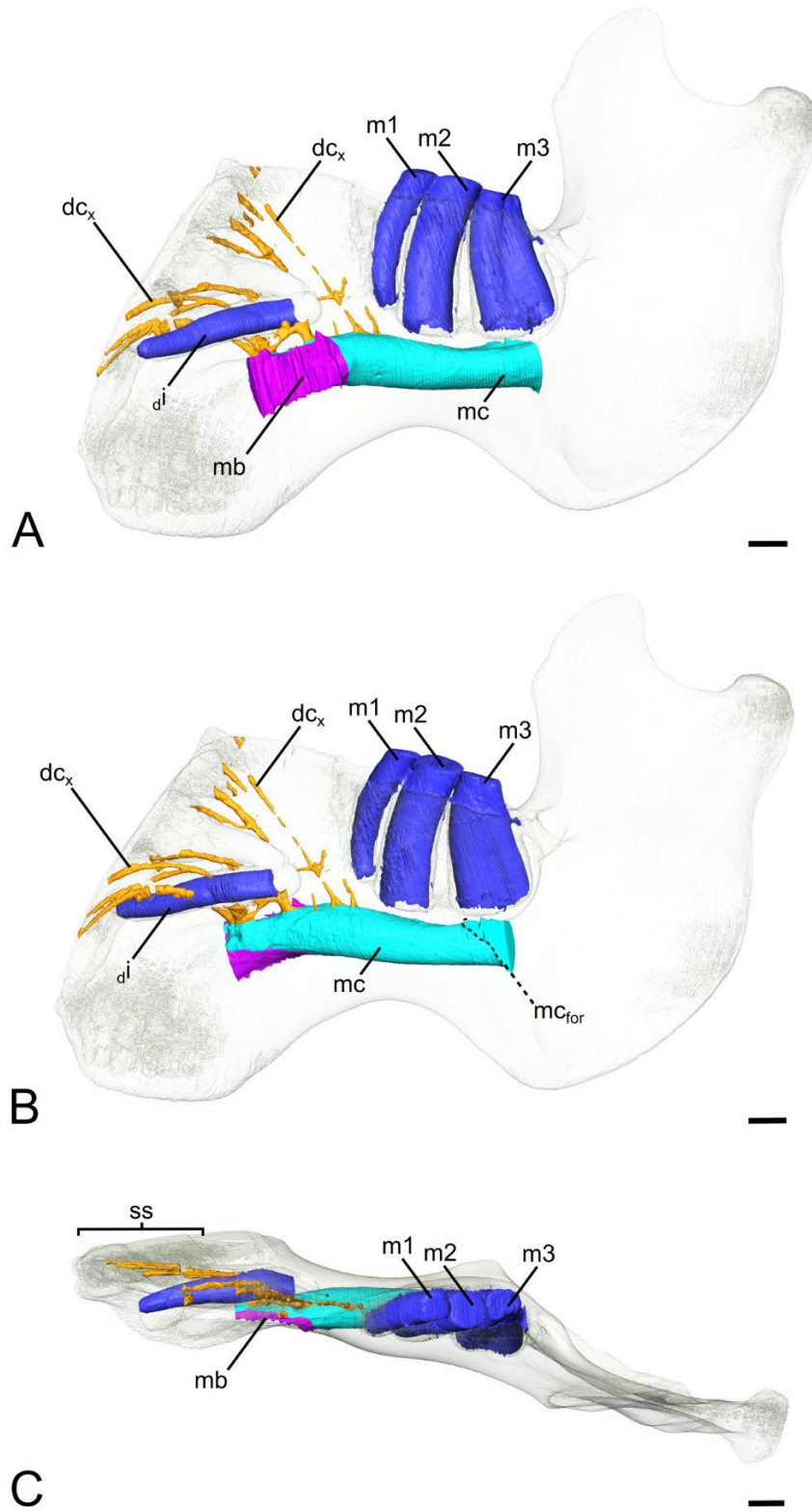


Figure 1. Internal mandibular morphology in *Dugong dugon* (NHMUK 1848.8.29.7). Orange = dorsal canaliculi; purple = mental branches; cyan = mandibular canal; dark blue = teeth.

Collection	Inv nr.	Taxon	Resolution (mm)
NHMUK	1946.8.6.2	<i>Dugong dugon</i>	0.08
NHMUK	2005.51	<i>Dugong dugon</i>	0.123
NHMUK	1991.427	<i>Dugong dugon</i>	0.127
NHMUK	1991.413	<i>Dugong dugon</i>	0.111
NHMUK	2023.66	<i>Dugong dugon</i>	0.127
UMZC	2017-3-9	<i>Dugong dugon</i>	0.25
NHMUK	1848.8.29.7/GERM 1027g	<i>Dugong dugon</i>	0.119
IRSNB	5386	<i>Dugong dugon</i>	0.077
NHMUK	65.4.28.9	<i>Trichechus sp.</i>	0.105
NHMUK	1864.6.5.1	<i>Trichechus manatus</i>	0.123
NHMUK	1950.1.23.1	<i>Trichechus manatus</i>	0.122
NHMUK	1843.3.10.12	<i>Trichechus manatus</i>	0.101
NHMUK	1868.12.19.2	<i>Trichechus manatus</i>	0.08
UM	ISEM V97	<i>Trichechus senegalensis</i>	0.12
NHMUK	1894.7.25.8	<i>Trichechus senegalensis</i>	0.064
NHMUK	1885.6.30.2	<i>Trichechus senegalensis</i>	0.116
NHMUK	OR.448976 Left md	<i>Prorastomus sirenoides</i>	0.121
NHMUK	OR.448976 Right md	<i>Prorastomus sirenoides</i>	0.121
NHMUK	M.45675	<i>Libysiren sickenbergi</i>	0.127
NHMUK	M.82429	<i>Libysiren sickenbergi</i>	0.101
NHMUK	1913-22	<i>Eosiren lybica</i>	0.12
MHNT	PAL2017-8-1	<i>Rytiodus capgrandi</i>	0.094
NHMUK	M.7073	<i>Ribodon limbatus</i>	0.115
MNHN	RGHP C001	<i>Halitherium taulannense</i>	0.107
MNHN	RGHP C009	<i>Halitherium taulannense</i>	0.141
NHMUK	1947.10.21.1	<i>Hydrodamalis gigas</i>	0.127
UMZC	C1021 Anterior part	<i>Hydrodamalis gigas</i>	0.125
UMZC	C1021 Posterior part	<i>Hydrodamalis gigas</i>	0.125
NHMUK	2023.67	<i>Hydrodamalis gigas</i>	0.127

Table 1. List of 3D models and associated information

the dorsal margin of the body of the mandible, which characterises dugongs. The symphysis is flattened anterodorsally. The mandibular canal foramen (mcfor) opens on the medial surface of the mandibular body, and is inclined anteriorly in lateral view. Posteriorly, the mandibular canal (mc) communicates directly with the third molar socket (m3). The mandibular canal projects anteriorly into the ventral part of the mandibular body. It is tall and thick, ellipsoidal in cross-section. The alveoli of the second and first molars are successively further away from the dorsal edge of the canal. Anteriorly, the mandibular canal terminates in the opening of a wide mental branch (mb), anterolaterally orientated, which is located on the lateral margin of the mandibular body. The mental foramen is elongated anteroposteriorly. The mandibular canal displays several dorsal branches of small diameter (dorsal canaliculi; dcx), anterior to the first molar (m1). The dorsal canaliculi (dcx) project dorsally or anterodorsally. The direction of inclination of the posterior dcx has a predominantly dorsal component, while the dcx anterior to the chin branch have a more anterior component. The most posterior canaliculus is ventral to the anterior border of the m1; it is short and terminates ventrally at the level of a trabecular mass anterior to the m1. The remaining canaliculi are longer and open outwards to form several foramina on the flattened region of the symphysis, which is covered by a keratinized pad. Most of the dcx lie anterior to the opening of the chin branch (mb). These canaliculi pass

through the tooth alveoli of the incisors before reaching the dorsal surface of the symphysis, which prevents us to follow them completely.

The other segmented specimens have a similar external morphology. UMCZ 2017-3-9 displays a greater number of chin branches, while NHMUK 1991-427 is characterized by an anteroventral projection of the mandibular canal. The number of dorsal canaliculi is much higher in the symphysis of NHMUK 1991-413, NHMUK 1991-427, and lower in UMCZ 2017-3-9. Moreover, some canaliculi with an anterodorsal orientation, with a large anterior component, are found ventrally to or in between the tooth alveoli.

Trichechus manatus

The mandible NHMUK 1843.3.10.12 (Fig. 2) is elongated anteroposteriorly, relatively low, and thicker transversely than that of dugongs. In the sagittal plane, the mandibular ramus is short and tall and the body is long and slender. Ventrally, the mandibular ramus has a broad lateral surface, with a rounded angular edge process. Five empty alveoli are visible anteriorly, but the two more posterior molar teeth are still present (mx6, mx7). Due to continuous tooth replacement, the teeth and alveoli can only be easily identified thanks to serial homologies. The empty alveoli likely correspond to molar loci. In contrast to *Dugong*, the molars are bilophodont, with long roots and short crowns.

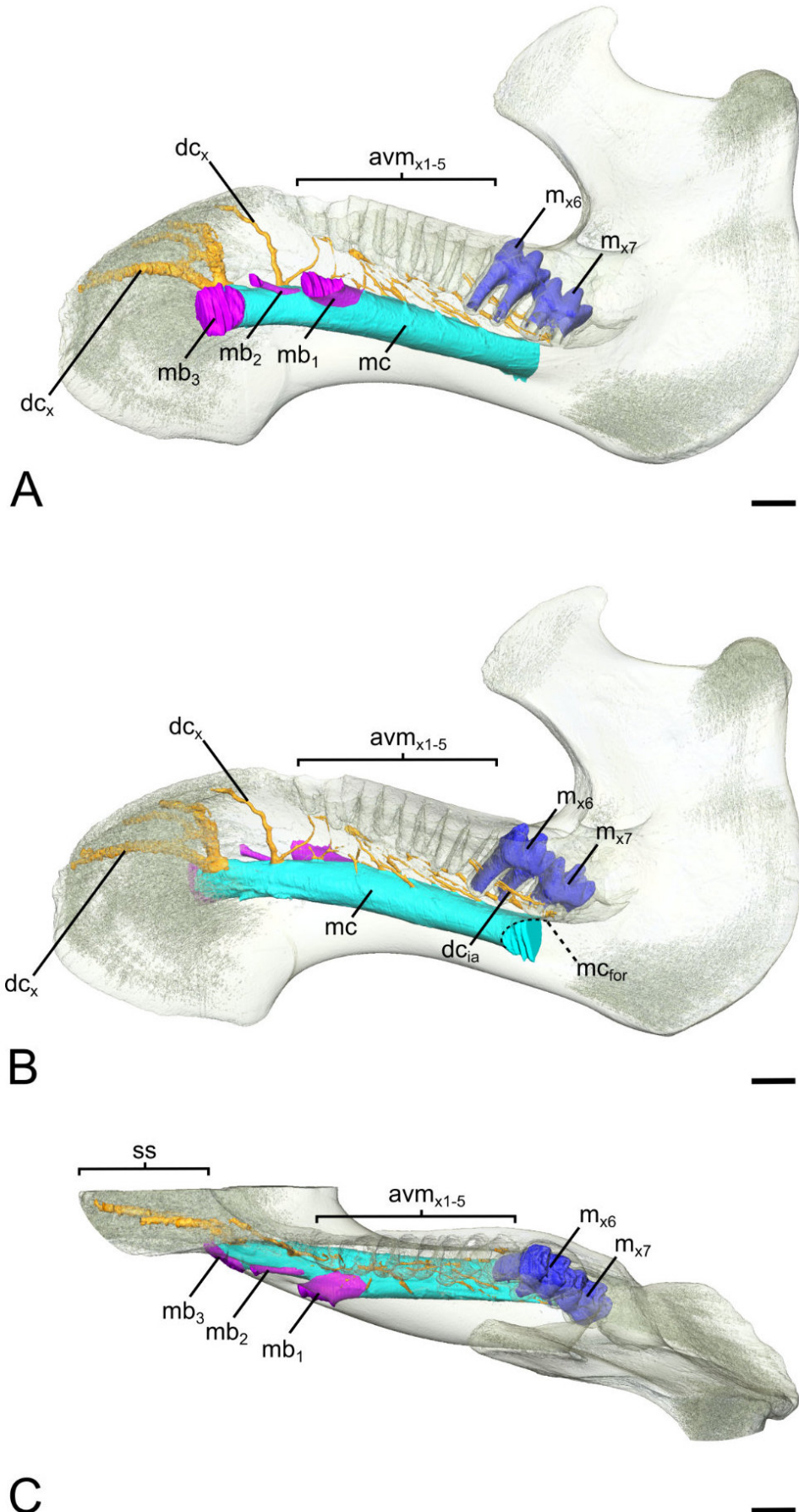


Figure 2. Internal mandibular morphology in *Trichechus manatus* (NHMUK 1843.3.10.12). Orange = dorsal canaliculi; purple = mental branches; cyan = mandibular canal; dark blue = teeth. Scale bars: 1 cm.

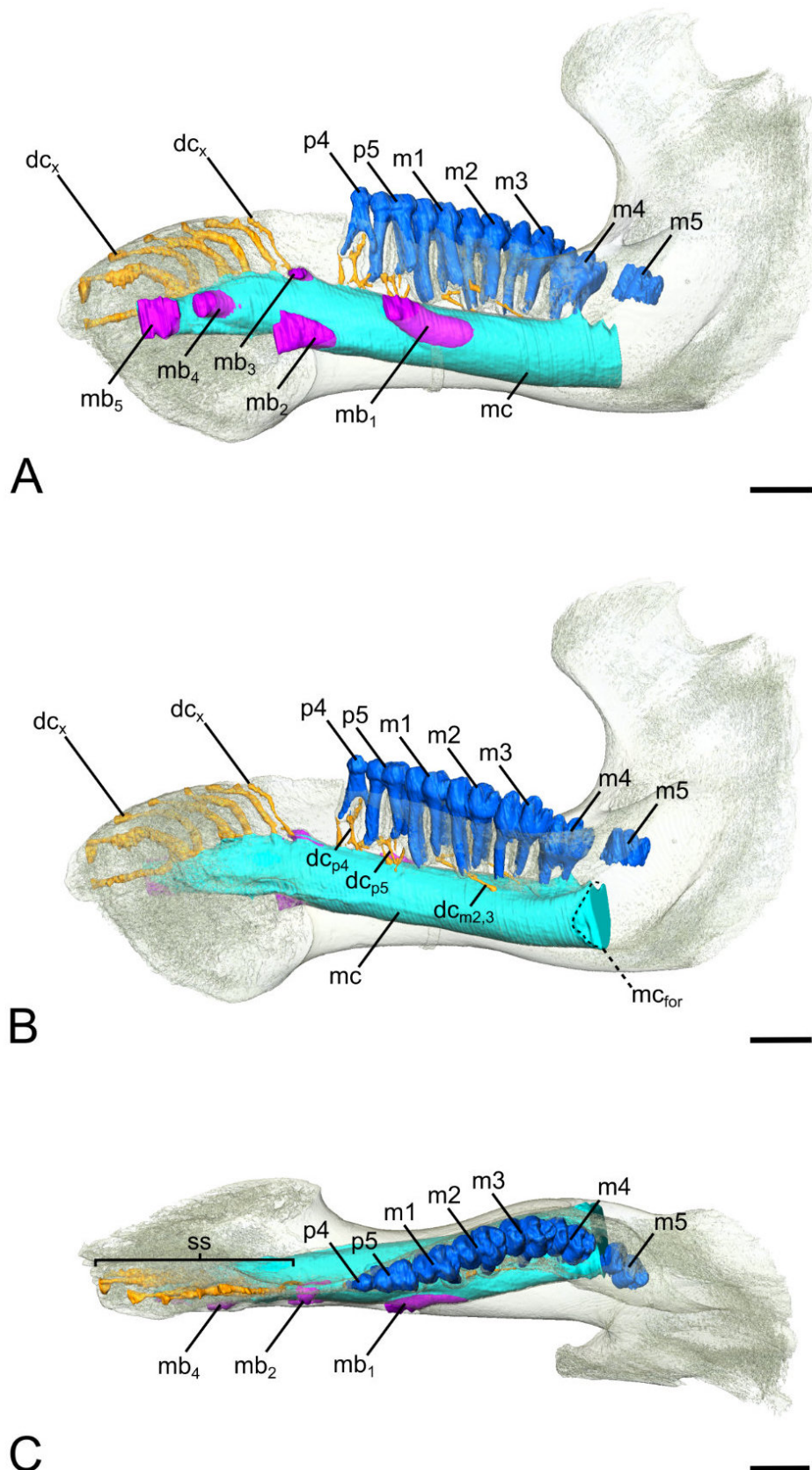


Figure 3. Internal mandibular morphology in *Trichechus inunguis* (NHMUK 1968.12.19.2). Orange = dorsal canaliculi; purple = mental branches; cyan = mandibular canal; dark blue = teeth. Scale bars: 1 cm.

The body of the mandible shows a concavity on the ventral edge, which ends anteriorly just anterior to the area of the symphysis. The concavity is more elongated anteroposteriorly than that present on the mandible of *Dugong* specimens. In lateral view, the symphysis forms a semicircle on the anterior surface of the mandible. The opening of the mandibular canal (mcfor) has a semi-ellipsoidal cross-section and its lateral margin is inclined posteriorly. The mcfor is located at the level of the seventh molar (mx7). The mandibular canal (mc) projects anteriorly, parallel to the edges of the mandibular body. As in the *Dugong* specimens, it is located in the ventral part of the mandible body. The mc posterior part is ventral to the molars, and the distance between these structures increases anteriorly. The most posterior mental branch (mb1) opens laterally at the level of the first two tooth alveoli (avmx1, avmx2). Anterior to it, the mb2 corresponds to the thinnest and longest mental branch. Its foramen outline is ellipsoid and more dorsal than those of mb1 and mb3. The most anterior mental (mb3) is located at the anterior end of the mc. The mental foramen is circular and located at the level of the long axis of the mandibular canal. The most posterior mb (mb1) has an ellipsoidal foramen and is located at the level of the long axis of the mandibular canal. The dorsal inter-alveolar canaliculi (dcia) project anterodorsally and connect the mandibular canal to the dental alveoli. The more anterior canaliculi (dcx), which are anterior to the intermediate mental branches (mb2, mb3), are anterodorsally projected, thicker, and exit at the level of the symphysis.

The mandible of NHMUK 1864-6-5-1 is similar to that of NHMUK 1843.3.10.12, but its symphysis is subtriangular and more jugal teeth are present (eight alveoli). The mental branch corresponding to mb2 of NHMUK 1843.3.10.12 is absent, but another mental branch is present posterior to that corresponding to mb1, below the tooth socket of the fourth molar. The only visible dorsal canaliculi are all located in the symphysis area and are shorter since the distance between the mandibular canal and the dorsal margin of the symphysis is smaller.

Trichechus inunguis

The mandible NHMUK 1968.12.19.2 (Fig. 3) is similar to that of other *Trichechus* species. However, the coronoid process of the ramus is slightly less anteriorly projected and less posteriorly curved. The teeth are all present, including two premolars (p4, p5) and five molars (m1-5). The concavity on the ventral margin is less pronounced than in *T. manatus* and *T. senegalensis*, and the body is more rectilinear anteroposteriorly than in *T. manatus*, similarly to the condition observed in *T. senegalensis*. The opening of the mandibular canal (mcfor) is semi-ellipsoidal in cross-section; its lateral edge is concave. The foramen lies ventral to the fifth molar (m5), which is not fully developed. The mandibular canal is much thicker and protrudes further anteriorly into the symphysis than those of other *Trichechus* species. As in *Dugong*, it is located in the ventral part of the mandibular body. Its position in relation to the teeth is similar to that of other *Trichechus* species. The mental branches (mb1, mb2, mb3, and mb4) open laterally. The most posterior mental branch (mb1) lies below the m1 and m2. The two most anterior

mental branches (mb4, mb5) are parallel to the mandibular canal. The intermediate mental branches (mb2, mb3) are positioned on the same dorso-ventral axis, but mb3 is dorsal to the mandibular canal while mb4 is ventral to it. The mb3 constitutes the thinnest and longest mental branch; its foramen is circular. The other mental branches are short and thick; their foramina are ellipsoid. The posterior dorsal canaliculi are connected to the teeth. The ones positioned ventral to the premolars (dcp4, dcp5) have a dorsal projection, while those positioned below the molars (m1-5) have an anterodorsal projection. The dorsal canaliculi (dcx), anterior to the intermediate mental branches (mb2, mb3), project anterodorsally, are thicker, and end at the level of the symphysis. They are more numerous and more vertical than those observed in other *Trichechus* species, except for the first anterior canaliculi which is almost perfectly horizontal.

Trichechus senegalensis

The external morphology of the mandible NHMUK 1885.6.30.2 (Fig. 4) is similar to that of *Trichechus manatus*. The coronoid process is prominent, pseudo-rectangular, and projected anteriorly at the same height as the condyle. The angular process forms an almost right angle. Eight molars are present (mx1-8). The opening of the mandibular canal (mcfor) is ventrolateral at the level of the seventh molar (mx7), semicircular in section and elongated anteroposteriorly. The mandibular canal (mc) projects anteriorly, parallel to the edges of the horizontal ramus; it lies at mid-height of the mandibular body. The posterior part is just ventral to the molars, and the distance between these structures increases anteriorly. The mental branches (mb1, mb2, and mb3) open laterally, parallel to the mandibular canal. The most posterior mental branch (mb1) lies under the molar mx3. The intermediate mental branch (mb2) lies under the molars mx1 and mx2. The most anterior mental branch is in continuity with the anterior projection of the mandibular canal. The three mental branches are short and thick; their foramina are ellipsoid in shape. The posterior dorsal canaliculi (dcx), which lie below the teeth (dcmx5,6), have an anterodorsal projection and are connected to the teeth. They diverge from the mandibular canal at the level of the mx7, but are connected to the mx5 and mx6. The dcia connects the alveoli of mx3 and mx4, and is not connected to the mandibular canal; it is horizontal, elongated anteroposteriorly and dorsal to the mandibular canal. The more anterior canaliculi (dcx) lie below the symphysis, anteriorly to the teeth and are thicker. They diverge from the mandibular canal between mb2 and mb3 with a dorsal direction, then form a right angle directed anteriorly, almost parallel to the mandibular canal, and then exit on the anterodorsal part of the symphysis. The dorsal canaliculus below mx1 has a more dorsal anterodorsal projection than the other canaliculi, and connects the mandibular canal to the tooth alveolus of mx1.

The specimen UM-V97 displays a similar external morphology as NHMUK 1868.12.19.2. It has one more tooth (nine molars) and more mental branches. Two mental branches are located at the anterior end of the mandibular canal, and are aligned anteroposteriorly and confluent: one is very anterior, longer, thinner, and opens dorsally; the other one is more posterior and shorter,

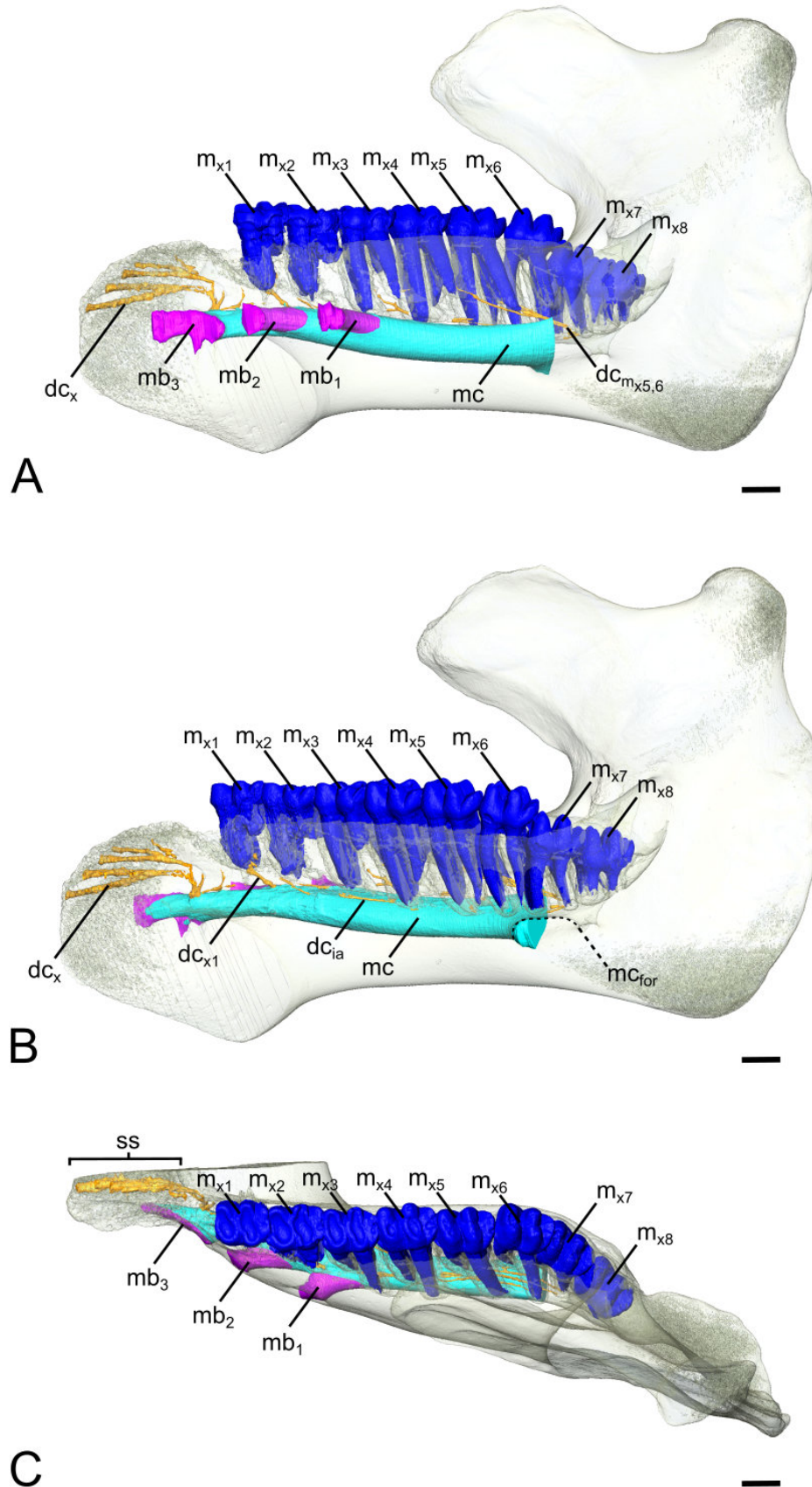


Figure 4. internal mandibular morphology in *Trichechus senegalensis* (NHMUK 1885.6.30.2). Orange = dorsal canaliculi; purple = mental branches; cyan = mandibular canal; dark blue = teeth. Scale bars: 1 cm.

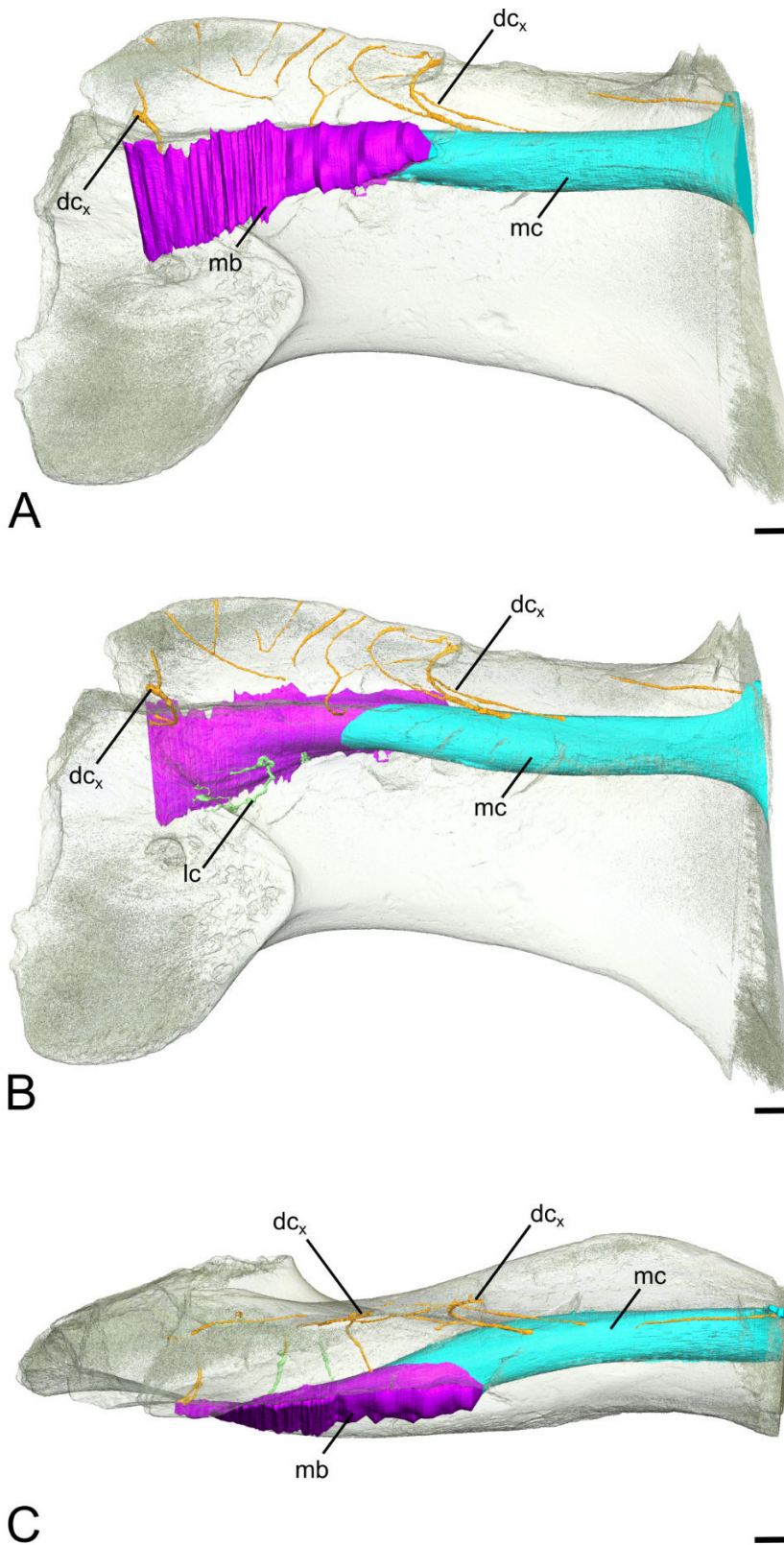


Figure 5. Internal mandibular morphology in *Hydrodamalis gigas* (UMZC 2023.67). Orange = dorsal canaliculi; purple = mental branches; cyan = mandibular canal. Scale bars: 1 cm

and opens dorsolaterally, below the anterior root of the third molar. NHMUK 1894-7-25-8 has a longer and thinner mandibular body. The posterior margin of the ascending ramus is damaged and only three teeth are present. Five tooth alveoli are visible. The anterior margin of the coronoid process is more rounded. The dorsal canaliculi of the symphysis are less numerous.

Hydrodamalis gigas

The mandible of NHMUK 2023-67 (Fig. 5) is damaged, but its CT data show a better resolution. The symphysis and the ascending ramus of the mandible are not preserved. All these elements are preserved in the specimen UMCZ C1021, which has a very high ascending ramus. The angular process is damaged on its most posterior edge, but it is prominent and projects posteroventrally. The horizontal ramus is high and thick; it shows no teeth or tooth alveoli. The symphysis, preserved only in UMCZ C1021, is sub-triangular, long and ventrally inclined; it displays a flattened area, similar to that observed in *Dugong* and *Trichechus*. The mandibular canal (mc) is longer than in *Dugong*, but relatively shorter than that of *Trichechus*. It is located dorsally in horizontal ramus. It is high and thick, circular in cross-section across its entire length. The posterior foramen of the mandibular canal is not preserved in NHMUK 2023-67, but its shape should be semi-ellipsoid, as in UMCZ C1021. The mandibular canal stops posteriorly to the symphysis and opens laterally into a wide mental foramen, which is elongated anteroposteriorly. The dorsal canaliculi are well marked on the laterodorsal surface of the mandible anterior to the mental foramen. They are homogeneous in thickness; the most posterior one is horizontally inclined in UMCZ C1021. Despite the complete absence of teeth, the mandible of *Hydrodamalis* displays dorsal canaliculi (dcx). The most anterior canaliculi are thicker, as in *Dugong* and *Trichechus*. However, this difference in thickness is much less pronounced than in other extant sirenian species. In contrast to *Dugong* and *Trichechus*, the most anterodorsal canaliculi (one in NHMUK 2023-67, two in UMCZ C1021) are posterior to the exit of the nerves through the mental canal. The most anterior half of the symphysis is devoid of dorsal canaliculi.

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