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Science history meets microtomography: Comparison of the world's oldest physical model of an extinct animal's inner ear with the 3D virtual counterpart

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ABSTRACT

In 1936 in Basel, Johannes Hürzeler isolated the bony labyrinth out of a fossil petrosal bone of the small artiodactyl Cainotherium from the 24 million years old French locality of Branssat (Hürzeler, 1936). To remove it from the bone, he filled the hollow chambers of the bony labyrinth with celluloid and then dissolved the petrosal bone itself to get its endocast. To the best of our knowledge, this is the first cast of an extinct animal inner ear ever produced and published. Here we compare this analog model still preserved in the collection of the Natural History Museum Basel with a 3D virtual model generated by a microtomography scan of another petrosal bone of the same taxon from the same locality. A previous study published the first digital data on the ear region of a cainotheriid but without segmenting the bony labyrinth and another one visualized an inner ear without describing it. Our 3D digital model expands our knowledge of the inner ear anatomy of Cainotherium by showing the so far unclear extent and course of the vestibular and cochlear aqueducts as well as by enabling volumetric data of the different labyrinth parts to be measured.

Keywords: bony labyrinth, artiodactyla, Cainotherium, Johannes Hürzeler, Oligocene, endocast, hard X-ray microtomography

1. INTRODUCTION

Cainotheriidae is a puzzling artiodactyl family that extends from the Middle Eocene to the Middle Miocene of continental Europe. It is often represented in fossil-bearing localities by very common remains of small rabbit-sized for which most of the skeletal parts are usually preserved. Despite the large amount of material available of this even-toed ungulate, its exquisite preservation and decades of studies, its affinities are still unclear. Various phylogenetic hypotheses were proposed placing Cainotheriidae either within the tylopod clade (i.e., a group including today's camels), or close to the base of ruminants, or even as a stem Artiodactyla being the sister group of the clade formed by Ruminantia + Tylopoda (see [1] for a review). Recent discoveries of very abundant material in the famous Quercy area in southern France enabled new phylogenetic analyses to be carried out and reinforced the hypothesis of a relationship with ruminants as a sister clade to anoplotheres, another puzzling Eocene-Oligocene endemic European artiodactyl family [2].

The history of studies on Cainotheriidae extends back to the 19th century until Johannes Hürzeler set a milestone by releasing a seminal work describing the whole skeleton of the late Oligocene and Early Miocene genus Cainotherium [3]. He had exquisite material from French localities of the Allier Basin at disposal at the Natural History Museum in Basel. His work offered a detailed description of the ear region of Cainotherium including for the time modern techniques, such as X-Ray shots, that enabled imaging internal structures. Along with these methods, Hürzeler decided to go a step further in producing a cast of the bony labyrinth of Cainotherium by filling the inner ear bony chambers of a petrosal bone from the late Oligocene of Branssat with celluloid and removing the later from its bony case by dissolving the petrosal bone itself with acids. This technique, well known since the middle of the 19th century and applied to many living vertebrates [4-6], seem to have been applied here for the first time on an extinct animal. This complex technique gave him access to the morphology of the bony labyrinth that he described in details, but without comparing it with other mammals. The

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celluloid cast produced in 1936 is still present in the collections of the Natural History Museum in Basel registered under the collection number NMB Bst.6140.

The bony labyrinth of artiodactyls has since proven to be of great phylogenetic interest, i.e. [7-8]. The advent of hard X-ray microtomography has largely contributed to the increase in the number of studies on the bony labyrinth anatomy in extinct animals, i.e. [9-14]. While the bony labyrinth of Cainotherium has been visualized together with the ear-region [1] or 3D rendered [15], it has never been described in details based on this new source of data so that the work of J. Hürzeler [3] remains the only description of its inner ear. Other parts of the ear region of Cainotherium are described in details (the petrosal bone [1] or the tiny ossicles [16]) but the bony labyrinth still awaits that level of details. Here we visualized a Cainotherium petrosal bone from Branssat, the same locality as for the celluloid cast of J. Hürzeler, and we compare the rendered bony labyrinth to the celluloid cast yielding more information on the anatomy of this structure.

2. MATERIALS AND METHODS

We here compare two specimens of the bony labyrinth of the artiodactyl mammal Cainotherium sp.. One is an old celluloid cast, numbered NMB Bst.6140, and made in 1936 for J. Hürzeler's seminal publication on this animal. It was produced by injecting celluloid inside a petrosal bone and dissolving the latter to isolate the endocast. The second is a digital model reconstructed from a petrosal bone, numbered NMB Bst.6139, of the same taxon and from the same locality, the French late Oligocene locality of Coderet-Branssat dated to about 24 million years. It is the reference locality for biochronological reference level MP30.

The celluloid cast NMB Bst.6140 was imaged using a Keyence binocular VHX-2000 at the Natural History Museum Basel (NMB). The microtomographs of the casts were produced by SKYSCAN 1275 (Bruker, Kontich, Belgium). 1440 equiangular radiographs were acquired over 360° using a selected acceleration voltage of 35 kV and a beam current of 231 µA. The effective pixel length of the detector was 7 µm.

The bony labyrinth of specimen NMB Bst.6139 was surrounded by hard tissues. Therefore higher energies were required for the visualization. For the experiments a phoenix|x-ray nanotom® (Waygate Technologies Wunstorf, Germany) with an acceleration voltage of 120 kV and a beam current of 200 µA was used. In order to increase the mean energy a Cu-filter with 0.25 mm thickness was introduced. 1440 equiangular projections over 360 ° were acquire with a pixel length of 11 µm. All scans were performed at the Core Facility "Micro- and Nanotomography" at the University of Basel.

Digital models were rendered using the visualization and segmentation editors of AVIZO 9.0 at the Natural History Museum Basel. Bony labyrinth (BL) measurements were done using the 3D measuring tools (length and angle) of AVIZO 9.0. They are given in Table 1.

Table 1. Measurements of both specimens. Length is measured from the top of the common crus to the apex, see [25].

Specimen	NMB Bst.6139	NMB Bst.6140
Number of cochlear turns	3.1	3.1
Volume [mm^3]	16.7	-
Stapedial ratio	1.95	1.92
Cochlear aspect ratio	0.81	0.87
ASC height [mm]	3.1	3.3
ASC width [mm]	3.1	2.9
PSC height [mm]	2.94	2.8
PSC width [mm]	2.12	1.9
LSC length [mm]	2.19	2.02
LSC width [mm]	2.53	2.4
Angle ASC-PSC [°]	91	92

Angle ASC-LSC [°]	90	80
Angle PSC-LSC [°]	84	90
Length [mm], sensu Ref. [25]	6.3	6.36

3. RESULTS

The celluloid cast of NMB Bst.6140 is still rather well preserved after more than 85 years of conservation at the Natural History Museum Basel (Fig. 1a-d). It shows most of the structures visible on a classical bony labyrinth except the most delicate or faint ones (vestibular and cochlear aqueducts, endolymphatic sac, and secondary lamina). The cochlea is very delicate too and has slightly more than 3 turns. A microtomography scan was performed but segmentation is rather difficult because of the very light celluloid material. No internal structures are visible. The cast is empty except the presence of some bony leftovers after dissolution of the enclosing petrosal bone. The vestibulo-cochlear nerve entrance within the cochlea is filled with indeterminate material that prevents further study of the internal wall of the cochlea. Table 1 gives the measurements of the BL. They are very similar to the ones taken on the digital model of NMB Bst.6139. The digital isosurface (Fig. 1d) of NMB Bst.6140 gives a little more details than its picture, although the interpretative drawings published in 1936 give a very good overview of its morphology. In particular, it shows that the lateral and posterior canals are not connected laterally and that the cochlea has clearly defined turns.

Figure 1 e-i illustrates the bony labyrinth of the petrosal NMB Bst.6139, extracted virtually. The cochlea completes a little more than 3 turns. It is close to the vestibule. The first third of the basal turn is slightly detached from the second one. The basal turn is much thicker than both others are. The secondary bony lamina is present and visible on about half of the basal turn, being quite deep on its first quarter. A primary bony lamina is visible inside the cochlea and turns along most of its course. The aspect ratio (total height divided by total width) is 0.81, which is high testifying to an elongate cochlea, even higher than in tragulid artiodactyl for which the cochlea completes more than 3 turns [17]. The cochlear aqueduct is long, flat in diameter with a slight thinning half way through, and straight (Fig. 1g, i).

The *fenestra cochleae* is round and bigger than the *fenestra vestibuli*. The latter is very ellipsoid in shape with a stapedial ratio of 1.95, being thus almost twice as long as large. It is slightly inclined rostrally (Fig. 1h). On the vestibule, both the *utriculus* and *sacculus* are well-defined (Fig. 1g), the latter is ellipsoid in shape. The *utriculus* seems to be small and round, much smaller than the *sacculus*.

The anterior semi-circular canal is by far the most dorsally expanded canal; it changes slightly its course after leavings the common crus and is round in shape. It shows a slight medial deflection, being thus bended over its course (Fig. 1h) accompanied by a sigmoid shape in dorsal view (Fig. 1i). The posterior semi-circular canal is much less expanded and has a more ellipsoid shape as the anterior semi-circular canal. The common crus is rather thin, quite long and keeps a similar diameter along its course. The lateral semi-circular canal is also rather ellipsoid but less so than the posterior semi-circular canal. It branches above the posterior ampulla between it and the base of the common crus. In lateral view (Fig. 1e), it extends through the plane of the posterior canal, a feature that was already described for tragulid ruminants [17]. The angles between the semi-circular canals all reach about 90° (Table 1). The ampullae at the base of the canals are round and bulged. The transition between the ampullae and the corresponding canals are not sharp but rather made by an enlargement of the canals themselves.

The vestibular aqueduct is a thin canal that runs dorso-ventrally, it is very tilted and short reaching out to the midpoint of the posterior semi-circular canal plane. Its origin lies around the base of the common crus laterally, not far from the medialmost extent of the *sacculus*. The vestibular aqueduct ends in a large pouch-like endolymphatic sac that is slightly depressed and tilted medially. The volume of NMB Bst.6139 is 16.7 mm3.

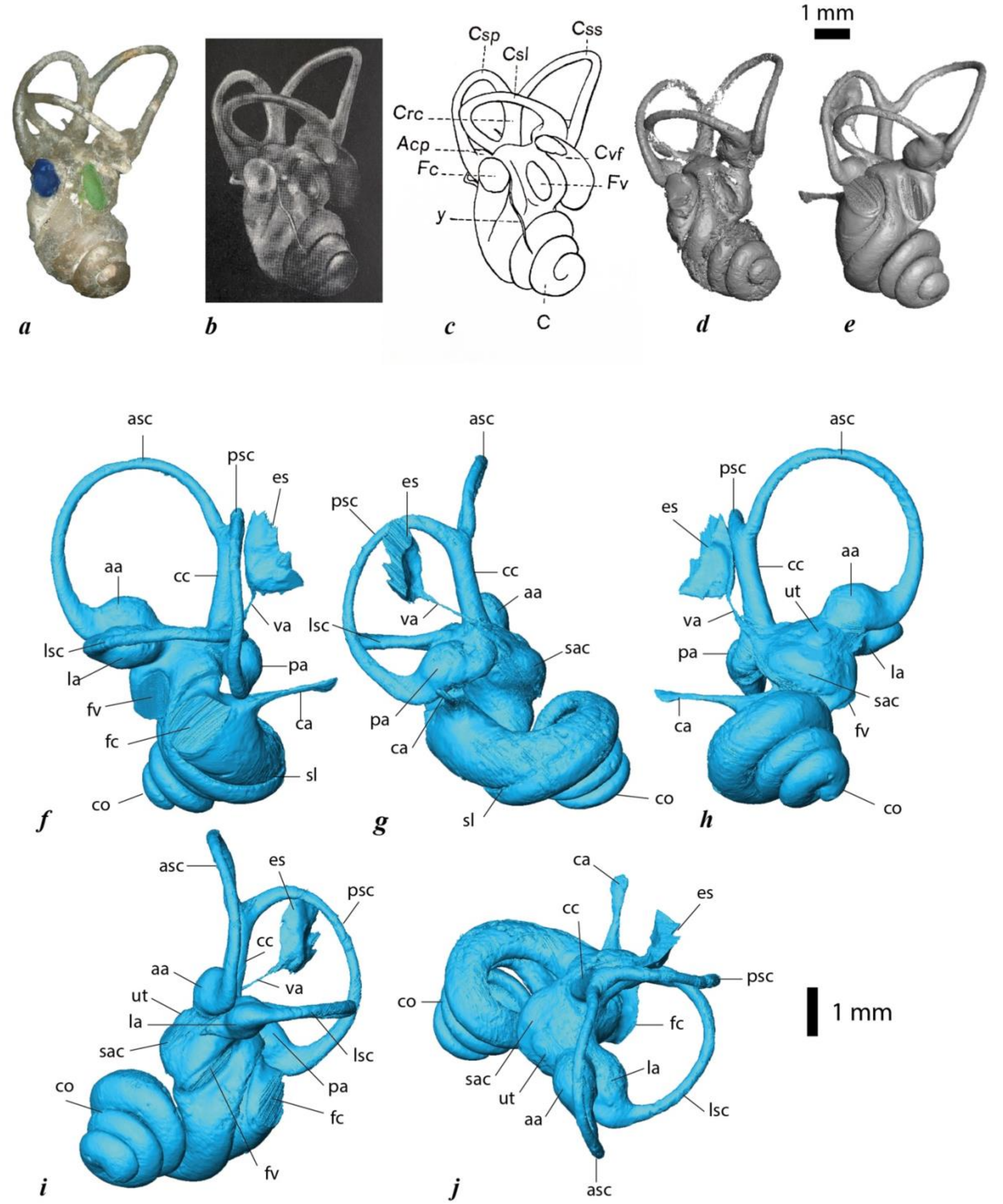

Figure 1. a-d: celluloid cast of the BL of Cainotherium from Coderet-Branssat (specimen NMB Bst.6140); a: picture taken in 2022 on a Keyence binocular VHX-2000, the stapedial window (fenestra vestibuli) was colored in green and the oval window (fenestra cochleae) in blue. b: drawing published in Hürzeler, 1936 (3, Fig. 27, p. 38). c: interpretative drawing with annotated morphological structures published in Hürzeler, 1936 (3, Fig. 28a, p. 39). d: digital isosurface model generated in 2022 through computed tomography. e-j, digital model of the BL of Cainotherium from Coderet-Branssat (specimen NMB Bst.6139) generated in 2022 through computed tomography and segmentation, in e: same orientation as the 1936 celluloid cast for comparison, f: lateral, g: occipital, h: medial, i: rostral, j: dorsal view.

4. DISCUSSION

Cainotherium's ear region has been the focus of research for more than 80 years, e.g. [1, 3, 16]. This area of the skull is rich in phylogenetic information and provides the basis of many studies investigating the affinities between extant and extinct mammals [7, 10, 12, 18-20]. In 1936, Johannes Hürzeler decided to physically model the bony labyrinth of *Cainotherium* sp. in order to describe the anatomy of the animal comprehensively. His description of the BL is still valid with nice illustrations albeit being short and not conclusive on any aspect of *Cainotherium*'s affinities. He only mentioned the possible head posture at rest derived from the horizontality of the lateral semi-circular canal but this relationship has recently been shown not to be significant over a large dataset of "ungulate" mammals [21].

Our comparison of the 1936 celluloid cast NMB Bst.6140 with a digital model of the BL of *Cainotherium* sp. (NMB Bst.6139) from the same locality shows how well preserved the +85 years old cast is and how relevant it remains. However, the digital model shows greater resolution and details, especially on the cochlea, vestibular aqueduct, and its accompanying endolymphatic sac. The orientation, length, and shape of the latter are of strong phylogenetic interest in a subset of the artiodactyl clade, namely in ruminants [7], which have been proposed to be phylogenetically be close to Cainotheriidae [2].

Cainotherium has a vestibular aqueduct originating slightly lateral from the base of the common crus and with a very much-tilted course. This is a somewhat derived condition with regards to early artiodactyls from the Eocene such as *Diacodexis* and others [10, 22] or from the Oligocene such as *Leptomeryx* [23] for which it seems to be more parallel to the common crus. Interestingly this condition is also present in *Hypisodus* (pers. obs. and [23]), a North American Oligocene diminutive early artiodactyl putatively attributed to the extinct stem ruminant family Hypertragulidae, but with debated affinities. It is out of the scope of this paper to describe the in BL of *Hypisodus* in details. Nonetheless, the BL of *Cainotherium* shares some similarities with that of *Hypisodus*, especially on its vestibular part: a high extension of the anterior semi-circular canal above the common crus, a high branching of the lateral semi-circular canal above (*Cainotherium*) or also high on the occipitalmost extent of the posterior ampulla (*Hypisodus*), a slight medial deflection of the anterior semi-circular canal. Typlopod affinities have been proposed for *Cainotherium* mostly on the basis of the external morphology of the ear region (e.g., see [1] for a review). *Cainotherium*'s BL is different from that of *Camelus*, which is much derived with a short common crus and very flat and expanded transitions between the ampullae and their corresponding semi-circular canals (pers. obs.). However, *Cainotherium* shares a high branching of the lateral semi-circular canal and a tilted vestibular aqueduct with Camelus although the furrow-shaped endolymphatic sac of the later is significantly different from that of *Cainotherium*.

It is too premature to draw any conclusion on these similarities or differences between *Cainotherium*, *Hypisodus*, and *Camelus*. It is worth noting that both *Hypisodus* and *Cainotherium* are among the smallest artiodactyls known, so structural constraints may also play a crucial role in shaping convergent morphological characters. Little is known as to how and to what extent structural constraints participate in shaping the inner ear. Morphological integration studies would help in disentangling the different factors influencing this region of the skull.

Finally, the microtomography scan of NMB Bst.6139 revealed a stapes preserved inside the BL, which is not going to be described here. The ossicular chain of earlier cainotheriids was recently published for the first time [16], including the stapes. The latter is known for its significant phylogenetic signal in ruminants for example [24]. Finding more middle ear bones along the history of the cainotheriid clade spanning more than 25 million years will undoubtedly lead to further critical information on their relationships.

5. CONCLUSIONS

Our new 3D digital model of *Cainotherium*'s BL enlarges the possibilities for comparisons that were so far available through a celluloid cast made by Johannes Hürzeler in 1936. J. Hürzeler dissolved the petrosal bone in the process of casting the enclosed BL, something that museums do not allow anymore, or only exceptionally. He took this decision because he knew that many other petrosal bones of the same animal and from the same locality were still available for studies in the collection of the Natural History Museum Basel. By doing so, he produced, to the best of our knowledge, maybe the first ever 3D physical model of an extinct mammal's inner ear paving the way for future developments. The destruction of the bone around the BL had no consequence on possible future studies, including the present one that he could not imagine back in 1936. Not only does the present comparison shows the now classical power of X-Ray CT-scanners to provide non-destructive data, but it also provides original data, that were not available on the 1936 celluloid cast. Altogether, this will participate in investigating the affinities of this enigmatic taxon. It will add to our understanding of the long evolutionary history of its family, the Cainotheriidae including why it lasted more than 25 million years, how it crossed the biological crisis of the Grande Coupure (Eocene-Oligocene transition), and why it became extinct in the Middle Miocene some 11 million years ago.

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