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Benoit, Julien

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Synchrotron scanning reveals the deep evolutionary root of the mammalian brain: the surprisingly advanced endocast morphology of *Lumkuia fuzzi* (Cynodontia: Probainognathia)

Julien Benoit* §

Evolutionary Studies Institute, University of the Witwatersrand, Private Bag 3, WITS, Johannesburg, 2050 South Africa
E-mail: julien.benoit@wits.ac.za

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The mammalian brain is very distinctive for its large relative size, enlarged olfactory bulbs, and layered isocortex. These defining traits likely evolved in the non-mammalian probainognathian cynodonts, although the timing and exact phylogenetic sequence in which these characters evolved is not well understood. The endocast of the brain cavity provides a unique window into the evolution of the central nervous system of extinct species. The endocast of the basal-most probainognathian, *Lumkuia fuzzi*, is here described for the first time. Its olfactory bulbs are relatively large despite that its encephalization quotient is lower than in Mammaliaformes. This contradicts the consensually established hypothesis that encephalization and olfaction evolved in concert. Moreover, the data presented here do not indisputably distinguish between the encephalization quotients of Mesozoic mammals, non-mammalian mammaliaforms, and non-mammaliaform cynodonts, which suggests that brain enlargement was gradual in this lineage. *Lumkuia* also possesses marked cerebral hemispheres, which is traditionally interpreted as the sign of the presence of an isocortex and hair. The enlarged olfactory bulbs and cerebral hemisphere in *Lumkuia* strongly support that the defining features of the mammalian brain began evolving in the last common ancestor of the Probainognathia clade, as early as the early Anisian.

Keywords: Cynodontia, endocast, brain, cerebral hemisphere, isocortex, Probainognathia, encephalization.

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INTRODUCTION

Mammals evolved some of the largest and most derived brains in the animal kingdom (Manger *et al*. 2013; Smaers *et al*. 2021, 2023; Bertrand *et al*. 2022). However, the evolutionary origin of key defining mammalian brain structures, such as the isocortex, remains unresolved. Therefore, documenting the evolution of the central nervous system among their ancestors, the non-mammalian cynodonts, is of utmost importance. It is also crucial to shed some light on the evolutionary origin of certain defining soft-tissue, behavioral, and physiological features such as endothermy, parental care, lactation, locomotion, hair, and gregariousness (see Benoit *et al*. 2022a; Rowe 2023 for reviews), which seldom preserve in the fossil record.

Non-mammalian cynodonts include the clade Eucynodontia and a basal grade of non-eucynodont cynodonts (Fig. 1), the latter of which includes all late Permian and some Triassic genera, such as *Thrinaxodon* and *Galesaurus* (Benoit *et al*. 2022b; Kerber *et al*. 2023). The Eucynodontia are themselves divided into two clades (Fig. 1), the Probainognathia and Cynognathia (Hopson & Kitching 2001; Liu & Olsen 2010). Mammalia, and the more inclusive clade Mammaliaformes, are directly descended from the Triassic Probainognathia (Fig. 1), and as such non-mammaliaform probainognathians have been, and are still, the focus point of many paleoneurological studies (Quiroga 1979, 1980, 1984; Rodrigues *et al*. 2014, 2018; Hoffman & Rowe 2018; Hoffmann *et al*. 2021; Kerber *et al*. 2021, 2023; see Benoit *et al*. 2022a; Rowe 2023 for reviews). Most of the apomorphies that define the early mammalian brain, such as the enlargement of the olfactory bulbs, division of the cerebral hemisphere, appearance of the vermis on the endocast, and the loss of the pineal body and unossified zone, originated among non-mammaliaform probainognathians (Rodrigues *et al*. 2018; Benoit *et al*. 2022a; Kerber *et al*. 2023).

Lumkuia fuzzi, from the Middle Triassic of South Africa, is currently considered the basal-most member of the Probainognathia, and is therefore crucial to understanding character polarity at the root of the mammalian clade (Hopson & Kitching 2001; Benoit *et al* 2022b). Synchrotron radiation computed tomography (SRCT) scanning has enabled the mining of an unprecedented wealth of data about this species' neurology and cranial and postcranial anatomy, that recently shed new light on its dental replacement, locomotion, physiology, and phylogeny (Benoit *et al*. 2017a, 2020, 2022b; Araújo *et al*. 2022). Here,

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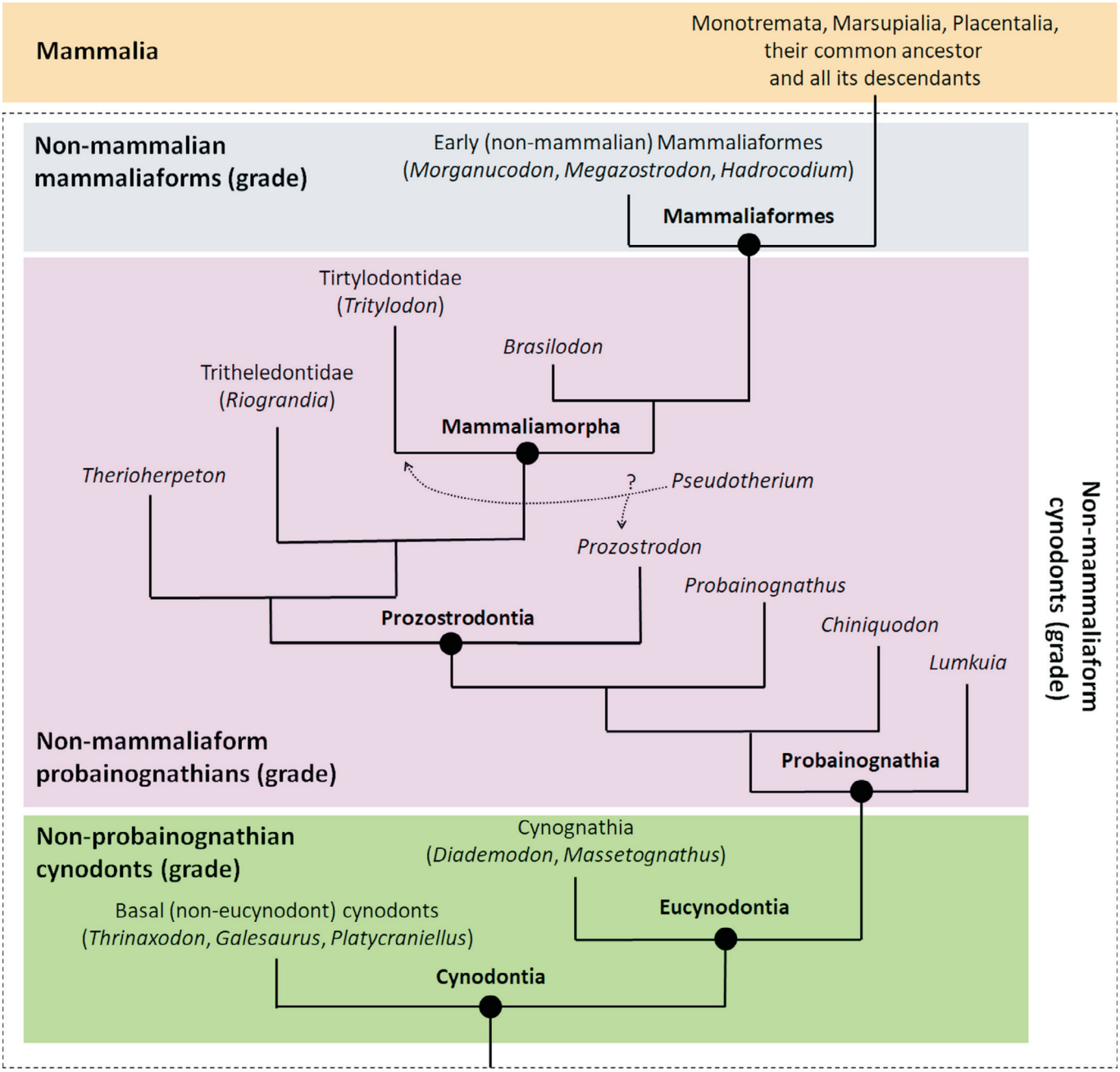


Figure 1. Phylogenetic tree of the taxa discussed in the text. Colour boxes : paraphyletic assemblages (grades). Tree topology after Benoit *et al.* (2022a) and Kerber *et al.* (2023).

Synchrotron Radiation CT scanning (SRCT) was used to access the brain cavity (endocast) of the holotype of *Lumkuia fuzzi*. In the context of synapsid neurological evolution, *Lumkuia* represents the baseline from which subsequent neurological traits evolved. Documenting the morphology of its endocast is crucial to help polarize the evolutionary changes that led to the onset of the unique mammalian nervous system.

MATERIAL AND METHODS

Specimen BP/1/2669 is from the Lumku Mission locality (Chris Hani District Municipality, Eastern Cape Province, South Africa), in the Burgersdorp Formation of the Beaufort Group, which is correlated to the *Trirachodon-Kannemeyeria* Subzone of the *Cynognathus* Assemblage Zone (about 247Ma) (Hopson & Kitching 2001; Hancox *et al*. 2020). The specimen includes a skull with occluded lower jaw (basal skull length: 59 mm) and a partial skeleton (Benoit *et al*. 2022b).

The specimen was scanned at the European Synchrotron Radiation Facility (Grenoble, France) on the ID19 beamline using propagation phase-contrast synchrotron X-ray microtomography (see details in Benoit *et al*. 2022b). The research at the ESRF was conducted under the South African Heritage Resources Agency export permit no. 2582, Case no. 11136. The SRCT scan of BP/1/2669 is available on the ESRF website: http://paleo.esrf.eu/. The 3D model of the endocast is available in the supplementary material (SI 1).

The endocast was reconstructed using manual segmentation in Avizo 9 (Thermo Fisher Scientific, Hillsborough, OR, U.S.A.). Unlike in mammals, the reconstruction of the digital endocast in non-mammalian cynodonts always poses a challenge as the ventral aspect of the braincase is

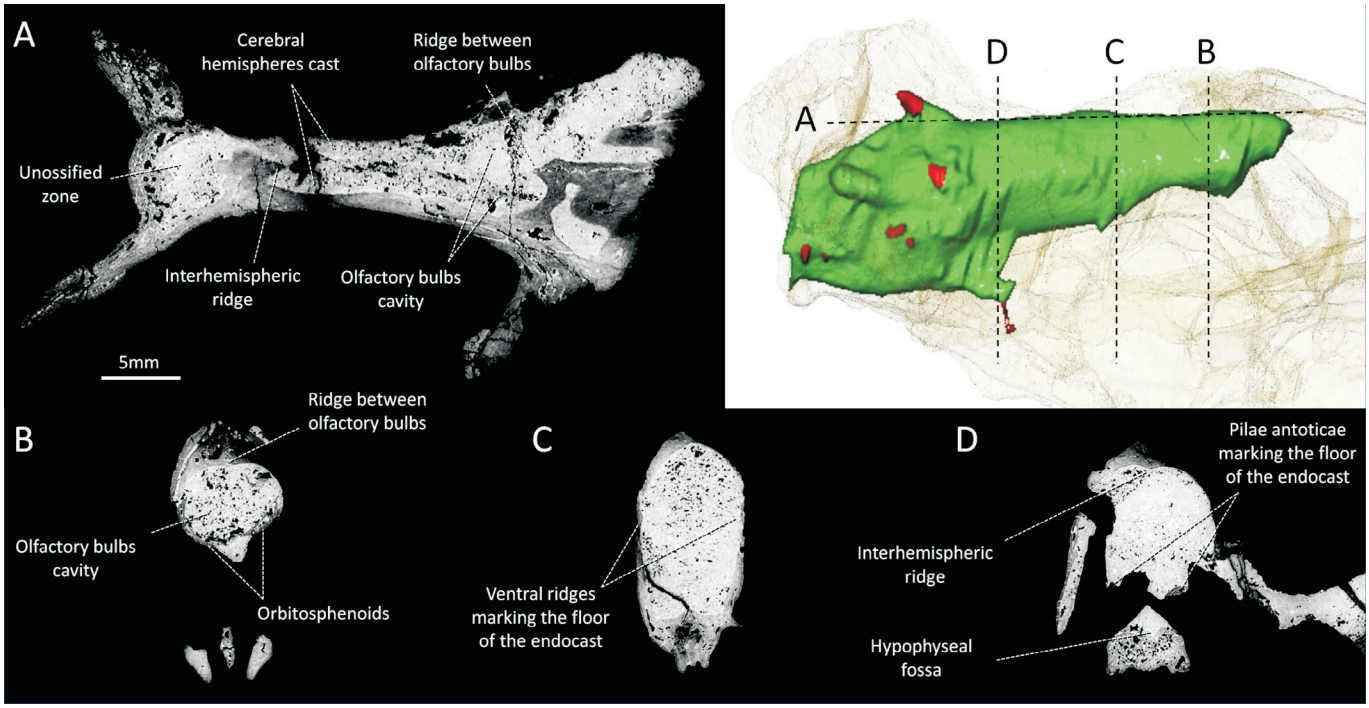


Figure 2. CT sections through the endocranial cavity of BP/1/2269. **A**, Coronal section through the cranial roof; **B**, **C** and **D**, cross-sections through the braincase at the level of the olfactory bulbs, anterior half of the cerebral hemispheres and hypophyseal fossa, respectively. The position of sections A, B, C, and D is indicated on the digital endocast in the top right (in lateral view, skull in transparent). Scale bar: 5 mm.

usually poorly ossified and the orbitosphenoids are rarely preserved *in situ* (Hopson 1979; Kielan-Jaworowska *et al*. 2004; Benoit *et al*. 2017b; Huttenlocker *et al*. 2020). Specimen BP/1/2669 preserves an *in situ* orbitosphenoid, as well as complete pila antoticae, and the unique combination of high resolution and phase contrast provided by SRCT images enables the observation of subtle ridges on the braincase wall that clearly mark the maximum possible expansion of the endocast (Fig. 2). As such, the ventral limits of the endocast could be reconstructed with reasonable accuracy, despite the absence of a continuous bony floor to the braincase. Damaged and unossified parts were reconstructed using the Avizo interpolation function.

Measurements of the endocast of *Lumkuia* were made using the 2D linear measurement tool of Avizo 9. The encephalization quotient adjusted to Paleozoic and Mesozoic Synapsida (EQS) introduced by Benoit *et al*. (2022a) was used to compare relative brain size in cynodonts (Table 1). The EQS of *Lumkuia* was calculated using a body mass of 645 g, which was estimated by Benoit *et al*. (2017a) using basal skull length. The EQS used for comparisons are from Benoit *et al*. (2022a). The EQS of *Pseudotherium*, *Prozostrodon*, *Chiniquodon*, and *Megazostrodon* were added to the dataset using the endocast volume and body mass from the literature (Jenkins & Parrington 1976; Wallace 2018; Hoffmann *et al*. 2021; Kerber *et al*. 2023; note that *Probelesodon* and *Chiniquodon* are here considered synonymous, following Abdala & Giannini 2002). All EQSs were calculated excluding the volume of the olfactory bulbs because it enables comparisons with more specimens (i.e. with those in which the lack of ossification of the floor of the olfactory area prevented reliable approximation of olfactory bulbs volume). In addition, since the volume of olfactory bulbs is notoriously difficult to estimate in non-mammalian cynodonts (Hopson 1979; Wallace 2018), variations in olfactory bulbs size are considered separately (ratio of olfactory bulb over endocast volumes in Table 1). The data

Table 1. The encephalization quotient adjusted to synapsids (EQS) and ratio of olfactory bulbs volume over the volume of the rest of the endocast (OB/EV) in Mesozoic Cynodontia. See the Material and Methods section for details.

Genus	EQS	OB/EV ratio
Galesaurus	1.68	0.14
Platycraniellus	1.78	0.10
Thrinaxodon	1.11	–
Diademodon	1.19	–
Lumkuia	0.98	0.16
Chiniquodon	1.21	0.06
Probainognathus	0.81	0.07
Prozostrodon	0.44	0.24
Therioherpeton	0.90	0.22
Riograndia	1.21	0.25
Pseudotherium	0.64	0.18
Tritylodon	1.00	0.05
Brasilodon	0.74	0.28
Morganucodon	1.54	–
Megazostrodon	1.15	0.09
Hadrocodium	1.30	0.15
Triconodon	2.00	–
Chulsanbaatar	2.56	–
Kryptobaatar	2.27	0.09
Vintana	2.35	0.17
Litovoi	1.57	0.17
Kennalestes	1.69	–
Vincelestes	1.89	0.12
Asioryctes	2.65	–
Zalambdalestes	3.50	–

for olfactory bulb volume is from Pavanatto *et al*. (2019) with the addition of data from Du Plessis (2010), Csiki-Sava *et al*. (2018), Hoffmann *et al*. (2014), and Kerber *et al*. (2023). Wallace (2018) argued that the volume of the olfactory bulbs in *Brasilodon* (including *Brasilitherium*, *sensu* Martinelli & Bonaparte 2011) was overestimated by approximately 25% to 50% in previous literature. Accordingly, the volume of the olfactory bulbs of *Brasilodon* was divided by two in the current dataset in order to use a more conservative value.

DESCRIPTION

In dorsal view, the endocast of BP/1/2669 is hourglass-shaped because the enlarged olfactory bulbs and cerebral hemispheres are separated by a narrow olfactory tract. In this respect, *Lumkuia* differs from all other non-mammaliaform cynodonts, which have a much more tubular-looking endocast in dorsal view (Hopson 1979; MacLean 1990; Macrini 2006; Kielan-Jaworowska *et al*. 2004; Rodrigues *et al*. 2018). The olfactory bulbs, cerebrum, midbrain, cerebellum, and spinal cord are linearly organized in the anteroposterior direction (Fig. 3). In lateral view, the endocast is mostly straight as there is little flexure between the fore- and hindbrain, unlike in many more basal synapsids such as dicynodonts and biarmosuchians (Benoit *et al*. 2017b; Laaß & Kaestner 2017). The total volume of the endocast is 1.31 cm^3. The volume of the endocast, excluding the olfactory bulbs is 1.13 cm^3. The resulting EQS is close to 1, similar to that of *Tritylodon* and *Thrinaxodon* (Fig. 4; Table 1). An EQS of about 1 indicates that the endocast of *Lumkuia* is of the volume expected for an early synapsid of its body size.

The olfactory bulbs are relatively large as their volume represents about 16% of the rest of the endocast (and 14% of the complete endocast) (Table 1). This is larger than in *Hadrocodium* and similar to the largest olfactory bulbs among crown Mesozoic Mammalia (Table 1). The olfactory bulbs are roughly oval in dorsal view as they are longer than wide (Fig. 3A). A deep longitudinal sulcus separates the two olfactory bulbs medially (Figs 2B, 3A), as in other probainognathians, except *Chiniquodon* (Quiroga 1979, 1980, 1984; Kemp 2009; Rodrigues *et al*. 2018; Kerber *et al*. 2023). This sulcus is usually faint or absent in non-probainognathian cynodonts, as in *Galesaurus* for example (Pusch *et al*. 2019). In lateral view, the olfactory bulbs taper anteriorly (Fig. 3B). The broad

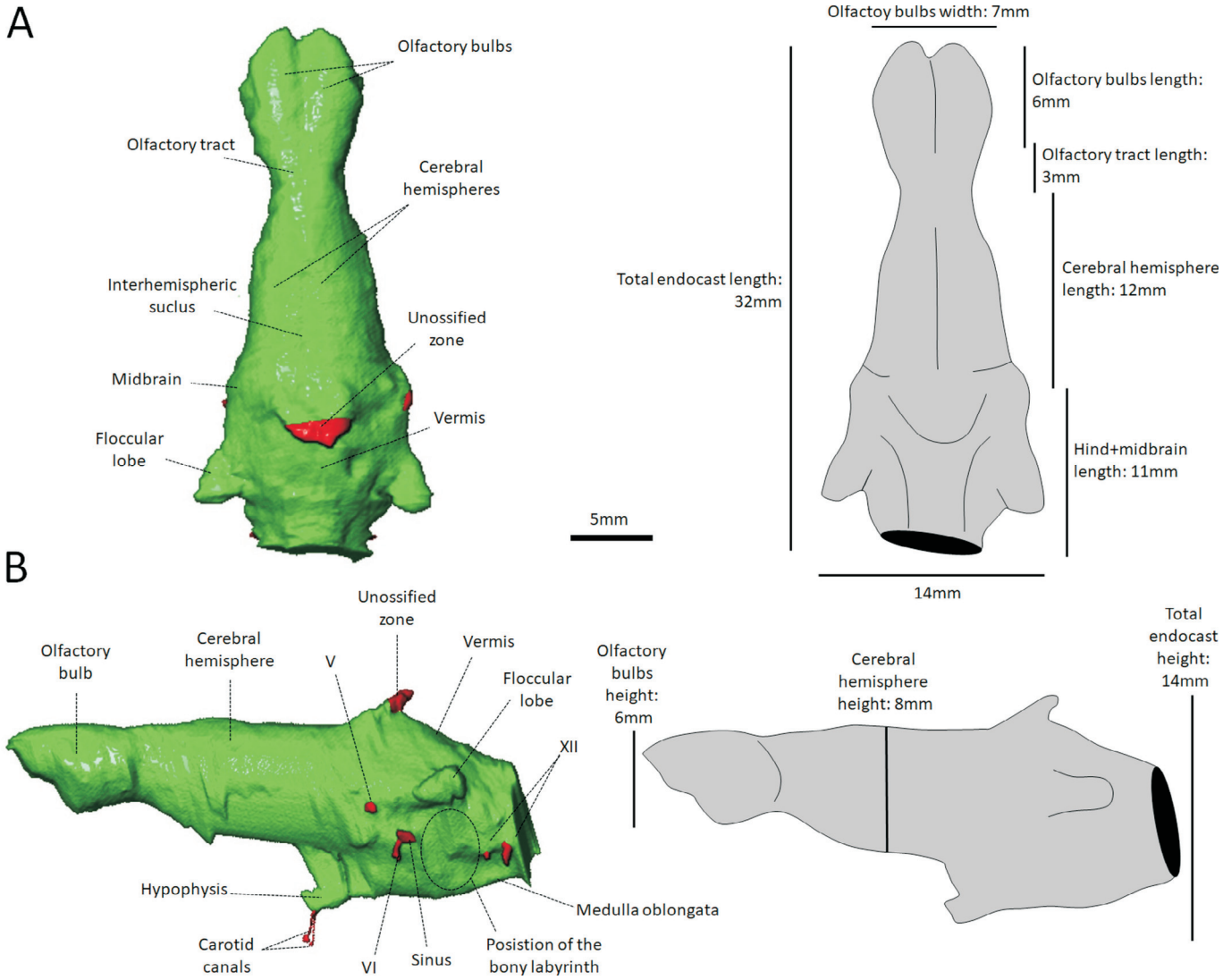


Figure 3. Digital reconstruction of the endocast of BP/1/2669, and interpretive drawings, in dorsal (**A**) and lateral (**B**) views. Measurements of the endocast are indicated on the interpretive drawings. Scale bar: 5 mm.

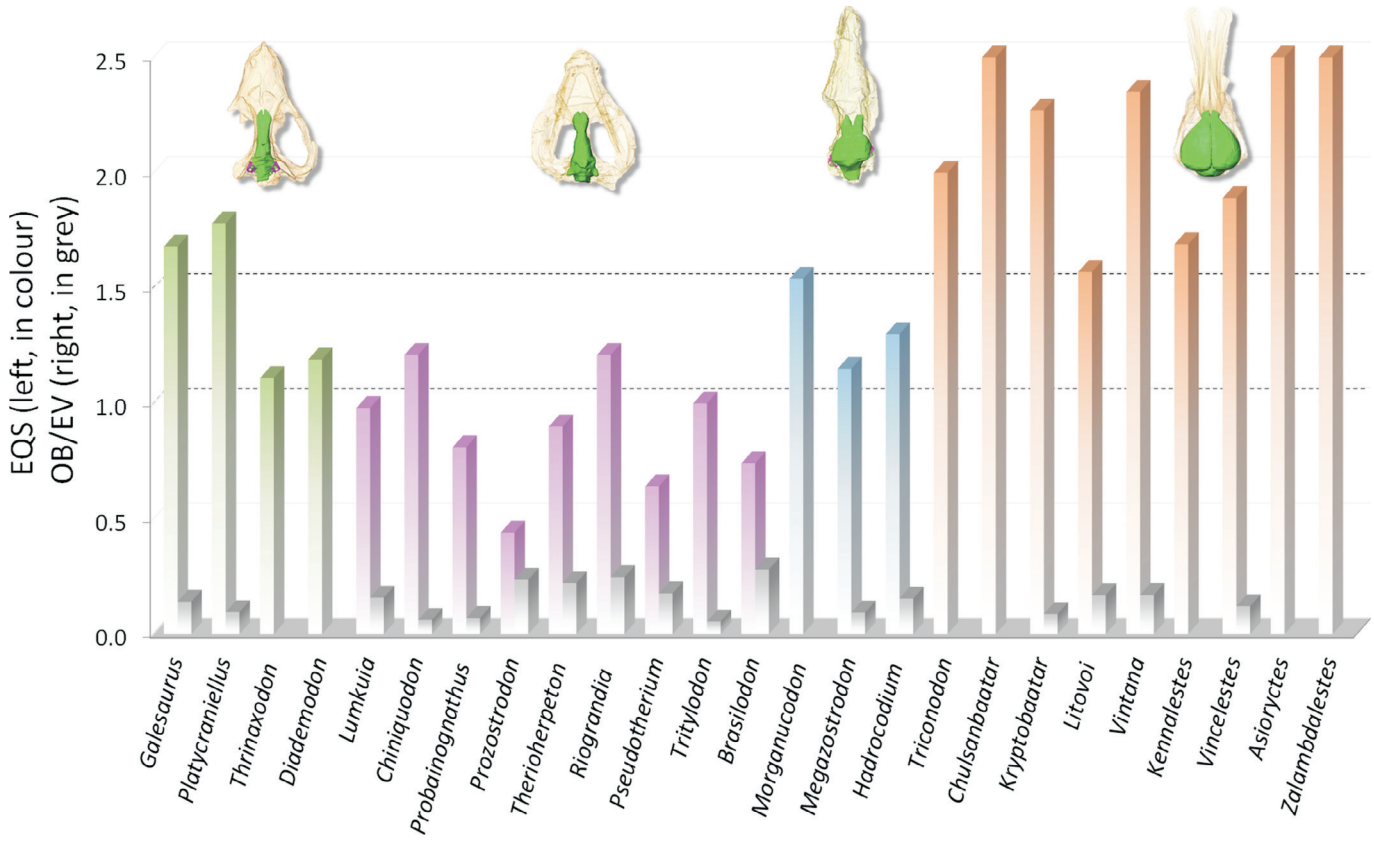


Figure 4. Bar chart of the encephalization quotient adjusted to synapsids (EQS) and ratio of olfactory bulbs volume over the volume of the rest of the endocast (OB/EV) in Mesozoic Cynodontia (see Table 1). Colour chart (same as in Fig. 1): Green, EQS in non-probainognathian cynodonts; Pink, EQS in non-mammaliaform probainognathians; Blue, EQS in non-mammalian mammaliaforms; Orange, EQS in Mammalia; Grey, OB/EV ratio. The same scale is used for the EQS and OB/EV ratio.

olfactory bulbs and cerebrum are connected through a relatively narrow olfactory tract.

The cerebrum is narrower anteriorly than posteriorly in dorsal view (Fig. 3A). Posteriorly, the cerebrum is only slightly broader than the olfactory bulbs. The two hemispheres of the cerebrum are separated by a long and deep longitudinal interhemispheric sulcus (Figs 2D, 3A), as in other probainognathians (Quiroga 1979, 1980, 1984; Rodrigues *et al*. 2018; Kerber *et al*. 2023). The hemispheres are narrow anteriorly and become gradually broader posteriorly, as in most non-mammaliaform probainognathians. Unlike the condition in *Probainognathus* and more derived taxa, in which the posterior part of the cerebral hemispheres diverge and are separated by the midbrain, the posterior-most part of both hemispheres still share a midline contact in *Lumkuia* (Figs 2, 3). This absence of divergence of the hemisphere is shared with *Chiniquodon*, *Prozostrodon* and non-probainognathian cynodonts (Quiroga 1979, 1980, 1984; Hopson 1979; MacLean 1990; Macrini 2006; Kemp 2009; Rodrigues *et al*. 2018; Pusch *et al*. 2019; Kerber *et al*. 2023), and suggests that the colliculi were not exposed. The ventral expansion of the hemispheres is not visible laterally.

The midbrain is covered by the unossified zone and possible accompanying vessels (see Kielan-Jaworowska 2004; Laaß *et al*. 2017). The unossified zone is the dorsalmost point of the endocast in lateral view (Fig. 3). The unossified zone disappears in *Probainognathus* and more derived probainognathians (Kerber *et al*. 2023). The hypophyseal fossa on the skull is located at the proximal end of the two carotid foramina (Fig. 3). The reconstructed cast of the hypophysis is very small, which is not unexpected in such a small animal (Edinger 1942). There is no trace of a pineal foramen or cast of a pineal body, as is usual in probainognathians (Benoit *et al*. 2016a, 2022a). In contrast, non-probainognathians that lost their pineal foramen usually preserve a cast of the pineal body, as exemplified by *Cynosaurus*, *Diademodon*, *Cistecynodon*, and *Massetognathus* (Quiroga 1979; Benoit *et al*. 2015, 2022a; Pavanatto *et al*. 2019, Hoffmann *et al*. 2021). There is a discrete, large foramen for the trigeminal nerve at midheight on the lateral aspect of the midbrain (Fig. 3B).

The canal for the facial nerve is located more caudally on the hindbrain. It is very small, and located just anterior to a small sinus of unknown function (Fig. 3B). Caudally, there are two very small canals that lead ventrally to two hypoglossal foramina, as is common among non-mammalian cynodonts and mammals (Estes 1961; Martinelli *et al*. 2016). In dorsal view, the most striking features of the hindbrain are the protruding floccular lobes attached to the cerebellum (Fig. 3A). Enlarged floccular lobes are ubiquitous among non-mammalian cynodonts and early mammals (Benoit *et al*. 2022a; Rowe 2023). Though prominent, they are not as well developed as in *Galesaurus* (Pusch *et al*. 2019). A low midline bulge may correspond to the cast of the vermis of the cerebellum (Fig. 3). A faint cast of the cerebellar vermis is also visible in *Probainognathus* and *Prozostrodon*, but not in *Chiniquodon* and non-probainognathian cynodonts (Quiroga 1979, 1980, 1984; Hopson 1979; MacLean 1990; Macrini 2006;

Kemp, 2009; Rodrigues *et al*., 2018; Pusch *et al*. 2019; Kerber *et al*. 2023). In contrast, *Therioherpeton*, Tritheledontidae and early Mammaliamorpha have a well-marked imprint for the vermis on their endocast (Quiroga 1979, 1980, 1984; Benoit *et al*. 2016b; Rodrigues *et al*. 2018; Kerber *et al*. 2021, 2023). In ventral view, the ventral aspect of the medulla oblongata of BP/1/2669 is mediolaterally pinched, resulting in the presence of a blunt ridge on the midline.

DISCUSSION

Evolution of encephalization

The timing and phylogenetic origin of brain enlargement in mammalian ancestors are of paramount importance in synapsid paleoneurology (Hopson 1979; Kielan-Jaworowska *et al*. 2004; Rowe *et al*. 2011; Rowe 2017, 2023; Benoit *et al*. 2022a). A dominant hypothesis in the literature is that brain expansion was, in large part, driven by the enhancement of the sense of smell in early Mammaliaformes (Rowe *et al*. 2011; Rowe & Shepherd 2016; Wallace 2018; Rowe 2017, 2023; Huttenlocker *et al*. 2018). As early mammaliaforms became nocturnal, olfaction compensated for the loss of visual cues (Jerison 1973), and encephalization seems to increase coincidentally with olfactory bulbs size and enhancement of the nasal capsule (Rowe *et al*. 2011; Rowe & Shepherd 2016; Wallace 2018). The data presented here, however, do not support this model for the evolution of encephalization.

Firstly, even though the EQS was specifically designed to enhance the contrast between these groups (Benoit *et al*. 2022a), the values of encephalization in Mesozoic Mammalia, early mammaliaforms, and non-mammaliaform cynodonts overlap strongly (Fig. 4). This is at odds with the hypothesis that a pulse of increased encephalization characterizes early mammaliaforms and mammals. Minor trends can be pointed out though. For instance, the EQS is always above 1 in early mammaliaforms and above 1.5 in Mesozoic crown Mammalia (Fig. 4); however, more Mesozoic endocasts will have to be studied to address whether these differences are significant. Given the data presented here (Table 1; Fig 4), it is safer to consider that encephalization did not increase suddenly from the non-mammaliaform cynodonts to early Mammalia, but that the changes were slower, more gradual and subject to important random variations.

Secondly, the volume of the olfactory bulbs relative to endocranial volume remains mostly constant across Mesozoic Mammalia, early mammaliaforms, and non-mammaliaform cynodonts (Fig. 4), as already pointed out by Du Plessis (2010). *Lumkuia* and many other non-mammaliaform probainognathians have, in fact, comparatively larger olfactory bulbs than most Mesozoic mammaliaforms (Fig. 4). This suggests that even if the subtle changes in EQS pointed out above are significant, it would not be safe to assume that they correlate with an increase in olfactory bulbs size.

The dataset presented here also does not support that miniaturization and nocturnality evolved in concert with encephalization. Extremely small body size and adaptation to a scotopic environment first appeared in the Mammaliamorpha (Angielczyk & Schmitz 2014; Lautenschlager *et al*. 2018) whereas here, only the Mammaliaformes reach an EQS value beyond 1 among Mammaliamorpha (Fig. 4). This suggests an enlarged brain may not be ancestral to this clade.

The advanced endocast of *Lumkuia*

Despite being the basal-most probainognathian (Benoit *et al*. 2022b), *Lumkuia* displays an unexpectedly advanced endocast morphology. Noticeably, it possesses enlarged olfactory bulbs separated by a deep longitudinal sulcus, distinct impressions of the cerebral hemispheres with a distinct interhemispheric sulcus, and possible presence of a vermis (Figs 2, 3). These characters are shared with modern mammals, and were believed to have originated in the last common ancestor of *Probainognathus* and more derived probainognathians (Quiroga 1980, 1984; Rodrigues *et al*. 2018; Kerber *et al* 2021, 2023; Benoit *et al*. 2022a). Their presence in *Lumkuia* suggests an earlier origin of these mammal-like brain features in the last common ancestor of all Probainognathia.

The absence of these traits in the phylogenetically more derived *Chiniquodon* (including *Probelesodon*, *sensu* Abdala & Giannini 2002) may result from a reversion or of misinterpretations of the endocasts studied so far (Quiroga 1979; Kemp 2009; Hoffman *et al*. 2021). Alternatively, adaptations to a subterranean habitat may account for the advanced endocast features observed in *Lumkuia*. Benoit *et al*. (2022b) suggested that *Lumkuia* may have been a non-obligatory fossorial animal, and a low-light underground environment could have stimulated the evolution of sensory and brain adaptations that mimicked those to a nocturnal environment in early mammals. This includes an enhanced sense of smell to compensate for the loss of visual cues and a larger cerebrum (Laaß & Kaestner 2017; Benoit *et al*. 2022a). However, this hypothesis is unlikely as it does not account for the fact that most non-probainognathian cynodonts (Cynognathia and basal cynodonts in Fig. 1) were evidently fossorial (Groenewald *et al*. 2001; Damiani *et al*. 2003; Fernandez *et al*. 2013).

The marks left by the cerebral hemispheres on the internal surface of the braincase (e.g. the interhemispheric sulcus) of *Lumkuia* are usually interpreted, when present in other synapsids, as resulting from the inflation of the corresponding part of the soft-tissue brain that filled up the endocranial cavity (Rowe *et al*. 2011; Laaß & Kaestner 2017; Kerber *et al*. 2023). These authors speculated that this might be the sign of the presence of a sizable mammal-like isocortex that covered the cerebral hemispheres. Under this assumption, the development of the isocortex was itself fuelled by the presence of a pelt that included sensory hairs that increased the somatosensory input into the hemispheres (Rowe *et al*. 2011; Rowe & Shepherd 2016; Rowe 2017, 2023). Although this is very speculative, it is noteworthy in this respect that the endocast of *Lumkuia* provides other paleoneurological evidence for the presence of hair. Coincidentally to the development of the cerebral hemispheres, *Lumkuia* lost the pineal foramen (and body) and may have had a larger cerebellar vermis than more basal cynodonts (Fig. 3). Both characters have

been correlated to a possible mutation of the homeogene *Msx2* that also controls for the maintenance of hair (Benoit *et al*. 2016b). *Lumkuia* also shows the beginning of a trend towards the simplification of the maxillary canal in the probainognathian lineage, which will ultimately result in its transformation into the mammalian infraorbital foramen in *Probainognathus* and the Prozostrodontia (Benoit *et al*. 2016b, 2020). The origin of this foramen is tightly correlated with that of sensory vibrissae (Benoit *et al*. 2016b; Miyamae & Bhullar 2017; Mulchinski *et al*. 2020).

In the absence of direct fossil evidence, reconstructing the evolution of hair in non-mammalian probainognathians is highly speculative; however, that so many paleoneurological proxies for hair evolved or began evolving in *Lumkuia* gives a consistent signal that strongly supports that the last common ancestor of the Probainognathia already had hair. This would be consistent with the subsequent evolution of whiskers and an elevated body temperature in more derived non-mammalian probainognathians (Benoit *et al*. 2020; Araújo *et al*. 2022).

CONCLUSION

The endocast of *Lumkuia* suggests that many advanced features such as the enlarged cerebral hemispheres and olfactory bulbs, and the possible presence of a cerebellar vermis did not evolve gradually across the probainognathian lineage, or suddenly in more derived forms closer to the origin of mammaliaforms, but were already present in the last common ancestor of the Probainognathia, as early as the early Anisian. The possible absence of these mammal-like features in chiniquodontids, if genuine, implies that these characters were not so firmly expressed at the evolutionary root of the clade. The appearance of these mammal-like brain traits early in the probainognathian lineage is consistent with many other paleoneurological traits that coincidentally indicate an origin of sensory and insulating hair at the same phylogenetic position. This would imply that hair, endothermy, and the isocortex originated in early probainognathians, as suggested by previous authors (Benoit *et al*. 2016b, 2020; Araújo *et al*. 2022).

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§ORCID iD

J. Benoit: orcid.org/0000-0001-5378-3940

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