ONTOGENY OF THE *MASSOSPONDYLUS* LABYRINTH: IMPLICATIONS FOR LOCOMOTORY SHIFTS IN A BASAL SAUROPODOMORPH DINOSAUR

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Abstract:

Ontogeny is a vital aspect of life history sometimes overlooked in palaeontological studies. However, the changing geometry of anatomical structures during growth can be informative regarding ecological and functional reconstructions. The inner ear, or labyrinth, is an ideal ontogenetic study system because it has a strong functional signal in its morphology that is linked to locomotor mode. Yet almost nothing is known about labyrinth development in dinosaurs. We quantified labyrinth scale and geometry through ontogeny in the Early Jurassic dinosaur *Massospondylus carinatus*, which has an exceptional fossil record and is hypothesised to have undergone a gait change, from quadrupedal juvenile to bipedal adult. To test whether this putative locomotor shift is reflected in labyrinth morphology, computed microtomography (μCT) and propagation phase-contrast synchrotron radiation microtomography (PPC-SRμCT) were used to obtain labyrinths from eight specimens, ranging from near-hatchling to adult. Labyrinths grow substantially but scale with slight negative allometry compared to skull length throughout ontogeny, the first time this has been documented in dinosaurs. Geometric morphometric analysis of the labyrinth using a sliding semilandmark approach shows some morphological change through ontogeny, but little evidence supporting a locomotor shift. These results have implications for our understanding of sauropodomorph development and provide a better understanding of dinosaur locomotory evolution.

Key words: Geometric Morphometrics, semilandmark, ontogeny, inner ear, semicircular canals, *Massospondylus*

Massospondylus carinatus Owen, 1854 is a species of sauropodomorph dinosaur from the Early Jurassic of southern Africa (e.g. Chapelle and Choiniere 2018). It is notable for its relative abundance in the upper Elliot and lower Clarens Formations of South Africa and Lesotho, and equivalent strata in the Tuli and Mid-Zambezi basins of Zimbabwe, which correspond to the Hettangian–Sinemurian stages (see Butler 2005). Excavations over the last 160 years have resulted in numerous specimens that represent a variety of ontogenetic stages, from embryos to large adults (Fig. 1) (Kitching 1979; Gow 1990; Gow *et al.* 1990; Sues *et al.* 2004; Reisz *et al.* 2005; Yates and Barrett 2010). Growth series are rare in the vertebrate fossil record and are important for the study of intraspecific phenotypic variation. Ontogenetic transformations in *M. carinatus* are therefore important for physiological and evolutionary studies, especially considering publications that stress the importance of understanding dinosaur development for the accurate reconstruction of life history and taxonomic relationships (e.g. *Tyrannosaurus* (Carr 1999), *Pachycephalosaurus* (Horner and Goodwin 2009), *Triceratops* (Scannella and Horner 2010), *Edmontosaurus* (Campione and Evans 2011), and *Parasaurolophus* (Farke *et al.* 2013)). *M. carinatus* is of particular importance for ontogenetic study as it has been hypothesised to undergo a major locomotory shift through growth, with hatchlings having a quadrupedal gait and adults becoming at least facultatively bipedal (Reisz *et al.* 2005, 2010). This transition has also been hypothesized in other dinosaurian lineages, with important implications for locomotory evolution, particularly those of Sauropodomorpha.

While previous ontogenetic studies have predominantly focused on osteology and histology, changes to the endocranial anatomy have not been studied in detail. We focus on the vestibular organ, which includes the semicircular canals, and is housed within the endosseous labyrinth of the inner ear. It comprises a series of ducts and sacs filled with endolymph, the movement of which stimulates sensory cilia in the ampullae (Fig. 1), providing sensory information on rotational accelerations of the head (Sipla and Spoor 2008). The displacement of endolymph in response to specific head motions depends on the geometry of the semicircular canals (Sipla and Spoor 2008; Malinzak *et al.* 2012). Numerous ecomorphological hypotheses have postulated that the geometry of the semicircular canals is fine-tuned to respond to specific sensory inputs imposed by environment, behaviour, and locomotion (e.g. Spoor 2003; Spoor *et al.* 2007; Malinzak *et al.* 2012; Ekdale 2016). The endosseous labyrinth is therefore an important tool in comparative, functional and evolutionary studies, particularly because it is frequently fossilised and can provide insight into the ecology, physiology and sensory abilities of extinct animals (e.g. Spoor *et al.* 1994; Witmer *et al.* 2003; Neenan and Scheyer 2012; Neenan *et al.* 2017).

Here we present the first labyrinth growth series (with n > 2) for any non-avian dinosaur. Our aim is to understand how labyrinth morphology, particularly the semicircular canals, changes through ontogeny. Because of the exceptional fossil record of *M. carinatus*, particularly considering the putative gait change that occurs during ontogeny, the labyrinth of this taxon is the perfect study system to examine the effects of locomotor style on the dinosaur vestibular apparatus. It has recently been shown that quadrupedal and bipedal tetrapods, and specifically non-avian dinosaurs, exhibit different semicircular canal proportions in comparison to skull mass, with bipedal taxa having larger vertical canals (Georgi *et al.* 2013). We therefore hypothesise that younger, putatively quadrupedal specimens of *M. carinatus* will have proportionally smaller vertical canals than the older, bipedal specimens. These data will have important connotations for our understanding of form-function interactions of the vestibular system and is the first study that attempts to track labyrinth morphology in a species with a hypothesised ontogenetic gait change.

MATERIAL AND METHODS

Dataset assembly

Eight specimens (Table 1) representing various ontogenetic stages were scanned using micro-computed tomography (µCT), with the exception of BP/1/4934 which was scanned with propagation phase-contrast synchrotron radiation microtomography (PPC-SRµCT). BP specimens (see institutional abbreviations below) were scanned at the Microfocus X-ray

Computed Tomography facility of the Evolutionary Studies Institute at the University of the Witwatersrand, using a Nikon Metrology XTH 225/320 LC dual source industrial CT system. SAM specimens were scanned at the Stellenbosch CT Facility using a General Electric V|TomeX L240 system. All µCT scan parameters can be found in Table 2.

BP/1/4934 was scanned at the BM05 beamline of the European Synchrotron Radiation Facility (ESRF, Grenoble, France). The setup consisted of a FReLoN-2k camera, a 0.3x magnification set of lenses, a scintillating fibre, a filtered white beam (Cu 2 mm, Al 2 mm) with a total integrated energy of 100 keV and a sample-detector distance of 4 m to perform Propagation Phase Contrast Synchrotron micro-computed Tomography (PPC-SRµCT). The tomography was computed based on 4998 projections of 0.1 seconds, each over 360 degrees, and resulting in data with a 45.78 µm isotropic voxel size. An attenuation protocol (Carlson *et al.* 2011; Sanchez *et al.* 2013) allowed us to increase the exposure time and compensate for X-ray attenuation by the sample, without saturating the detector. Additionally, the centre of rotation was shifted by ~35 mm to increase the horizontal field of view in the reconstructed data (i.e., half acquisition protocol). Given the limited vertical field of view, 153 scans were necessary (30% of vertical overlap between two consecutive scans) to cover the full height of the sample. The tomographic reconstruction was performed using the single distance phase retrieval approach of the software PyHST2 (Paganin *et al.* 2002; Mirone *et al.* 2014). The resulting 32 bit data were converted to a stack of 16 bit tiffs using 0.001 % min and max saturation values from the 3D histogram generated by PyHST2. Individual scans were stacked along the vertical axis using a weighted average for duplicated slices. Finally, a ring correction was applied.

Endosseous labyrinths were segmented and reconstructed using VGStudio MAX 3.1 (Volume Graphics, Heidelberg, Germany). All specimens had at least one fully-articulated labyrinth, with the exception of BP/1/5231 (the youngest specimen), where both are completely disarticulated. In that case, the prootic, opisthotic and supraoccipital portions of the left labyrinth were reconstructed individually and then reorientated accurately into life-position in VGStudio MAX 3.1 using the contacts between these bones with reference to the anatomy of other specimens in the study. We are confident that the specimens are minimally distorted because our personal observations from segmentation of non-mammalian labyrinths suggest that they tend to disarticulate, rather than distort, when subjected to compression. Also, where possible, we segmented labyrinths from both sides and found them to be highly symmetrical.

Since the vestibular organ of the inner ear, i.e. membranous labyrinth, is a soft tissue structure and not preserved in fossil specimens, we use the virtual endocast of the osseous labyrinth (i.e. endosseous labyrinth) as a proxy for our analyses. Numerous important and useful structures are preserved in the osseous labyrinth, including the length, courses and curvatures of the semicircular canals, as well as the angles between them (e.g. (David *et al.* 2010)), which are still useful in comparative studies (Grohé *et al.* 2016; Walsh *et al.* 2009; Walsh *et al.* 2014; Alloing-Séguier *et al.* 2013; Benson *et al.* 2017; Neenan*, et al.* 2017).

Labyrinth scaling relationships

Tetrapod labyrinth size has been shown to scale positively with head mass (Jones and Spells 1963), although modifications to this scaling relationship occur from influences such as agility (Spoor *et al.* 2007; Benson *et al.* 2017), aquatic lifestyles (Spoor *et al.* 2002; Spoor and Thewissen 2008; Ekdale 2013; Neenan *et al.* 2017), and other aspects of locomotion, such as gait (Georgi *et al.* 2013). As recently noted by Neenan et al. (Neenan*, et al.* 2017), the size of the skull is the most useful comparative measure of size, because the labyrinth provides sensory information specifically for head stabilisation (Georgi *et al.* 2013). We tested for allometry using Ordinary Least Squares regression (OLS) in R version 3.4.3 (R Development Core Team 2008) using both labyrinth centroid size and labyrinth anteroposterior length against skull anteroposterior length (note that only five specimens were complete enough for this measurement to be taken). In addition we tested for allometry in PC axes against centroid size using the same framework.

Geometric morphometrics

Similar to the method used by Neenan *et al*. (2017), we used IDAV Landmark (Wiley *et al.* 2005) to place two series of semilandmarks (Gunz and Mitteroecker 2013) along the midline of each semicircular canal as determined by the dorsal view for the vertical semicircular canals, and lateral view for the lateral canal (electronic supplementary material, Fig. S1). This captured the shortest route along the internal surface of each canal, and the longest, external surface of each canal. This method is ideal, as it not only captures important semicircular canal geometries (i.e. radius of curvature, canal length and angles between the canals), but also encompasses the ampullae and crus communis (Fig. 1). After semilandmark placement, they were subsampled to 14 equally-spaced sliding semilandmarks for each curve using the digit.curves() function of the geomorph 3.0.6 package in R (R Development Core Team 2008; Adams *et al.* 2018). All landmark sets were treated as open-ended curves and Procrustes superimposition was applied to remove the effects of scale, orientation, and the arbitrary spacing of semilandmarks. Principal component analysis (PCA) was then used to quantify labyrinth shape distribution (Zelditch *et al.* 2004).

Institutional abbreviations. BP, Evolutionary Studies Institute (formerly the Bernard Price Institute), University of the Witwatersrand, Johannesburg, South Africa; SAM, South African Museum, Cape Town, South Africa.

RESULTS

Labyrinth morphology

The labyrinth morphology of *M. carinatus* was recently described by Chapelle & Choiniere (2018). However, the addition of multiple new specimens allows for a more thorough description of several salient points. Although large portions of the sacculus and lagena are not preserved in most specimens, BP/1/4934 exhibits a prominent lagena that projects ventrally. This may be due to its greater age (being the largest specimen of our sample) and thus higher degree of cranial ossification. Ampullae are more generally present in the sample, and are located on the distal end of the anterior canal and the anterior end of the lateral canal (Figs. 1 and 2). The ampulla for the posterior canal is not represented in the endosseous labyrinth, but would have been positioned at the distal end of the posterior semicircular duct, ventral to the lateral canal. Also, the posterior canal has a distinct sinusoidal curvature (i.e. diversion from planarity), which is particularly pronounced in the youngest individuals (BP/1/5231 and SAM/PK/7904; Fig. 2). This curvature creates an acute angle between the anterior and posterior canals at the point where they meet the crus communis, although this angle becomes closer to perpendicular in older individuals.

Labyrinth scaling through ontogeny

M. carinatus labyrinth anteroposterior length grows with slight negative allometry in comparison to skull length (OLS, n = 5, slope = 1.2, std error = 0.06, adjusted r^2 = 0.99, $p<0.001$; Fig. 3; Table 3). This allometric relationship is also seen when using centroid size instead of anteroposterior length, though with slightly less significance and greater error (OLS, n = 5, slope = 1.4, std error = 0.22, adjusted r^2 = 0.91, p = 0.008; Table 3).

Labyrinth geometry though ontogeny

The geometric morphometric analysis shows that, through ontogeny, semicircular canal (including the crus communis) geometry does change, albeit to a minor degree (Fig. 4). PC1, which explains 44.0 % of the variance, is not significantly related to ontogeny (Table 1), and it separates two of the taxa as outliers to the remainder of the sample: BP/1/4930 and BP/1/4779. These taxa are characterised by negative values on PC1, which represent, in comparison to positive PC1 values, a more vertically orientated crus communis in lateral view, a taller anterior canal (albeit with comparable relative arc length to positive PC1 values), a far more acute angle between the vertical canals, larger ampullae, narrower lumen size in the posterior canal compared to the anterior canal, and a shorter lateral canal (Fig. 4; electronic supplementary material, Fig. S2).

PC2 explains 19.5 % of the variance but is the most important axis for displaying ontogenetic shape change, with a significant relationship between PC score and labyrinth centroid size, thus capturing an allometric signal (OLS, n = 8, slope = -0.004, std error<0.001, adjusted r^2 = 0.75, p = 0.003; Table 3). Taxa are generally distributed according to growth stage along this axis, with negative values being the oldest individuals and positive ones being the youngest. The only exceptions are BP/1/5241, the second largest individual, which clusters with the oldest individuals but it has the most extreme negative value; and BP/1/4779 which, as the third-smallest labyrinth in terms of antero-posterior length, also clusters closely with the largest individuals. However, the relatively unusual morphology of the BP/1/4779 labyrinth resulted in a larger than expected centroid size (53.3, the fourth largest value; Table 1), which may account for this position. The shape change that occurs along PC2 is therefore our best indicator for how semicircular canal geometry changes through ontogeny. The largest difference between young and old individuals is the relative angle of the crus communis, the ventral point of which is much more laterally placed (i.e. closer to the lateral canal) in older individuals. This means that older individuals have progressively more dorsomedially inclined crus communi (Fig. 4; electronic supplementary material, Fig. S2).

While there is relatively little difference between the arc lengths of the semicircular canals, the lateral canals and, to a lesser degree, the anterior canals, are more strongly curved in the younger individuals. The posterior canals, on the other hand, have stronger sinusoidal curvature in the younger specimens, creating a more acute angle between the two vertical canals at the point where they meet the crus communis. The ampullae are also relatively larger in younger specimens. PC3, like PC1, is not significantly related to allometry

(Table 3), and displays a seemingly random distribution of taxa along its axis. This is likely due to intraspecific variation within our sample, and possibly due to small preservational differences. Negative values, which are particularly relevant for the youngest specimen (BP/1/5231) and the second oldest specimen (BP/1/5241), have an anterior canal which is taller but extends less far laterally, a less-curved posterior canal, and a more curved lateral canal that extends further laterally (Fig. 4; electronic supplementary material, Fig. S2).

DISCUSSION

By studying the relative size and geometry of the *M. carinatus* labyrinth, we present two main results. Firstly, labyrinth growth continues throughout ontogeny and is proportional to head size, with a slightly negative allometric relationship. Secondly, although there is a slight change in labyrinth geometry over ontogeny, there is little evidence to support a gait change according to previously published metrics.

Labyrinth growth

The labyrinth of *M. carinatus* continues to grow throughout postnatal ontogeny with a highly significant and slightly negative allometric relationship to skull length (Fig. 3). With a slope of 1.2, this correlation is almost isometric, the signal of negative allometry possibly being due to the fact that relative rostral length increases through ontogeny (i.e. the rostrum grows at a faster rate than the rest of the skull; Fig. 1) (Marugán-Lobón and Buscalioni 2003). This result differs somewhat from what has already been reported for the Jurassic ornithischian dinosaur, *Dysalotosaurus lettowvorbecki*, where little to no anteroposterior labyrinth growth occurs between a juvenile (3-4 years) and subadult specimen (12-13 years; Lautenschlager and Hübner 2013). However, the authors do note differences in semicircular canal width, length and shape (circular in the juvenile and elliptical in the subadult). In that taxon, semicircular canals grow in length between 15.3 % (anterior canal) and 30.6 % (posterior canal), despite a 118.2 % increase in skull length. This is to a much lesser degree than in our sample, where semicircular canal lengths remain, in relative terms, the same length throughout ontogeny and are strongly correlated to skull length. However, *M. carinatus* semicircular canals are somewhat more rounded in younger individuals, mirroring the condition in *D. lettowvorbecki* (Figs. 1 and 2).

Little research has been published on proportional growth of the labyrinth in other non-mammalian tetrapods, although it has been suggested that the labyrinth also ossifies and attains adult proportions early in chicken (*Gallus*, Knowlton 1967). Generally, in both eutherian and marsupial mammals, there is also little growth of the labyrinth after the otic capsule has ossified despite substantial overall skull growth, although there are often some changes in semicircular canal dimensions (e.g. *Monodelphis*, Ekdale 2010). However, this is not the case in all mammals, as in *Oryctolagus* (rabbit) and *Homo* (humans), the labyrinth attains full adult size and dimensions during gestation, once again being decoupled from overall skull growth (Hoyte 1961; Jeffery and Spoor 2004).

Intra/Interspecific variation

PC axes 1 and 3 are not correlated with allometry, but rather demonstrate morphological variation from other sources. PC1 essentially separates two outlier specimens from the rest of the sample: BP/1/4930 and BP/1/4779 (Fig. 4). This is most likely due to either a large-scale preservational bias that is skewing the results, or because one or both of these specimens are, in fact, a different taxon. This latter possibility is currently being investigated by the authors using other sources of evidence pertinent to this taxonomic distinction.

M. carinatus specimens are spread along PC3 in a seemingly random manner. This would seem to indicate that it is detecting intraspecific variation or minor preservational differences between the specimens, mostly confined to small variances between the curvature of the semicircular canals. This phenomenon has been well documented in some mammals (Ekdale 2010), and should be taken into consideration when interpreting labyrinth morphology in functional and taxonomic studies. We account for this in our interpretation of the ontogenetic changes that we observe on PC2, which do show slight variation in semicircular canal curvature, particularly regarding the lateral canal. For this reason, we discount this variation from our interpretation of labyrinth geometry through ontogeny.

Implications for locomotion

Taking the intraspecific variation displayed on PC3 into account, there is still some change in geometry between the youngest and oldest specimens in our sample, which may have functional connotations. The base of the crus communis shifts radically from a lateral position that creates a large angle relative to the vertical canals, to a more vertical position in the older specimens. There is also a greater degree of sinusoidal curvature of the posterior canal in younger individuals, creating a more acute angle between the anterior and posterior canal where they meet the crus communis. Interpretation of these changes can be made in one of two ways: either there is a spatial constraint within the cranium that influences labyrinth shape; or there is a functional reason. After the discovery of embryo-containing eggs, limb proportions indicated that *M. carinatus* hatchlings were quadrupedal (Reisz *et al.* 2005, 2010), while adults were at least facultatively bipedal. Since the labyrinth organ helps to stabilise the head during locomotion, a gait change of this nature could indeed affect labyrinth geometry. To our knowledge, no previous study has attempted to quantify labyrinth shape change with respect to a gait change through ontogeny. However, a series of studies have used labyrinth geometry in an attempted to understand the evolution of bipedality in humans from mostly quadrupedal primate ancestors (Spoor 2003; Spoor*, et al.* 1994, 1996, 2003) and, importantly, whether semicircular canal geometries differ significantly between quadrupedal and bipedal tetrapods, particularly in non-avian dinosaurs (Georgi *et al.* 2013). Studies in both taxonomic groups found evidence for an associated labyrinth geometry change with bipedal locomotor modes.

In humans, the anterior and posterior canals are larger than in non-hominin apes, which is interpreted as a functional adaptation for certain bipedal abilities, namely running and jumping. A similar result was obtained for dinosaurs, where Georgi et al. (2013) found that vertical canal size compared to skull mass and to skull length significantly distinguished between quadrupedal and bipedal taxa, with the latter also having larger vertical canals. Our results show that adult *M. carinatus* do not have larger vertical canals compared to skull length than young *M. carinatus* do, so the same paradigm cannot be applied here. We therefore reject our hypothesis that a gait change is detectable by the relative sizes of the vertical canals.

This apparent absence of a gait modification is supported by a lack of a change in the relative 'alert' head posture observed between juvenile and adult individuals (Fig. 1). This concept (see Witmer *et al.* 2003, 2008 for a summary) postulates that an animal is at its most alert and comfortable when the orientation of the head lies at an angle where the lateral semicircular canal is roughly horizontal. While this hypothesis was recently called into question by Marugán-Lobón *et al*. (2013), it stands to reason that a gait change would be expected to coincide with a modification of the lateral canal position, as each locomotor style requires the head to lie at a different angle to the neck.

If we are not observing a gait change through ontogeny according to our hypothesis, then our results may be due to: (1) our monospecific ontogenetic series is not a comparable dataset to studies that use multiple species that have evolved disparate locomotion types, so a similar pattern would not be expected; (2) the pattern observed by Georgi *et al*. (2013) does apply to *M. carinatus*, but vertical canal size is optimised very early in ontogeny for the eventual adult locomotory posture. However, this would seem unlikely due to the amount of overall growth observed, which would enable high-magnitude changes in the labyrinth to occur; (3) *M. carinatus* achieves the same functional adaptation to a bipedal gait, but through the relative position of the crus communis and curvature of the posterior canal, rather than by enlarging the vertical canals. However, there is no published evidence that would support this to date; or (4) the observed shape change is not related to gait, but to other factors such as cranial spatial constraints. This last point is plausible, as a recent study by Benson *et al*. (2017) on modern birds revealed only a limited relationship between labyrinth geometry and locomotor mode. In that analysis, shape is instead more strongly influenced by restricted space in the skull, a theory originally proposed by Muller (1999) (but see Spoor 2003).

CONCLUSION

We present the first study that attempts to unravel how the labyrinth grows and changes geometry through ontogeny in any dinosaur. We find that the overall size of the labyrinth grows throughout ontogeny with a highly significant and slightly negative allometric relationship with skull length, unlike some birds and mammals. The geometry of the labyrinth changes modestly through ontogeny, although this is more subtle than the size changes and is mostly with regard to the angle and position of the crus communis and to the degree of sinusoidal curvature of the posterior canal. It is unlikely, but possible, that this is the result of a unique functional change related to a gait shift during ontogeny that has not been recognized in other studies investigating transitions from bipedality to quadrupedality (Georgi*, et al.* 2013). A lack of gait change is also supported by the relative similarity in 'alert' head posture between juveniles and adults. Outliers in our analysis may possibly represent other closely related taxa.

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DATA ARCHIVING STATEMENT

All surface models from this study are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.pj5952j.

SUPPORTING INFORMATION

Additional Supporting Information can be found in the online version of this article:

Appendix S1. Supplementary figures.

FIGURES

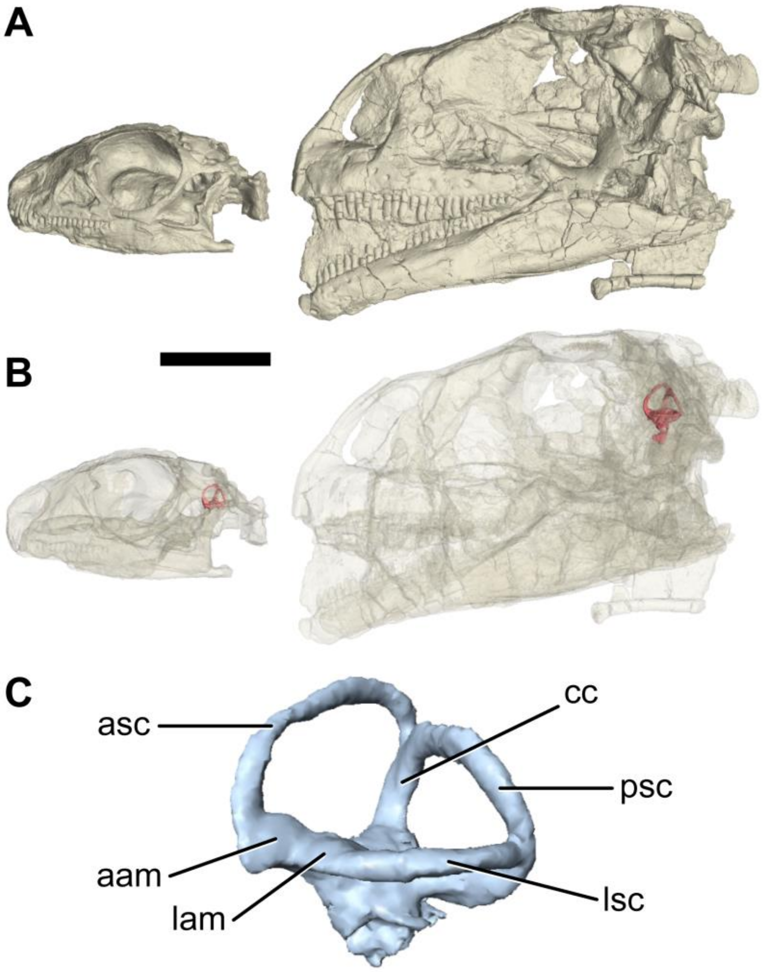

FIG. 1. Comparison between juvenile and adult *Massospondylus carinatus* specimens in 'alert' head position, and overview of labyrinth anatomy. A, *M. carinatus* skull reconstructions in left lateral view, with the most juvenile representative with a complete skull on the left (SAM/PK/7904) and the largest representative on the right (BP/1/4934). Note that the youngest specimen in our sample is BP/1/5231, but is reconstructed from disarticulated elements and does not have a complete skull. B, Transparent reconstructions of A, revealing the position and size of the labyrinth. Both lateral canals are orientated in a horizontal position, and there is no appreciable difference in 'alert' head posture. C, Labyrinth of SAM/PK/1314 (reflected right labyrinth, not to scale). Scale bar represents 50 mm (does not apply to C). Abbreviations: aam, ampulla of the anterior semicircular canal; asc, anterior semicircular canal; cc, crus communis; lam, ampulla of the lateral semicircular canal; lsc, lateral semicircular canal; psc, posterior semicircular canal. See also Table 1.

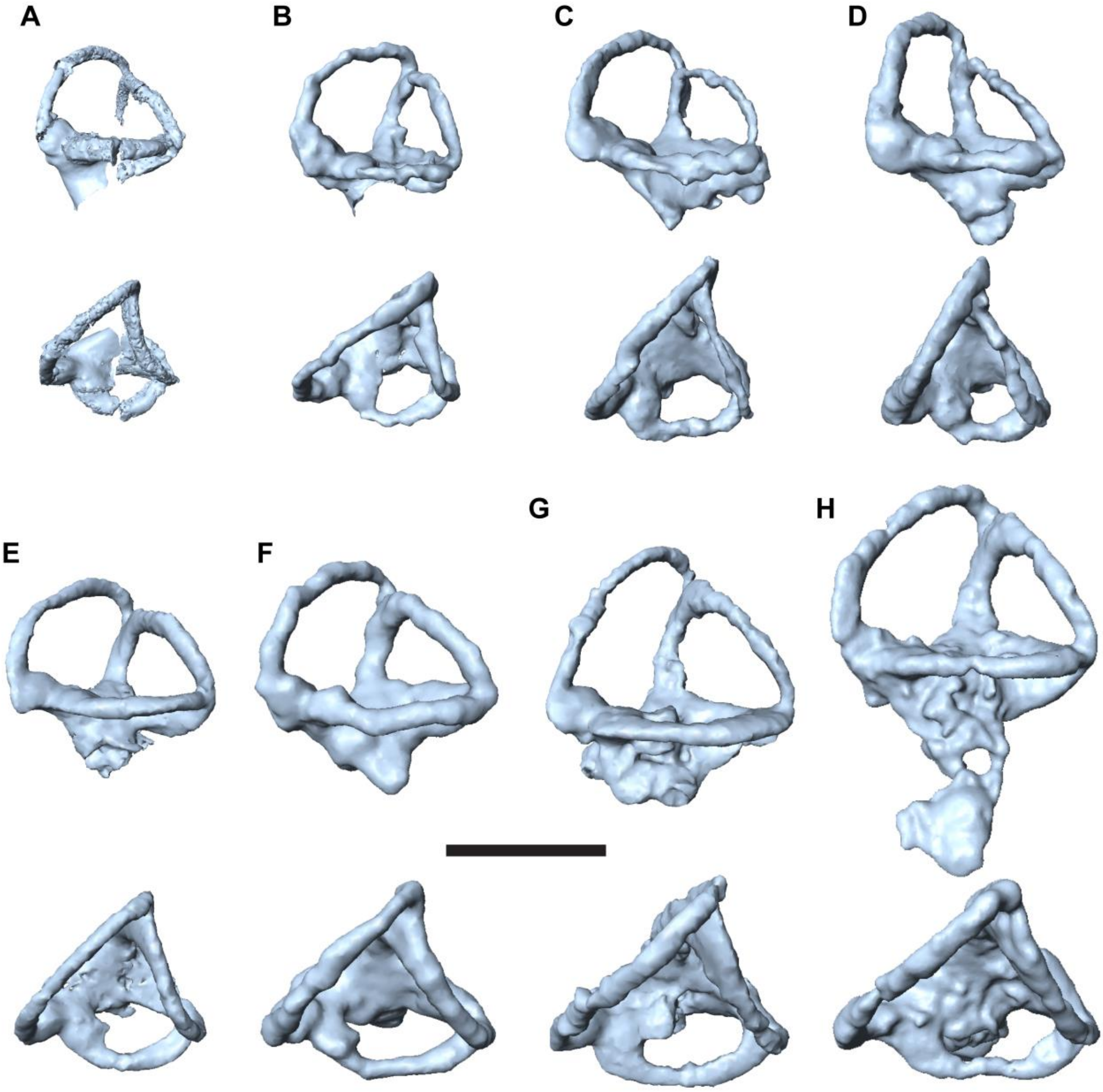

FIG. 2. Lateral (first and third rows) and dorsal (second and fourth rows) views of the 8 *Massospondylus carinatus* left labyrinths used in our analyses. A, BP/1/5231. B, SAM/PK/7904. C, BP/1/4779. D, BP/1/4930. E, SAM/PK/1314 (right labyrinth reflected). F, BP/1/4998. G, BP/1/5241 (right labyrinth reflected). H, BP/1/4934. Scale bar represents 10 mm. See also Table 1.

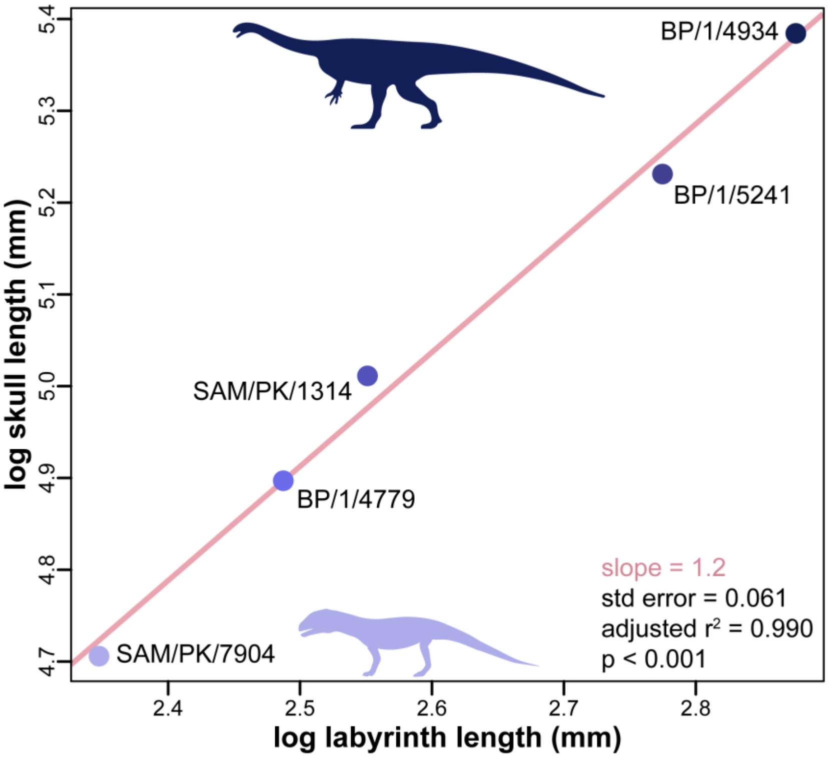

FIG. 3. Plot of log transformed labyrinth and skull anteroposterior lengths in *Massospondylus carinatus*. The labyrinth of *M. carinatus* grows throughout ontogeny with slight negative allometry in relation to skull length. Lighter colours indicate younger, putatively quadrupedal individuals and get darker with increasing age and hypothetical bipedality. See also Tables 1 and 3.

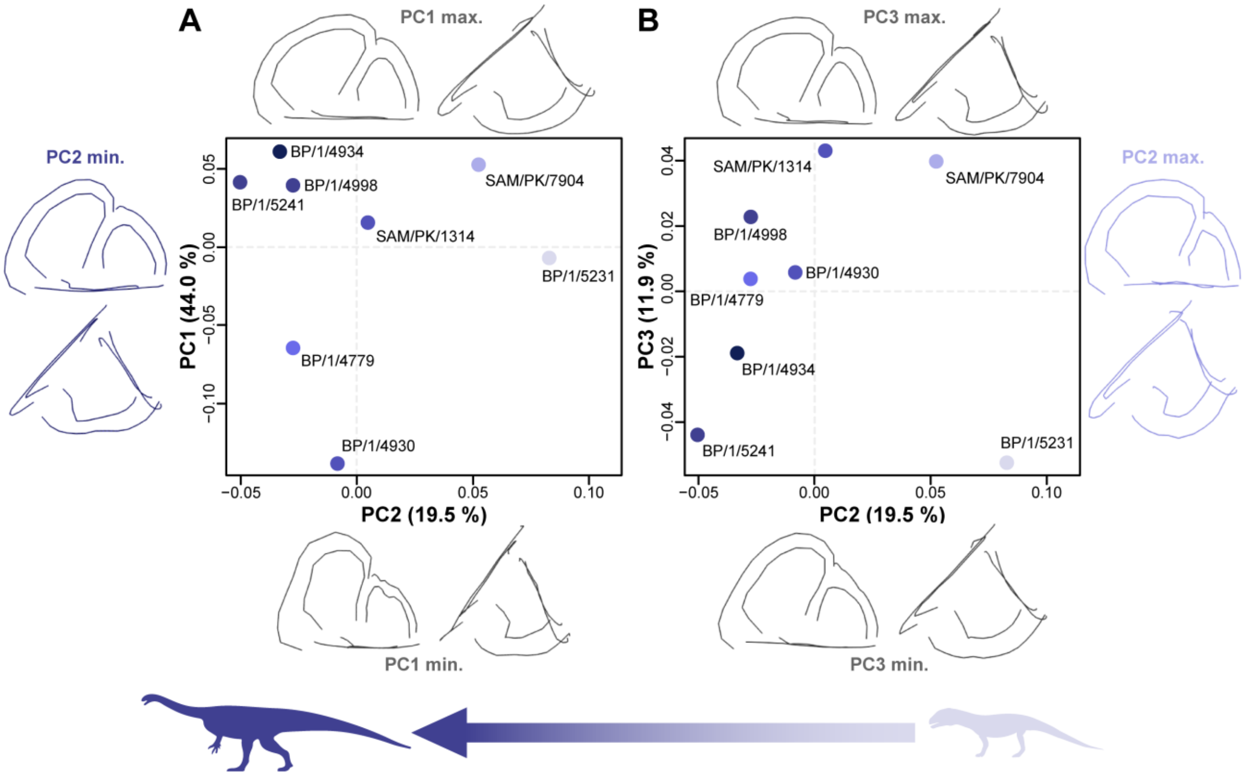

FIG. 4. Plots of principal components 1, 2 and 3, which together account for 75.4 % of the total shape variance. A, PC1 vs. PC2. B, PC3 vs. PC2. Labyrinth diagrams represent anatomical extremes of each PC axis in lateral and dorsal views. Colour scheme follows Fig. 3. See also electronic supplementary material, Figs. S1 and S2.

TABLES

TABLE 1. Specimen information with associated morphological metadata.

Specimen Number	Basicranial Length (mm)	Labyrinth Anteroposterior Length (mm)	Centroid Size	PC1 Score	PC2 Score	PC3 Score
BP/1/4934	218.05	17.74	69.177	0.060976474	-0.032318123	-0.018891640
BP/1/5241	187.04	16.03	66.201	0.041366000	-0.049476530	-0.043998981
BP/1/4998	Incomplete	15.86	58.827	0.039400951	-0.026576472	0.022842986
SAM/PK/1314	150.10	12.82	51.089	0.015549584	0.005532687	0.043043953
BP/1/4930	Incomplete	12.49	49.901	-0.138475122	-0.007492696	0.005824770
BP/1/4779	133.87	12.03	53.345	-0.064530976	-0.026614167	0.003874503
SAM/PK/7904	110.57	10.46	44.458	0.052668535	0.053265886	0.039765277
BP/1/5231	Incomplete	9.50	39.307	-0.006955446	0.083679415	-0.052460869

TABLE 2. Computed microtomography (μCT) scan parameters. For propagation phase-contrast synchrotron radiation microtomography (PPC-SRμCT) parameters, see Material and Methods.

Specimen Number	Scan Type	Voxel Size (mm)	Voltage (kV)	Current (μA)	Filter type and thickness (mm)
BP/1/5241	μCT	0.1069	105	680	Cu 1.8
BP/1/4998	μCT	0.0932	160	100	Cu 1.2
SAM/PK/1314	μCT	0.0900	160	200	Cu 1.0
BP/1/4930	μCT	0.0916	100	540	Cu 1.8
BP/1/4779	μCT	0.0946	120	250	Cu 1.2
SAM/PK/7904	μCT	0.0705	160	200	Cu 1.0
BP/1/5231	μCT	0.0333	85	95	Al 1.7

TABLE 3. Ordinary least squares regressions.

Model	N	Adjusted R^2	Slope	2.5% CI Slope	97.5% CI Slope	P-value	Intercept	P-value
log$_{10}$(Skull Length) ~ log$_{10}$(Labyrinth Length)	5	0.990	1.2438	1.0490	1.4390	0.0003	1.803	0.002
log$_{10}$(Skull Length) ~ log$_{10}$(Centroid Size)	5	0.908	1.4014	0.7005	2.1022	0.0080	-0.597	0.549
PC1 ~ Centroid Size	8	-0.015	0.002	-0.0039	0.0087	0.381	-0.131	0.388
PC2 ~ Centroid Size	8	0.751	-0.004	-0.0061	-0.0019	0.003	0.215	0.004
PC3 ~ Centroid Size	8	-0.127	-0.0006	-0.0041	0.0028	0.663	0.035	0.668
PC4 ~ Centroid Size	8	-0.158	0.0003	-0.0029	0.0035	0.842	-0.015	0.844
PC5 ~ Centroid Size	8	-0.159	0.0002	-0.0026	0.003	0.848	-0.012	0.85
PC6 ~ Centroid Size	8	-0.142	0.0003	-0.0017	0.0024	0.731	-0.016	0.735

REFERENCES

ADAMS, D. C., COLLYER, M. L. and KALIONTZOPOULOU, A. 2018. Geomorph: Software for geometric morphometric analyses. R package version 3.0.6. https://cran.r-project.org/package=geomorph.

ALLOING-SÉGUIER, L., SÁNCHEZ-VILLAGRA, M. R., LEE, M. S. Y. and LEBRUN, R. 2013. The bony labyrinth in diprotodontian marsupial mammals: diversity in extant and extinct forms and relationships with size and phylogeny. *Journal of Mammalian Evolution*, **20**, 191-198.

BENSON, R. B. J., STARMER-JONES, E., CLOSE, R. A. and WALSH, S. A. 2017. Comparative analysis of vestibular ecomorphology in birds. *Journal of Anatomy*, **231**, 990-1018.

BUTLER, R. J. 2005. The ‘fabrosaurid’ ornithischian dinosaurs of the Upper Elliot Formation (Lower Jurassic) of South Africa and Lesotho. *Zoological Journal of the Linnean Society*, **145**, 175-218.

CAMPIONE, N. E. and EVANS, D. C. 2011. Cranial growth and variation in edmontosaurs (Dinosauria: Hadrosauridae): implications for latest Cretaceous megaherbivore diversity in North America. *PLoS ONE*, **6**, e25186.

CARLSON, K. J., STOUT, D., JASHASHVILI, T., DE RUITER, D. J., TAFFOREAU, P., CARLSON, K. and BERGER, L. R. 2011. The endocast of MH1, *Australopithecus sediba*. *Science*, **333**, 1402-1407.

CARR, T. D. 1999. Craniofacial ontogeny in Tyrannosauridae (Dinosauria, Coelurosauria). *Journal of Vertebrate Paleontology*, **19**, 497-520.

CHAPELLE, K. E. J. and CHOINIERE, J. N. 2018. A revised cranial description of *Massospondylus carinatus* Owen (Dinosauria: Sauropodomorpha) based on computed tomographic scans and a review of cranial characters for basal Sauropodomorpha. *PeerJ*, **6**, e4224.

DAVID, R., DROULEZ, J., ALLAIN, R., BERTHOZ, A., JANVIER, P. and BENNEQUIN, D. 2010. Motion from the past. A new method to infer vestibular capacities of extinct species. *Comptes Rendus Palevol*, **9**, 397-410.

EKDALE, E. G. 2010. Ontogenetic variation in the bony labyrinth of *Monodelphis domestica* (Mammalia: Marsupialia) following ossification of the inner ear cavities. *The Anatomical Record*, **293**, 1896-1912.

--- 2013. Comparative anatomy of the bony labyrinth (inner ear) of placental mammals. *PLOS ONE*, **8**, e66624.

--- 2016. Form and function of the mammalian inner ear. *Journal of Anatomy*, **228**, 324-337.

FARKE, A. A., CHOK, D. J., HERRERO, A., SCOLIERI, B. and WERNING, S. 2013. Ontogeny in the tube-crested dinosaur *Parasaurolophus* (Hadrosauridae) and heterochrony in hadrosaurids. *PeerJ*, **1**, e182.

GEORGI, J. A., SIPLA, J. S. and FORSTER, C. A. 2013. Turning semicircular canal function on its head: dinosaurs and a novel vestibular analysis. *PLOS ONE*, **8**, e58517.

GOW, C. E. 1990. Morphology and growth of the *Massospondylus braincase* (Dinosauria Prosauropoda). *Palaeontologia Africana*, **27**, 59-75.

--- KITCHING, J. W. and RAATH, M. A. 1990. Skulls of the prosauropod dinosaur *Massospondylus carinatus* Owen in the collections of the Bernard Price Institute for Palaeontological Research. *Palaeontologia Africana*, **27**, 45-58.

GROHÉ, C., TSENG, Z. J., LEBRUN, R., BOISTEL, R. and FLYNN, J. J. 2016. Bony labyrinth shape variation in extant Carnivora: a case study of Musteloidea. *Journal of Anatomy*, **228**, 366-383.

GUNZ, P. and MITTEROECKER, P. 2013. Semilandmarks: a method for quantifying curves and surfaces. *Hystrix, the Italian Journal of Mammalogy*, **24**, 103-109.

HORNER, J. R. and GOODWIN, M. B. 2009. Extreme cranial ontogeny in the Upper Cretaceous dinosaur *Pachycephalosaurus*. *PLOS ONE*, **4**, e7626.

HOYTE, D. A. N. 1961. The postnatal growth of the ear capsule in the rabbit. *Developmental Dynamics*, **108**, 1-16.

JEFFERY, N. and SPOOR, F. 2004. Prenatal growth and development of the modern human labyrinth. *Journal of Anatomy*, **204**, 71-92.

JONES, G. M. and SPELLS, K. E. 1963. A theoretical and comparative study of the functional dependence of the semicircular canal upon its physical dimensions. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **157**, 403-419.

KITCHING, J. W. 1979. Preliminary report on a clutch of six dinosaurian eggs from the Upper Triassic Elliot Formation, Northern Orange Free State. *Palaeontologia Africana*, **22**, 41-45.

KNOWLTON, V. Y. 1967. Correlation of the development of membranous and bony labyrinths, acoustic ganglia, nerves, and brain centers of the chick embryo. *Journal of Morphology*, **121**, 179-208.

LAUTENSCHLAGER, S. and HÜBNER, T. 2013. Ontogenetic trajectories in the ornithischian endocranium. *Journal of Evolutionary Biology*, **26**, 2044-2050.

MALINZAK, M. D., KAY, R. F. and HULLAR, T. E. 2012. Locomotor head movements and semicircular canal morphology in primates. *Proceedings of the National Academy of Sciences*, **109**, 17914-17919.

MARUGÁN-LOBÓN, J. and BUSCALIONI, A. D. 2003. Disparity and geometry of the skull in Archosauria (Reptilia: Diapsida). *Biological Journal of the Linnean Society*, **80**, 67-88.

MARUGÁN-LOBÓN, J., CHIAPPE, L. M. and FARKE, A. A. 2013. The variability of inner ear orientation in saurischian dinosaurs: testing the use of semicircular canals as a reference system for comparative anatomy. *PeerJ*, **1**, e124.

MIRONE, A., BRUN, E., GOUILLART, E., TAFFOREAU, P. and KIEFFER, J. 2014. The PyHST2 hybrid distributed code for high speed tomographic reconstruction with iterative reconstruction and a priori knowledge capabilities. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, **324**, 41-48.

MULLER, M. 1999. Size limitations in semicircular duct systems. *Journal of Theoretical Biology*, **198**, 405-437.

NEENAN, J. M., REICH, T., EVERS, S. W., DRUCKENMILLER, P. S., VOETEN, D. F. A. E., CHOINIERE, J. N., BARRETT, P. M., PIERCE, S. E. and BENSON, R. B. J. 2017.

Evolution of the sauropterygian labyrinth with increasingly pelagic lifestyles. *Current Biology*, **27**, 3852–3858.

--- and SCHEYER, T. M. 2012. The braincase and inner ear of *Placodus gigas* (Sauropterygia, Placodontia)—a new reconstruction based on micro-computed tomographic data. *Journal of Vertebrate Paleontology*, **32**, 1350-1357.

OWEN, R. 1854. *Descriptive catalogue of the fossil organic remains of Reptilia and Pisces contained in the Museum of the Royal College of Surgeons of England*. Taylor & Francis, London.

PAGANIN, D., MAYO, S. C., GUREYEV, T. E., MILLER, P. R. and WILKINS, S. W. 2002. Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. *Journal of Microscopy*, **206**, 33-40.

REISZ, R. R., EVANS, D. C., SUES, H.-D. and SCOTT, D. 2010. Embryonic skeletal anatomy of the sauropodomorph dinosaur *Massospondylus* from the Lower Jurassic of South Africa. *Journal of Vertebrate Paleontology*, **30**, 1653-1665.

REISZ, R. R., SCOTT, D., SUES, H.-D., EVANS, D. C. and RAATH, M. A. 2005. Embryos of an Early Jurassic prosauropod dinosaur and their evolutionary significance. *Science*, **309**, 761-764.

SANCHEZ, S., FERNANDEZ, V., PIERCE, S. E. and TAFFOREAU, P. 2013. Homogenization of sample absorption for the imaging of large and dense fossils with synchrotron microtomography. *Nature Protocols*, **8**, 1708-1717.

SCANNELLA, J. B. and HORNER, J. R. 2010. *Torosaurus* Marsh, 1891, is *Triceratops* Marsh, 1889 (Ceratopsidae: Chasmosaurinae): synonymy through ontogeny. *Journal of Vertebrate Paleontology*, **30**, 1157-1168.

SIPLA, J. S. and SPOOR, F. 2008. The physics and physiology of balance. 227-232. *In* THEWISSEN, J. G. M. and NUMMELA, S. (eds). *Sensory Evolution on the Threshold: Adaptations in Secondarily Aquatic Vertebrates*. University of California Press, Berkeley, pp. Custom 7.

SPOOR, F. 2003. The semicircular canal system and locomotor behaviour, with special reference to hominin evolution. *Courier Forschungsinstitut Senckenberg*, **243**, 93-104.

--- BAJPAI, S., HUSSAIN, S. T., KUMAR, K. and THEWISSEN, J. G. M. 2002. Vestibular evidence for the evolution of aquatic behaviour in early cetaceans. *Nature*, **417**, 163-166.

--- GARLAND, T., KROVITZ, G., RYAN, T. M., SILCOX, M. T. and WALKER, A. 2007. The primate semicircular canal system and locomotion. *Proceedings of the National Academy of Sciences*, **104**, 10808-10812.

--- HUBLIN, J.-J., BRAUN, M. and ZONNEVELD, F. 2003. The bony labyrinth of Neanderthals. *Journal of Human Evolution*, **44**, 141-165.

--- and THEWISSEN, J. G. M. 2008. Comparative and functional anatomy of balance in aquatic mammals. 257-284. *In* THEWISSEN, J. G. M. and NUMMELA, S. (eds). *Sensory Evolution on the Threshold: Adaptations in Secondarily Aquatic Vertebrates*. University of California Press, Berkeley, pp. Custom 7.

--- WOOD, B. and ZONNEVELD, F. 1994. Implications of early hominid labyrinthine morphology for evolution of human bipedal locomotion. *Nature*, **369**, 645-648.

--- 1996. Evidence for a link between human semicircular canal size and bipedal behaviour. *Journal of Human Evolution*, **30**, 183-187.

SUES, H.-D., REISZ, R. R., HINIC, S. and RAATH, M. A. 2004. On the skull of *Massospondylus carinatus* Owen, 1854 (Dinosauria: Sauropodomorpha) from the Elliot and Clarens formations (Lower Jurassic) of South Africa. *Annals of the Carnegie Museum*, **73**, 239-257.

R DEVELOPMENT CORE TEAM. 2008. R: a language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria (2008) (ISBN 3-900051-07-0)*, http://www.R-project.org.

WALSH, S. A., BARRETT, P. M., MILNER, A. C., MANLEY, G. and WITMER, L. M. 2009. Inner ear anatomy is a proxy for deducing auditory capability and behaviour in reptiles and birds. *Proceedings of the Royal Society B*, **276**, 1355-1360.

--- LUO, Z.-X. and BARRETT, P. M. 2014. Modern imaging techniques as a window to prehistoric auditory worlds. 227-261. *In* KÖPPL, C., MANLEY, G. A., POPPER, A. N. and FAY, R. (eds). *Insights from Comparative Hearing Research. Springer Handbook of Auditory Research 49*. Springer, London, pp. Custom 7.

WILEY, D. F., AMENTA, N., ALCANTARA, D. A., GHOSH, D., KIL, Y. J., DELSON, E., HARCOURT-SMITH, W., ROHLF, F. J., JOHN, K. S. and HAMANN, B. 2005. Evolutionary morphing. 431-438. *VIS 05. IEEE Visualization, 2005.*

WITMER, L. M., CHATTERJEE, S., FRANZOSA, J. and ROWE, T. 2003. Neuroanatomy of flying reptiles and implications for flight, posture and behaviour. *Nature*, **425**, 950-953.

--- RIDGELY, R. C., DUFEAU, D. L. and SEMONES, M. C. 2008. Using CT to peer into the past: 3D visualization of the brain and ear regions of birds, crocodiles, and nonavian dinosaurs. 67-87. *In* ENDO, H. and FREY, R. (eds). *Anatomical Imaging. Towards a New Morphology*. Springer, Tokyo, pp. Custom 7.

YATES, A. M. and BARRETT, P. M. 2010. *Massospondylus carinatus* Owen 1854 (Dinosauria: Sauropodomorpha) from the Lower Jurassic of South Africa: Proposed conservation of the usage by designation of a neotype. *Palaeontologia Africana*, **45**, 7-10.

ZELDITCH, M. L., SWIDERSKI, D. L., SHEETS, H. D. and FINK, W. L. 2004. *Geometric morphometrics for biologists: a primer*. Elsevier Academic Press, Amsterdam, 443 pp.

Appendix 1: Supplementary Figures.

ONTOGENY OF THE *MASSOSPONDYLUS* LABYRINTH: IMPLICATIONS FOR LOCOMOTORY SHIFTS IN A BASAL SAUROPODOMORPH DINOSAUR

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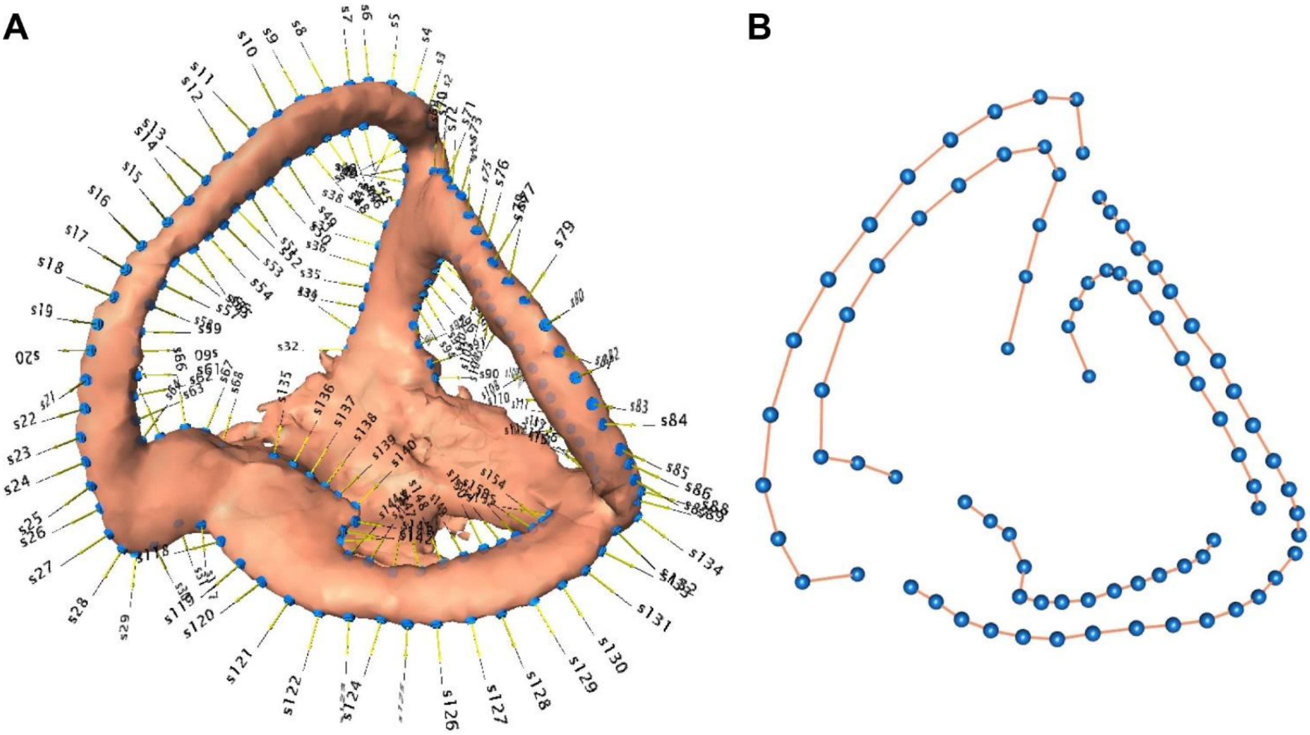

FIG. S1. The landmarking method employed in this study. A, semilandmarks are placed along the exterior and interior margins of each semicircular canal, additionally capturing the geometries of the crus communis as well as the anterior and lateral ampullae. B, semilandmark sequences are resampled in R to 14 equally-spaced points along each margin.

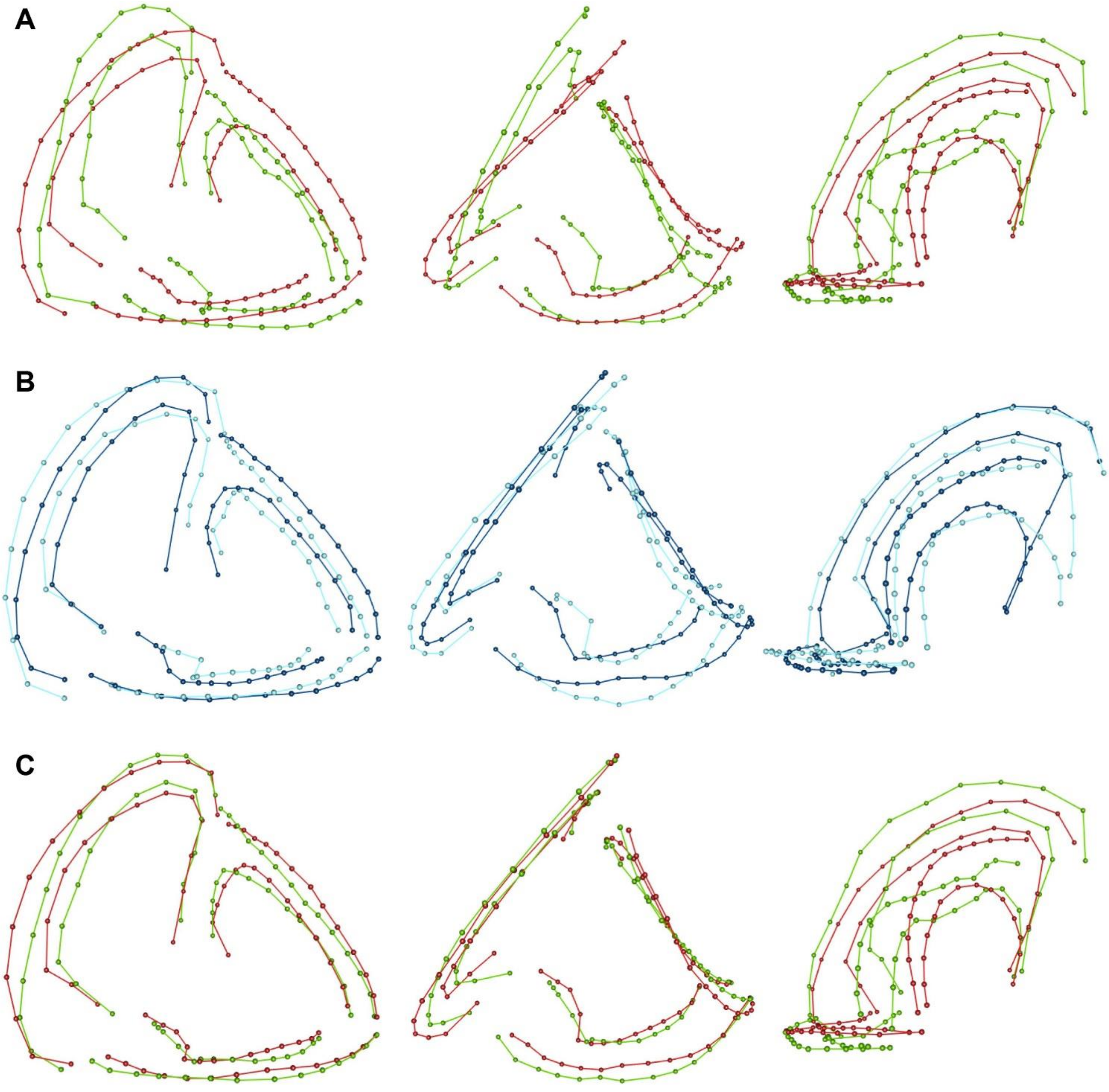

FIG. S2. Extreme shapes from PC axes overlain to demonstrate geometry change in lateral (left), dorsal (middle) and posterior (right) views. A, PC1. Negative values are in green and positive values in red. B, PC2, the axis that shows ontogenetic shape change. Colour scheme follows figure 3, with negative values in dark blue and positive values in light blue. C, PC3. Colour scheme follows A.