



INNER EAR MORPHOLOGY OF DIADECTOMORPHS AND SEYMOURIAMORPHS (TETRAPODA) UNCOVERED BY HIGH-RESOLUTION X-RAY MICROCOMPUTED TOMOGRAPHY, AND THE ORIGIN OF THE AMNIOTE CROWN-GROUP

Journal:	<i>Palaeontology</i>
Manuscript ID	PALA-01-19-4428-OA.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	22-May-2019
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Key words:	Diadectomorpha, Seymouriamorpha, inner ear, fossa subarcuata, origin of amniotes, amniote phylogeny

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47 **Abstract:** The origin of amniotes was a key event in vertebrate evolution, enabling tetrapods
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49 to break their ties with water and invade terrestrial environments. Two pivotal clades of early
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51 tetrapods, the diadectomorphs and the seymouriamorphs, have played an unsurpassed role in
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53 debates about the ancestry of amniotes for over a century, but their skeletal morphology has
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55 provided conflicting evidence for their affinities. Using high-resolution X-ray microcomputed
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57 tomography, we reveal the three-dimensional architecture of the well preserved endosseous
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3 labyrinth of the inner ear in representative species belonging to both groups. Data from the
4
5 inner ear are coded in a new cladistic matrix of stem and primitive crown amniotes. Both
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7 maximum parsimony and Bayesian inference analyses retrieve seymouriamorphs as derived
8
9 non-crown amniotes and diadectomorphs as sister group to synapsids. If confirmed, this sister
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11 group relationship invites re-examination of character polarity near the roots of the crown
12
13 amniote radiation. Major changes in the endosseous labyrinth and adjacent braincase regions
14
15 are mapped across the transition from non-amniote to amniote tetrapods, and include: a
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17 ventral shift of the cochlear recess relative to the vestibule and the semicircular canals;
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19 cochlear recess (primitively housed exclusively within the opisthotic) accommodated within
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21 both the prootic and the opisthotic; development of a distinct fossa subarcuata. The inner ear
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23 of seymouriamorphs foreshadows conditions of more derived groups, whereas that of
24
25 diadectomorphs shows a mosaic of plesiomorphic and apomorphic traits, some of which are
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27 unambiguously amniote-like, including a distinct and pyramid-like cochlear recess.
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35 **Key words:** Diadectomorpha, Seymouriamorpha, inner ear, fossa subarcuata, origin of
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37 amniotes, amniote phylogeny.
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42 CROWN-GROUP amniotes – the clade that includes Sauropsida (reptiles, birds, and their extinct
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44 relatives), Synapsida (mammals and their extinct relatives), the most recent common ancestor
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46 of those two groups, and all its descendants – are estimated to have originated approximately
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48 320 million years ago (median estimate; Shedlock & Edwards 2009; Benton 2014; Benton *et*
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50 *al.* 2015; Jones *et al.* 2018; for alternative estimates, see www.timetree.org). However, the
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52 ancestry of amniotes, the taxonomic composition of this clade, and the interrelationships of its
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54 constituent subclades are contentious, for four main reasons. Firstly, the origin of the unique
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56 physiological and reproductive characteristics of amniotes, especially the production of eggs
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3 with mineralized or leathery shells in which the developing embryos are surrounded by extra-
4 embryonic membranes, has not yet been documented in the fossil record (e.g. Skulan 2000;
5 Wilkinson *et al.* 2002; Laurin 2004, 2005; Piñeiro *et al.* 2012). Secondly, the long-established
6 dichotomy of crown amniotes between sauropsids and synapsids (e.g. see Case 1907; Watson
7 1957; Modesto & Anderson 2004; Benton 2014) has sometimes been called into question. For
8 example, Vaughn (1960) postulated that microsaur – the most species-rich group within the
9 informal assemblage of early tetrapods known as the ‘lepospondyls’ (Carroll *et al.* 1998; see
10 also Clack *et al.* 2019 for a rebuttal of lepospondyl monophyly) – were related to sauropsids.
11 Recently, Pardo *et al.* (2017) provided formal cladistic support for a sister group relationship
12 between microsaur and sauropsids. Thirdly, traditional branching patterns in several lineages
13 of primitive crown amniotes have been challenged. As an example, Ford and Benson (2019)
14 put forward a new phylogenetic hypothesis in which varanopids, usually regarded as a clade
15 of basal synapsids, are nested within diapsids. Fourthly, the identity of the closest relatives of
16 crown amniotes from among the diverse array of early tetrapods has long been debated (e.g.
17 Cope 1880, 1886; Lee & Spencer 1997; Ruta *et al.* 2003; Reisz 2007; Ruta & Coates 2007;
18 Klembara *et al.* 2014; Marjanović & Laurin 2019), and only partial consensus has emerged.

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40 In the present paper we focus on the most widely discussed of all candidate groups of
41 early tetrapods implicated in the amniote origin debate, namely the Diadectomorpha and the
42 Seymouriamorpha. The Carboniferous and Permian diadectomorphs are recognizable by their
43 deep and heavily ossified skulls, robust lower jaws, differentiated marginal dentition with
44 various degrees of tooth molarization, sturdy ribs, stocky limbs, and massive pectoral and
45 pelvic girdles (e.g. Case 1911; Olson 1947; Berman *et al.* 1992, 1998, 2004; Reisz 2007;
46 Kissel 2010; Liu & Bever 2015). Diadectomorphs were the first group of early tetrapods to
47 evolve high-fibre herbivory (e.g. Sumida & Martin 1997; Reisz 2007; Sues 2008; Anderson *et*
48 *al.* 2013). Members of the family Diadectidae, in particular the genus *Diadectes*, were once
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3 hypothesized to be true amniotes, although presumed to have retained amphibian-like aspects
4 of reproduction (e.g. Case 1911; Olson 1947). For instance, Case (1907, 1914) postulated a
5 close relationship between diadectomorphs and synapsids. In most phylogenetic analyses,
6
7 however, diadectomorphs form the monophyletic sister taxon to crown amniotes (but see Ruta
8 & Coates 2007), and are often employed as the ‘default’ outgroup to polarize characters near
9 the roots of this clade (e.g. Müller & Reisz 2006; Benson 2012; Ford & Benson 2019). To the
10 best of our knowledge, Berman (2000, 2013) was the first author to retrieve diadectomorphs
11 as the sister taxon to synapsids based upon a formal character-based analysis. This hypothesis
12 (see especially Berman 2013) was based upon a small number of taxa (mostly supraspecific
13 units) and a restricted set of characters. However, it has received further support in at least
14 one recent study that utilized a much larger cladistic data set (Marjanović & Laurin 2019).

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The Permian seymouriamorphs were superficially lizard-like animals with a distinctive
dermal sculpture consisting of pits and ridges on the skulls of mature individuals, moderately
elongate trunks, and short and robust limbs (e.g. Špinar 1952; Laurin 1996; Klembara 1997,
2011; Klembara & Ruta 2004a, b, 2005a, b). Various species are known from very abundant
larval stages preserving delicate impressions of filamentous external gills as well as sensory
canals. Seymouriamorphs have generally been considered to be either proximal to, or the
immediate sister taxon to the clade encompassing diadectomorphs plus crown amniotes (e.g.
Sumida & Martin 1997; Ruta *et al.* 2003; Ruta & Coates 2007; Klembara *et al.* 2007, 2014;
Pardo *et al.* 2017). In some analyses, however, they have been assigned to the tetrapod stem-
group (Marjanović & Laurin 2019), implying *ipso facto* no special relationship with either
major branch – amniotes or lissamphibians – of crown tetrapods (but see Clack *et al.* 2019).

Although several cranial and postcranial characteristics in both seymouriamorphs and
diadectomorphs are reminiscent of amniote conditions (e.g. Klembara 1997, 2011; Sumida &
Martin 1997; Klembara & Ruta 2004a, b, 2005a, b; Reisz 2007; Klembara *et al.* 2007, 2014),

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3 neither group shares unambiguous features with amniotes. In this respect, therefore, additional
4 information may acquire relevance. In particular, neuroanatomical characters provide a rich
5 but as yet largely unexplored source of data for comparative morphological and phylogenetic
6 investigation. The endosseous labyrinth that surrounds the membranous inner ear is especially
7 complex and variable, and thus potentially significant for examining patterns of character
8 acquisition and transformation near the base of the amniote radiation. However, with some
9 exceptions (e.g. Robinson *et al.* 2005; Maddin *et al.* 2012; Pardo *et al.* 2015; Pardo &
10 Anderson 2016, and references therein) data on the endosseous labyrinth are inadequate in
11 most early tetrapods because of the generally small size, fragile nature, poor ossification, and
12 difficult anatomical location of this structure. In addition, its intricate architecture is often lost
13 or damaged as a result of fossil preservation.

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15 In this context, it is noteworthy that specimens of *Diadectes* from the Early Permian of
16 North America examined over a century ago (Watson 1916; Olson 1966) yielded preliminary
17 data on the vestibule, the tubular connection between the vestibule and the fenestra vestibuli,
18 the semicircular canals with putative associated ampullae (housing the sensory structures that
19 register the rotational movements of the head), and a lagena (the terminal end of the cochlear
20 duct, housing the hearing organ) which was figured in a single transverse section but without
21 details of its shape, size, and position (see Olson 1966). However, the accuracy of these early
22 observations has never been substantiated (see discussion below).

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24 Here we employ high-resolution X-ray microcomputed tomography to document for the
25 first time the three-dimensional architecture of the endosseous labyrinth in diadectomorphs
26 and seymouriamorphs. The new data allow us to reject and/or amend to a considerable degree
27 previous accounts of the diadectomorph labyrinth and to provide critical new information on
28 its morphology and variation. In addition, they permit in-depth scrutiny of plesiomorphic and
29 apomorphic conditions of the stato-acoustic apparatus through detailed comparisons with the
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3 labyrinth of seymouriamorphs and primitive crown amniotes. Furthermore, characters of the
4 labyrinth are coded in a new cladistic matrix which we use to inspect the polarity of inner ear
5 traits near the roots of the amniote radiation. Finally, the results of the phylogenetic analyses
6 inform a short discussion of the interrelationships of near-amniote and basal crown amniote
7 tetrapods, a detailed analysis of which is currently being undertaken (M.R., work in progress).
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17 MATERIAL AND METHOD

18 *Taxon sample for microcomputed tomography*

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21 The following species were examined: the seymouriamorphs *Seymouria baylorensis* and *S.*
22 *sanjuanensis* (Laurin 1996), the diadectomorphs *Diadectes absitus* (Berman *et al.* 1998) and
23 *Orobates pabsti* (Berman *et al.* 2004), and the captorhinid eureptile *Labidosaurus hamatus*
24 (Modesto *et al.* 2007). Literature data on the inner ear of extant tetrapods were complemented
25 by information obtained from a μ CT-scanned specimen of the extant European Glass Lizard,
26 *Pseudopus apodus*.
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42 *Specimen preservation*

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45 In specimen MNG 8747 of *Diadectes absitus*, the right-hand side of the braincase is preserved
46 in great detail. In contrast, the two specimens of *Seymouria baylorensis* (MCZ 1081, 1086)
47 reveal good preservation of the left-hand side. Therefore, for ease of direct comparisons the
48 virtual 3D models and transverse sections of the inner ear of *S. baylorensis* (especially MCZ
49 1086) are illustrated in reversed (right-left) orientation throughout.
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57 Only in specimen CM 73371 of *Labidosaurus hamatus* is the supraoccipital visible,
58 while completely preserved right prootic and opisthotic occur in CM 73370. Therefore, for
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3 3D reconstruction purposes we assembled the CT-scans of those three bones so as to reflect
4 their mutual anatomical orientations. To this end, the supraoccipital of CM 73371 was scaled
5 to the size of the prootic and opisthotic in CM 73370. Following this procedure, the bones
6 were joined together, such that the cavity of the endosseous labyrinth could be segmented.
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14 *Institutional abbreviations.* CM, Carnegie Museum of Natural History, Pittsburgh, USA; DE,
15 Department of Ecology, Faculty of Natural Sciences, Bratislava, Slovakia; FMNH UR, Field
16 Museum of Natural History, Chicago, USA; MCZ, Museum of Comparative Zoology,
17 Harvard University, Cambridge, USA; MNG, Museum der Natur, Gotha, Germany.
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26 *Age, stratigraphy, and locality data*

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30 *Seymouria baylorensis* MCZ 1081 (almost complete adult skull) and 1086 (partial adult
31 skull): early Permian, lower Clear Fork Formation (following Nelson *et al.*, 2013, this
32 corresponds to the Arroyo Formation of older literature), West Coffee Creek, Baylor County,
33 Texas, USA; *S. sanjuanensis* CM 28597 (complete adult skull): early Permian, Arroyo del
34 Agua Formation, Arroyo del Agua #10, Rio Arriba County, New Mexico, USA; *Diadectes*
35 *absitus* MNG 8747 (incomplete subadult skull) and *Orobates pabsti* MNG 10181 (complete
36 adult skull): early Permian, Tambach Formation, Bromacker locality, near Tambach-Dietharz,
37 Thuringia, Germany; *Labidosaurus hamatus* CM 73370 (disarticulated adult skull) and CM
38 73371 (complete adult skull), and FMNH UR 161 (partial adult skull): early Permian, lower
39 Clear Fork Formation, *L. hamatus* Pocket, northcentral Texas, USA; *Pseudopus apodus* DE 8
40 (complete adult skull): Recent, Balkan Peninsula.
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CT-scan data visualization

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3 The scans of *Diadectes absitus* (MNG 8747), *Labidosaurus hamatus* (FMNH UR 161, CM
4 73370), and *Pseudopus apodus* (DE 8) were performed using a GE Phoenix Nanotom 180
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6 facility at the Institute of Measurement Science, Slovak Academy of Sciences, Bratislava,
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8 Slovakia, with the following settings: 170 kV accelerating voltage; 0.055 mA current; 1800
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10 X-ray projections; 500 ms acquisition time for each projection; 0.06 mm voxel size; use of 0.2
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12 mm thin copper plate for cutting off low energy photons from the X-ray beam. To expand the
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14 measurement range, multiscan and virtual sensor regimes were used. 3D volume assembly
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16 and the rendering and segmentation of image data were carried out in VGStudio Max 2.1.
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21 Specimen CM 73371 of *Labidosaurus hamatus* was scanned using a GE Phoenix CT
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23 v|tome|x L240 at the Institute of Geosciences, Slovak Academy of Sciences, Banská Bystrica,
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25 Slovakia, with the following settings: 220 kV accelerating voltage; 0.220 mA current; 2600
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27 X-ray projections; 700 ms acquisition time for each projection; 0.1 mm voxel size; use of 0.2
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29 mm thin copper plate for X-ray filtering. The CT data were analysed in VGStudio Max 2.2.
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33 Specimens MCZ 1081, 1086 of *Seymouria baylorensis* were scanned using a Bruker
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35 SkyScan 1173 at the Museum of Comparative Zoology, Harvard University, USA, with the
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37 following settings for MCZ 1081 specimen: 130 kV accelerating voltage; 0.061 mA current;
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39 600 X-ray projections; 1200 ms acquisition time for each projection; 0.026 mm voxel size;
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41 use of 0.25 mm thin brass plate for X-ray filtering. For MCZ 1081 specimen, the following
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43 settings were used: 130 kV accelerating voltage; 0.061 mA current; 720 X-ray projections;
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45 1200 ms acquisition time for each projection; 0.035 mm voxel size; 0.25 mm thin brass plate
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47 filter. In both specimens, the assembly of 3D volumes, rendering, and segmentation of image
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49 data were performed using VGStudio Max 2.1.
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53 The following settings were employed for specimen MNG 10181 of *Orobates pabsti*
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55 (Nyakatura *et al.* 2015): 240 kV accelerating voltage; 0.140 mA current; 1440 X-ray
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3 projections; 0.075 mm voxel size; use of 0.2 mm thin copper plate for X-ray filtering. The
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5 data were analysed in Avizo 8.1.
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10 *Phylogenetic analysis*

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13 We built a new data matrix consisting of 288 osteological characters coded in 53 species
14 (Dryad Digital Repository, character list and data matrix): 11 outgroups, 34 amniote-like taxa
15 (from among chroniosuchians, embolomeres, gephyrostegids, seymouriamorphs, and
16 diadectomorphs), and eight crown amniotes (four synapsids; four eureptiles). The matrix was
17 processed under maximum parsimony and Bayesian optimality criteria. Parsimony analyses
18 were executed in PAUP* v. 4.0a build 165 (Swofford 1998; <https://paup.phylosolutions.com>).
19 A preliminary search for “rogue” taxa (*sensu* Wilkinson 1996) using the package *strap* (Bell
20 & Lloyd 2015) in the R environment for statistical computing and graphics ([https://cran.r-](https://cran.r-project.org)
21 [project.org](https://cran.r-project.org)) showed that no taxa could be safely deleted. Parsimony analyses were run with
22 equally weighted characters (i.e. all characters were assigned equal unit weight) as well as
23 with simple reweighting and implied weighting regimes. A strict consensus tree was used to
24 summarize the multiple shortest trees from the initial analysis with equal weights. The simple
25 reweighting procedure employed the maximum value (best fit) of the rescaled consistency
26 index of each character across all most parsimonious trees from the equally weighted analysis.
27 The implied weighting analysis (Goloboff 1993), carried out in PAUP*, followed the simple
28 protocol outlined by Congreve and Lamsdell (2016; see also Clack *et al.* 2019). Specifically,
29 we used different integer values of Goloboff’s K constant of concavity, with $1 \leq K \leq 10$. For
30 each K value, we saved the most parsimonious tree(s). We summarized group relationships
31 from the trees generated by all implied weighting runs in the form of a strict consensus and a
32 maximum agreement subtree (i.e. a taxonomically pruned topology that contains the largest
33 possible set of taxa showing the same relative relationships in all original trees).
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3 For all parsimony analyses, and under each of the three weighting schemes, optimal tree
4 searches employed identical settings, as follows: collapsing branches with minimum length of
5 zero; heuristic search method; tree bisection-reconnection branch-swapping algorithm based
6 upon 5000 random stepwise taxon addition sequences and holding one tree in memory at each
7 replicate; five consecutive branch-swapping rounds applied to all trees in memory from this
8 initial search, but with the option of saving multiple trees. Neither shorter nor additional trees
9 were obtained at the end of these five branch-swapping rounds in any of the unweighted and
10 weighted analyses. Tree node support was evaluated with bootstrapping (Felsenstein 1985)
11 and jackknifing (Farris *et al.* 1996), in each case using 5000 random replicates of character
12 resampling (with jackknifing, 50% of all characters were resampled in each replicate) under
13 the fast stepwise addition option in PAUP*.
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28 The Bayesian analysis was carried out in MrBayes v. 3.2.6 (Ronquist & Huelsenbeck
29 2003) with the standard data type option (morphological characters), with characters treated
30 as informative (following removal of uninformative characters in the original matrix), under
31 an equal-rate model of state change, running four chains with 10^7 generations, sampling every
32 1000 generations, and discarding 25% of the obtained samples. At the end of the searches the
33 branch lengths were saved alongside the clade credibility values. Convergence was checked
34 through inspection of Gelman and Rubin's (1992) Potential Scale Reduction Factor (PSRF).
35 Convergence was deemed satisfactory (PSRF approaching 1).
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47 A final note concerns the exclusion of microsauria from our data matrix. The status and
48 affinities of microsauria are especially important in the context of early tetrapod relationships,
49 certainly given their newly proposed assignment to crown amniotes in Pardo *et al.*'s (2017)
50 analysis, and the fact that they have appeared in various positions along the amniote stem in
51 various earlier studies (e.g. Ruta & Coates 2007; Clack *et al.* 2016, 2019). Amniote-like
52 affinities for microsauria have also been proposed in light of skeletal developmental data
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3 (Olori 2013). A review of early tetrapod phylogeny, including the status of microsaur, and a
4 re-evaluation of recently published character matrices are currently under way (M. R., work in
5 progress). Pending further scrutiny, microsaur will not be considered further in this paper.
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10 11 12 **RESULTS**

13 14 15 16 17 18 *The endosseous labyrinth of Diadectomorpha*

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21 *Diadectes absitus*. The skull of *D. absitus* specimen MNG 8747 (Fig. 1A, B; see also Berman
22 *et al.* 1998) belongs to a subadult individual. Most of the endosseous labyrinth is enclosed
23 within three bones, i.e. the opisthotic, the prootic, and the supraoccipital (Fig. 1B-D, 2; Dryad
24 Digital Repository, video 1). However, a large portion of the vestibule and several sections of
25 the semicircular canals are not fully enclosed. In life, the entire anteromedial wall of the inner
26 ear cavity would consist of a membrane extending between the medial wall of the prootic, the
27 ventral crest of the supraoccipital, and the anterior margin of the opisthotic (representing the
28 internal auditory meatus). The endosseous labyrinth consists of the vestibule, and the anterior,
29 posterior, and lateral semicircular canals (hereafter ASC, PSC, and LSC), which lie anterior to
30 the cochlear recess housing the lagenar macula (Figs 1C, 3A, B, 4B). The ASC and PSC meet
31 in the medially positioned crus commune, which opens into the vestibule ventrally (Figs 1D,
32 5B). Immediately anteromedial to the crus commune, the canal for the endolymphatic duct
33 exits the vestibule and opens into the cranial cavity (Figs 1C, 2A, 3A, B). The posterior end of
34 the PSC enters the anterodorsal portion of the cochlear recess, whereas the posterior end of
35 the LSC enters the anteroventral portion of the cochlear recess; from this point, the cochlear
36 recess freely communicates with the vestibule anteriorly (Figs 3A, B, 4B). A distinct
37 dorsoventrally orientated fissure occurs between the cochlear recess and the vestibule. The
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3 LSC runs lateral to the otic tube, which connects the fenestra vestibuli to the vestibule (Fig.
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5 1D). The cochlear recess is completely enclosed in the opisthotic (Figs 1C, 2B) and resembles
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7 a mediolaterally flattened triangle with a posteriorly directed apex (Figs 1C, 2B, 3A, B, 4B).
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9 The fossa subarcuata (a bony depression housing a small process of the dura mater as well as
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11 the cerebellar flocculus in extant amniotes; see below) is entirely enclosed in the
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13 supraoccipital (Fig. 5) and situated on its ventral wall at the posterior end of the skull. Most of
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15 the fossa is anteromedial to the anterior portion of the ASC. At this level, it forms a shallow
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17 and elongate depression.
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24 *Orobates pabsti*. The endosseous labyrinth of *O. pabsti* is contained within the opisthotic,
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26 prootic, and supraoccipital (Fig. 6). It is dorsoventrally flattened, with the semicircular canals
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28 orientated horizontally (Figs 3C, D, 4A, 6). The dorsal wall of the vestibule reaches the level
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30 of the dorsal walls of the canals. The otic tube is short, and the fenestra vestibuli opens
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32 ventrally. The cochlear recess is posterior to the vestibule and completely enclosed in the
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34 opisthotic. The recess is shaped like a dorsoventrally orientated and slightly mediolaterally
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36 flattened tube that communicates freely with the vestibule both dorsally and ventrally (Fig.
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38 4A). Between these two levels a fissure is visible between the recess and the vestibule. The
39
40 fossa subarcuata forms a depression situated on the anteromedial wall of the supraoccipital
41
42 and lies dorsomedial to the anterior portion of the ASC (Fig. 7).
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49 *The endosseous labyrinth of Seymouriamorpha*

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52 The preserved portion of the endosseous labyrinth in *Seymouria baylorensis* includes the
53
54 cochlear recess, the otic tube, the LSC, the anterior portion of the ASC, and most of the
55
56 vestibule (Figs 3E, F, 4C; Dryad Digital Repository, video 2). The subtriangular and
57
58 dorsoventrally elongate cochlear recess forms a distinct posterior extension of the vestibule
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1
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3 situated medial to the otic tube and fully enclosed in the opisthotic, as in *Diadectes* and
4
5 *Orobates* (Figs 8, 9). The narrow and elongate otic tube in *Seymouria* is comparatively much
6
7 longer than in *Diadectes*. It is situated anterior to the cochlear recess and posterior to the
8
9 semicircular canals, and connects the fenestra vestibuli to the vestibule. The LSC joins the
10
11 anterior end of the ASC. However, only the anterior section of the ASC is visible, and this is
12
13 enclosed in the prootic. We hypothesize that the posterior section of the ASC was
14
15 accommodated within an unossified supraoccipital in life. However, additional data from
16
17 *Seymouria* are needed to confirm this. In this respect, we note that in *Diadectes*, *Orobates*,
18
19 *Labidosaurus*, and several other extinct and extant amniotes, the supraoccipital is fully
20
21 ossified. The position of the anterior section of the ASC in *Seymouria* indicates that the ASC
22
23 was at least slightly curved, perhaps not dissimilar from the condition seen in *Diadectes* (Fig.
24
25 1C, 3A). The LSC runs lateral to the vestibule, and its posterior end is confluent with the
26
27 vestibule immediately anterior to the proximal portion of the otic tube.
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36 *The endosseous labyrinth of Labidosaurus hamatus*

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40 The general morphology of the endosseous labyrinth in the captorhinomorph *Labidosaurus* is
41
42 very similar to that of other extinct and extant crown amniotes (Figs 3G, H, 4D, E, 10, 11,
43
44 12A-C; Dryad Digital Repository, video 3). In lateral aspect, its vestibule is tube-like and
45
46 communicates freely with the cochlear recess ventrally (Figs 3G, H, 4D, 11). All three canals,
47
48 including their ampullae, appear well developed. The ASC and PSC are slightly curved and
49
50 located in the supraoccipital (Fig. 12B, C). The ampullae of the LSC and ASC are situated
51
52 within the prootic (Fig. 10C), whereas that of the PSC occurs within the opisthotic (Fig. 10A).
53
54 The apex of the triangular cochlear recess is directed ventrally, as is characteristic for crown
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56 amniotes. However, the recess in *Labidosaurus* is slightly larger – relative to the size of the
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2
3 vestibule – than in other crown amniotes (Fig. 4D, E), and occurs within the opisthotic and
4
5 the prootic, a condition also seen in Recent amniotes, such as the anguine reptile *Pseudopus*
6
7 *apodus* (Fig. 4E). The orientated posterolaterally fenestra vestibuli is located in the cochlear
8
9 recess. A deep fossa, the recessus scala tympani, is visible on the anteromedial surface of the
10
11 opisthotic (Fig. 10A). It opens dorsally through the perilymphatic foramen into the vestibule,
12
13 immediately lateral to the recess of the ampulla of the PSC, and ventromedially into the
14
15 subarachnoidal space (Fig. 10A). In the supraoccipital, immediately medial to the crus
16
17 commune of the ASC and PSC, is a long tube extending dorsally and medially, which
18
19 represents the canal for the endolymphatic duct (Fig. 3G, H). The fossa subarcuata in
20
21 *Labidosaurus* is comparatively deeper than its homologue in *Diadectes* (Figs 5, 12B-E), has a
22
23 subcircular outline, and is clearly recognizable on transverse and horizontal braincase sections
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25 (Fig. 12B-E).
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35 *Phylogenetic results*

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39 We illustrate the results of different phylogenetic analyses in Figures 13–17. The maximum
40
41 parsimony analysis with equally weighted characters results in 421 trees (length = 1222 steps;
42
43 ensemble consistency index (C.I.) with uninformative characters excluded = 0.2736; ensemble
44
45 retention index (R.I.) = 0.5768). The strict consensus of these trees (Fig. 15A) shows poor
46
47 resolution, although diadectomorphs and crown amniotes are consistently retrieved as a clade.
48
49 Within that clade, diadectomorphs emerge as the sister taxon to synapsids in 60 percent of all
50
51 shortest trees. Reweighting characters by the maximum values of their individual consistency
52
53 indices yields a single shortest tree (length = 226.28398 steps; C.I. without uninformative
54
55 characters = 0.4574; R.I. = 0.772) in which, again, diadectomorphs form the sister taxon to
56
57 synapsids (Fig. 13). This relationship is also found in all trees generated from taxon deletion
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3 experiments. These experiments sought to characterize the main source of conflict given the
4 absence of rogue taxa (see above). While several deletions are possible, the following are of
5 particular interest, as they result in greater resolution and negligible or no impact on the
6 placement of other taxa. Thus, when *Proterogyrinus pancheni* and *Eobaphetes kansensis* are
7 removed from the matrix, either individually or together, many fewer trees are obtained (four
8 trees without *P. pancheni* and without *E. kansensis* and *P. pancheni*; 12 trees without *E.*
9 *kansensis*). Indeed, these two species are chiefly responsible for the greatest loss of resolution
10 in the initial analysis inclusive of all taxa. In each of the analyses with deleted taxa, the strict
11 consensus topologies are well resolved (Figs 14, 16). Each of the implied weighting analyses
12 (with values of the K constant of concavity ranging from one to ten) yielded a single tree. The
13 ten trees resulting from these analyses show slightly different branching patterns, summarized
14 as a strict consensus in Figure 17A. The apical portion of this consensus has a large polytomy
15 involving *Solenodonsaurus*, *Westlothiana*, diadectomorphs, synapsids, and sauropsids. This
16 polytomy results exclusively from the unstable position of *Solenodonsaurus*. The maximum
17 agreement subtree (Fig. 17B) shows that in all implied weighting trees, diadectomorphs form
18 the sister taxon to synapsids.

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Given the large amount of homoplasy in the matrix, node support is invariably poor and
most nodes are collapsed in the 50 percent majority-rule bootstrap and jackknife consensus
topologies (Fig. 15A). The results of the parsimony analyses are not altered after exclusion of
the neurocranial characters (discussed below).

Unlike in the parsimony analyses, the diadectomorph-synapsid relationship is strongly
supported in the Bayesian analysis, in which it receives a credibility value of 92 (Fig. 15B). If
corroborated by future studies (M. R. in preparation), this relationship will force a rethinking
of the character polarity and sequence of branching events near the roots of the crown amniote
radiation. We think it useful to offer a preliminary account of the character-state changes

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2
3 along the diadectomorph-synapsid branch. For simplicity, we employ the single tree obtained
4 from the reweighted analysis to inspect such changes (Fig. 13). A total of 20 changes occur on
5 the branch in question under the accelerated transformation (ACCTTRAN) and 10 under the
6 delayed transformation (DELTRAN). Seven of the DELTRAN changes are also found under
7 ACCTTRAN. Under both ACCTTRAN and DELTRAN transformations, however, most of the
8 characters are heavily homoplastic, some of them change within each of the diadectomorph
9 and synapsid clades, and several result from the optimization of missing entries.

19 ACCTTRAN changes include: 1 (c.i. = 0.273), nostril outline elliptical and elongate; 27
20 (c.i. = 0.111), total length of the lacrimal less than two and a quarter times its maximum pre-
21 orbital length; 40 (c.i. = 0.5), presence of lateral parietal lappets; 41 (c.i. = 0.333), postparietal
22 unpaired; 88 (c.i. = 0.25), quadrate with dorsal process; 90 (c.i. = 0.167), naris size equal to or
23 greater than 50% the size of the choana; 91 (c.i. = 0.5), naris height equal to or less than the
24 distance from the naris ventral rim to the upper jaw margin; 101 (c.i. = 0.5), pineal foramen
25 diameter 33% or greater than the anteroposterior length of the midline parietal suture; 103
26 (c.i. = 1), posttemporal fenestra is a small fossa present near occiput ventrolateral corner,
27 bordered laterally by tabular ventromedial flange, delimited dorsally by dorsal portion of the
28 lateral margin of the supraoccipital–opisthotic complex and floored by lateral extension of
29 opisthotic; 135 (c.i. = 0.333), transverse flange of pterygoid with row of small teeth; 137 (c.i.
30 = 0.167), pterygoid with a posterolateral flange; 151 (c.i. = 0.333), opisthotic forming a
31 thickened plate fused together with supraoccipital, preventing exoccipitals from contacting
32 the skull table; 157 (c.i. = 0.143), parasphenoid without a single median depression; 169 (c.i. =
33 0.143), dentary with anterior fangs generally comparable in size with, or greater than, other
34 dentary teeth, lying close to symphyseal region, and usually mesial to marginal dentary teeth;
35 205 (c.i. = 0.222), less than four premaxillary teeth; 208 (c.i. = 0.667), cleithrum stem is a
36 flattened oval in cross-section; 229 (c.i. = 0.2), portion of humeral shaft length proximal to
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3 entepicondyle less than humerus head width; 237 (c.i. = 0.333), posterior iliac process
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5 subhorizontal, stout, abbreviated posteriorly and tapering rearward in lateral aspect; 241 (c.i.
6
7 = 0.125), internal trochanter not raised as a distinct protuberance; 281 (c.i. = 1), either the
8
9 entire ASC or its posteromedial section as well as the entire PSC lie in the horizontal plane
10
11 and delimit an approximate right angle between them in the region of the crus commune.
12
13

14 The conditions of characters 40, 88, 103, 135, 137, 205, and 229 are also found under
15
16 DELTRAN, along with the following additional changes: 193 (c.i. = 0.333), posterodorsal
17
18 process of posterior coronoid contributing to tallest point of lateral margin of adductor fossa;
19
20 204 (c.i. = 0.222), less than 30 maxillary teeth; 243 (c.i. = 0.167), fourth trochanter of the
21
22 femur with a distinct rugose area.
23
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25

26 Some final remarks on the overall tree topology are warranted. The branching sequence
27
28 of stem amniotes is largely in agreement with those found in some previous analyses (e.g.
29
30 Ruta & Coates 2007; Klembara *et al.* 2014; Clack *et al.* 2019), but it also reveals the
31
32 instability of certain key taxa. Both seymouriamorphs and, in most analyses, anthracosaurs
33
34 (i.e. Eoherpetontidae plus Embolomeri) appear monophyletic, but we note the variable
35
36 position of *Silvanerpeton*, which either forms the sister taxon to other anthracosaurs or is
37
38 immediately anti-crownward of seymouriamorphs. Crownward of anthracosaurs, the
39
40 gephyrostegids (*Gephyrostegus*; *Brukererpeton*) emerge either as a clade (Figs 14A, 15B, 16)
41
42 or as a paraphyletic array (Figs 13, 14B). In one analysis (Fig. 14B), the chroniosuchians
43
44 (represented by *Chroniosaurus*) are nested within the anthracosaurs, while in others they are
45
46 either phylogenetically close to (Fig. 13) or the sister taxon to (Figs 14A, 16) anthracosaurs.
47
48 Finally, *Westlothiana* and *Solenodonsaurus* are the first and second most proximal sister taxa,
49
50 respectively, to the diadectomorphs-crown amniotes clade. A re-evaluation of the affinities of
51
52 these tetrapods is part of ongoing investigation (M. R., work in progress).
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DISCUSSION

The most striking feature of the endosseous labyrinth in *Diadectes* is its well-developed and sub-pyramidal cochlear recess. The recess narrows posteriorly and is partially separated from the vestibule (Figs 3A, B, 4B). In both these features, the labyrinth of *Diadectes* resembles closely that in extinct (where observations are possible) and extant amniotes (Fig. 4D, E) and differs significantly from that of amphibians (Baird 1970; Wever 1985; Robinson *et al.* 2005). However, as a plesiomorphic condition the cochlear portion of the labyrinth in *Diadectes* lies posterior to the vestibule, as seen also in *Orobates*, *Seymouria*, and extant amphibians (Wever 1985). In contrast, in crown amniotes the cochlear portion of the labyrinth varies in position relative to the vestibule. Thus, it may occur either posteroventral or entirely ventral to the vestibule and the semicircular canals (Fig. 4) (Baird 1970; Butler & Hodos 2005; Cuthbertson *et al.* 2015; Laaß 2016). The derived configuration also characterizes the captorhinomorph eureptiles *Captorhinus aguti* (Price 1935) and *Labidosaurus* Fig. 4D).

Aside from a posteriorly narrowing cochlear recess partly separated from the vestibule, other features point to amniote-like affinities for *Diadectes*. A feature of particular historical interest is the presence of a fossa subarcuata. Although Case (1911) reported this structure in *Diadectes* (albeit a different species from *D. absitus*) more than a century ago, its anatomical and evolutionary significance has not been recognized until now. Thus, in all extant amniotes the fossa subarcuata accommodates a process of the dura mater membrane (the outermost of the three meninges surrounding the central nervous system) and the cerebellar flocculus. The flocculus is concerned (among other functions) with equilibrium and receives sensory input from the vestibular portion of the inner ear situated immediately lateral to it (Butler & Hodos 2005). In contrast, amphibians do not have a distinct subarcuate fossa. In this group, the poorly developed homologue of the amniote flocculus consists of a small accumulation of cells (the auricle) dorsal to the acoustico-lateralis nucleus of the inner ear (Wever 1985).

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3 It is noteworthy that *Orobates* differs from *Diadectes* in its anteroposteriorly shorter
4 cochlear recess, similar to the plesiomorphic condition in *Seymouria*, and its more abbreviated
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It is noteworthy that *Orobates* differs from *Diadectes* in its anteroposteriorly shorter cochlear recess, similar to the plesiomorphic condition in *Seymouria*, and its more abbreviated otic tube (Figs 3A-F, 4A-C). In both *Orobates* and *Diadectes*, at least some semicircular canals show a straight course (Fig. 3A-D). This condition characterizes all three canals in *Orobates*, as well as the posterior portion of the ASC and the entire PSC in *Diadectes*. In addition, in both taxa the canals are mostly aligned in a sub-horizontal plane except for the anterior part of the ASC in *Diadectes*. Neither *Diadectes* nor *Orobates* show evidence of ampullae, *contra* previous reports (Watson 1916; Olson 1966). However, the specimens of *Diadectes* studied by Olson (1947, 1966) are larger than the subadult specimen of *D. absitus* studied here, and it is therefore impossible at present to confirm whether the ampullae are fully developed only in larger, more mature individuals. In contrast to *Diadectes* and *Orobates*, the crus commune of crown amniotes is situated distinctly more dorsally relative to the LSC (Fig. 4). Furthermore, in the majority of crown amniotes all the canals are more or less strongly arcuate (Baird 1970; Butler & Hodos 2005), although in *Labidosaurus* the ASC and PSC appear only gently curved (Fig. 3G).

Key changes in the endosseous labyrinth at the evolutionary transition from near-amniote to crown amniote tetrapods can now be considered in greater detail. Character 276 describes the most general aspect of the amniote inner ear, namely the occurrence of a large cochlear recess of approximately triangular shape in lateral view. Under ACCTAN, the apomorphic state is mapped on the branch subtending *Solenodonsaurus* and all more derived taxa, whereas DELTRAN places the state transformation at the base of the amniote crown.

Character 277 describes the position of the cochlear recess relative to the vestibule, its posteroventral or ventral position, as a derived condition, being confined to diapsids under both ACCTAN and DELTRAN. Modifications in the size, shape, and position of the cochlear recess across the transition from near-amniote to amniote tetrapods (Figs 3, 4, 13)

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2
3 are especially complex and can be summarized as follows (see also the descriptions above).
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5 The triangular recess in *Seymouria baylorensis* and the dorsoventrally elongate recess in
6
7 *Orobates pabsti* are similar in size to the vestibule. However, in *O. pabsti*, as well as in
8
9 *Diadectes absitus*, the recess is partially separated from the vestibule by a narrow fissure. In
10
11 addition, the triangular recess in *D. absitus* is much enlarged relative to the vestibule, as
12
13 observed also in *Labidosaurus hamatus* and *Pseudopus apodus*. Furthermore, in *S.*
14
15 *baylorensis*, *O. pabsti*, and *D. absitus* the recess is entirely posterior to the vestibule and the
16
17 canals. Although part of the recess is posteroventral to the vestibule in *L. hamatus*, its anterior
18
19 half is distinctly ventral to it (Figs 3, 4, 13). Finally, in several synapsids the triangular recess
20
21 is orientated slightly posteroventrally, but its extension is confined to the anteroposterior
22
23 length of the vestibule (Case 1914; Castanhinha *et al.* 2013; Laaß 2016; Benoit *et al.* 2017).
24
25 According to some researchers (see Benoit *et al.* 2017 and reference therein), the cochlear
26
27 recess is present in cynodont synapsids only, in which it constitutes the precursor to the
28
29 mammalian coiled cochlea, and there is ongoing debate about the possible convergent origin
30
31 of the recess among synapsids. However, our finding that a cochlear recess occurs near the
32
33 roots of the crown amniote clade suggests that this structure may in fact be plesiomorphic for
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35 synapsids.
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42 As for the derived condition of character 278, which concerns a distinctly developed
43
44 subarcuate fossa shaped like a shallow depression, the derived condition is optimised under
45
46 both ACCTAN and DELTRAN as two parallel acquisitions, i.e. in *Diadectes absitus* and
47
48 along the branch subtending *Captorhinus* (see Price 1935) and *Labidosaurus*. However, we
49
50 note the occurrence of various intervening taxa with unknown conditions for the character in
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52 question, implying that it may have a much wider distribution than revealed by our taxon
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54 sample. It is noted that a distinct fossa is also present in *Orobates pabsti*.
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3 Concerning the apomorphic states of an otic tube and otic trough (characters 279, 280),
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5 they appear as secondary losses in diapsids among crown amniotes under both optimization
6
7 regimes. The otic trough (Berman *et al.* 1998) and otic tube (Watson 1916; Heaton 1980;
8
9 Laurin 1996) have long been regarded as diagnostic for seymouriamorphs and
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11 diadectomorphs. To the best of our knowledge, an otic tube has not been described in
12
13 synapsids, but we think it plausible that the homologue of this structure in some
14
15 representatives of this group is the so-called vestibular tube, which connects the fenestra
16
17 vestibuli with the vestibule (e.g. Case 1914; Benoit *et al.* 2017). An otic trough occurs in
18
19 “pelycosaur”-grade synapsids, such as *Varanops* and *Dimetrodon* (Berman *et al.* 1992).
20
21 However, neither the otic trough nor the otic tube are observed in eureptiles, as far as we can
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23 concern.
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28 Finally, character 281 describes the shape of the semicircular canals. We note that in
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30 diadectomorphs the entire PSC and either the entire ASC or its posteromedial portion are for
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32 the most part straight, occur on a sub-horizontal plane, and meet at an approximately right
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34 angle in the region of the crus commune. In basal synapsids, such as *Edaphosaurus* and
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36 *Dimetrodon*, the ASC and PSC are almost straight (Case 1914), which is also the condition
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38 observed in *Orobates* and, in part, *Diadectes*. In the advanced synapsids, the canals are curved
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40 to various degrees (Castanhinha *et al.* 2013; Laaß 2016; Benoit *et al.* 2017). This condition is
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42 also observed in *Seymouria*, *Captorhinus* (Price 1935), and *Labidosaurus*, as well as in the
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44 temnospondyl amphibian *Dendrerpeton acadianum* (Robinson *et al.* 2005). Thus, at present,
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46 changes in the degree of curvature of the canals do not follow a simple evolutionary course
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48 when mapped onto the phylogeny, and the condition of diadectomorphs may well turn out to
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50 be autapomorphic, at least under ACCTRAN.
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58 **CONCLUSIONS**

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5 The present study has revealed substantial differences in the construction of the endosseous
6 labyrinth of the inner ear, both in the most crownward part of the amniote stem and, as well
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8 as, at the base of the amniote crown, providing some unique insights into the sequence of
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10 character acquisition and transformation in the assembly of the amniote stato-acoustic
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12 apparatus. Major modifications in the labyrinth include the development of a distinct
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14 triangular cochlear recess, the ventral repositioning of this recess in relation to the vestibule
15
16 and semicircular canals, an increase in the curvature of the canals, the dorsal displacement of
17
18 the anterior and posterior canals relative to the lateral canal, and the appearance of a distinct
19
20 subarcuate fossa housing the cerebellar flocculus. Diadectomorphs reveal a mosaic of inner
21
22 ear characters, some clearly primitive (e.g., cochlear recess lying posterior to vestibule and
23
24 housed entirely only in opisthotic, in contrast to crown amniotes in which the cochlear recess
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26 lies ventral to the vestibule and is housed in opisthotic and prootic as in *Labidosaurus*), others
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28 derived (e.g., a distinctly developed cochlear recess shaped like a pyramid, as in recent
29
30 reptiles) (Fig. 4). The neurocranial anatomy of diadectomorphs is transitional between that of
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32 seymouriamorphs and that of basal amniotes (Fig. 13), and reveals a complex mosaic of
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34 plesiomorphic and apomorphic traits, the polarity of several of which necessitates a broader
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36 sample of taxa. Although neuroanatomical analyses of early tetrapods still remain in their
37
38 infancy, we hope that the data presented here will stimulate further explorations into the
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40 palaeoneurology of early tetrapods and the evolution of sensory perception during vertebrate
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42 terrestrialization.
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53 *Acknowledgements.* J. K. and M. H. acknowledge financial support from the Scientific Grant
54
55 Agency of Ministry of Education of Slovak Republic and Slovak Academy of Sciences
56
57 (Grants No. 1/0228/19 to J. K. and APVV 14-0719 to M. H. for CT-scan data). For
58
59
60

1
2
3 permission to access, borrow, and CT-scan specimens, and to reproduce CT-scan data, we
4
5 thank: Drs T. Martens (Gotha), T. Hübner, and O. Wings (Stiftung Schloss Friedenstein
6
7 Gotha, Museum der Natur, Gotha, Germany) (*Diadectes absitus*); Dr. J. Nyakatura
8
9 (Humboldt Universität zu Berlin, Germany) (*Orobates pabsti*); Drs C. Capobianco and J.
10
11 Cundiff (Museum of Comparative Zoology, Harvard University, Cambridge, USA)
12
13 (*Seymouria baylorensis*); Drs W. F. Simpson (Field Museum of Natural History, Chicago,
14
15 USA) and Professor Z. Luo (University of Chicago, USA) (*Labidosaurus hamatus*). Dr. A.
16
17 Čerňanský (Comenius University in Bratislava, Slovakia) drew figures 3A-H, 4A-D, and 5B.
18
19 We thank Mrs. E. Kováčiková (Slovakia) for help in obtaining permissions to borrow several
20
21 specimens for this study.
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28 *Author contributions.* J. K. conceived and designed the project; M. H. took measurements
29
30 from CT-scans; M. H. and J. K. segmented the CT-scan data; J. K., M. H. and D. S B.
31
32 analysed the CT-scan data; M. R. performed the phylogenetic analyses; J. K. and M. R. wrote
33
34 the text; J. K., M. R., and M. H. assembled the figures; S. E. P. arranged for the CT-scans of
35
36 *Seymouria baylorensis*; A. C. H. prepared the specimens of *Diadectes absitus* and *Orobates*
37
38 *pabsti*. All authors contributed to writing the manuscript, reviewed manuscript drafts, and
39
40 approved the final version.
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47 **DATA ARCHIVING STATEMENT**

48 Data in support of this study are available at the Dryad Digital Repository:

49
50
51 <https://datadryad.org/review?doi=doi:10.5061/dryad.4j2tp4s>
52
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FIGURES

FIG. 1. Skull and endosseous labyrinth in *Diadectes absitus* MNG 8747. A, Skull in dorsal view. B, Virtual 3D reconstruction of partially transparent skull and endosseous labyrinth in dorsal view. C, 3D virtual model of endosseous labyrinth in partially transparent three braincase bones in dorsal view. D, Transverse section through braincase and inner ear cavity (yellow dotted line) in *D. absitus* at the level of crus commune and otic tube. ced, canal for endolymphatic duct. Scale bars represent 20 mm (A, B) and 7 mm (C, D).

FULL SIZE WIDTH

FIG. 2. Transverse sections in *Diadectes absitus* MCZ 8747 at the level of vestibule (A) and cochlear recess (B). Scale bars represent 5 mm.

FULL SIZE WIDTH

FIG. 3. Inner ear morphology of A, B, *Diadectes absitus*; C, D, *Orobates pabsti*; E, F, *Seymouria baylorensis*; and G, H, *Labidosaurus hamatus*. Drawings of virtual 3D reconstructions of right endosseous labyrinths in dorsal (A, C, E, G) and ventral (B, D, F, H) views. The position of the recessus scala tympani is indicated in *L. hamatus*. All scale bars represent 3 mm.

FULL SIZE WIDTH

FIG. 4. Comparative inner ear anatomy in non-amniote and amniote tetrapods. Drawings of virtual 3D reconstructions of right endosseous labyrinths in right lateral view in A, *Orobates pabsti*; B, *Diadectes absitus*; C, *Seymouria baylorensis*; D, *Labidosaurus hamatus*; and E, *Pseudopus apodus*. Scale bars represent 3 mm (A-D) and 2 mm (E).

FULL SIZE WIDTH

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5 **FIG. 5.** Morphology and position of subarcuate fossa and associated inner ear structures in
6 *Diadectes absitus*. A, virtual 3D reconstruction of right half of supraoccipital in ventral view.
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8 B, drawing of right half of supraoccipital in ventral view. C, D, transverse sections through the
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10 braincase and inner ear cavities at the level of the subarcuate fossa. Scale bars represent 5 mm.
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19 **FIG. 6.** Horizontal sections of skull in *Orobates pabsti* MNG 10181. Horizontal sections in
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21 dorsal (A) to ventral (C) sequence. Scale bar represents 15 mm.
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24 FULL SIZE WIDTH
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29 **FIG. 7.** Supraoccipital, endosseous labyrinth and horizontal sections of skull in *Orobates*
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31 *pabsti* MNG 10181. A, supraoccipital in ventral view; B, supraoccipital and endosseous
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33 labyrinth in ventral view. C, D, horizontal sections at level of fossa subarcuata. Scale bars
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35 represent 15 mm.
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38 FULL SIZE WIDTH
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42 **FIG. 8.** Virtual 3D reconstruction of posterior half of skull in *Seymouria baylorensis* MCZ
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44 1086. A, skull in dorsal view; B, skull in ventral view. C, D, transparent skull with 3D virtual
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46 reconstruction of endosseous labyrinth enclosed in fused opisthotic and prootic in dorsal (C)
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48 and ventral (D) views. Scale bar represents 10 mm.
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51 FULL SIZE WIDTH
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56 **FIG. 9.** Transverse sections in posterior (A) to anterior (B) sequence in *Seymouria baylorensis*
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58 MCZ 1086 at the level of cochlear recesses and otic tube. Scale bars represents 4 mm.
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3 SINGLE COLUMN WIDTH
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8 **FIG. 10.** Braincase bones in *Labidosaurus hamatus* CM 73370. 3D virtual reconstructions of
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10 (A) opisthotic in anterolateral view, and prootic in medial (B) and dorsal (C) views. Scale bar
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12 represents 6 mm.
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15 FULL SIZE WIDTH
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19 **FIG. 11.** 3D virtual reconstruction of braincase bones and inner ear structures in *Labidosaurus*
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21 *hamatus* CM 73370. A, three braincase bones in right lateral view with partially visible
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23 cochlear recess and fenestra vestibuli. B, same bones with prootic and supraoccipital
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25 transparent showing the inner ear structures. Scale bar represents 6 mm.
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34 **FIG. 12.** Skull and sections of skull in *Labidosaurus hamatus* CM 73370. A, skull in
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36 posterior view; segmented portion of supraoccipital in red. Transverse (B, C) and horizontal
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38 (D, E) sections of skull at level of fossa subarcuata. Scale bars represent 20 mm (A) and 7 mm
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40 (B-E).
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7 **FIG. 13.** Single shortest tree obtained from parsimony analysis of new data matrix following
8 reweighting of characters by the maximum value of their rescaled consistency index from the
9 initial unweighted analysis. The names of the taxa discussed in the text are in bold. Drawings
10 of virtual 3D reconstructions of the right endosseous labyrinths of those taxa are shown in
11 right lateral view.
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18 FULL SIZE WIDTH
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25 **FIG. 14.** Phylogeny of major Palaeozoic tetrapod lineages illustrating the relationships of
26 stem and crown amniotes. A, B, strict consensus topologies from parsimony analyses with
27 equally weighted characters, following deletion of *Proterogyrinys pancheni* (A) and
28 *Eobaphetes kansensis* (B).
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34 FULL SIZE WIDTH
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39 **FIG. 15.** A, strict consensus topology from parsimony analysis with equally weighted
40 characters; pairs of numbers along branches represent bootstrap (to the left of the forward
41 slash) and jackknife percentage support; note: the clade Eureptilia is collapsed, but receives
42 bootstrap and jackknife support of 53%. B, Bayesian topology showing clade credibility
43 values appended to branches.
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50 FULL SIZE WIDTH
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3 **FIG. 16.** Strict consensus topology from parsimony analysis with equally weighted
4 characters, following deletion of *Proterogyrinus pancheni* and *Eobaphetes kansensis*.
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7 SINGLE COLUMN WIDTH
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14 **FIG. 17.** A, strict consensus of all trees obtained from parsimony analyses with implied
15 weighting, using the first ten integer values of the K constant of concavity. B, maximum
16 agreement subtree of all implied weighting trees.
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Dear Editor,

Thank you very much for the insightful comments that you, you, the Technical Editor, and the referees provided. We are delighted to learn that the topic of our research was generally regarded as having broad appeal. Please find below a detailed response and commentary to the referee's remarks. We have endeavoured, so far as possible, to follow the advice that was given to us. We attempted to clarify certain points raised by Referee 4, the essence of which we simply failed to grasp. However, we have tried to address such points in the best way we could. We append our remarks and answers to the letter sent to us.

We do hope you, the Technical Editor, and the referees find our replies satisfactory.

Yours sincerely
Jozef Klembara (on behalf of all authors)

Referee: 1

Comments to the Author

The paper describes the bony labyrinth (inner ear) morphology of 4 species of basal tetrapods. The relevance of the study comes from 1) the rarity of published descriptions, ii) the phylogenetic position of the studied taxa (i.e. close to the Amniotes evolutionary root) and iii) the originality of the phylogenetic results, which suggest a sister group relationship between Synapsida and Diadectomorpha.

The paper is overall well written. The scientific question is well defined, the material and methods to tackle it are adequate and well presented. The results are sufficiently new and the conclusions are measured and adapted to the results.

ANSWER: We thank the referee for their praising remarks and for highlighting the thrust, scope and merits of our work.

The figures are difficult to interpret, but this is more due to the quality of the material than to anything else since it is evident that a lot of effort were put to make the anatomical structures as clear as possible, despite the poor state of ossification of the bony labyrinth.

ANSWER: We have provided a repository of images in Dryad that will complement the illustrations in the main text. Indeed, as the referee points out, the material is difficult but mostly because of the intricate morphology of the labyrinth. The specimens are well preserved and although, as we expound below, we have fine-tuned our original statements about their quality, we emphasize that the amount of morphological detail they provide is indeed conspicuous and highly informative. We also point out that the reconstructions that we supply are based upon of the 3D rendering of the scans, and take into account all the observed features. Similarly, the selected scans that we include in the main text appear crisp and detailed, certainly at the high resolution that we supply. To assist the viewers in locating structures of interest that we illustrate in the main text, we have superimposed semi-transparent and colour-coded areas on the original scans. The sole purpose for superimposing coloured areas is to assist the reader in locating the extension and boundaries among regions.

The bibliography is incomplete (see below).

ANSWER: This is now rectified through addition of all recommended papers and, where appropriate, inclusion of further comments in the main text.

1
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3 This is an interesting and worthwhile paper that I would recommend for publication to
4 Palaeontology.

5 **ANSWER:** We are happy to know the referee is supportive of our research.
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8 However, I would strongly recommend some major modifications before acceptance:

9 - First, the authors overstate the quality of preservation of their material (e.g. p.1, l. 59:
10 Exceptionally well preserved; p.5, l.38: Exquisitely preserved). Figures 3 and 4 show that the
11 anterior and posterior semicircular canals are broken in some specimens and that the lateral
12 canal is often barely distinguishable from the vestibule. Also, the bony labyrinths had to be
13 re-drawn because (I suppose) the 3D models were probably too difficult to interpret. Finally,
14 one of the specimens is a composite bony labyrinth made from two different specimens. I
15 would suggest the author be more humble regarding the quality of their material, which is just
16 well preserved enough to enable anatomical description.

17 **ANSWER:** We deleted the words “exceptionally” and “exquisitely”. We understand that the
18 emphasis we originally gave in describing the material may have come across as being
19 excessive. However, the material is very good, and eminently tractable. It is not clear to us
20 why the referee states that figures 3 and 4 show that the two semicircular canals are broken in
21 some specimens. Assuming that the referee is talking about the hatched oval area on the
22 anterior semicircular canal of the *Seymouria* inner ear, we point out that this does not indicate
23 breakage, but that the canal continued in cartilage (we explain in the main text that the
24 supraoccipital was probably cartilaginous in adult *Seymouria*, but we also temper that very
25 remark by stating that additional observations are needed to confirm the nature of the
26 supraoccipital). Furthermore, the lateral semi-circular canal is poorly distinguishable only at
27 its posterior extremity and this is indicated by a hatched line (Fig. 4A). But that the semi-
28 circular canals are not always completely surrounded by bone is a normal anatomical
29 condition, especially in juveniles and subadults of various extant amniotes. The scanned
30 specimen of *Diadectes* is a subadult. These canals are not broken in any of the specimens
31 studied and this is demonstrated on the raw and segmented data added as supplementary files
32 to Dryad.
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38 - Second, it appears to me that some important literature has been overlooked during the
39 writing of this manuscript. For instance, I am surprised that the authors do not mention the
40 work by Cope (1886: <https://www.jstor.org/stable/pdf/983234.pdf>) in the sentence about
41 previous works done on diadectid ear region. This work make the study of diadectid inner ear
42 significantly older than what is stated in the manuscript. In addition, the drawings of
43 diadectid bony labyrinth made by Cope (1886) are much more informative than the single
44 transverse section from Olson (1966) that is described p. 5. Perhaps the specimens used by
45 Cope have been re-attributed to non-diadectomorphs (which would make Cope's work
46 irrelevant), but in this case this has to be stated clearly in the manuscript.

47 **ANSWER:** We added the paper by Cope (1886), but we note that it is not “significantly older
48 than what is stated in the manuscript”, because we mention Cope (1880), so we are aware of
49 older literature on the topic. The specimen described by Cope (1886) is now recognized as
50 belonging to *Diadectes sideropelicus*.
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54 - Similarly, I was also surprised to read that placing diadectomorphs close to synapsids in a
55 phylogeny was "a challenging new hypothesis" (p.4, l.15). Historical works by Case (1907,
56 1914) exemplify that placing diadectomorphs close to synapsids was actually very common a
57 100 years ago, but these works are not cited in this manuscript. It is noteworthy that Case
58 (1914) also figured the same inner ear of a diadectid than Cope (1886), as well as the bony
59 labyrinth of a Dimetrodon and an edaphosaurid, two basal pelycosaur taxa for which the
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3 authors declare that data are missing (see p. 18, l. 52-53; p. 20, l.3). The authors seemingly
4 missed these references during their bibliographic review and I would strongly suggest that
5 they take them into account to improve the coding of their dataset.

6
7 Case, E. C. 1907. Revision of the Pelycosauria of North America. Carnegie Institution
8 of Washington Publication 55: 1-176.

9 Case, E.C. 1914. On the structure of the inner ear in two primitive reptiles. Biological
10 Bulletin, 27:213-216.

11 **ANSWER:** We have added these two papers into the manuscript and the data gleaned from
12 them to the dataset. The results are the same. Yes, it is true that around the time when Case
13 and others wrote, a close relationship between diadectids and synapsids had been posited, and
14 we have acknowledged this in full in the revised manuscript. As a note, with the advent of
15 cladistics, most authors (except Berman, Marjanovic and Laurin, and ourselves in this paper)
16 had not retrieved a diadectids-synapsids sister group relationship. We have clarified this.
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19 There are also other, minor modifications that I would suggest:

- 20 - p2 110: "derived stem amniote": this is confusing as the two words "derived" and "stem"
21 seem contradictory. Perhaps try "derived non-crown amniotes" to avoid confusion.
 - 22 - p2 129-30: "and the development of a distinct subarcuate fossa for the cerebellar flocculus."
23 This should be in a separate sentence as this sentence is about bony labyrinth characters and
24 the subarcuate fossa does not belong to the bony labyrinth.
 - 25 - p3 120: "Pardo et al. (2017)". On the same topic, see also the provoking hypothesis of Ford
26 and Benton 2018: A redescription of Orovenator mayorum (Sauropsida, Diapsida) using
27 high-resolution μ CT and the consequences for early amniote phylogeny.
 - 28 - p4 159: "endosseous labyrinth of the inner ear" change to "endosseous labyrinth for the
29 membranous inner ear"
 - 30 - p5 110-13: "The endosseous labyrinth are inadequate in most early tetrapods because of the
31 generally small size, fragile nature, and difficult anatomical location of this structure." This
32 is, in my opinion, more a problem of poor ossification of the structure in basal tetrapods.
 - 33 - p9: Phylogenetic analysis: The link to access the supplementary information on Dryad
34 should be provided in the "Material and methods" section.
 - 35 - p10 137-38: "Diadectes absitus" should be italicized.
 - 36 - p13-15: the whole paragraph entitled "Remarks" is fascinating but clearly represent
37 "Discussion" material rather than "Results". I suggest it should be moved into the
38 "Discussion" section of this manuscript.
 - 39 - p14 142-43: "Noteworthy" would read better if changed to "It is noteworthy that"
 - 40 - p17 18: "posttemporal fenestra a small fossa" change to " posttemporal fenestra is a small
41 fossa"
 - 42 - p17 129: "cleithrum stem a flattened" change to "'cleithrum stem is a flattened"
 - 43 - p17 143: "make an approximate a right angle" change to "make an approximate right angle"
 - 44 - p18: Many researchers consider the cochlear recess as a character found in cynodonts only,
45 in which it constitutes the precursor to the mammalian coiled cochlea. There is an ongoing
46 debate about its possible convergent evolution among synapsids (see Benoit et al., 2017 and
47 reference therein). The presence of a cochlear recess at the evolutionary root of amniote thus
48 has huge implications since it would settle the debate by evidencing that it is actually
49 plesiomorphic! This should be, if not discussed, at least mentioned here.
 - 50 - p19 126: "this structure in this group" change to "this structure in some representatives of
51 this group" as a vestibular tube is present only in some biarmosuchians, gorgonopsians and
52 dicynodonts according to Benoit et al. (2017)
 - 53 - p20 142-43: "hosed" change to "housed"
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3 **ANSWER:** We accepted all the comments and suggested amendments listed above, and
4 corrected all typos. In particular: we have added a commentary on the recent analysis by Ford
5 and Benson (and added relevant reference); we have changed the wording of the characters;
6 we have mentioned the issue of the distribution of the cochlear recess, with added remarks.
7 Although we accepted the suggested change from “derived stem amniotes” to “derived non-
8 crown amniotes”, we note these two phrases mean exactly the same thing and we would have
9 preferred our former version, but we guess this is not greatly relevant.
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13 Referee: 2

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16 Comments to the Author

17 Please see technical comments in the attached file: Klembara_4428_TE

18 Sally Thomas

19 editor@palass.org

20 **ANSWER:** We thank our Technical Editor for all her remarks, which we have taken into
21 account, especially the repository of data issue.
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24 Referee: 3

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26 Comments to the Author

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28 GENERAL COMMENTS AND CONCERNS

29 The approach to phylogenetic analysis is thorough and thoughtful, and up the most modern
30 standards of analysis.
31

32 **ANSWER:** We thank the referee for their encouraging and supportive remark.
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35 To be clear, this is a thoroughly illustrated submission, containing a treasure trove of new
36 morphological information, much of it in figures 5-12. That said, the data associated with
37 those images are virtually ignored other than brief mention of figures 5 and 7. Information on
38 Orobates (Figures 6-7) Seymouria (Figures 8-9), and Labidosaurus (Figures 10-12) are not
39 even discussed, and each could warrant a short paper in and of themselves. I do not take issue
40 with the anatomical interpretations presented, but it is not clear why so much anatomical
41 information was illustrated, but not then described or discussed. For a moment, I even
42 wondered if perhaps my review PDF might be missing something. But I could find no
43 mention of those figures in the discussion as well.
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45 **ANSWER:** We were a little confused after an initial reading of these comments. It may be
46 that this is probably a slight oversight, because all structures seen on Figs 6-12 are described
47 (see pages 10 – 14 in the manuscript). The descriptions contain all anatomical data needed for
48 the understanding of the topics expounded in the paper. Our morphological descriptions are
49 succinct but comprehensive and cover all key points of interest. We emphasized structures
50 and comparisons that are of immediate relevance to the discussion. The labelled illustrations
51 and diagrams show other details.
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54 Another concern/question regards the conspicuous absence of a basal synapsid in the
55 analysis. The “challenging new hypothesis” (authors own words) of placement for
56 Diadectomorpha by Berman (2004, 2013) suggests that [Diadectomorpha+Synapsida] is the
57 basal-most clade of Amniota. Thus, I am puzzled as to why a basal synapsid is not included
58 in this analysis. I would presume that co-author Pierce must have a reasonable amount of
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3 basal pelycosaurian material at her disposal (unless it is all on loan to the Riesz lab, which is
4 entirely possible).

5 This study would benefit tremendously from such inclusion. This could send this reviewer
6 in one of two directions:
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8
9 This is a very important paper, finally beginning to use modern technological tools to tease
10 apart the relationships of basal amniote taxa and their relatives. Accept pending major
11 revision of inclusion of micro-CT scan data of a basal synapsid.
12

13 Or
14

15
16 This is a very important paper, finally beginning to use modern technological tools to tease
17 apart the relationships of basal amniote taxa and their relatives. Accept with very little
18 revision with the understanding that the authors should be following up with analysis of
19 micro-CT scan data of a basal synapsid.

20 So, I am torn. But in the end, I will leave it to the editors to decide. I will say I think this
21 paper is very important and I would like to see it published sooner as opposed to later, and
22 minimally I encourage the authors strongly to follow up with basal synapsids.
23

24 **ANSWER:** We prefer the second option indicated by the referee. With Stephanie Pierce, we
25 have assembled a set of CT scans and a virtual 3D model of an *Edaphosaurus* braincase
26 (deposited in MCZ). Although the prootic, opisthotic and supraoccipital are visible in this
27 specimen, their preservation is, unfortunately, very poor (there are too many fractures and
28 disruptions). Thus, it was not possible to segment the endosseous labyrinth to any satisfactory
29 degree. But, several structures are of interest and usable, but additional and better-preserved
30 material is needed. Therefore, although we will continue with the study of basal synapsids,
31 including *Edaphosaurus*, at present it is not possible to amalgamate any new data to the
32 existing ones. Concerning further analyses of CT-scans, these are part of ongoing work and
33 some still require negotiations with several host institutions. The time required by further
34 analyses would be too long and we would like to present the results on near-amniote and
35 selected basal amniotes as soon as possible, given the elements of originality of our research.
36 For this paper, however, it is sufficient to use the literature data. And these show (e.g., Case,
37 1914, etc.) that basal synapsids have a cochlear recess located ventral to the vestibule, as in
38 *Labidosaurus* or *Captorhinus*. Thus, the absence of the segmented basal synapsid is not likely
39 to influence any of the major conclusions presented in our study. Indeed, as the referee
40 suggests, we will follow up with basal synapsid studies, so long as no one else is undertaking
41 this on material that we would like to investigate, and assuming of course it is not spoken for.
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46 There is little doubt that there will be some entertaining backlash to the phylogenetic
47 conclusions presented in this submission. This reviewer has no personal quibbles with the
48 results, and given the careful anatomical work supporting them, they are difficult to dismiss.
49

50 **ANSWER:** We thank the referee for their supportive remark. Some backlash may ensue,
51 which we see as a positive thing, and we will be pleased to exchange with other researchers
52 on this topic. We predict that the base of the amniote crown (as well as the apical part of the
53 stem) will be substantially “shaken” by future studies, as Pardo *et al.* and Ford and Benson
54 have already shown.
55

56 It is clear that partial data sets or those focused on specific regions do not a complete
57 phylogenetic analysis make, and they take care to indicate the otic data here are part of a
58 larger data set, despite that the focus of the paper is narrower. I am sure the authors are aware
59 of this as some of them have already done so (Berman *et al.* 1992 on the temporal and
60

occipital regions), and Sumida, Berman, and others (1992 on the atlas-axis complex). I presume they will address all data available to them in the upcoming analysis. With such a large data set however I would prefer to see key new characters generated by this analysis give discussion in the body of the paper itself

ANSWER: The key new characters that the referee refers to are, we presume, those relevant to our investigation. We have included those and we have discussed their distribution (and potential implications for the evolution of the stato-acoustic system). A detailed investigation of the data set and associated cladistic results is part of work in progress by one of the authors (M. R.) and will tackle precisely the issue of character evolution (not just the stato-acoustic apparatus) near the evolutionary origins of major amniote lineages. We point out that characters listed by the referee, taken from previous studies, are already in the matrix, in some format or another, either matching original coding or presented with a modified coding. However, these characters are beyond the scope of our paper. They do deserve a separate paper, currently being written up. It is true that our focus is narrow, and this is precisely what was intended from the outset of our investigation.

Specific and Editorial Comments

INTRODUCTION:

Page 3, lines 10-17. The authors state that features of an amniotic egg “cannot be determined from the fossil record”. Whereas this is true in terms of extra-embryonic membranes, many fossilized amniotic eggs have been recovered from Mesozoic and later groups. Might the term “have not yet been” be better applied than “cannot”?

Page 3, lines 19. Although Pardo et al (2017) have recently championed microsaur as the sister taxon to sauropsids, they were not the first. The Idea has been floated many times, somewhat more formally by Vaughn in 1960. (Credit where credit is due and all that.)

Vaughn, P. P. 1960. The Paleozoic Microsaur as Close Relatives of Reptiles, Again. *The American Midland Naturalist*, 67:79-84

ANSWER: All comments accepted and Vaughn reference included.

Page 4, last paragraph. This statement is nominally correct, and not unreasonable in the context of the paper. However, it is worth noting that Sumida (1997), in Sumida and Martin (1997) as referenced by the authors did suggest some characters that united Seymouriamorpha and Diadectomorpha + Amniota. Although this may not reflect on the current paper, phylogenetic analyses using more than only inner ear morphology (as appear to be in process by the authors) may benefit.

Seymouriamorpha and Diadectomorpha + Amniota:

☐ Swollen neural arches (though some microsaur do this as well) ☐ Dorsal and posterior processes of iliac blade no longer distinct. Diadectomorpha + Amniota:

☐ 8-10 additional features

ANSWER: Those characters are already in the data set and many more are being scrutinized for a future investigation of stem amniote interrelationships (work in progress by M. R)

MATERIALS AND METHODS:

Pages 6-7. Why the choice of Labidosaurus? Admittedly, it is comparable in size and preservation to the other taxa. But, given author Berman’s hypotheses of basal amniote

relationships, why not include a pelycosaur? If there are compelling logistic or phylogenetic reasons, then a couple/few sentences of explanation should be added here.

ANSWER: In the analysis we have used the data of the “pelycosaurs” *Dimetrodon* and *Eothyris*. We had ready access to *Labidosaurus*, a very interesting taxon in its own right given that it “mimics” some features of some diadectomorphs. Indeed many more taxa are currently being under study and time constraints and availability of specimens (currently under study by other researchers) meant that we had to be realistic about the breadth of our work.

Age, Stratigraphy, and locality data:

Pages 7-8. The authors continue to use Romer (1973), based on the Plummer and Moore (1921) stratigraphic scheme. Hentz (1988) revised the north-central Texas considerably. And, whereas some authors persist in using that scheme, most have move to the more up to date analysis of Hentz. If the authors choose to ignore Hentz, they should state why. Or, they can site both older and newer equivalent interpretations. Hook (1989) provided a useful key to formational equivalents between Hentz’s (1988) work and earlier designations.

ANSWER: We have updated the section on stratigraphic age with side-by-side older and newer stratigraphic schemes.

RESULTS:

Page 11, Figures 1 & 2. The figures are nicely drafted. However, I feel figure 1 or 2 would benefit significantly if an image similar to 1B was added in occipital view to either 1, or 2.

ANSWER: We did not use a picture of the *Diadectes absitus* skull in posterior view, because such a view does not illustrate any anatomical data relevant to the issue of the structure of the endosseous labyrinth.

REMARKS:

Page 13, line 43. The authors state that the most striking feature of the endosseous labyrinth in *Diadectes* is the sub-pyramidal cochlear recess. They cite figures 3A,B, 4B. In this regard it would then be indicated that they label it in 3B.

ANSWER: This has been done.

(Section between Remarks and Phylogenetic Results?)

To be clear, this is a thoroughly illustrated submission, containing a treasure trove of new morphological information, much of it in figures 5-12. That said, the data associated with those images are virtually ignored other than brief mention of figures 5 and 7. Information on *Orobates* (Figures 6-7) *Seymouria* (Figures 8-9), and *Labidosaurus* (Figures 10-12) are not even discussed, and each could warrant a short paper in and of themselves. I do not take issue with the anatomical interpretations presented, but it is not clear why so much anatomical information was illustrated, but not hen described or discussed. For a moment, I even wondered if perhaps my review PDF might be missing something. But I could find no mention of those figures in the discussion as well.

ANSWER: This must be a misunderstanding - see our comments above.

REFERENCES:

BERMAN ~~KELMBARA~~ et al (2004); Page 23, line 34. “Central” should not be capitalized.

ANSWER: But Central is already capitalized in the original paper, so we cannot correct it here.

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4 Page 24, line 38. GELMAN et al. 1992. runs into previous reference.
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6 **ANSWER:** We have corrected this.
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9 Page 27, line 31. In NAYAKATURA et al 2015, “Fischer” should be capitalized.
10

11 **ANSWER:** We have corrected this.
12

13 Page 27, line 343. In OLORI 2013, *Microbrachis* and *Hyloplesion* should be italicized.
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15 **ANSWER:** We have corrected this.
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19 Referee: 4
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FINAL ASSESSMENT OF THE PAPER

I am afraid that the observations resulting from this manuscript are not well-supported by the raw CT-scan data. The scans are not of good quality and/or the specimens did not scan well, thus resulting in questionable interpretations of the inner anatomy and phylogenetic results based on those observations.

ANSWER: The referee does not write what anatomical data are incorrect or what is incorrect in the phylogeny. We are concerned about his take on our work as his statements appear vague in places. However, we have tried to address, so far as possible, all his remarks. To begin with, the scan data, now on Dryad, reveal much valuable information and these are now accessible. We reject his claim that our interpretations are questionable

The original raw data is not of high-quality enough to actually show what the shape of the inner ear system in diadectomorphs and seymouriamorphs, except maybe for some portions of the canals/vestibule. Because the data is not of good quality there seems to be some overinterpretation of the inner ear morphology. Just by looking at the slices there is a lot of noise-to-signal ratio, for example.

ANSWER: We firmly reject the referee's assessment. Perhaps the original submitted files were not of sufficiently high quality in the original submission, but we have rectified this. The referee does not explain what he means by "high noise-to-signal ratio". We explain why, where applicable (if at all), partial portions of certain structures are visible. Perhaps not everything could be accessed in the scans, but what we were able to glean from them is considerable. It is true, that the density indices of the bones and matrix are close, however it this does not mean it was not possible to recognize the borders of the bones and to perform reliable segmentation, and our Figures (e.g., 2, 5, 7, 9, 12) show evidence of this.

I also like to declare absolutely no conflict of interests. Although I have segmented inner ears of overlapping taxa with this present manuscript, the purpose of my study is radically different from this.

ANSWER: We appreciate the candid and sincere statement about conflict of interest, but we fail to grasp the implications of it in the context of the referee's evaluation. He states he has segmented specimens belonging to some of the species that we studied, but for different purposes. This is fine. We do not have access to his data, and we welcome with eager anticipation the publication of his results. No doubt these will be interesting and will complement/refine/amend our own observations. But until then, we have no way to counteract his dismissive statements.

MAJOR POINTS

Positive:

- Original and interesting topic with possible broad implications for the origins of the inner ear system in amniotes
- Potentially controversial conclusions particularly with the re-arrangement of diadectomorphs as sister to Synapsida.

ANSWER: We are pleased to hear these positive remarks. Yes, we agree that the results may be controversial, but we are encouraged by the observation that there are at least two preceding examples of analyses that have retrieved the diadectomorphs as sister taxon to synapsids. One study (Berman) was based on a very small matrix (taxon- and character-wise), the other (Marjanovic and Laurin) used tweaking of a much larger matrix based on Ruta and Coates (2007). So there appears to be some retrievable signal from three different data sets that target different taxon sample and different character lists and address different research questions. Our matrix is the most detailed study so far that includes near-amniote

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3 and basal crown amniote taxa. We are confident that support for the proposed sister group
4 relationship has some justification. Crucially, as we state in an added note, these results, at
5 least using our matrix, are not contingent upon inner ear data. The latter add strength to
6 results that are also retrievable after exclusion of those inner ear data, which in turn gives us a
7 further incentive to map character-state changes relevant to the endosseous labyrinth.
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10 **Negative:**

11 - The major evolutionary trends proposed lack comparison with relevant outgroup taxa.

12 **ANSWER:** We are unclear as to the meaning of this. However, we note that inner ear data
13 are not known in many taxa, but the sequence of changes from seymouriamorphs to basal
14 crown amniotes provides useful preliminary way to establish the major re-patterning that
15 occurred in the stato-acoustic apparatus of these taxa. Many other early tetrapods are simply
16 not conducive to the analyses of CT-scan data.
17
18

19
20 - The results are solidly different from what I have been getting for diadectomorphs (can't
21 comment on seymouriamorphs though)

22 **ANSWER:** We have no way to compare and contrast our results with the referees'. We think
23 it reasonable to wait until the referee delivers his results and we are in no doubt they will be
24 interesting and transformative. At present, we can only do our best to deliver the results of
25 our own observations.
26
27

28 - The segmentation data is not shown in the paper, which immediately casts doubts about the
29 interpretations done. It seems that the interpretative figures are trying to hide the bad(?)
30 quality of the scan raw data and subsequent interpretation.
31

32 **ANSWER:** This comment is slightly judgmental and mildly offensive. We think the referee
33 did not mean this, but a cursory reading taken out of context would suggest to us that we have
34 been accused of fabricating the data. We are not trying to hide anything. Data (including
35 segmentation) are available in Dryad and we are very open about this. We have a 3D model
36 of the braincase of *Diadectes*, generated from the data. The sequence of methodological
37 protocols from raw data acquisition through to assembly of scans, to reconstruction follows
38 protocols. We do not claim it is perfect, and this applies to all or most palaeo studies that
39 have used CT scan data. Indirectly, what we did find makes perfect sense in the context of the
40 evolutionary history of amniotes. As a trivial and hypothetical example, we would have been
41 extremely surprised to find a long and tightly coiled mammal-like cochlea in a
42 seymouriamorph. What we did find is amenable to a highly reasonable – we hope –
43 interpretation and one that can be perfected time and again through further data acquisitions.
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47 - I think that there are two papers: one dealing with the inner ear anatomy and another on the
48 phylogeny. The few characters from the inner ear that can help in resolving the phylogeny do
49 not seem particularly relevant in the “phylogenetics results” section.

50 **ANSWER:** There is only one paper, as far as we can see, built upon a specific research
51 programme that explicitly seeks to make sense of the morphology of the endosseous
52 labyrinth. We see our paper as one narrative. Splitting it up would deprive of its connotations
53 – a comparative morphological investigation with an evolutionary treatment of an important
54 morpho-functional complex. Contrary to the referee's opinion, we think that our discussion of
55 key changes in this system is highly relevant. A single phylogenetic exercise, even with the
56 new and expanded data set we produced, would not pass muster for the journal.
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60 **Concerns about this paper:**

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2
3 Most of the characters probably lack a robust comparison with relevant extant taxa. For
4 example:

5 - The origin of the ampullae is just a bony feature. Meaning that it is more of a result of an
6 architectural constraint rather than a feature with any functional relevance. You cannot,
7 unfortunately measure features of the cupula by looking at the ampullae. So, I really cast my
8 doubts on the effective functional importance of this feature. Many non-amniotes don't have
9 developed ampullae which does not mean that they don't have a fully functional large cupula.

10 **ANSWER:** True, we cannot measure features of the cupula and we would be very surprised
11 if it did leave any traces in the fossil record. But the swellings of the semicircular canals
12 (ampullae) can be dealt with, minimally by stating whether we retrieve them or not. We do
13 not analyze the function of the ampullae in the present paper, as the palaeophysiology of the
14 sensory function – a worthwhile research agenda in its own right – is not our focus, at least
15 not in this paper. So whether a functional cupula is present or absent in increasingly more
16 outlying early tetrapods is irrelevant. We only wanted to address, among other things, the
17 occurrence of ampullae.
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4 - Amphibians, the extant relevant outgroup, have perfectly curved canals.... How could this
5 be a character that shows the amniote/non-amniote transition?
6

7 **ANSWER:** This is a misunderstanding and we corrected it in the manuscript.
8

9
10 - Again, a subarcuate fossa or floccular fossa is the result of a pure architectural constraint
11 during development, that has been shown to not have any link to ecology or behavior
12 (Ferreira-Cardoso et al. 2017). The brain is already there when ossification starts, so through
13 development it is the bones that accommodate to the brain and not the other way around.

14 **ANSWER:** We are fully aware of this. What we write is that in diadectids the subarcuate
15 fossa is present (marked by a distinct pit in the bone) – this is typical only of amniotes, in
16 contrast to Anamnia where the clusters of cells (flocculus) with the same function as the cells
17 accommodated in the subarcuate fossa of amniotes, is present; however, in anamniotes these
18 cells are not housed in a distinct bony structure (pit). Thus, this feature suggests Diadectes
19 and Orobates are closer to amniotes than previously thought or, minimally, that they share
20 this character with them. Regardless of details of neuroanatomical and neurocranial
21 development, we demonstrated through CT scans the occurrence of the fossa and the
22 relevance of this feature, where present, in some of the fossils we studied.
23
24

25 Other thing that concerns me is that the only characters mentioned are inner ear characters,
26 but aren't there more cranial/postcranial characters that resulted as apomorphies from the
27 phylogenetic analysis?
28

29 **ANSWER:** We are not sure what the referee is addressing here. Presumably he refers to other
30 skeletal characters supporting the relationships we propose. If so, we have listed this, for
31 nodes of interest in the phylogeny.
32

33 Introduction

34 It is not clear why the inner ear should help resolving the phylogenetic placement of
35 seymouriamorphs and diadectomorphs. This is a good introduction for a phylogenetic study,
36 which does not seem to be the central goal of this paper.
37

38 **ANSWER:** We are sorry, but this is clearly explained in the present paper. We never claimed
39 that inner ear – alone – can resolve relationships. In fact, we found that the major branching
40 pattern is not contingent upon those. But, minimally, we have a set of new characters that can
41 now be used in similar studies. What we stated in the introduction is merely a preamble to our
42 research questions: first and foremost, we are interested in the comparative morphology of
43 the endosseous labyrinth. We review briefly history of research in this area, but we also
44 introduced the candidate taxonomic groups of interest – not all palaeontologists may be
45 familiar with such groups. We actually think that the introduction, certainly in its rewritten
46 form for the revised text, achieves a decent balance of all its components and other reviewers
47 on this paper have praised this.
48
49

50 Again, there are two papers here: one dealing with the new phylogenetic hypothesis and
51 another with the anatomy of the inner ear.
52

53 **ANSWER:** We simply beg to differ – see above.
54

55 Material and Method

56 *Taxon sample for microcomputed tomography*

57 - I really have a problem with this sample. I do not think that there are enough extant taxa to
58 show what is going on and to polarize the characters properly. Namely, there is not a single
59 modern amphibian.
60

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3 **ANSWER:** The Anamnia-Amniota transition occurred within the informal grade-group
4 Reptiliomorpha. Thus, the anatomy of the extant taxa is irrelevant from this aspect.
5 Realistically, obtaining a sample size for modern taxa would be a daunting task, well beyond
6 the focused research questions we address. Time and specimen availability were constraints,
7 but we are making progress in the data collection for future studies. The temnospondyl taxa
8 in the data (admittedly, Dendrerpeton, for which good ear data are available) are sufficient to
9 polarize characters and the ear is fundamentally amphibian-like as countless researchers
10 (Anderson, Clack, Maddin, Milner, Schoch inter alia) have already noted.

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14 - Plus, why the legless lizard? It sounds just like a convenience sample that adds nothing to
15 the purpose of the paper.

16 **ANSWER:** We realize the choice of an anguine squamate may look odd. But precisely
17 because of its extreme specialization, this amniote (readily available to one of us (J. K.),
18 served a dual purpose. It both revealed substantial similarities, at a gross level, in the amniote
19 ear construction and, indirectly, also added to existing databases. So, all in all, its use was not
20 wasted. It informs the anatomy of amniote conditions at reptile level, especially shape, size
21 and position of the cochlear recess.

22 *Phylogenetic analysis*

23
24 Using reweighted characters has been questioned before as a valid procedure. Re-run the
25 analysis under TNT using implied weighting.

26 **ANSWER:** We are aware of (ill-founded) criticism of simple weighting, but at least one of us
27 (M. R.) disagrees at a fundamental level with the reasoning behind this criticism. In his
28 experience with countless data matrices processed for several studies, he found that, more
29 often than not, simple weighting simply “cleans” the signal retrieved from initial unweighted
30 parsimony runs. Experimenting with other character weighting regimes is beyond the scope
31 of our work but is part of work in progress (already mentioned above). Nevertheless, we did
32 and added the implied weighting analysis. We have added a short section covering it in the
33 methods and results section. Implied weighting can be undertaken in PAUP*, which we used.

34 *CT-scan data visualization*

35
36 The scanning parameters seem ok. Specify which techniques were being use to segment the
37 inner ears. Under VG studio, was it region growing, dilate/erode, brush? Under Avizo, was it
38 thresholding, magic wand, brush? This is important because I am (somewhat) surprised of the
39 results you are getting are so distinctly different from what I got for *Diadectes*, which makes
40 me think that either the segmentation was not well-done or that the raw data was simply not
41 good enough. From my experience of literally segmenting hundreds of taxa, including from
42 difficult taxa (from which diadectomorphs were among the most difficult) a great deal of
43 manual segmentation is necessary.

44 **ANSWER:** In VG Studio several segmentation tools were used depending on the contrast of
45 the segmented structures: region growing, erode/dilate, opening/closing and rarely draw and
46 polyline tools.

47 Results

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3 I won't comment on the details of the description because the actual segmentation data and
4 respective renderings are not shown. Why? Rather, some seemingly overinterpreted figures
5 are shown. Without actually seeing the segmentation, and believe me, I know how difficult it
6 is to segment these creatures, I cannot trust the present interpretations.

7
8 **ANSWER:** The raw data of each taxon are added to Dryad. We are sorry to hear the referee
9 is skeptical, but we have made our data available.

10
11 Discussion and Conclusions

12 All this hinge on the results.

13 **ANSWER:** Well, they would, would they not? We are satisfied with the results we obtained.

14
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16 Figures (general)

17 No segmented data is shown.

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19 **ANSWER:** They are now in Dryad.
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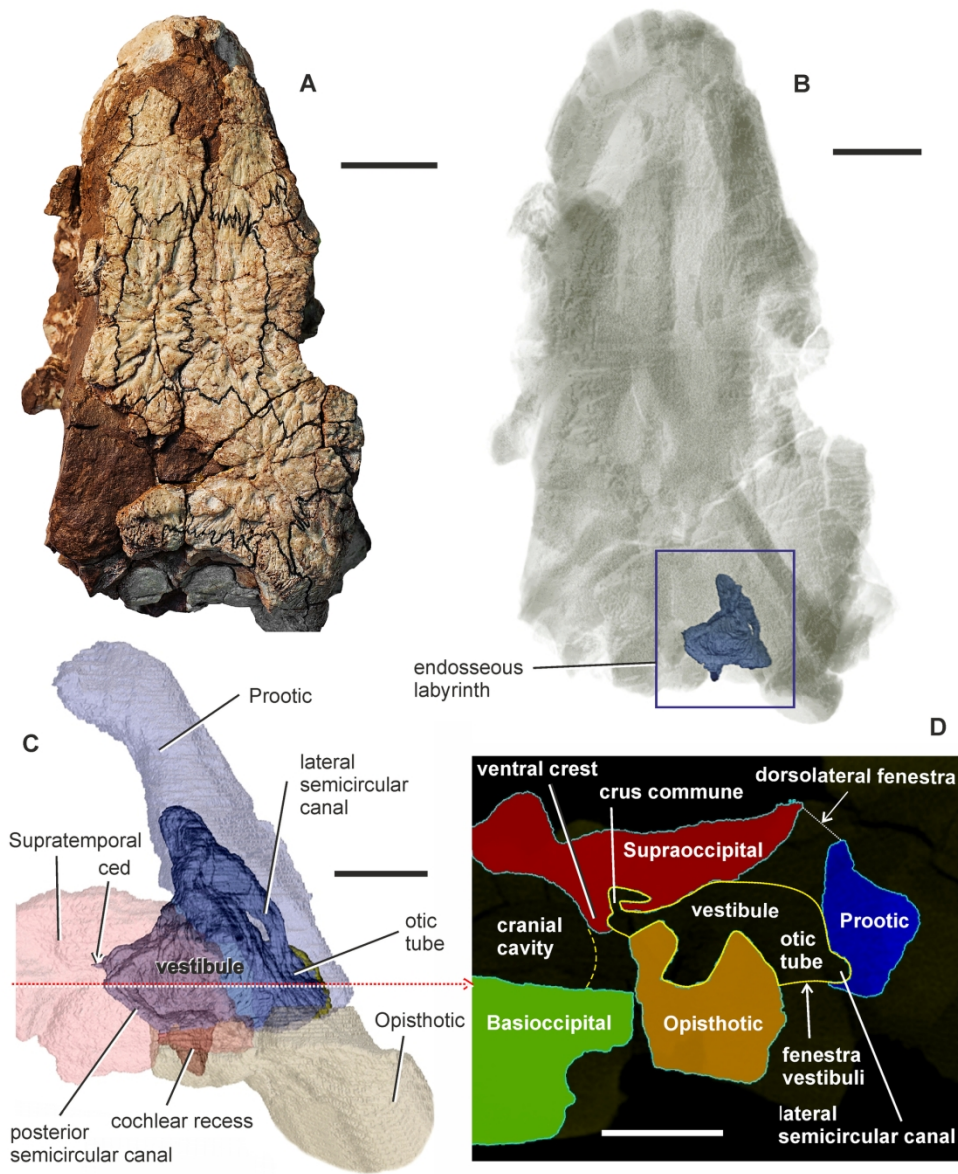


FIG. 1. Skull and endosseous labyrinth in *Diadectes absitus* MNG 8747. A, Skull in dorsal view. B, Virtual 3D reconstruction of partially transparent skull and endosseous labyrinth in dorsal view. C, 3D virtual model of endosseous labyrinth in partially transparent three braincase bones in dorsal view. D, Transverse section through braincase and inner ear cavity (yellow dotted line) in *D. absitus* at the level of crus commune and otic tube. ced, canal for endolymphatic duct. Scale bars represent 20 mm (A, B) and 7 mm (C, D). FULL SIZE WIDTH

168x208mm (300 x 300 DPI)

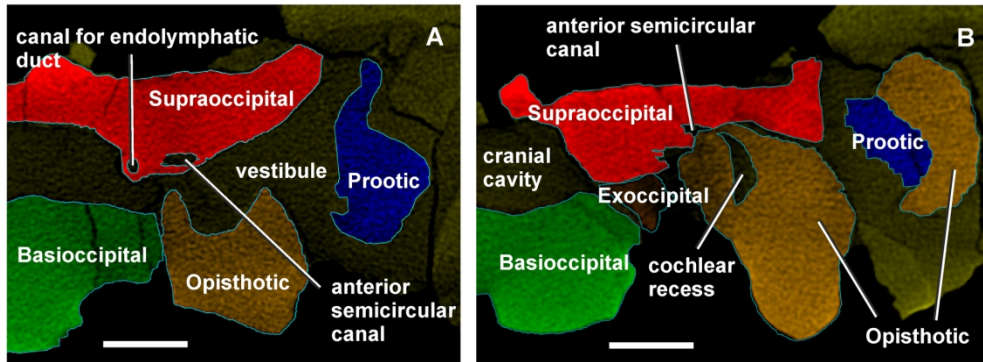


FIG. 2. Transverse sections in *Diadectes absitus* MCZ 8747 at the level of vestibule (A) and cochlear recess (B). Scale bars represent 5 mm.
FULL SIZE WIDTH

166x61mm (300 x 300 DPI)

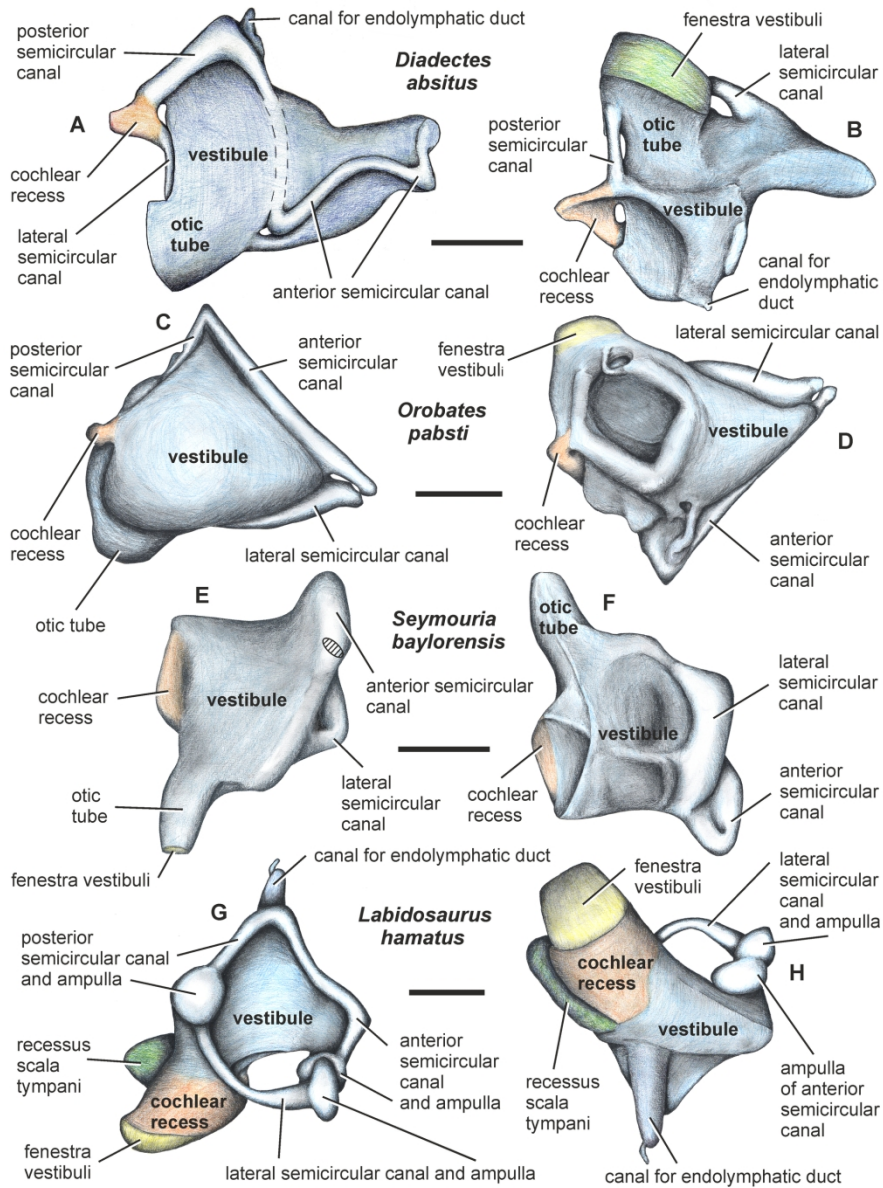


FIG. 3. Inner ear morphology of A, B, *Diadectes absitus*; C, D, *Orobates pabsti*; E, F, *Seymouria baylorensis*; and G, H, *Labidosaurus hamatus*. Drawings of virtual 3D reconstructions of right endosseous labyrinths in dorsal (A, C, E, G) and ventral (B, D, F, H) views. The position of the recessus scala tympani is indicated in L. hamatus. All scale bars represent 3 mm.

FULL SIZE WIDTH

168x228mm (300 x 300 DPI)

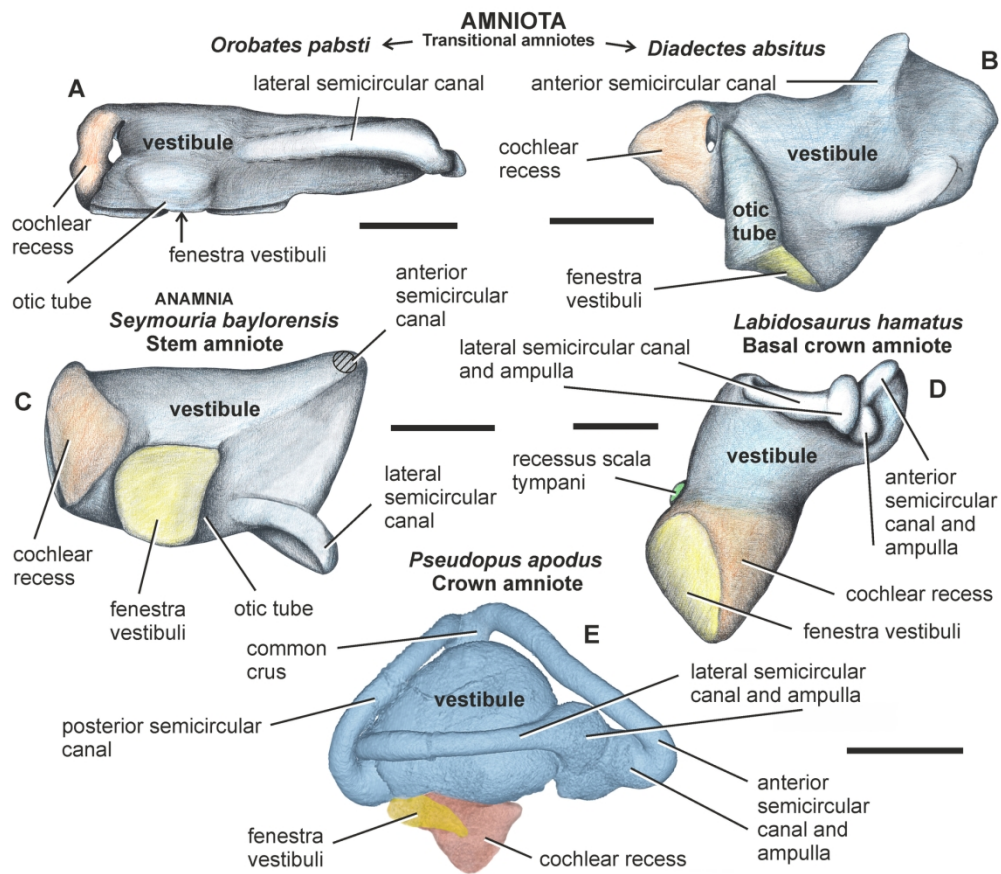


FIG. 4. Comparative inner ear anatomy in non-amniote and amniote tetrapods. Drawings of virtual 3D reconstructions of right endosseous labyrinths in right lateral view in A, *Orobates pabsti*; B, *Diadectes absitus*; C, *Seymouria baylorensis*; D, *Labidosaurus hamatus*; and E, *Pseudopus apodus*. Scale bars represent 3 mm (A-D) and 2 mm (E).

FULL SIZE WIDTH

168x153mm (300 x 300 DPI)

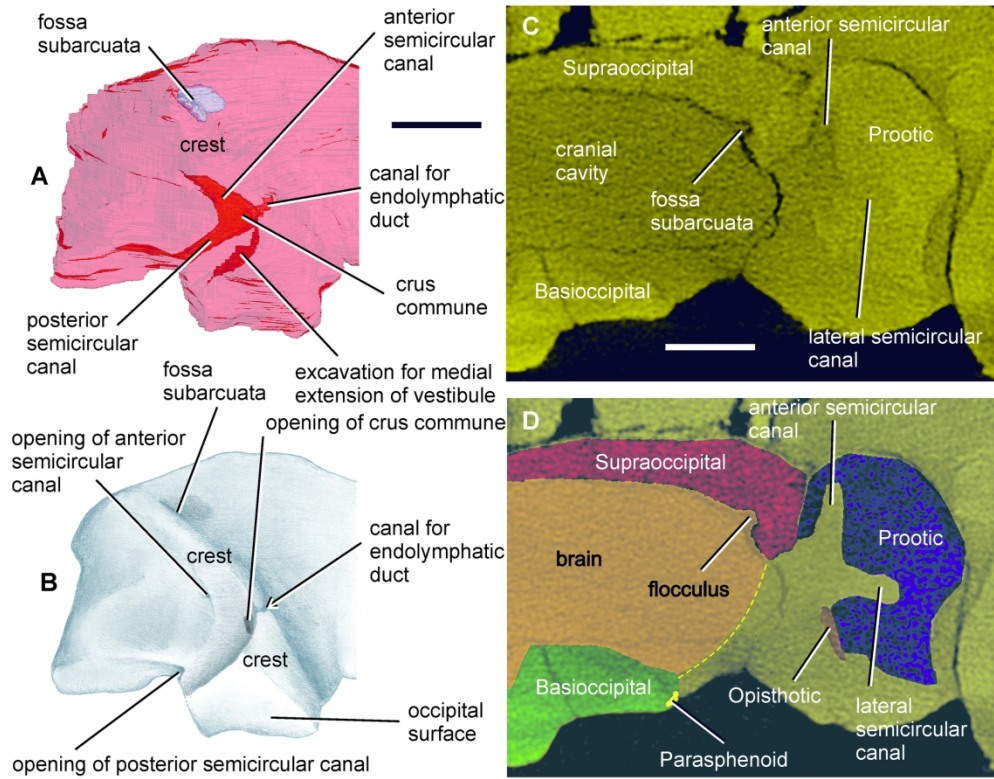


FIG. 5. Morphology and position of subarcuate fossa and associated inner ear structures in *Diadectes absitus*. A, virtual 3D reconstruction of right half of supraoccipital in ventral view. B, drawing of right half of supraoccipital in ventral view. C, D, transverse sections through the braincase and inner ear cavities at the level of the subarcuate fossa. Scale bars represent 5 mm.

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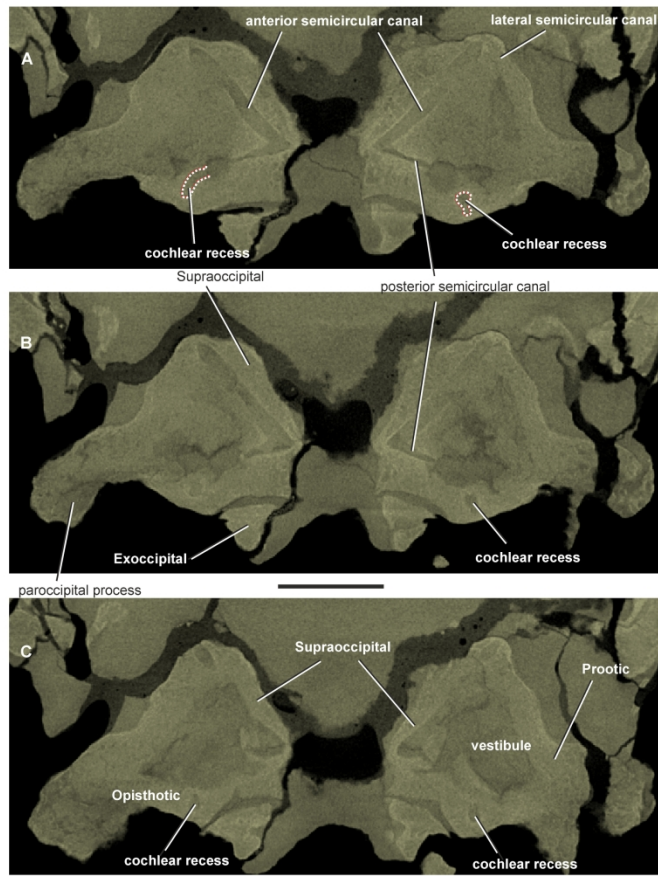


FIG. 6. Horizontal sections of skull in *Orobates pabsti* MNG 10181. Horizontal sections in dorsal (A) to ventral (C) sequence. Scale bar represents 15 mm.
FULL SIZE WIDTH

196x303mm (300 x 300 DPI)

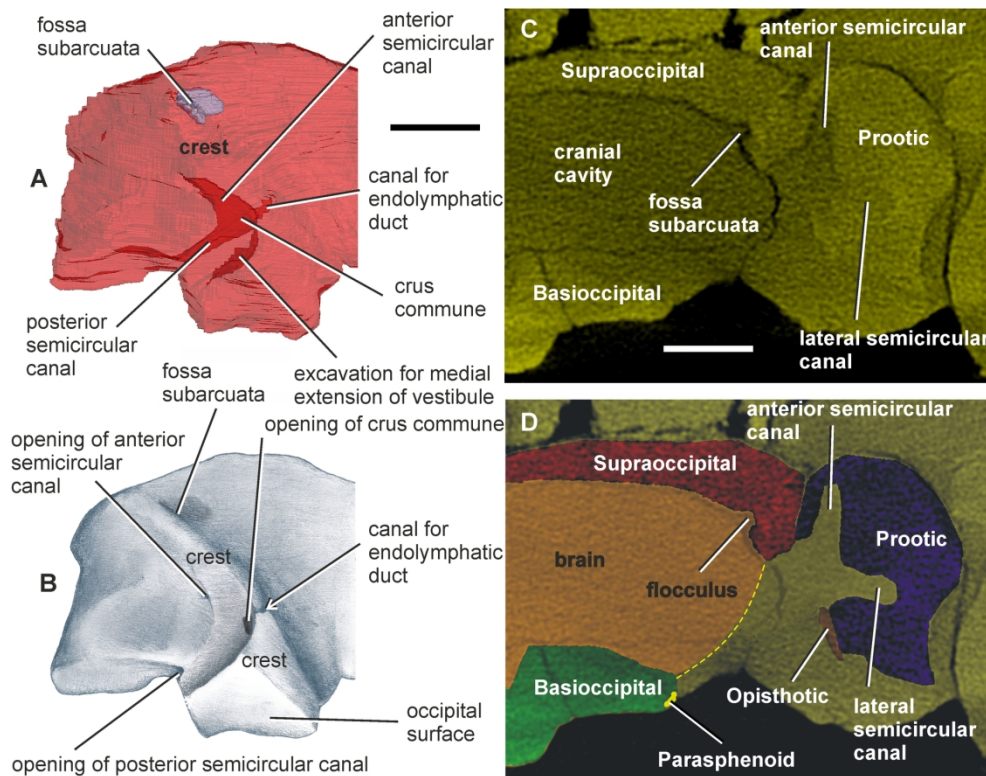


FIG. 7. Supraoccipital, endosseous labyrinth and horizontal sections of skull in *Orobates pabsti* MNG 10181. A, supraoccipital in ventral view; B, supraoccipital and endosseous labyrinth in ventral view. C, D, horizontal sections at level of fossa subarcuata. Scale bars represent 15 mm.

FULL SIZE WIDTH

169x132mm (300 x 300 DPI)

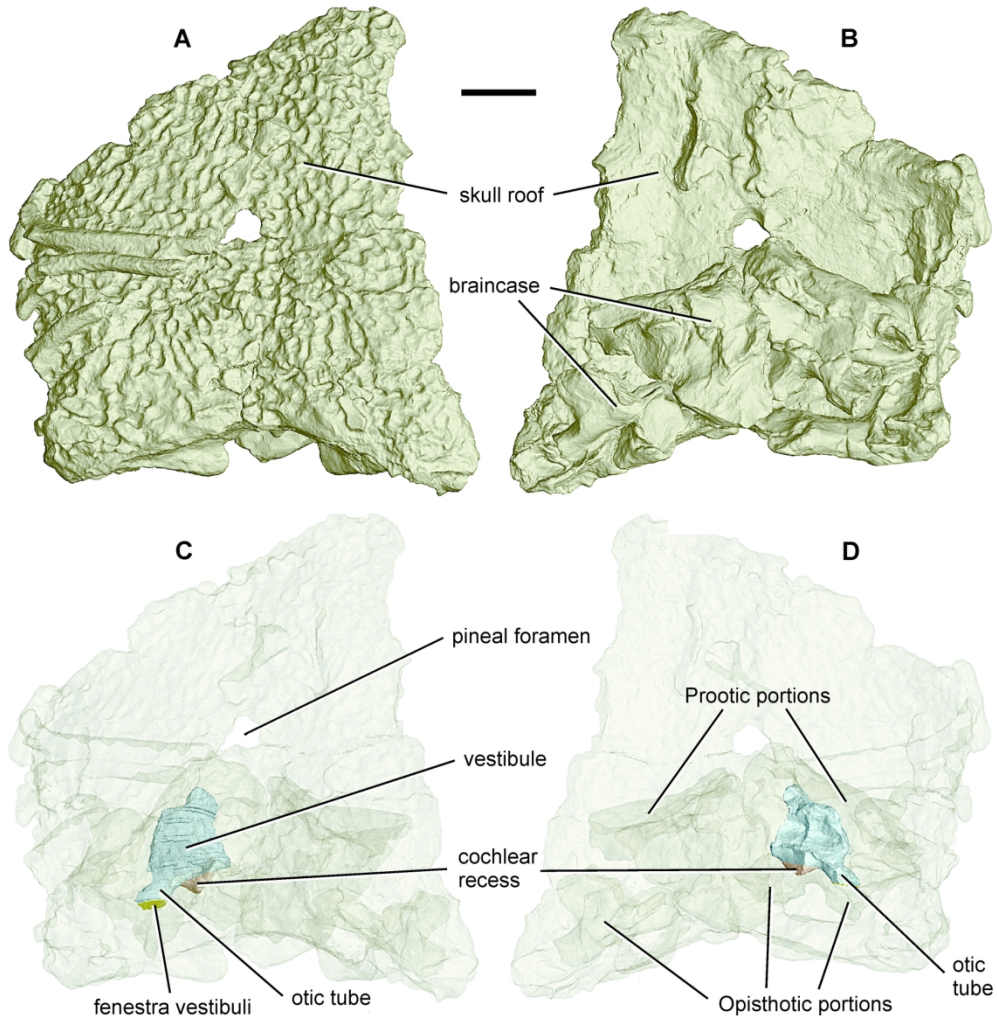


FIG. 8. Virtual 3D reconstruction of posterior half of skull in *Seymouria baylorensis* MCZ 1086. A, skull in dorsal view; B, skull in ventral view. C, D, transparent skull with 3D virtual reconstruction of endosseous labyrinth enclosed in fused opisthotic and prootic in dorsal (C) and ventral (D) views. Scale bar represents 10 mm.

FULL SIZE WIDTH

167x172mm (300 x 300 DPI)

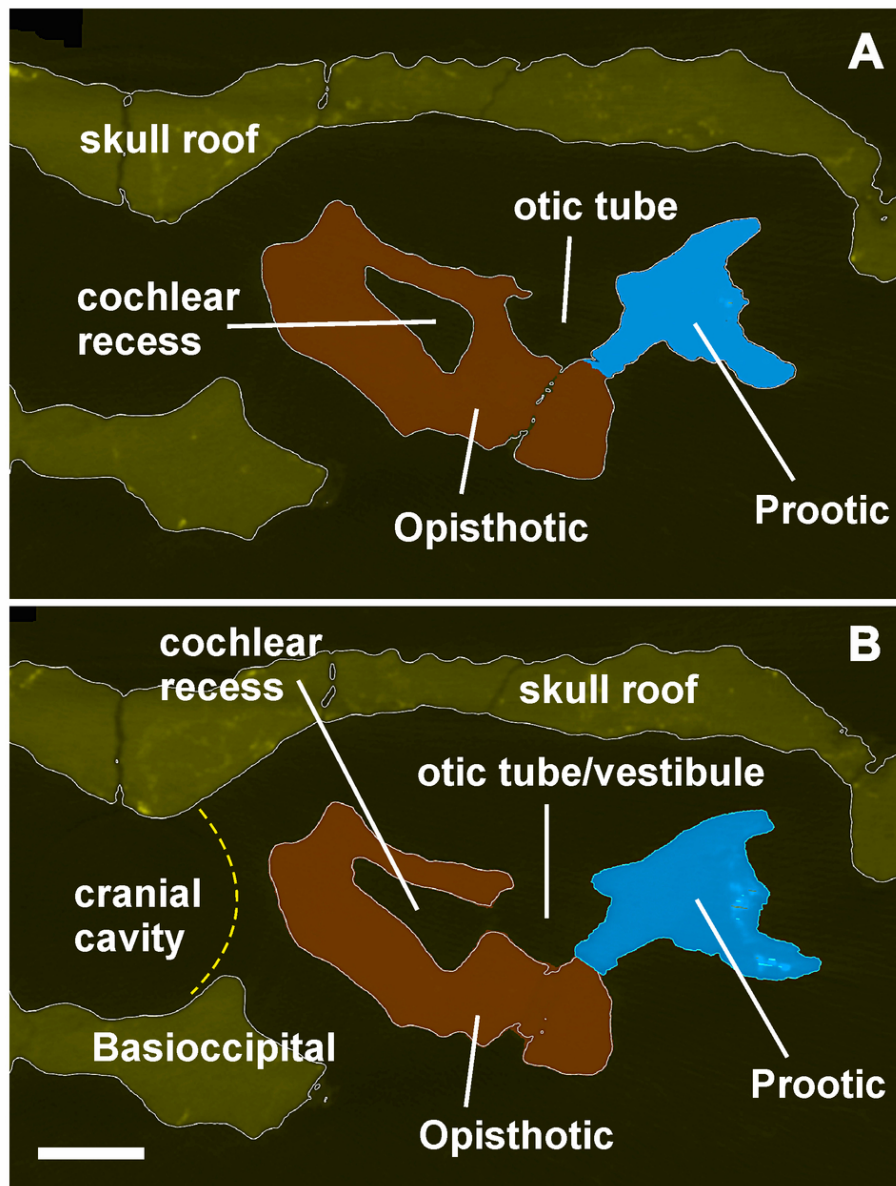


FIG. 9. Transverse sections in posterior (A) to anterior (B) sequence in *Seymouria baylorensis* MCZ 1086 at the level of cochlear recesses and otic tube. Scale bars represents 4 mm.
SINGLE COLUMN WIDTH

81x106mm (300 x 300 DPI)

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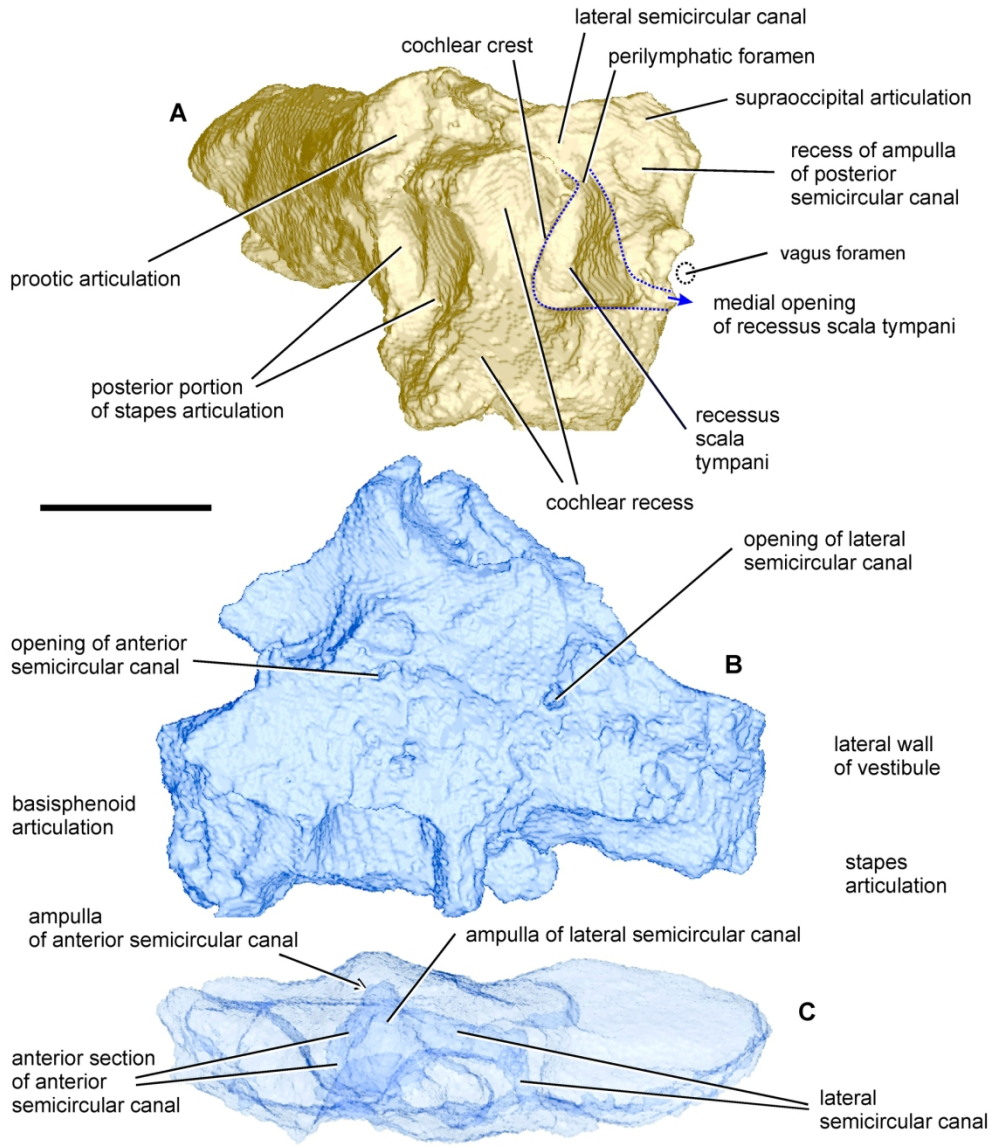


FIG. 10. Braincase bones in *Labidosaurus hamatus* CM 73370. 3D virtual reconstructions of (A) opisthotic in anterolateral view, and prootic in medial (B) and dorsal (C) views. Scale bar represents 6 mm. FULL SIZE WIDTH

167x195mm (300 x 300 DPI)

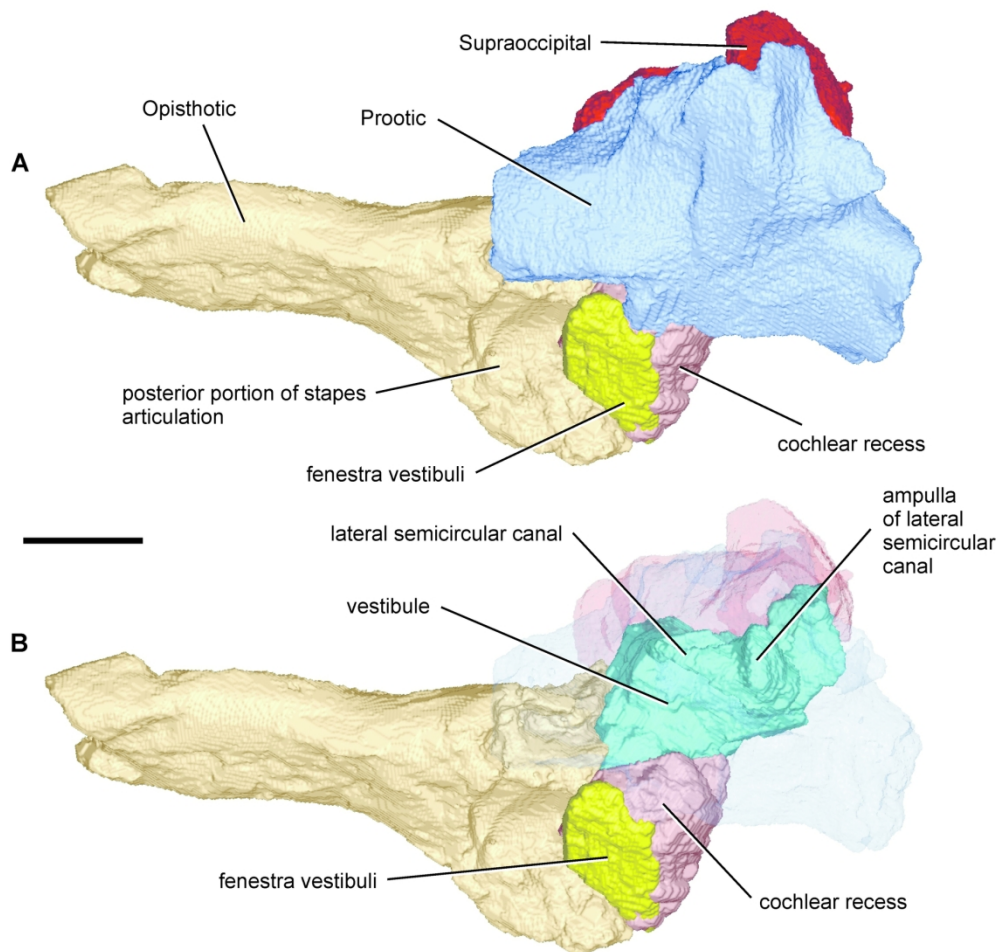
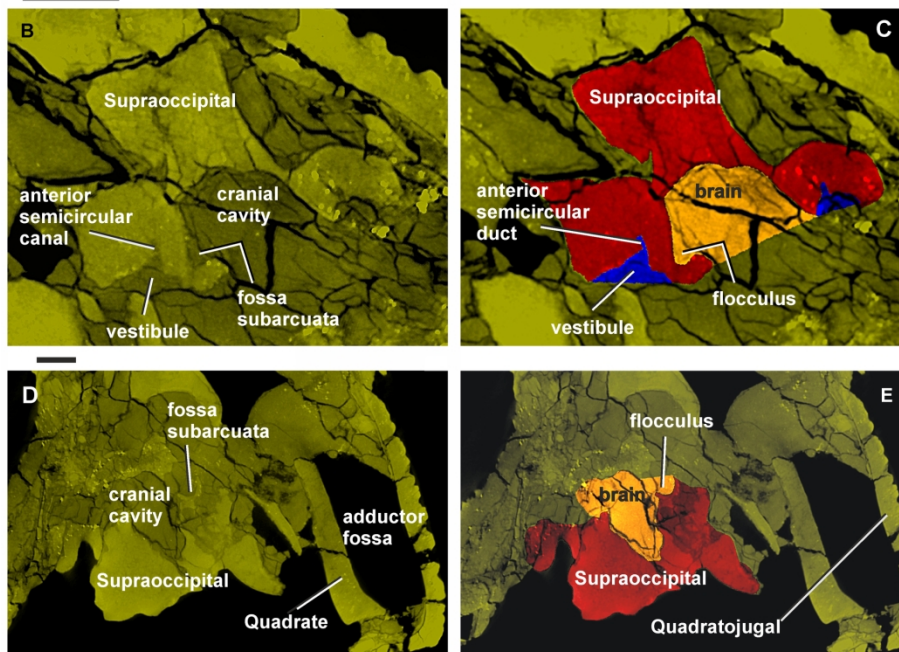
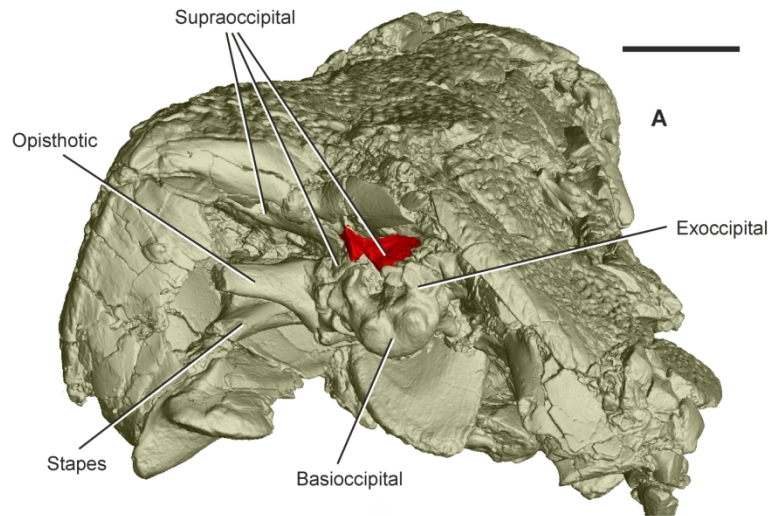


FIG. 11. 3D virtual reconstruction of braincase bones and inner ear structures in *Labidosaurus hamatus* CM 73370. A, three braincase bones in right lateral view with partially visible cochlear recess and fenestra vestibuli. B, same bones with prootic and supraoccipital transparent showing the inner ear structures. Scale bar represents 6 mm.
FULL SIZE WIDTH

167x162mm (300 x 300 DPI)



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FIG. 12. Skull and sections of skull in *Labidosaurus hamatus* CM 73370. A, skull in posterior view; segmented portion of supraoccipital in red. Transverse (B, C) and horizontal (D, E) sections of skull at level of fossa subarcuata. Scale bars represent 20 mm (A) and 7 mm (B-E).

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168x220mm (300 x 300 DPI)

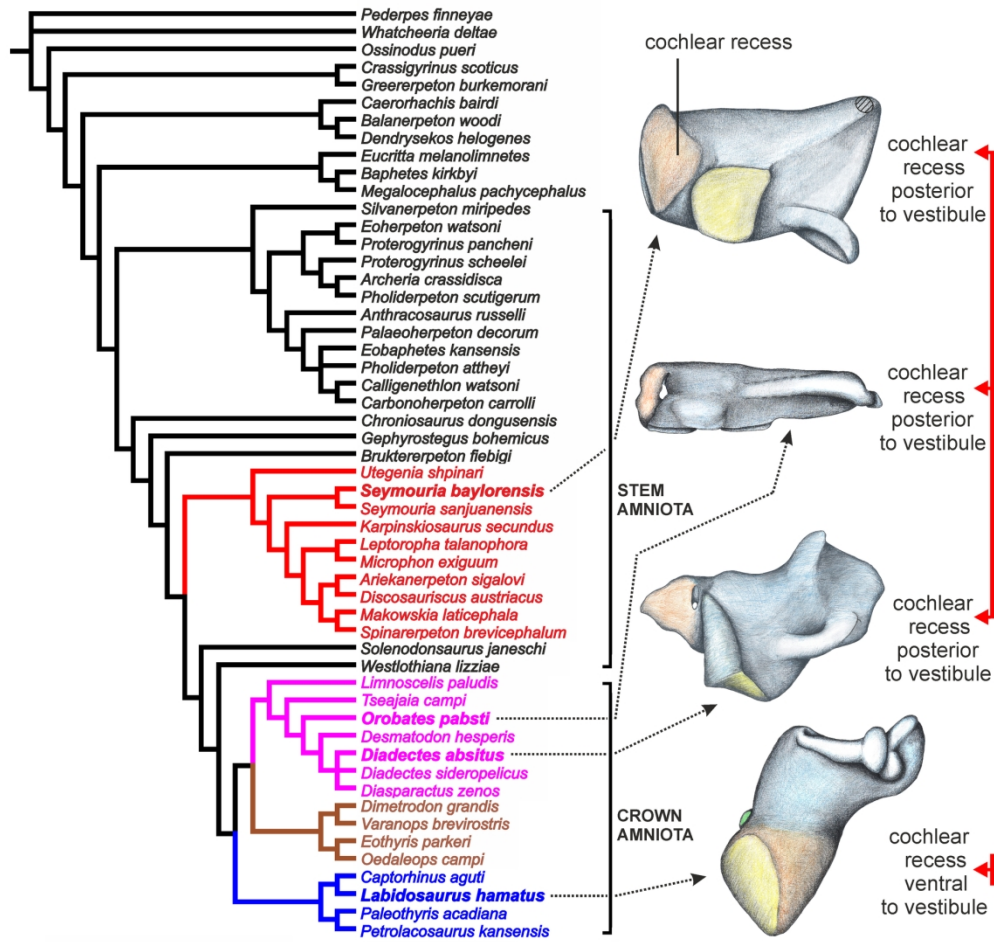


FIG. 13. Single shortest tree obtained from parsimony analysis of new data matrix following reweighting of characters by the maximum value of their rescaled consistency index from the initial unweighted analysis. The names of the taxa discussed in the text are in bold. Drawings of virtual 3D reconstructions of the right endosseous labyrinths of those taxa are shown in right lateral view.

FULL SIZE WIDTH

167x161mm (300 x 300 DPI)

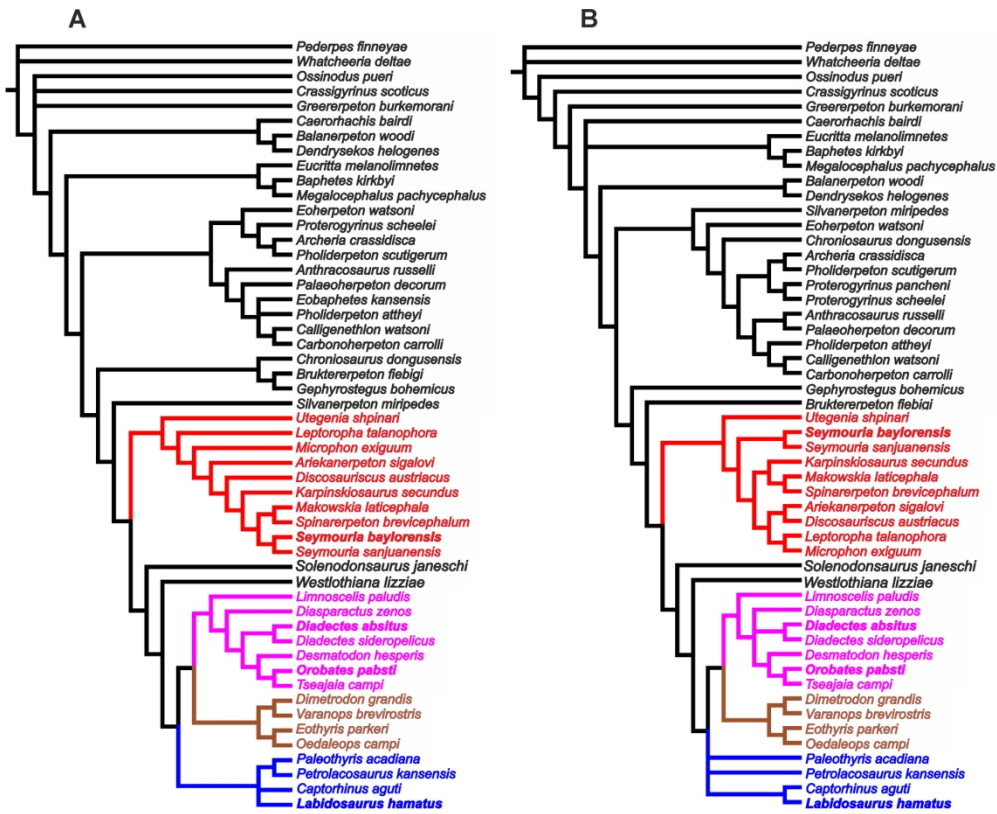


FIG. 14. Phylogeny of major Palaeozoic tetrapod lineages illustrating the relationships of stem and crown amniotes. A, B, strict consensus topologies from parsimony analyses with equally weighted characters, following deletion of *Proterogyrinus pancheni* (A) and *Eobaphetes kansensis* (B).
FULL SIZE WIDTH

166x135mm (300 x 300 DPI)

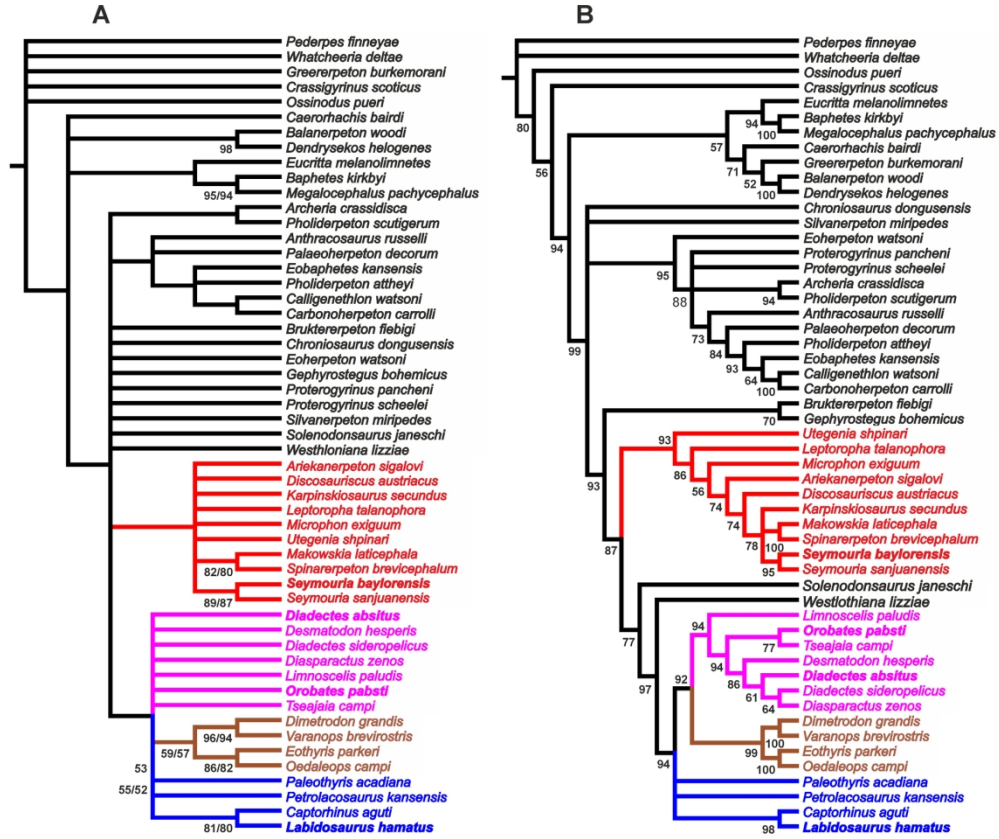


FIG. 15. A, strict consensus topology from parsimony analysis with equally weighted characters; pairs of numbers along branches represent bootstrap (to the left of the forward slash) and jackknife percentage support; note: the clade Eureptilia is collapsed, but receives bootstrap and jackknife support of 53%. B, Bayesian topology showing clade credibility values appended to branches.

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167x142mm (300 x 300 DPI)

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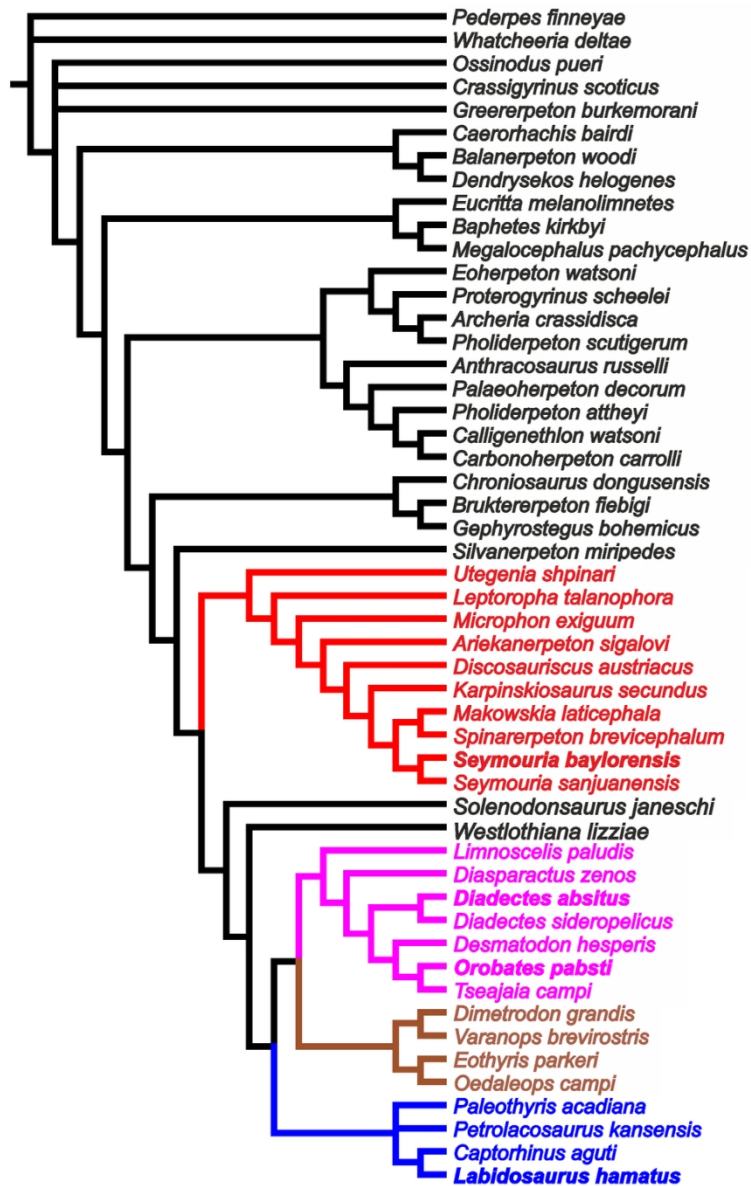


FIG. 16. Strict consensus topology from parsimony analysis with equally weighted characters, following deletion of *Proterogyrinus pancheni* and *Eobaphetes kansensis*. SINGLE COLUMN WIDTH

82x131mm (300 x 300 DPI)

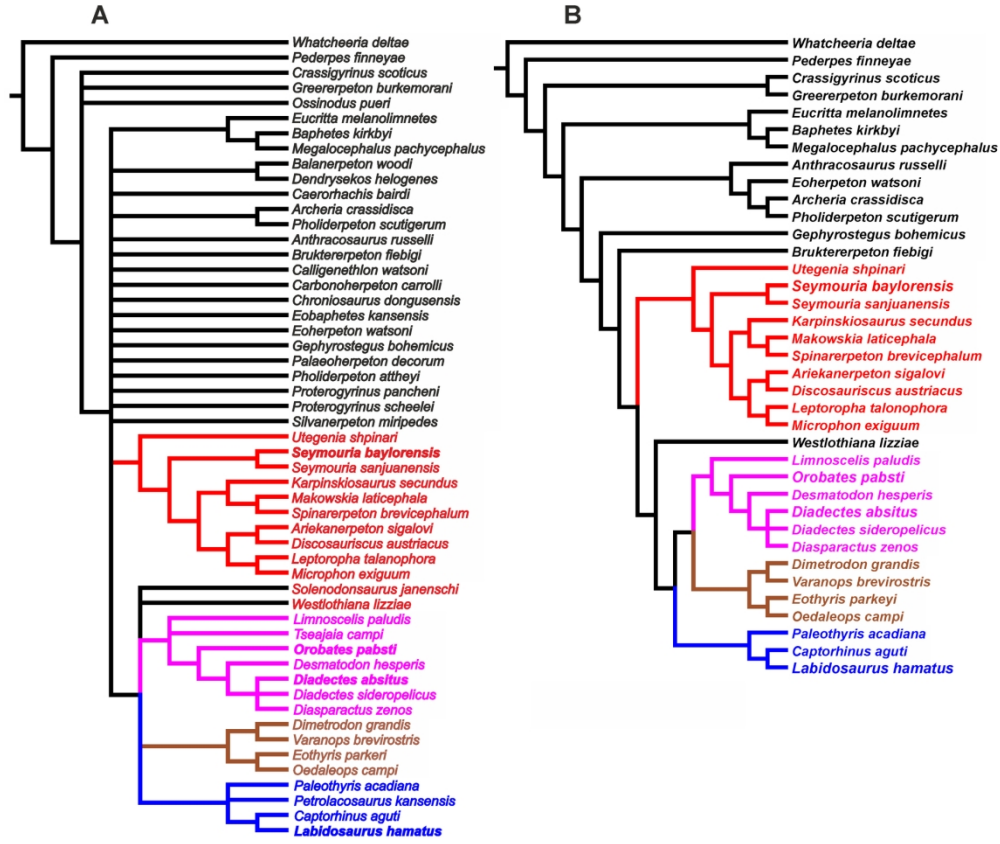


FIG. 17. A, strict consensus of all trees obtained from parsimony analyses with implied weighting, using the first ten integer values of the K constant of concavity. B, maximum agreement subtree of all implied weighting trees. FULL SIZE WIDTH

167x141mm (300 x 300 DPI)