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Fossil evidence on evolution of inner ear cochlea in Jurassic mammals

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The coiled cochlea is a key evolutionary innovation of modern therian mammals. We report that the Late Jurassic mammal *Dryolestes*, a relative to modern therians, has derived bony characteristics of therian-like innervation, but its uncoiled cochlear canal is less derived than the coiled cochlea of modern therians. This suggests a therian-like innervation evolved before the fully coiled cochlea in phylogeny. The embryogenesis of the cochlear nerve and ganglion in the inner ear of mice is now known to be patterned by neurogenic genes, which we hypothesize to have influenced the formation of the auditory nerve and its ganglion in Jurassic therian evolution, as shown by their osteological correlates in *Dryolestes*, and by the similar base-to-apex progression in morphogenesis of the ganglion in mice, and in transformation of its canal in phylogeny. The cochlear innervation in *Dryolestes* is the precursory condition in the curve-to-coil transformation of the cochlea in mammalian phylogeny. This provides the timing of the evolution, and where along the phylogeny the morphogenetic genes were co-opted into patterning the cochlear innervation, and the full coiling of the cochlea in modern therians.

Keywords: Mammalia; inner ear; evolution; Jurassic

1. INTRODUCTION

The snail-shaped cochlea with its hearing organ (Organ of Corti) is a key evolutionary innovation in the inner ear of modern marsupial and placental mammals. Coiling of the cochlea is a major feature distinguishing the modern marsupials and placentals from all other mammals [1–5]. The cochlea coils into a spiral to compact itself into a smaller space in the skull for efficient innervation and blood supply in marsupials and placentals [6,7]. Elongation of the cochlea with more spiral turns is correlated with increased resolution of sound frequencies [2,8]. The curved gradient of the coiled cochlear canal wall focuses acoustic energy towards the apex of the cochlea, the most sensitive region for the low-frequency sound [9]. The key innovation in the fully coiled cochlea, including its auditory innervation, is correlated with the earliest diversification of metatherians and eutherians in the Cretaceous, and has led to many spectacular functional adaptations in hearing in Cenozoic and living marsupials and placentals [3,5,10–16]. However, evolution of this important ear structure cannot be fully deciphered until the precursory condition of its main characters can be mapped from the fossil record of early therians, and their phylogenetic transformation can be correlated with the morphogenesis patterned by developmental genes.

Here, we report the discovery of the precursory structures of the fully coiled cochlea of modern therians in the inner ear of the Late Jurassic mammal *Dryolestes leiriensis* [17], a 150 Myr old fossil mammal in the cladotherian clade, as defined by the common ancestor of dryolestoids + extant therians (e.g. [3]). *Dryolestes* is a stem taxon characterized by plesiomorphic dental features, and a near relative to the modern marsupials and placentals. The fine inner ear structures are preserved in a petrosal bone that houses the inner ear in the skull. Through the high-resolution micro-computer tomography (CT) scanning and comparative analysis, we obtained new information on its morphological features that represent the evolutionarily ancestral condition of extant therian mammals. This is relevant to the understanding of the evolution of the coiled cochlear canal and the innervation of its hearing organ.

2. MATERIAL AND METHODS

The petrosal specimen (Guimarota Collection of Museo Geológico (Lisboa, Portugal), specimen number SGP 6807) shows similar apomorphic features as the known petrosal of the paurodontid *Henkelotherium* (Paurodontidae, Dryolestoidea) [5,18]. The inner ear inside the petrosal was observed by our high-resolution micro-CT scanning and visualized by three-dimensional virtual endocasts from the CT data.

The first scanning of X-ray CT was by the OMNI-X Universal HD600 Scanner at the Center of Quantitative Imaging (CQI), Pennsylvania State University, State College, Pennsylvania, USA. The images have a 1024 pixel resolution and voxel size of $0.025 \times 0.025 \times 0.028891$ mm. This scanning allowed us to initially identify this fossil. The second scanning for greater morphological details was by scanner v|tome|x s (GE Sensing & Inspection Technologies GmbH phoenix|x-ray) at the Steinmann-Institut für Geologie, Mineralogie und Paläontologie, Universität Bonn, Germany. The images have a 1024 pixel resolution and a voxel size of $0.010158 \times 0.010158 \times 0.010158$ mm. To increase the resolution of the region of interest (cochlear canal), we used the software datos|x-reconstruction (GE Sensing & Inspection Technologies GmbH phoenix|x-ray)

Table 1. Measurement of bony labyrinth structures of *D. leiriensis* (SGP 6807). (Endocast measurements from Avizo 5.1 based on the second CT scanning in the scanner in Steinmann-Institut, Universität Bonn. Scanned resolution 0.010158 mm; virtually half voxel size 0.005079 mm.)

	coiling along length		estimated length (mm)	
cochlear canal	∼270°		3.3	
primary bony lamina length			1.6	
secondary bony lamina length			1.18	
	long diameter (mm)		short diameter (mm)	
fenestra vestibuli	0.53		0.47	
	estimated diameter (width–height average mm)		width (mm)	height (mm)
apical turn diameter	0.77		0.85	0.68
basal turn diameter	0.99		0.96	1.2
angle of basal turn to lateral semicircular canal	145°			
semicircular canals	width (mm)	height (mm)	arc radius (inner curvature mm)	lumen diameter (mm)
anterior	2.31	2.22	1.13	0.21
posterior	1.56	1.50	0.77	0.22
lateral	1.55	1.46	0.75	0.22
average radius			0.88	

for virtually halving the voxel size (0.005079 mm) from the raw dataset, which has further increased the resolution of fine structures (electronic supplementary material, figures S2–S3). Virtual reconstructions of the petrosal bone and the inner ear bony labyrinth were completed by manual segmentation function of the software Avizo 5.1. Linear measurements were from the inner ear bony labyrinth endocast with Avizo (table 1).

3. DESCRIPTION AND COMPARISON

(a) *Vestibule and semicircular canals*
On the three-dimensional virtual endocasts visualized from the CT scanning, the vestibular part of the inner ear shows a discernible separation between the utricle and the saccule of the vestibule (figure 1*d–f*), the fenestra vestibuli (with a stapedial ratio of 1.2), which transmits sound into the inner ear, and the fenestra cochleae for releasing the sound pressure (figure 1). The anterior, posterior and lateral semicircular canals for the detection of motion and equilibrium function have their respective arc radii at 1.13, 0.75 and 0.77 mm (figure 1: asc, lsc and psc). The plane of the lateral semicircular canal forms a 145° angle to the axis through the basal cochlear canal (table 1). The lateral and posterior semicircular canals form the secondary crus commune, similar to the condition of the related dryolestoid *Henkelotherium* [5].

The semicircular canals of *Dryolestes* can provide the inference for the locomotor agility of this extinct mammal [19–21]. The agility scores derived from the sizes of the canals for *Dryolestes* range from 3.16 to 3.67 (electronic supplementary material). This shows that *Dryolestes* had a nearly average degree of agility of motion, according to scales of extant mammals [19], and interpreted for fossil mammals [20,21]. Based on extant mammals with the same range of agility scores [19] as *Dryolestes*, it is certain that *Dryolestes* did not

have gliding, flying, saltatorial or fully aquatic locomotion. *Dryolestes* was either a generalized terrestrial, or a scansorial mammal, neither of which can be ruled out because its postcranial skeleton is not preserved, except for a single humerus [22]. But the latter possibility of a scansorial mammal is more likely by comparison to the closely related dryolestoid *Henkelotherium*, which has many scansorial skeletal features [18].

(b) *Cochlear canal structure*
The cochlear canal in *Dryolestes* is 3.3 mm long and partly coiled through about 270° or three-quarters of a complete turn, starting from the proximal entry point of the cochlear nerve (figures 1, 2 and table 1), according the measurement landmarks suggested by West [8] (see also [5,23]). The canal is relatively straight and has a circular cross section near the base. Its distal half-turn forms an arc around the distal point of the cochlear nerves and has an oval cross section in the apical part. The curvature of the cochlear canal corroborates previous observations that the pre-tribosphenic mammals, such as *Henkelotherium* [5] and *Vincelestes* [24], have a consistent, plesiomorphic pattern of 270° curvature of cochlea (three-quarters of a turn). This represents the precursor condition from which the fully coiled cochlea (360° or more or one full turn) of modern marsupials and placentals probably evolved.

In contrast to the plesiomorphic and under-coiled cochlear canal of *Dryolestes*, the interior bony structures for innervation have derived and functionally significant characteristics in *Dryolestes*, which were previously unknown in the pre-tribosphenic mammals (not preserved in *Henkelotherium* and unknown in *Vincelestes*). *Dryolestes* shows a curved track of fine cochlear foramina in CT scans (down to 5 μm resolution), which are represented on the exterior surface of the endocast (figure 2*g–i*: iam[cn8], tsf[fcn]). Each of the foramina is a separate entrance of an individual fascicle of cochlear
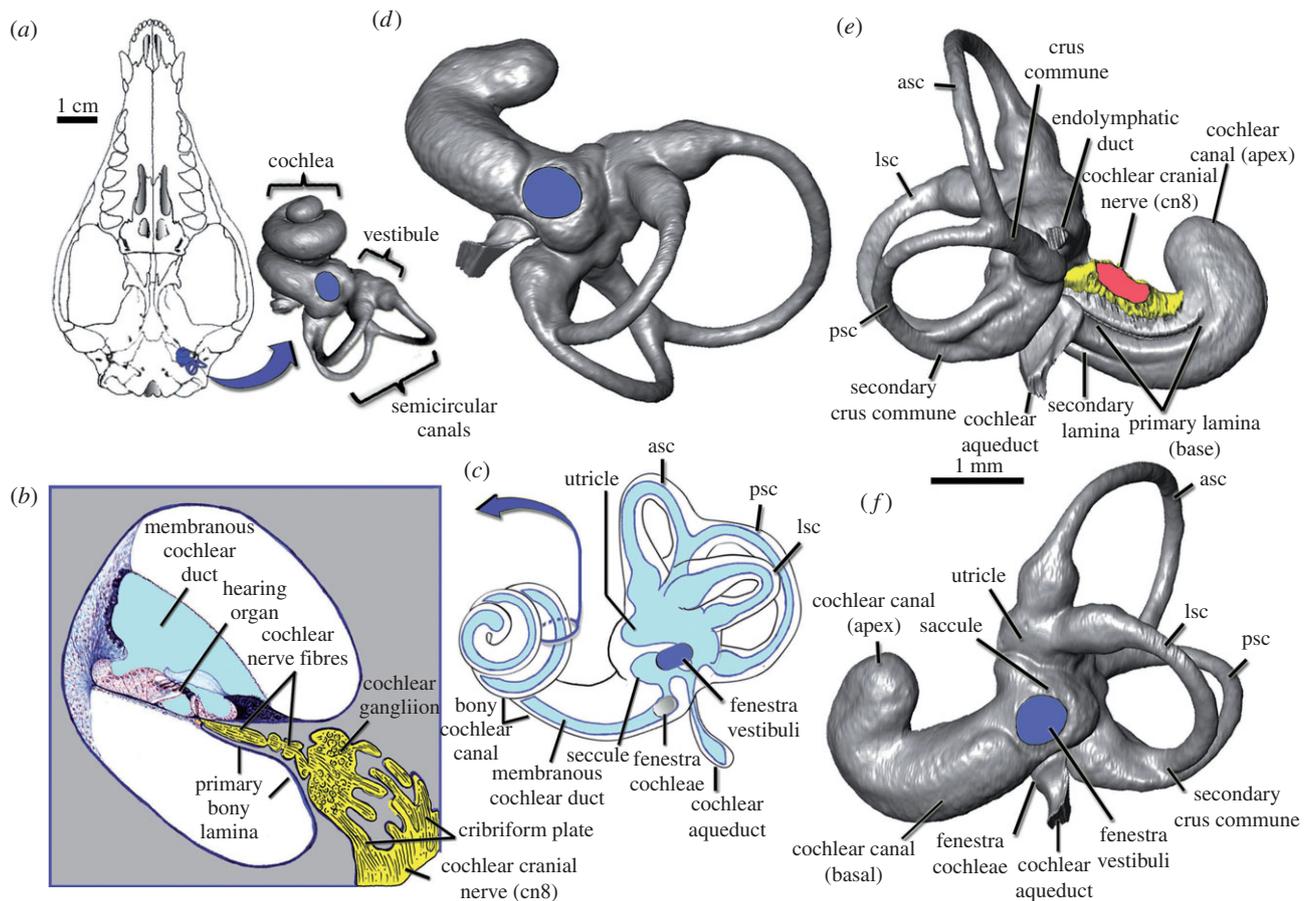
Figure 1. Inner ears of the Jurassic cladotherian mammal *D. leiriensis* and modern therians. (*a*) Marsupial *Didelphis*: skull and inner ear endocast. (*b*,*c*) Human inner ear and cochlear section structures (external view in orientation of human anatomy [7]). Light blue, endolymphatic membranous labyrinth. (*d*–*f*) *Dryolestes* inner ear (endocasts of SGP 6807; Museo Geológico Lisboa, Portugal). (*d*) Ventral view (approx. as in skull (*a*)). (*e*) Internal view as in standard orientation in human anatomy. (*f*) External view in standard orientation. Colour codes and abbreviations: asc, lsc and psc, anterior, lateral and posterior semi-circular canals, respectively. Red and yellow, cut-off and restoration of cochlear cranial nerve (viii) in cribriform plate; blue, fenestra vestibuli.

nerve (cranial viii), and these foramina collectively form the sieve-like cribriform plate in the internal acoustic meatus in marsupials and placentals [6,7]. Previously, the earliest known of this type of structures first appeared in the Cretaceous metatherians and eutherians (10–13,16,25,26), but not in any fossil mammals that are phylogenetically more primitive than the pre-tribosphenic therians, such as multituberculates [27], triconodontids and spalacotheroid symmetrodonts [28,29] (Z.-X. Luo 2009, personal observation) and in the pre-mammalian mammaliaforms [30,31].

Dryolestes has a bony canal for the cochlear ganglion, similar to modern marsupials and placentals, but different from monotremes (figure 2 and electronic supplementary material, figure S3). The CT scans and the virtual endocast show the base of the primary bony lamina for the basilar membrane. In extant mammals (figure 1*b*), this lamina forms the bony conduits for individual cochlear nerve fibres, and it insulates the fibres connecting hair cells to the ganglion. It also supports the medial edge of the basilar membrane on which the hair cells are positioned. The ganglion canal is embedded in the base of the primary bony lamina, and the two structures are interrelated [6,7]. These reliable osteological correlates of modern therian-like cochlear innervation

suggest that the latter originated in stem taxa in the cla-dotherian (dryolestids + marsupials + placentals) clade, with the clade's first appearance in the Middle Jurassic [3,4,17,32].

Dryolestes provides new evidence to better understand how therian-like innervation transformed along the length of the cochlear canal in phylogeny. In *Dryolestes*, the primary bony lamina and its associated ganglion canal extend along the basal half-turn (the first 180°) of the cochlear canal, but do not reach the apical quarter cochlear turn (figure 2*g*–*i*). In extant marsupials and placentals, the primary bony lamina and the ganglion canal extend to the apex of the entire coiled cochlea (figure 2*l*). Obviously, the phylogenetic transformation of the cochlear ganglion canal proceeded in the base-to-apex direction along the cochlea, during the evolutionary descent of marsupials and placentals from their cladotherian ancestry.

Bony structures of cochlear innervation are evolutionary novelties that first appeared in dryolestids, and are apomorphies of the cladotherian clade (figure 3: node 5), in contrast to the plesiomorphic condition documented extensively for about 20 taxa of primitive mammaliaforms, eutriconodonts, multituberculates and spalacotheroids [1,27,30]. The primitive condition of
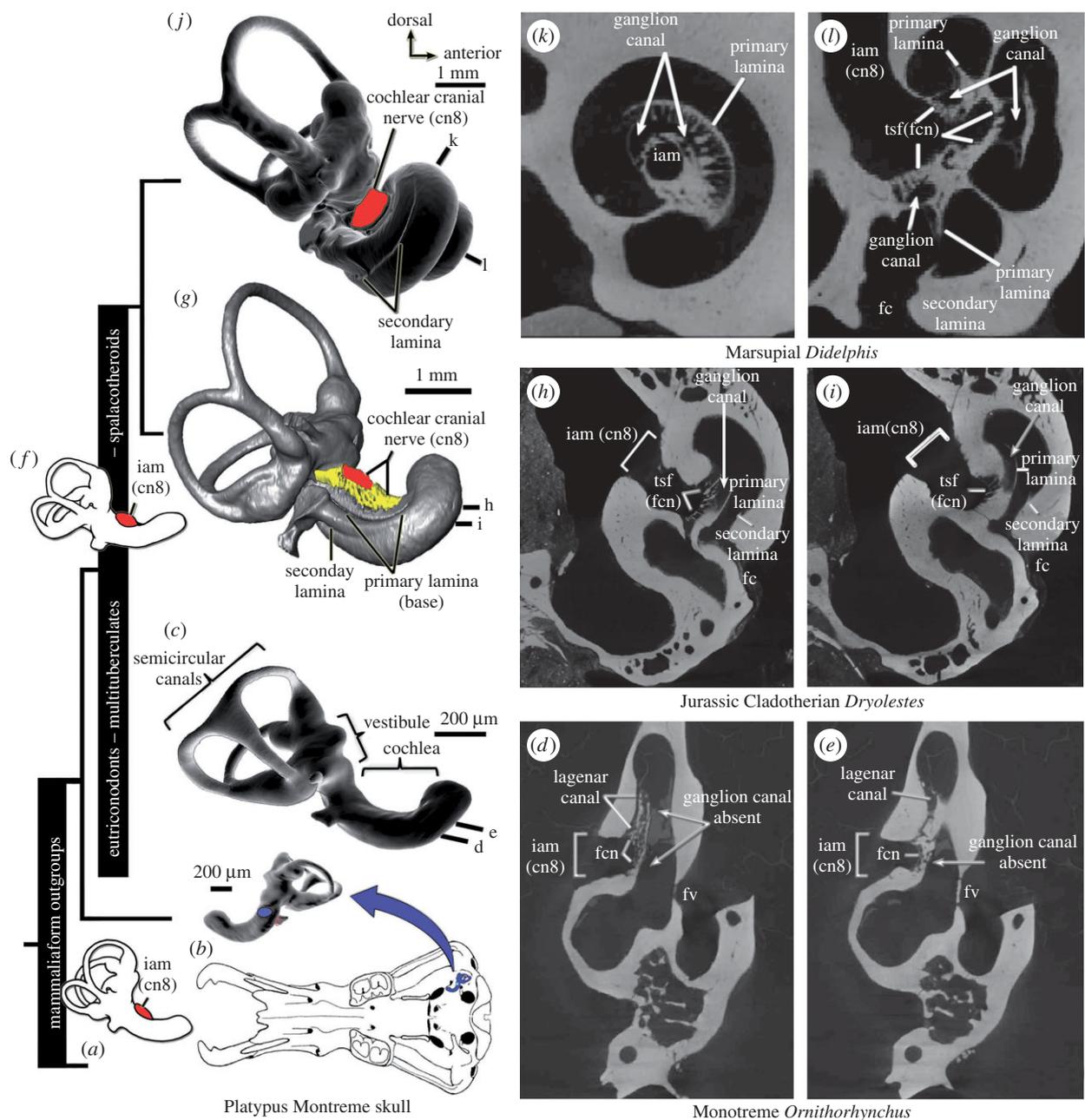
Figure 2. Inner ear endocast and structures for cochlear nerve innervation in the Jurassic *Dryolestes*, monotremes and marsupials, mapped on a phylogenetic tree (topology from [3,4]). (*a*) Jurassic *Morganucodon* for the ancestral condition. (*b*) Monotreme *Ornithorhynchus* skull and inner ear (ventral view). (*c*) Inner ear (medial view); (*d,e*) horizontal sections (histological sections of monotremes illustrated in electronic supplementary material, figure S2). (*f*) Multituberculate [1,27], representing the simple tubular cochlear canal of euriconodonts, multituberculates and spalacotheroids. (*g*) Late Jurassic cladotherian *Dryolestes* inner ear (endocast medial view). (*h,i*) Horizontal sections. (*j*) Marsupial *Didelphis* inner ear (medial view). (*k*) Horizontal section through basal cochlear turn. (*l*) Transverse section (perpendicular to (*k*)). Colour codes and abbreviations: yellow and blue, restoration of cochlear cranial nerve (viii) in tractus spiralis foraminosus (tsf) and cut-off of nerve trunk; fc, fenestra cochleae; fcn, foramina of cochlear nerves; fv, fenestra vestibuli; iam(cn8), internal acoustic meatus (iam) containing the acoustico-vestibular cranial nerve (viii); tsf(fcn), tractus spiralis foraminosus (cribriform plate) (spiral foramina of cochlear nerve fibres).

both mammaliaforms and crown Mammalia is that the cochlear canal is a simple tube, straight or slightly curved, with a single large opening for the cochlear cranial nerve in the internal acoustic meatus, but without any interior bony structures for cochlear innervation (figures 2 and 3: iam[cn8]).

The characteristics for cochlear innervation differ between *Dryolestes* and extant monotremes. The bony cochlear ganglion canal and primary bony lamina for

nerve fibres between the ganglion and hair cells are present in *Dryolestes* but absent in monotremes. The only similarity between monotremes on the one hand and cladotherians on the other is in the presence of cochlear nerve foramina of the sieve-like cribriform plate (reviewed by Fox & Meng [1]; figure 2: fcn). This is a convergence because no such structures are present in the intervening clades (euriconodonts, multituberculates and spalacotheroids) between monotremes and cladotherians
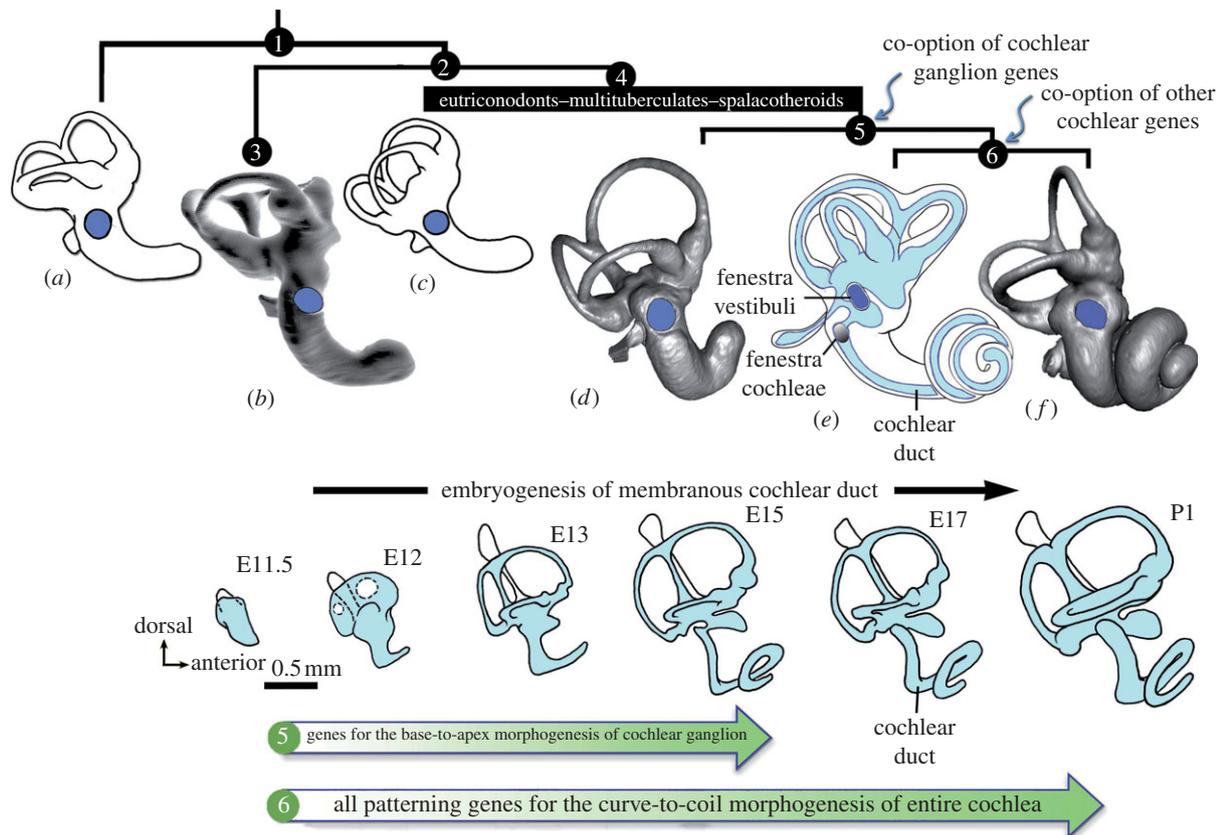
Figure 3. Evolution (upper) and morphogenesis (lower) of the curve-to-coil cochlea in therian mammals. (1) mammaliaforms: straight or slightly curved, tubular cochlear canal [30]. (2) Mammalia: straight or slightly curved canal (without the interior ganglion canal). (3) Monotremata: coiled apex of membranous cochlear duct but uncoiled bony cochlear canal, (convergent) cribriform plate without ganglion canal and bony lamina (new CT data; also see [1]). (4) Many intermediate clades with the ancestral mammaliaform condition. (5) Cladotherians: fully developed ganglion canal (patterned by genes for cochlear ganglion and related bony structures induced by epithelio-mesenchyme interaction), primary and secondary bony laminae, but not yet fully coiled cochlear canal. (6) Modern therians: fully coiled (beyond 360°) cochlear canal (requiring all genes for full curve-to-coil cochlear development). (a) *Morganucodon*. (b) *Ornithorhynchus*. (c) A generalized multituberculate (for euriconodonts, multituberculates and spalacotheroids) with similar simple tubular cochlear canal. (d) *Dryolestes*. (e) *Homo* (blue, endolymphatic membranous cochlear duct). (f) *Didelphis*. Lower panel: ontogeny of coiling the endolymphatic membranous labyrinth in the laboratory mouse *Mus* (redrawn from [34,35]).

[1,27,30]. The case of convergence in the cribriform plate is consistent with other major differences in hair cells and the innervation of the cochlea [33] (see also the electronic supplementary material, figure S3).

The inner ear characters of *Dryolestes* also show that it had a better hearing function for high-frequency sound than some other Mesozoic mammals, such as euricono-donts, multituberculates and spalacotheroids with a plesiomorphous cochlear structure [1,27,30]. *Dryolestes* has a secondary bony lamina for the basilar membrane (figure 2), although it is less developed than in the related *Henkelotherium* and the pre-tribosphenic *Vincelestes* [5,24], and the Cretaceous metatherians and eutherians [10–12,16]. The primary and the secondary bony laminae are for a more rigid support of the basilar membrane for a greater sensitivity to higher frequency sound. This corroborates an earlier observation that the important hearing of high-frequency sound in extant marsupials and placentals evolved earlier in basal cladotherians [5].

4. DISCUSSION
To understand the origins of complex structures and evolutionary novelties is a central quest of evolutionary

biology. The snail-shaped cochlea with its interior complexity is one of the most prominent features of marsupial and placental mammals with significant function and evolutionary consequence. Because dryolestoids are phylogenetically basal to extant marsupials and placentals, the combination of an ancestral and uncoiled cochlear canal and the neomorphic bony features of cochlear innervation is important for inferring the ancestral condition, from which the more derived and sophisticated ear structures of marsupials and placentals must have evolved [3,17,18]. The therian-like cochlear innervation in this group begins in the basal turn in *Dryolestes*, and the neomorphic innervation progressed, base to apex, in phylogenetic evolution from the cladotherians (including *Dryolestes*) to modern therians (figure 3: from node 5 to 6).

Developmental studies of laboratory *Mus* have characterized a network of genes for the morphogenesis of the cochlea [34–36], for specifying sensory and neural progenitors and for patterning the sensory epithelium [35,36]. Some genes are specifically patterning the cochlear nerve and its ganglion [37,38]; still other genes are involved in epithelio-mesenchymal interaction, in which the sensory epithelial and the neural tissues

induce the morphogenesis of their surrounding mesenchymes that are precursors to the bony structure through chondrogenesis and osteogenesis [7,38].

In the embryogenesis of *Mus* [34,35], the hearing organ (the Organ of Corti) becomes a distinctive entity in a short and curved ('L-shaped') cochlear duct in embryonic days (E)11–13 and elongates from the base to the apex, before the coiling of the cochlear duct to 1.75 turns between E13 day and maturity (figure 3). Concurrently, differentiation of the cochlear ganglion starts from the cochlear base in E11–13 and progresses towards the apex through E16 [37]. Differentiation of hair cells in the hearing organ also shows a base-to-apex gradient [36] (figure 3: gene patterning 5). Formation of the cochlear ganglion and the coiling and elongation of the cochlear duct are intricately linked in late (E13 to maturity) embryogenesis. These processes further induce the embryonic mesenchyme differentiation that leads to the surrounding osteological structures [7,38].

All these patterning genes and their related signalling pathways are required for the cochlear elongation and the curve-to-coil development of the cochlear duct beyond the curved 'L-shaped' cochlea of E11-13 stages (reviewed by 35). Mutant mice with knockouts of these genes for inner ear development show an ontogenetic arrest of cochlear coiling and their cochleas become under-coiled, more or less similar to the curved (or partially coiled) cochlear canal in stem cladotherian and pre-tribosphenic mammals (figure 3). This network of morphogenetic genes must have been co-opted to form the derived characters of coiling, in the evolution from basal cladotherians with a curved cochlear canal, as seen in *Dryolestes* (figure 3, node 5), to the fully coiled cochlear canals of the Cretaceous relatives to marsupials and placentals. The full coiling of the cochlear canal follows the curved precursory condition, both in phylogeny and in embryogenesis (E13 day to maturity; figure 3, node 6).

New fossil evidence from *Dryolestes* shows that the phylogenetic transformation of the therian-like ganglion, as shown by its ossified canal, occurs from the base to the apex. This is congruent with the base-to-apex progression of morphogenesis of the cochlear ganglion in mice, the genetic controls of which have been deciphered in recent years [37]. The morphogenesis of the ganglion in mice requires *Neurogenin-1* (*Ngn1*) for the progenitor determination of ganglion neurons, *NeuroD* (*Neurod1*) for forming and maintaining the ganglion neurons, *Brain Derived Neurotrophic Factor* (*BDNF*), and *Neurotrophin-3* (*NT3*) for supporting the ganglionic innervation to hair cells [37,38], plus other genes with global influence for the inner ear morphogenesis that also impact on the cochlear ganglion [34–37]. We hypothesize that this suite of genes (gene pattern 5) must have been co-opted for the formation of a therian-like ganglion and cochlear innervation, in the Jurassic cladotherian evolution, as evidenced by their osteological correlates in *Dryolestes* (figure 2), which must have been accompanied by chondrogenesis and osteogenesis of bony structures through epithelio-mesenchyme interaction influenced by additional genes (e.g. [38]). Possibly, similar developmental processes for the base-to-apex growth of cochlear ganglion [37], and for the base-to-apex differentiation of hair cells in mice [35,36], may have similarly underlined the base-to-apex evolution of the neomorphic and therian-like innervation in phylogeny, as indicated by the direction of transformation of the cochlear ganglion canal in fossils.

The fossil record provides the phylogenetic scope and geological time scale for developmental mechanisms of the inner ear cochlea of therian mammals. In morphogenesis of extant marsupials and placentals, the full coiling of the cochlear duct is inextricably linked with the formation of the cochlear ganglion, and both are also linked with chondrogenesis and osteogenesis of complex bony labyrinth structures, all during the late embryogenesis. According to phylogeny (figure 3: nodes 5 and 6), formation of cochlear ganglion by co-option of such genes as *Ngn1*, *Neurod1*, *BDNF* and *NT3*, and the genes for the related epithelio-mesenchyme interaction (figure 3: gene pattern 5) [37,38], had occurred first in evolution, no later than the first appearance of the cladotherian clade in the Middle Jurassic [3,4,32]. That occurred before the full complement of patterning genes were co-opted for full cochlear coiling in the modern marsupial and placental lineages dated to the Early Cretaceous (figure 3: gene pattern 6) [10–16]. This sheds light on the evolutionary assembly of such an intricate structure as the coiled cochlear canal with all of its interior complexity. This provides the timing of the evolution, and where along the phylogeny the morphogenetic genes were co-opted into patterning the cochlear innervation, and the full coiling of the cochlea in modern therians.

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