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## 2.14.1 Abstract

Mammalian ears differ from those of other vertebrates, and have distinctive hearing functions. Their evolution can be traced into mammals of the Mesozoic Era and their predecessors. In mammaliaforms, the middle ear is attached to the jaw via the ossified Meckel's cartilage. Monotremes, marsupials and placentals, known as the crown mammals, are more derived in having a middle ear that is disconnected from the jaw. However, this disconnected ear evolved independently in Mesozoic multituberculates, in therians, and lastly in monotremes, from their respective predecessors. The ectotympanic and the malleus supporting the tympanum are less developed in Mesozoic mammals than in living mammals. This is consistent with their simple cochleae, suggesting that the ancestral, less sensitive middle ear was correlated with limitation of their inner ear. Neomorphic inner ear structures of mammaliaforms include the petrosal fused from the periotics, the promontorium for the longer cochlea, and a vasculature similar to those of extant mammals. Fossil trechnotherians are the earliest to show a therian-like perilymphatic conduit configuration. Cladotherians (closer to living therians) are the earliest to have developed the canal for the spiral ganglion, the tractus foraminosus for the cochlear nerve, and the primary and secondary bony lamina supporting the organ of Corti. This also occurred in parallel in gondwanatherians. The tractus foraminosus evolved independently in monotremes. A much better fossil record in the last 20 years revealed that ear structures of different groups are not only disparate in morphologies, but also different in the timing of their evolution. Their functional convergence is often incomplete. Nonetheless, the modern placentals and marsupials have developed broadly-similar ear structures and hearing function, despite the different paths of their phylogenetic evolution. The ears of extant monotreme mammals, by contrast, differ in a number of respects.

## 2.14.2 Introduction

Modern mammals are distinct from other extant vertebrates in their ear structures and hearing capability (Manley, 2000; Luo et al., 2016). Living mammals have a unique middle structure with a ring-shaped ectotympanic ("tympanic ring"), a malleus ("hammer bone" with its handle) and the incus ("anvil bone") that are evolutionarily derived, plus the stapes ("stirrup" of many placentals) that is an ancestral structure shared by other vertebrates. If the three extant mammal groups - egg-laying monotremes, pouched marsupials, and placentals - were to be considered alone without their fossil relatives, these middle ear bones, are seemingly quite different from their homologs ("evolutionary counterparts") in all other extant vertebrates. Extant mammals are also distinct from other vertebrates in that their inner ear cochlea has the specialized structures of the Organ of Corti, and a spiral cochlear gangion. Both of these sensory and innervating structures are supported by intricate osteological structures in the cochlear canal, collectively known as the bony labyrinths, as exemplified by the snail-shaped cochlea of marsupials and placentals.

By a simple comparison to other living vertebrates such as reptiles and birds, modern mammals can be distinguished by their unique characters of the middle and inner ears. However, the evolution of the mammalian middle ear is more interesting, and far more informative, than can be inferred from extant mammals alone. There is a rich record of fossil mammals of the Mesozoic Era, and their near relatives, known as the mammaliaforms, plus fossils of cynodonts (the ancestral clade including modern mammals) (Fig. 2). Some of the better-preserved fossils have provided direct evidence of how the fundamental transformation of mammalian ears occurred in evolution. The evolutionary history of mammalian ears can be traced back along the phylogenetic tree well into their synapsid relatives that first appeared about 310 million years ago (ma) (Allin and Hopson, 1992; Clack and Allin, 2004).

When mapped on phylogenies, the seemingly unique characters of the middle ear of modern mammals clearly show a stepwise evolution, by a piecemeal assembly of precursor characters through the cynodonts (Figs. 1 and 2). The structural changes have been acquired by the three living mammal groups along their respective long branches well into the Jurassic Period of the Mesozoic Era (Fig. 2). Furthermore, the evolution of ear structures in the synapsid-mammal lineage is not a simple linear progression, but a remarkable process of many successive transformations within many separate lineages (Angielczyk and Kammerer, 2018; Martin, 2018). The family tree of mammaliaforms is bush-like, with numerous diverse fossil groups that are anatomically different not only from extant mammals, but also from each other in their ear structures (Luo, 2007). The extinct side branches of the mammal evolutionary tree can have unique combination of features of ears that are not represented in the three main extant mammalian groups. The obvious similarities in ears of extant mammals have evolved by convergence, and these convergences can be clearly traced on the mammaliaform evolutionary tree (Fig. 3) (Urban et al., 2017; Zhou et al., 2019; Wang et al., 2019).

Discoveries of abundant new fossils over the last 20 years have filled many gaps in our previous knowledge of early mammal history (Kielan-Jaworowska et al., 2004; Martin, 2018). New studies by high-resolution computed tomography (CT) scans and synchrotron beamline scans have also revealed much new information from fossils. These new findings have provided a more fine-grained understanding of mammalian ear evolution, but also posed new questions and led to new hypotheses that need to be addressed.

A relatively new understanding of ear evolution on a large scale across 200 million years of geological history of mammaliaforms is the idea that repetitive and iterative evolutionary changes occurred in disparate Mesozoic clades of mammals and their mammaliaform relatives (Luo, 2007). Significant homoplasies, both evolutionary convergencies and reversals, occurred not only in the ears of the Mesozoic mammal groups, but also in the transition to stem mammaliaforms from cynodonts. Those homoplasies are analogous to evolutionary experiments in form and – presumably - function during the Mesozoic Era, and they can serve to test the mechanistic hypotheses of the macroevolutionary processes that gave rise to the patterns of living mammals (Luo et al., 2011, 2016).

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Figure 1 Evolution of mammalian middle ear and jaw hinge from cynodonts to mammaliaforms and modern mammals. (A). Cynodont Thrinaxodon from the Early Triassic. In nonmammaliaform cynodonts (node 1), which are the ancestral clade the include mammaliaforms, the middle ear bones are attached to the mandible. This is the Mandibular Middle Ear of Cynodonts (MMEC), as exemplified by Thrinaxodon. The functioning craniomandibular joint (CMJ) is formed by the quadrate bone (homolog of the incus) and the articular (homolog of the malleus). This ancestral configuration is also known as the primary jaw joint. This jaw hinge has a dual function, and is involved both in jaw movement, and concurrently in sound conduction from the plate-like angular bone (homolog to the ectotympanic hook). (B). Sinoconodon from the Early Jurassic, one of the most primitive stem mammaliaforms, with a fully mammal-like cranio-mandibular joint formed by the dentary condyle and the squamosal glenoid (= the "secondary" jaw joint). The incus and the malleus are smaller than in Thrinaxodon, but their articulation is still mobile. This joint is side-by-side with the derived dentary-squamosal joint, and both are functioning as a compound jaw hinge (also known as the double joint). In mammaliaforms (node 3), as exemplified by Sinoconodon, the ear bones are far more slender than the plate-like ear bones in cynodonts. These bones are fully attached to the postdentary trough in the dentary, and also anteriorly connected by the Meckel's element to the Meckel's sulcus on the dentary, as in cynodonts. (C). Yanoconodon from the Early Cretaceous, a basal member of the extinct eutriconodontan clade nested within crown Mammalia (node 5). In eutriconodontans, the dentary has lost the postdentary trough, and the ectotympanic-malleus complex is medially displaced from the posterior region of the mandible, made feasible by the curved Meckel's cartilage. The middle ear is, however, still connected anteriorly to the mandible by an ossified Meckel's cartilage that is fitted into the Meckel's sulcus. This type of partly connected middle ear is evolutionarily more derived than those of stem mammaliaforms, and is known as the Partial Mammalian Middle Ear (PMME) (or "Transitional Mammalian Middle Ear" - TMME). (D). Extant mammal Monodelphis of crown Theria (node 14). In embryo and neonate stages, the ontogenetic precursors to middle ear bones are still connected to the dentary by the Meckel's cartilage, but in the adult, the middle ear is fully disconnected from the dentary after the resorption of this cartilage. Phylogenetic nodes are the same in all figures.

## 2.14.2.1 Evolutionary Tree of Cynodonts and Mammals

Evolutionary trees are the roadmaps for tracing how ear structures of modern mammals evolved since the synapsid-mammal lineage split from other vertebrates (Fig. 2). Modern mammals, also known as crown Mammalia, are defined by the common ancestor of monotremes, marsupials, and placentals, plus other extinct clades nested within these extant mammals as indicated by phylogenetic estimates (Fig. 1: Node 5). Mammalia are a subclade of the more inclusive clade of Mammaliaformes, which consists of crown Mammalia plus their proximal relatives of the Mesozoic (Rowe, 1988, as modified by Kemp, 2005 and others). Mammaliaforms (Fig. 2: Node 3) are a subclade of cynodonts, but they are distinctive from the pre-mammaliaform cynodonts in many anatomical characters (hereafter referred to as "cynodonts", defined at node 1 of Fig. 2). Cynodonts, in turn, are a subclade of all synapsids (Sidor, 2001; Angielczyk and Kammerer, 2018).

The novel features (apomorphies) of mammaliaforms include a derived jaw joint formed between the dentary and the squamosal, a more fully developed braincase and orbital wall, a determinate ("plateau-like") pattern for skull growth, and a diphyodont



**Figure 2** Evolutionary sequence of anatomical transformation and functional novelties of mammaliaforms, on a time-calibrated phylogenetic tree. Middle ear evolution (marked in blue): The three convergent evolutions that disconnect the middle ear from the mandible, by resorption of Meckel's cartilage, occurred in different lineages in different geological times: the earliest-known disconnected middle ear evolved in multituberculates no later than the Late Jurassic (around 160 million years ago, ma). The disconnected middle evolved independently in metatherians and eutherians in the Early Cretaceous (circa 125 ma). The last and convergent evolution of disconnected middle ear occurred in monotremes, in (or after) the Late Cretaceous. Inner ear evolution (marked in red): elongate cochlear canal (to about 2 mm) with development of the organ of Corti on a basilar membrane and with a vascular supply for the stria vascularis likely originated in the ancestor of mammaliaforms (Node 3). Development of the tractus foraminosus and the related bony labyrinth structures, supporting the basilar membrane and innervating the organ of Corti, occurred twice: the first time in the common ancestor of cladotherians by the Late Jurassic, and second time in gondwanatherians in the Late Cretaceous. Phylogenetic tree nodes: Node (1) Cynodonts, of which *Thrinaxodon* is a well-known representative taxon; this large clade includes mammals. Node (2) Mammaliaforms/, which are advanced cynodonts. The ear structures of this clade are known from the brasilodontid *Brasilitherium*, the tritylodontid *Yunnanodon*, and *Pseudotherium*. Node (3) Mammaliaforms – *Sinoconodon*, *Morganucodon*, docodontans (*Agilodocodon*, *Borealestes* and *Microdocodon*), haramiyidans (*Haramiyavia* and *Vilevolodon*), and *Hadrocodium*. Node (4) clade of haramiyidans including Triassic haramiyids and

("two-generation") replacement of deciduous teeth (Crompton and Parker, 1978; Kielan-Jaworowska et al., 2004), all of which are more modern mammal-like, and are rather different from the plesiomorphies (primitive features) of cynodonts. Stem mammalia-forms are rich in fossil representatives, spanning over 120 million years from about 220 ma to about 100 ma, and many groups are taxonomically diverse (Kielan-Jaworowska et al., 2004; Martin, 2018). More relevant to the assessment of the evolution of ears, many of these fossil groups are specialized in their own right, and disparate from each other. A full and informative understanding of the early evolution of mammal middle ears must therefore take into account the disparate morphologies of middle ears among the stem mammaliaforms (Fig. 3).

# 2.14.3 Middle Ears of Cynodonts and Mammaliaforms

Extant mammals are evolutionarily more derived than cynodonts, as defined by the several apomorphic features of the middle ear (Fig. 1) (Allin and Hopson, 1992; Luo et al., 2016). Middle-ear evolution occurred hand-in-hand with the changes in the craniomandibular joint, namely, the jaw hinge. Characters of the ear and the jaw hinge that made mammals unique can be easily understood by comparing mammals to our successively more distant evolutionary cousins – extinct Mesozoic mammals such as eutriconodonts from the Cretaceous, stem mammaliaforms from the Early Jurassic, and cynodonts, such as *Thrinaxodon*, from the Early Triassic (Fig. 1). The co-transformation of the middle ear and the jaw joint occurred in an incremental pattern, as shown by mapping these structures and their precursor conditions on a large-scale synapsid phylogeny (Sidor, 2001; Luo, 2007).

In cynodonts (Fig. 1: node 1), the primary jaw hinge serving a feeding function is formed by the articular (homolog to the malleus of mammals) and the quadrate bone (homolog to the incus of mammals). The angular bone (homolog to the ectotympanic of mammals) is assumed to have been capable of receiving sound, both through bone conduction from the entire mandible, and by air-borne sound directly impinging on the tympanic membrane suspended by the postdentary bones (the angular and the articular). From there, the sound vibration was transmitted from the postdentary bones to the articular-quadrate joint, and then through the quadrate-stapes contact into the fenestra vestibuli (the oval window). The quadrate and the articular are relatively massive structures serving the masticatory function of the jaw hinge, but also doubling up for the conduction of the sound. All bones that have presumptive hearing function (Kermack et al., 1973; Allin, 1975) are extensively attached to the rest of the mandible; thus the middle ear is known as the Mandibular Middle Ear of Cynodonts (MMEC) (Luo, 2011).

The ancestral MMEC, as seen in *Thrinaxodon* and *Cynognathus*, is characterized by large presumptive middle-ear bones that are still fully attached to the mandible. The hook-like angular bone has a strap-shaped posterior process. The surangular and the anterior limb of the malleus are also strap-like (Fig. 3). A consensus in the literature suggests that these middle-ear structures functioned as a receiver of direct air-borne sound (Allin, 1975; Maier and van der Heever, 2002), or perhaps both a receiver of direct airborne sound by a tympanum, and a bony conduit for conducting low-frequency ground vibration through the mandible (Kermack and Mussett, 1983; Luo et al., 2016).

In the common ancestor of mammaliaforms, a key evolutionary transformation changed the jaw hinge, and simultaneously also the middle ear. Mammaliaforms, as exemplified by *Sinoconodon* and *Morganucodon* (Figs. 1 and 2: node 3) have a secondary jaw hinge formed between the squamosal glenoid (a concave structure opposing the end of dentary) and the dentary, which developed a spindle-shaped or ball-shaped condyle. Both the glenoid and the condyle are novel structures evolved within mammaliaforms (Kermack et al., 1973; Crompton and Parker, 1978). This "secondary" jaw joint becomes the main jaw hinge that bears most of the force of mastication, relieving the primary joint (of the malleus and incus) from load bearing, although the malleus initially remains fully connected by an ossified Meckel's element to the lower jaw, and the malleus and incus are still mobile relative to each other (Fig. 1: *Sinoconodon*) (see Luo, 2011).

The craniomandibular joint of the stem mammaliaforms consists of both an "ancestral" malleus-incus joint, and the novel joint between dentary and squamosal – i.e., a double joint (Crompton and Parker, 1978; Kermack et al., 1981; Luo, 2011). The ectotympanic, the surangular, and the malleus are attached side by side to the mandible, the same as in the mandibular middle ear of cynodont ancestors. Furthermore, the middle ear is also connected anteriorly to the mandible, by a long Meckel's element permanently housed by the Meckel's sulcus on the mandible.

Jurassic eleutherodontids (which may be alternatively placed with gondwanatherians, *sensu* Krause et al., 2020). Node (5) Mammalia (crown mammals), defined by the common ancestor of living monotremes, marsupials and placentals, plus extinct Mesozoic clades that are also nested in this clade by phylogenetic analyses. Node (6) Australosphenida clade that first appeared in the late Early Jurassic (following Kielan-Jaworowska et al., 2004). Node (7) Monotremata – stem monotremes extend to the Early Cretaceous; the Mesozoic and extant monotremes are a subclade of the autralosphenidan group. Node (8) Theriimorpha, a clade defined by the common ancestor of eutriconodontans, *Fruitafossor* and living therians, to the exclusion of the australosphenidan-monotremes clade. Node (9) Theriiformes – defined as the common ancestor of multituberculates and living therians. Node (10) "Allotherian" clade consists of multituberculates and gondwanatherians (according to Krause et al., 2014, 2020). Node (11) Multituberculates. Node (12) Trechnotherians, defined by the common ancestor of *Dryolestes* and living therians. Node (14) crown Theria. Node (15) Eutherian-Placental clade. Node (16) Metatherian-Marsupial clade. Phylogenetic clade definition and nomenclature following Rowe (1988, 1993), amended by others. Abbreviations: IHC and OHC, inner hair cells and outer hair cells; ma, million years ago.

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**Figure 3** Structural diversity of the middle ears, in stem mammaliaforms and cynodont outgroups. Stem mammaliaforms (Node 3) and cynodont outgroups (Node 2) show disparate morphologies of the postdentary trough and Meckel's sulcus on the mandibles for holding the middle ear. Haramiyidans (Node 3) show a pattern of decreasing size in postdentary trough and Meckel's sulcus. In stem mammaliaforms, the ectotympanic has a short, hook-like ventral process (= base of the ectotympanic ring), but long anterior and posterior processes, all ancestral features of cynodont outgroups. The malleus lacks the gracile manubrium of crown mammals. The haramiyidan lineage (Node 3) of *Haramiyavia – Megaconus – Vilvolodon* based on Huttenlocker et al. (2018). All phylogenetic nodes are the same as in **Fig. 2**. (A). *Cynognathus*, a cynodont from the Early Triassic; the ectotympanic (= angular bone) has a ventral hook, homologous to the base of the tympanic ring of extant mammals; it is hypothesized to have a presumptive Meckel's cartilage, but not ossified in adult (Kermack et al., 1993). (B). Exaeretodon, a cynodont from the Mid-to-Late Triassic (*E. riograndensis* is Late Triassic). (C). Tritylodontid *Kayentatherium* from the Early Jurassic, a very derived cynodont, also known as mammaliamorph. (D). *Sinoconodon*. (E). *Morganucodon*. (F). *Haramiyavia* of the Late Triassic. (G). *Megaconus* of the Middle Jurassic. (H). *Vilevolodon* of the Late Jurassic. (I). Agilodocodon, a docodont from the Middle Jurassic. (J). *Hadrocodium* of the Early Jurassic. (K). *Sinobaatar*, a multituberculate mammal from the Early Cretaceous, as a simplified representation of crown mammals.

The Meckel's element, the malleus and the incus are all ossified from the posterior section of the Meckel's cartilage (Fig. 1). As established since Gaupp (1913), the malleus and the incus in adult mammals and the articular and the quadrate in reptiles are homologs, as these bones are ossified from the posterior end of the embryonic Meckel's cartilage of the first pharyngeal arch, common to all jawed vertebrates (Goodrich, 1930; De Beer, 1937). While the Meckel's cartilage is retained in adults in reptiles (reviewed in Anthwal et al., 2017), much of the posterior section of the embryonic Meckel's cartilage of mammals is resorbed at a late ontogenetic stage, and the fibrous remnent of the posterior section of the Meckel's cartilage becomes the sphenomandibular ligament in adults (Harada and Ishizeki, 1998; Tucker, 2017).

In mammaliaforms, the Meckel's element, which is an anterior extension of (and continuous from) the malleus, is fully ossified and permanently retained in adults (Kermack et al., 1981; Luo, 2011). This is in contrast to the resorption of the embryonic Meckel's cartilage in adults of extant mammals (Rowe, 1996; Sánchez-Villagra et al., 2002). After the resorption of the Meckel's cartilage, there is a further decrease in size of the middle ear bones relative to the size of the jaw, as seen in extant marsupials (Ramírez-Chaves et al. 2016). A key difference between extant mammals and stem mammaliaforms (and some Mesozoic mammals) (Fig. 3) is that much of the posterior section of the Meckel's cartilage (*sensu* Harada and Ishzeki, 1998) is resorbed in extant mammals, but permanently retained in mammaliaforms. In cynodonts, however, the posterior section of Meckel's element was likely cartilaginous and unossified (Kermack et al., 1973; Kemp, 2005, 2007), but the Meckel's sulcus is present, usually covered by the splenial bone (Figs. 1 and 3) (Allin and Hopson, 1992; Bonaparte et al., 2005). In summary, the ossified posterior section of Meckel's cartilage of stem mammaliaforms is an apomorphy of mammaliaforms, if compared to the Meckel's cartilage that is presumably unossified in cynodonts (Fig. 3A). But it is concomitantly also a plesiomorphy as compared to the Meckel's cartilage resorbed in adults of extant mammals.

There is, however, a major evolutionary change – a conspicuous reduction in size and thickness of the ear bones relative to the tooth-bearing dentary and the skull as a whole: the stapes, the incus (= quadrate), and the malleus (= the articular) all became much smaller. The ectotympanic became a thin rod, with a ventral hook homologous to the base of the ectotympanic of living mammals, and bearing a partial resemblance to the modern ectotympanic ring.

This size reduction of the ear bones facilitated a reduction in the strain imposed by jaw muscle force on the "double" craniomandibular joint, as cynodont-mammal evolution went through an overall body-size reduction (Lautenschlager et al., 2018). Concurrently, the contact of the middle ear to the base of the cranium via the incus was also simplified, enhancing the movement flexibility of the middle ear (Luo and Crompton, 1994). These evolutionary changes all appear to be influenced by the miniaturization of the skulls of early mammaliaforms, subjugated by a macroevolutinary bottle-neck in body-size reduction in the broader cynodont-mammal transition (Lautenschlager et al., 2018).

## 2.14.3.1 Major Patterns of Middle Ears

The Mandibular Middle Ear of Cynodonts (MMEC) (*sensu* Luo, 2011) (Fig. 1: node 1) are distinctively different from the Definitive Mammalian Middle Ear of extant mammals (DMME). DMME was originally defined by Allin and Hopson (1992), and is alternatively called Detached Mammalian Ear (DME) (Harper and Rougier, 2019) (Figs. 1 and 2). MMEC and DMME are the two divergent patterns or end points of a wide morphological continuum that emerged from the long evolution of cynodonts over 250 million years. This morphological continuum of cynodont-to-mammal ear evolution also included another configuration – the Partial Mammalian Middle Ear (PMME) shown by two clades of Mesozoic mammals (Figs. 1-5: node 5). In the PMME (Fig. 5), the middle ear is medio-laterally displaced from the posterior part of the mandible, thereby adding a space between the dentary bone and the malleus and the ectotympanic of the ear; yet anteriorly, these ear bones remain connected to the mandible by the ossified Meckel's cartilage (Fig. 1: node 5). This is a precursor evolutionary pattern to the DMME, and is alternatively called the "Transitional Mammalian Middle Ear" – TMME – Meng et al., 2011). This configuration of the middle ear has now been found to be present in two extint groups of Mesozoic Mammals that are phylogenetically intermediate between extant therians and extant monotremes (Figs. 4 and 5).

# 2.14.3.2 Mammaliaform Ancestral Morphotype

Stem mammaliaforms have the same basic structure as the mandibular middle-ear of the cynodont outgroups (MMEC) (Kermack and Mussett, 1983; Luo, 2011), although the mandibular components of ear (surangular, malleus, ectotympanic) are more slender than in cynodont outgroups. Relative to the skull size and as a consequence of the miniaturization of overall body size, these mandibular ear elements, as well as the incus and stapes are much smaller than their counterparts in cynodonts (Lautenschlager et al., 2018). The pleisiomorphic features of stem mammaliaforms include: the malleus, the angular, and the surangular are still attached side-by-side to the mandible, as in cynodonts. By retention of this ear-mandible attachment, stem mammaliaforms retain a more ancestral pattern than crown mammals (Fig. 2). A new and important observation since Allin's (1975) classic study is that the ectotympanic is a short hook and the malleus "manubrium", if present at all, is tiny in these mammaliaforms. This has functional implications for hearing, and must be taken into consideration in estimating the hearing capacity of mammaliaforms of the Triassic and Jurassic.



Figure 4 Independent evolution of Definitive Mammalian Middle Ear (DMME) among major clades of crown mammals (node 5): disconnections of the middle ear from the mandible evolved independently in extant monotremes, in multituberculates, and in extant therians. The preservation of intact middle ear elements connected to the ossified Meckel's cartilage are very rare in Mesozoic mammaliaform fossils. A conservative assessment of the disconnection of the ear from the mandible can only be based on the well-preserved fossils with middle ears and proven absence of the Meckel's element, or on unambiguous absence of any mandibular structures for attachment of the Meckel's element. It is relatively easier to assess the attachment and connection of middle ear in stem mammaliaforms (those positioned between node 3 and node 5) - the presence of a mandibular middle ear can be inferred reliably from the presence of the postdentary trough that is commonly preserved in the mandibular fossils. For Mesozoic clades, that are nested in the crown mammals (between node 5 and node 12), the presence of a connected middle ear may be inferred from the Meckel's sulcus alone, but this is dependent on quality of the preservation, and can be less reliable. On such evidence, it can be inferred that the middle ear connected to the mandible by an ossified Meckel's cartilage (Partial Mandibular Middle Ear) is an ancestral condition for common ancestors of crown Mammalia (Node 5), Australosphenidans (node 6) and toothed monotremes, through Trechnotheria (Node 6). Cladogram nodes are the same as in Fig. 2 and defined in the caption to Fig. 2. (A). Morganucodon. (B). Haramiyavia. (C). Haldanodon - stapes only. (D). Hadrocodium. (E). Henosferus. (F). stem monotreme Teinolophos. (G). the extant monotreme Tachyglossus (stapes on a different scale, original drawing). (H). Fruitafossor. (I). eutriconodont Yanoconodon. (J). multituberculate Sinobaatar. (K). the multituberculate Pseudobolodon. (L). spalacotherioid Maotherium. (M). marsupial Monodelphis: left - mandible; middle - ear bones; right - stapes of a juvenile of Didelphis (on a different scale). (C), Based on Ruf et al. (2013); Panciroli et al. (2018). (D), From Luo et al. (2001a,b). (E), Courtesy of Dr. G. W. Rougier, used by permission; Rougier et al. (2007). (F), Adapted from Rich et al. (2016), with permission. (H), From Luo and Wible (2005). (I), From Luo et al. (2007). (J), From Luo et al. (2017). (K), Redrawn from Schultz et al. (2018). (L), Redrawn by Luo, from Zhou et al. (2019). (M), From Luo et al. (2016).



**Figure 5** Comparison of mandibular middle ear (MMEC) of mammaliaforms and the partial mammalian middle ear (PMME) of Mesozoic clades of crown Mammalia. (A). *Morganucodon*: the main model of MMEC. (B). *Vilevolodon* (a haramiyidan): a variant pattern of MMEC. The mandibular middle ear (MMEC) as exemplified by *Morganucodon*: the ectotympanic hook (= reflected lamina of the angular) attached to the concavity of the angular process of dentary, the surangular and the prearticular (gonial) part of malleus attached in the postdentary trough under the medial ridge, the Meckel's element extends from the postdentary trough into the Meckel's sulcus. The mandibular middle ear of *Vilevolodon* represents a variant pattern of the MMEC: the Meckel's element and the anterior limb of ectotympanic are shorter (more derived), and the girth of the surangular and malleus are much wider (more plesiomorphic) than those of other mammaliaforms. (C). *Yanoconodon* (a crown mammal) and the partial mammalian middle ear

## 2.14.3.2.1 Ectotympanic Hook

Mammaliaforms are characterized by several neomorphic characters of the ectotympanic bone that are absent in primitive cynodonts. The ventral limb of the ectotympanic is the homolog of the reflected lamina of the angular bone in cynodonts (Allin, 1975). The reflected lamina of the angular of cynodonts is shaped like an oar-paddle (being slightly wider distally), as seen in *Thrinaxodon* and *Cynognathus*, or is short and rod-like (slender), as seen in the derived cynodonts *Exaeretodon* and *Kayentatherium* (Figs. 1 and 3). In *Probainognathus, Pachygenelus*, and *Brasilodon*, which are phylogenetically more crownward, the ventral limb of the ectotympanic is very small and has a similar (plesiomorphic) hook shape (Martinelli, 2017; Hopson and Kitching, 2001: Fig. 5). It is also important to note that *Probainognathus* – a key cynodont for reconstructing mandible-ear evolution – has only a short and rodlike ectotympanic ventral limb (Allin and Hopson, 1992; Hopson and Kitching, 2001), not a long rod as previously reconstructed (Allin, 1975). The short rod-like or the oar-paddle-like morphologies of the angular bone indicate a wide range of variation of this bone among different cynodonts (Figs. 1 and 3).

By comparison to the straight rod (plesiomorphic) in cynodonts, the ectotympanic ventral limb of mammaliaforms shows a slight curvature as the rod tapers toward its apex, as in the exemplary *Sinoconodon* and *Morganucodon* (Luo, 2011), and as widely documented in docodontans (Meng et al., 2015; Zhou et al., 2019) (Figs. 1 and 3). During the rise of mammaliaforms, the ecto-tympanic underwent a transformation from a paddle-shaped ectotympanic bone, supplemented by a small tympanic membrane in cynodonts, to a slender ectotympanic bone suspending a proportionately larger tympanic membrane in mammaliaforms. This transformation likely occurred through some precursor character states in phylogenetically intermediate mammaliamorphs (trity-lodontids, trithelodontids and brasilodontids), all of which have a straight and thin reflected lamina of the angular, nearly identical to the ectotympanic hook of mammaliaforms, except being shorter (Fig. 3) (Sues, 1986; Allin and Hopson, 1992; Hoffman and Rowe, 2018; Martinelli, 2017).

The gracile and slender ectotympanic hook of mammaliaforms is more similar to the ectotympanic of mammals than to those of most cynodonts (Fig. 3). Nonetheless, the hook is relatively short – it is only half the length of the dorsal limb of the angular in *Sinoconodon, Morganucodon*, and docodonts (Ji et al., 2006; Meng et al., 2015), and even shorter in the mammaliaform *Vilevolodon* (Fig. 3). Relative to the dorsal limb length, the hook is shorter and less curved than those of crown mammals (Fig. 4), such as eutriconodonts (also known as "triconodonts") and spalacotherioids (or "acute-triangled symmetrodonts," or "stem therians" of the Mesozoic) (Luo et al., 2007; Meng et al., 2011; Zhou et al., 2019; Mao et al., 2020).

The ectotympanic hook of stem mammaliaforms is far shorter and less curved than those of extant platypus (*Ornithorhynchus*), let alone those of the echidna (*Tachyglossus*) and of all extant marsupials and placentals (Doran, 1879; Fleischer, 1973). The hook-like ectotympanic of stem mammaliaforms does not even approximate the 270 degrees of arc of the tympanic ring as seen in extant therians (Fig. 4) (Fleischer, 1973; Novacek and Wyss, 1986). The putative reconstruction of the tympanic membrane will be discussed below.

#### 2.14.3.2.2 Malleus

The malleus (articular) of *Sinoconodon, Morganucodon*, and haramiyidans is triangle-shaped and has a short retroarticular process with a pointed ventral apex (Figs. 1 and 2) (Crompton and Luo, 1993; Luo, 2011). This is similar (plesiomorphic) to the retroarticular process of cynodonts (Fig. 1), although it is slightly larger (apomorphic) than the homologs of cynodonts, as exemplified by the early cynodont *Cynognathus* (Fig. 3). It is possible that the body of the malleus in most cynodonts is short and small because it was incompletely ossified. However, there are some exceptions to this: the well-preserved articular (malleus) in the tritylodontid *Kayentatherium* is perfectly like those of mammaliaforms (Fig. 3) (Sues, 1986). In two other cynodonts - *Diademodon* and *Exaeretodon* – the base of the retroarticular process supports a curved hook that bears some resemblance to the mammalian malleus manubrium – the latter structure becomes a rod extending distally toward the center of the tympanic membrane in extant mammals (Fig. 3) (Brink, 1963; Liparini et al., 2013).

The triangular retroarticular process of mammaliaforms (and some cynodonts) corresponds to the base for the manubrium of the malleus, but the malleus lacks the delicate arm of the manubrium of extant mammals (Fig. 4) (Luo, 2011). In most stem mammaliaforms examined so far, the malleus apex does not show any elongate distal arm that could be interpreted as a manubrium. A gracile manubrium-like structure has not been found in the currently available fossils of *Morganucodon* (Crompton and Luo, 1993). A conservative interpretation is that the distal arm of the manubrium is not present in these mammaliaforms, with the possible exception of docodontans. There are two possibilities: either this delicate structure was cartilaginous and not ossified, or else it was not developed at all, which is indicated by the well-preserved periosteal surface of the retroarticular process in *Sinoconodon*, and haramiyidans.

## ◀

(PMME): the mandible has a neomorphic (and much expanded) pterygoid fossa in replacement of the postdentary trough of stem mammaliaforms; the trough and ridge structures for ear attachment are lost; the ectotympanic-malleus complex is medio-laterally bent away from the pterygoid fossa of mandible, made feasible by curvature of the ossified Meckel's cartilage. The middle ear elements are still connected anteriorly to the mandible by an ossified Meckel's cartilage fitting into the Meckel's sulcus. (D). *Maotherium* (a spalacotherioid "stem therian") with a similar middle ear that is bent medially by the curved Meckel's element, away from the mandible, but Meckel's cartilage is fully connected to the middle ear (Ji et al., 2009; Zhou et al., 2019; Schultz, 2020). This reconstruction is a correction on an interpretative error of a fossil fracture as an anatomical gap by Mao et al. (2020). (A), From Luo (2007) and Luo et al. (2016); (B), From ;Luo et al. (2017); (C), From Luo et al. (2007); (D), From Ji et al. (2002); Zhou et al. (2019).

A short manubrium of the malleus, demarcated by a distinctive bend from the base of the malleus, is known for docodontans (Ji et al., 2006; Meng et al., 2015). This morphology suggests that manubrial support in the central part of the tympanic membrane, as seen in extant mammals, may be partly developed in docodontans (Luo, 2011). The haramiyidan *Megaconus* also appears to have a manubrium (Zhou et al., 2013). Thus, the manubrium of the malleus evolved in some (although not all) mammaliaforms preceding the ancestor of crown Mammalia.

### 2.14.3.2.3 Ossified Meckel's Cartilage ("Prearticular")

In the historical literature on stem mammaliaforms, the ossified Meckel's cartilage (often abbreviated as "OMC" in literature) of crown mammals (*sensu* Li et al., 2001) was alternatively called the prearticular (e.g., Kermack et al., 1973). These redundant terms have now been unified (Luo, 2011; Meng et al., 2011). By historical convention of terminology for stem mammaliaforms, however, the ossified Meckel's cartilage happened to be called the "pre-articular", in keeping with the term "articular" (= malleus) for cynodont anatomy (Allin, 1975; Kermack et al., 1973; Sues, 1986). Also by fortuitous convention in stem mammaliaform anatomy, the groove on the dentary that houses the prearticular was always called Meckel's sulcus or groove (e.g., Kermack et al., 1973). The pre-articular is the anterior extension of the articular (= malleus) as historically used in the cynodont-mammaliaform literature, and Meckel's cartilage or Meckel's element for mammaliaforms and derived cynodonts, instead of the "prearticular" (Fig. 3).

It is a consensus (Kermack et al., 1973; Allin, 1975) that while the posterior part of the Meckel's cartilage became the malleus, the anterior part of this cartilage was present in ontogeny (equivalent to the intra-membranous part of Meckel's element in extant mammal) but did not fully ossify in adult in cynodonts (Fig. 3A), as is the case in extant reptiles (Anthwal et al., 2017). The cartilage was lost in fossilization due to lack of ossification. Nonetheless, the presence of the Meckel's can be inferred from a prominent (Meckel's) groove on the dentary, as evidenced by *Thrinaxodon* and *Cynognathus* (Kermack et al., 1973). Meckel's cartilage and its sulcus were interpreted as having been covered by the splenial bone in such cynodonts as *Thrinaxodon* and *Cynognathus* (Kermack et al., 1973; Allin, 1975). In derived cynodonts, the splenial bone was greatly reduced or even lost, resulting in an open groove along much of Meckel's sulcus (Sues, 1986; 2001; Hoffman and Rowe, 2018). In these derived cynodonts, such as *Kayentatherium*, the anterior part of Meckel's sulcus is an open groove, presumably occupied by the anterior part of the Meckel's cartilage that did not ossify. But the posterior part of the Meckel's groove is occupied by a short Meckel's cartilage that is ossified in the adult (a.k.a. the prearticular) (Fig. 3) (Sues, 1986; Hoffman and Rowe, 2018).

Proportionately to the mandible's length, the ossified part of Meckel's cartilage is longer in stem mammaliaforms than its counterpart in cynodonts. The Meckel's element reaches further forward along the Meckel's sulcus in mammaliaforms, than it does in advanced cynodonts (Fig. 3) (Kermack et al., 1973; Pancirolli et al., 2019). Also, the Meckel's element is much longer relative to the size of the middle ear (the ectotympanic and the malleus) in mammaliaforms, than in cynodonts. This is a derived feature of mammaliaforms.

Although the Meckel's sulcus is a universal and ancestral feature in cynodonts and mammaliaforms, this structure shows wide variation in its size and length. The anterior part of the Meckel's sulcus is a permanent groove in *Sinoconodon* and *Morganucodon* (Figs. 1 and 3) (Kermack et al., 1973; Crompton and Luo, 1993), in Haramiyavia (Luo et al., 2015a), and also in *Kuehneotherium* (Gill et al., 2014). In docodontans, the anterior part of the sulcus is present in young individuals and lost in older individuals (Schultz et al., 2017a; Panciroli et al., 2018, 2019). This pattern also occurs in the Jurassic haramiyidans (Fig. 3). The disparity of Meckel's sulcus among different mammaliaforms will be further discussed in the section on "Disparity of Meckel's cartilage in Mesozoic mammals."

#### 2.14.3.2.4 Tympanum Suspension by Ectotympanic Hook

An important new insight from the last three decades is that all advanced cynodonts and all stem mammaliaforms have a hook-like ventral limb of the ectotympanic (Figs. 1 and 3), but it was never a ring-shaped structure, despite its homology to the ectotympanic of extant mammals. The hook-like morphology of the mammaliaform ectotympanic is a more plesiomorphic state in character evolution to the more curved ectotympanic in more crownward clades of Mesozoic mammals.

There is now overwhelming evidence that the hook-like ectotympanic annulus provided suspension for a soft-tissue tympanic membrane, an anatomical reconstruction first proposed for *Morganucodon* and then adopted for other mammaliaforms (Kermack and Mussett, 1983; Allin and Hopson, 1992; Luo, 2011). The ectotympanic and the malleus that suspended the membrane are gracile, but the presumptive tympanic membrane would be larger (relative to skull size) in mammaliaforms than its counterpart in derived cynodonts, such as *Exaeretodon, Probainognathus and Kayentatherium* (Fig. 3) (Sues, 1986; Hopson and Kitching, 2001). In the primitive cynodonts *Thrinaxodon* and *Cynognathus*, the paddle-shaped reflected lamina still played a role in sound reception and conduction, as originally hypothesized by Allin (1975), but the reflected lamina further restricts the space allowable for the tympanic membrane. It is generally accepted that the relatively larger tympanic membrane in mammaliaforms would enable more air-borne sound to be directly received by the membrane (Allin, 1975; Luo and Crompton, 1994; Kemp, 2016). The entire mandible would still be capable of serving as a route for bone-conduction of low-frequency sound to the ear, by the side-to-side contact of the middle ear to the dentary (Luo et al., 2016). This will be further discussed below, in section on "Routes of Sound Conduction to Inner Ear".

It is important to point out an incorrect fossil reconstruction in the literature of the 1970s, in which the ectotympanic of mammaliaforms was reconstructed to be ring-shaped (e.g., Allin, 1975; Kermack and Mussett, 1983). This was purely hypothetical, since no actual fossil data had been available to those earlier studies. The well-preserved, newer fossils available since the 1990s have

provided overwhelming anatomical data that mammaliaforms had only a hook-like structure to suspend the tympanic membrane, but not a ring-like structure as previously reconstructed for *Morganucodon* (Allin, 1975; Kermack and Mussett, 1983). Allin's (1975) landmark study on the homology of the cynodont mandibular ear was so influential that, despite E. F. Allin himself already changing the *Morganucodon* reconstruction to a hook-like structure (e.g., Allin and Hopson, 1992, Fig. 4), others authors have continued to use the incorrect reconstruction as an idealized ancestral model of the mammaliaform middle ear (Han et al., 2017; Wang et al., 2019). The latter reconstructions are not supported by actual fossils of *Morganucodon*. Similarly, the well preserved middle ear bones of docodonts also show a short and hook-like ectotympanic and thus for this clade as whole (Fig. 3J) (Ji et al., 2006; Zhou et al., 2019), and not a ring-shaped structure as earlier postulated for docodonts (Lillegraven and Krusat, 1991).

#### 2.14.3.2.5 Incus

In all cynodonts and mammaliaforms, the incus consists of a dorsal plate for its cranial contact and suspension and a ventral cylindrical trochlea that articulated with the malleus. Both components underwent major changes in cynodont-mammaliaform evolution, plus a neomorphic stapedial process (Fig. 6).

The dorsal plate of the mammaliaform incus is homologous to the pointed short process (the crus brevis) of the incus of extant mammals (Fig. 6H and I). The dorsal plate is reduced to a small process brevis in crown mammals – the extreme reduction of this process has occurred in monotremes (Zeller, 1993; Novacek, 1993; Luo and Crompton, 1994).

The ventral trochlea of the mammaliaform incus corresponds to the malleus facet of the incus of extant mammals. While the trochlea of the incus maintained a mobile joint with the malleus in the "primary joint" of mammaliaforms (Luo and Crompton, 1994), the incus in therians has a saddle-shaped contact surface for the malleus for a synovial joint between the incus and malleus, but this joint is no longer a fully mobile hinge as in mammaliaforms (Doran, 1879; Williams et al., 1989; Bastl et al., 2017). By comparison to the cylindrical shape of the trochlea of mammaliaforms, the malleus contact of the incus is highly transformed



Figure 6 Evolution of mobile suspension of incus and the incus leverage for impedance match through cynodont-mammaliaform transition. (A). Thrinaxodon and (B). Probainognathus: the guadrate and guadratojugal (in purple color) extracted from their articulation with the squamosal. The quadrate-quadratojugal complex articulated in notches of squamosal (box: Probainognathus) helps to reinforce the quadrate-articular joint for craniomandibular function. (C). tritylodontid Bienotherium (a mammaliamorph, quadrate-quadratojugal complex). (D). tritheledontid Pachygenelus (quadrate only). (E). Brasilitherium (quadrate-quadratojugal complex). (F). mammaliaform Sinoconodon (quadrate only). (G). Morganucodon, the incus extracted and shown in inverted, dorso-posterior view, and the articulation of the incus to the crista parotica of the petrosal (box), a modern mammal-like mobile suspension. (H). placental Vulpes to show the derived characters of the incus in therians corresponding to plesiomorphic characters of mammaliaforms. (I). the monotreme Tachyglossus to show the derived characters of the flattened incus, and loss of saddle-shaped malleus contact, and even more reduced short process, as compared to therians. The primitive pattern of dual quadrate-quadratojugal is present in Brasilitherium, but absent in the tritheledontid Pachygenelus, and in mammaliaforms. The loss of the quadratojugal enhanced the agility of the incus, for ear function. The stapedial process is present in Morganucodon, Brasilitherium and in tritylodontids with some structural variations in the latter. But it is absent in tritheledontids and likely also absent in Sinoconodon. The stapedial process is a part of the incus leverage system, serving the function as a secondary transformer for impedance match. However, the evolutionary loss of the guadratojugal (black triangles) and the evolutionary gain of the stapedial process of the incus (blue arrows) are in conflict with each other, and show homoplasies on the cynodont-mammaliaform phylogeny. Different clades show parallelly derived structures of the incus, as evolutionary experiments for better functions in sound transition. (A), (B), (C), (D), (E), (F) and (G) drawings from Luo (1994); Luo and Crompton (1994); Luo (2007); (H), redrawn by Luo from Melkemper et al (2020); (I) redrawn by Luo from Zeller (1993) and Novacek (1993).

and varied in different crown mammal groups (Fig. 6). The incudo-malleolar contact became saddle-shaped in most therians (Evans and de LaHunta, 2013; Malkemper et al., 2020), or completely flat in monotremes (Zeller, 1993; Novacek, 1993; Luo and Crompton, 1994). These two bones become secondarily fused in some extant mammals (Tucker, 2017).

Another major evolutionary transformation of the incus through the cynodont-mammaliaform transition is a re-configuration of the cranial suspension of the incus (Luo and Crompton, 1994; Kemp, 2005). In cynodonts, the quadrate (=incus) is articulated with the quadratojugal, and the two bones are juxtaposed together (Fig. 6). The quadrate-quadratojugal complex is housed by two notches in the squamosal bone (Luo and Crompton, 1994; Kemp, 2005). These articulations helped to strengthen the quadrate-articular joint (the "primary jaw hinge") for the feeding function of the lower jaw. However, the extensive contacts of the quadrate with the cranium also reduce the mobility of the quadrate in transmitting sound.

Through the cynodont-mammaliaform transition, the quadratojugal is lost in *Pachygenelus* (a mammaliamorph cynodont), and in all mammaliaforms (Fig. 5) (Luo, 2007). Further, the dorsal plate of the mammaliaform incus is straddled onto the crista parotica of the petrosal, as in extant mammals (Luo and Crompton, 1994; Zhou et al., 2019). The cranial suspension for the incus had shifted from a plesiomorphic articulation through both the quadratojugal and the squamosal, to a simplified contact of the incus to the petrosal. This re-configuration of the incus articulation to the cranium contributed to a greater mobility of the middle-ear bones, which is essential for hearing sensitivity (Fig. 6) (Luo and Crompton, 1994).

The stapedial process is a new development of the incus in some cynodonts and mammaliaforms. The stapedial process of the incus is a part of the middle ear lever system for transmitting sound with impedance matching (Durrant and Lovrinic, 1984; Clack and Allin, 2004). This novel structure of the incus contributes to the impedance match of the middle ear, with the incus as a secondary transformer (Kermack and Mussett, 1983). The stapedial process of the incus is absent in most nonmammaliaform cynodonts except tritylodontids and brasilodontids (Sues, 1986; Bonaparte et al., 2005). By comparison, most mammaliaforms have this stapedial process (Kermack et al., 1981; Luo and Crompton, 1994), except *Sinoconodon* (Luo, 2007).

Each of these evolutionary changes of the incus helps to gain some biomechanical functions of the middle ear, collectively contributing to a better capacity for hearing. However, these functionally relevant characters of the incus did not evolve in unison in cynodont-mammaliaform phylogeny (Liu and Olsen, 2010; Huttenlocker et al., 2018; Wallace et al., 2019). For example, in *Pachygeneus*, a derived and crownward mammaliamorph (Fig. 6), the quadrate has no stapedial process (primitive) but likely has lost its quadratojugal (derived) (Luo and Crompton, 1994). Tritylodontids and brasilodontids have a stapedial process (derived) but also retain a very large quadratojugal (primitive). The middle ear as a whole and also parts of the incus, show an incongruent and homoplastic phylogenetic pattern among the transitional mammaliamorphs to mammaliaforms. Thus an inevitable inference is that these characters of the incus, as mapped on an evolutionary tree, show certain homoplasy (Fig. 6) (Luo, 2007; Luo et al., 2016). A plausible evolutionary scenario is that different clades developed some (but not all) novel structures of the incus, as evolutionary experiments. The evolution of the stapedial process, and the greater mobility of the incus via a simplified cranial suspension, evolved at a different pace in different mammaliaform clades (Fig. 6).

# 2.14.3.2.6 Stapes

Most cynodonts, ranging from the early Triassic *Thrinaxodon* to the late Triassic *Brasilodon*, have a relatively large stapes with a prominent stapedial foramen bound by two crura. The proximal (fenestra) end and the distal (incus) ends of the stapes are of approximately the same size (Allin and Hopson, 1992), although individuals of the same clade can show significant variations in the size of the stapedial foramen, thickness of the crura, and extent of the postero-dorsal process (Gaetano and Abdala, 2015). This type of stapes is called a symmetrical bicrural stapes (*sensu* Schultz et al., 2018) (= "columelliform-perforate stapes" of Novacek and Wyss, 1986).

*Brasilitherium*, a crownward mammaliamorph cynodont very close to mammaliaforms (Bonaparte et al., 2005; Liu and Olsen, 2010), has such a symmetrical bicrural stapes (Fig. 1) (Rodrigues et al., 2013). This is an ancestral condition from which the asymmetrical bicrural stapes evolved in mammaliaforms (*sensu* Schultz et al., 2018). It is from the latter pattern of the asymmetrical bicrural stapes that the crown mammals evolved their very diverse stapes structures (Duran, 1879; Schultz et al., 2018).

The asymmetrical bicrural stapes of stem mammaliaforms shows several derived characters that are absent in cynodonts. The most prominent feature is that the lateral (incudal) end of the stapes is substantially smaller than the larger proximal stapedial footplate that fits into the fenestra vestibuli, as exemplified by *Sinoconodon* (Fig. 3) (Luo, 2007). Although an earlier study had reconstructed a cynodont-like "columelliform" stapes for *Morganucodon* (Kermack et al., 1981; Novacek and Wyss, 1986), the more accurate (and recent) reconstruction of a later study (Allin and Hopson, 1992) shows a substantially smaller stapedial head compared to the footplate for *Morganucodon*. The most convincing and new evidence comes from docodontans, which demonstrates a size disparity between a smaller stapedial head and much larger footplate in *Haldanodon* and *Borealestes* (Ruf et al., 2013; Panciroli et al., 2018). This is a major apomorphy for mammaliaforms (Fig. 3), which is also present in basal members of multituberculates, a major Mesozoic clade of crown mammals (Schultz et al., 2018). This feature is also present in spalacotherioids (Mao et al., 2020). The disparity of the lateral end and the large footplate and the two crura give the stapes the familiar and iconic appearance of a stirrup, as well known for placental mammals. The stapedial head vs. footplate size disparity became even more pronounced and varied in many clades of crown mammals (Doran, 1879; Novacek and Wyss, 1986).

Another apomorphy of mammaliaforms is the asymmetrical placement of the two crura on the foot plate: the anterior crus is connected to (or near) the center of the footplate, while the posterior crus is connected to the posterior margin (or nearly so), as best exemplified by docodontans (Fig. 4) (Ruf et al., 2013; Panciroli et al., 2018). This asymmetry is also present in the less well

preserved stapes of *Morganucodon* and *Sinoconodon* (Kermack et al., 1981; Luo et al., 2016), and is likely the most prevalent morphology of all mammaliaforms (Schultz et al., 2018).

The third derived feature of mammaliaforms is that the footplate has a precise fitting to the grooved rim of the fenestra vestibuli, although this appears to be already partially developed in the derived mammaliamorph cynodont *Brasilitherium* (Rodrigues et al., 2013), possibly also in tritylodontids (Luo, 2001). This is in contrast to the fenestra vestibuli of cynodonts, which is characterized by a thickened and elevated rim around the periphery of the fenestra.

The stapedial footplate has a circular outline in the stem mammaliaforms examined so far (Kermack et al., 1981; Panciroli et al., 2018). This is a plesiomorphic feature of cynodonts (Gaetano and Abdala, 2015; Pusch et al., 2019), and also a common feature of multituberculates (Meng, 1992; Schultz et al., 2018) and monotremes (Fleischer, 1973). The stapedial footplate of docodonts has a bullate shape that is slightly flaring outward around the periphery and convex in the center towards the fenestra vestibuli (Ruf et al., 2013; Panciroli et al., 2018). This is also known in some extant marsupials (Schmelzle et al., 2005). The footplate can also be nearly flat, as seen *Sinoconodon* and *Morganucodon* (Kermack et al., 1981; Luo, 2007). Recently, the stapes of the two species of haramiyidan *Arboroharamiya* have been described to be symmetrical bicrural stapes with a large posterior process, similar to those of cynodonts (Meng et al., 2016; Han et al., 2017). However, Schultz et al. (2018) noted that the proximo-distal orientation of these stapes (*sensu* Meng et al., 2016) should be the opposite. After this correction, the stapes of *Arboroharamiya* would have the asymmetrical bicrurate pattern (Schultz et al., 2018).

Among Mesozoic clades of crown mammals, the Jurassic multituberculate *Pseudobolodon* has an asymmetrical bicrural stapes (Schultz et al., 2018). The more derived Cretaceous multituberculate *Kryptobaatar* also appears to have this stirrup morphology (Rougier et al., 1996a). The Paleocene multituberculate *Lambdopsalis* was previously interpreted to have a columnar microperforate stapes with a single crus to the center of the footplate (Meng, 1992), but this has been reinterpreted to be an incomplete preservation of just one remaining crus of the two presumptive crura of the stapes. Thus *Lambdopsalis* should have an asymmetrical bi-crurate stapes, as well (Schultz et al., 2018).

For crown mammals, monotremes have columelliform stapes (Fig. 5G), Marsupials show both columelliform and bicrurate stapes (Novacek and Wyss, 1986; Schmelzle et al., 2005), whereas placentals mostly have bicrurate stapes. There also exists, however, a vast range of other forms, which have been extensively documented in the literature (Doran, 1879; Novacek and Wyss, 1986). It has been proposed that the bicrurate stapes in a stirrup shape is evolutionarily ancestral for crown mammals, while the columelliform stapes of monotremes and some marsupials are secondarily derived, as is the symmetrical, bicrurate stapes of many placentals (Schultz et al., 2018). A recent finding that the spalacotherioid *Origolestes* has a bicrurate stapes (Mao et al., 2020) is consistent with this interpretation.

#### 2.14.3.3 Disparity Among Mammaliaforms

In contrast to crown mammals, stem mammaliaform clades have many plesiomorphic features consistent with each other, which can unify an ancestral middle ear morphotype. However, these do not preclude the middle ears of stem mammaliaform from being morphologically disparate from each other. Stem mammaliaforms evolved divergently, in their own right. The highly specialized docodontans and haramiyidans show anatomical disparity in their middle ears, and each mammaliaform clade can be specialized in its own way, consistent with the fact that these groups are very different in their jaws and teeth, and in their skeletons. The disparity of middle ear bones are manifest in their size and shape, and in their attachment to the mandible (Fig. 3) (Meng et al., 2015; Luo et al., 2017).

Major clades of mammaliaforms exhibit a range of variation in the mandibular structures for the middle ear: size difference of the postdentary trough, variation of grooves and fosse in this trough, curvature of the Meckel's sulcus and its ontogenetic changes (Fig. 3). Even though the delicate ear bones are not preserved in the majority of mammaliaform fossils, the mandibular structures, which are proxies to the middle ear, are more often preserved. The variation of these mandibular structures clearly indicates that the ear-mandible attachment is far from uniform, and can vary to a significant extent across stem mammaliaform groups.

The mandibles with middle ears of *Sinoconodon, Morganucodon*, and docodontans are familiar examples (Fig. 3). These mandibles have a pointed angular process, which bears a concavity that is continuous with the posterior opening of the postdentary trough (*Morganucodon*, Fig. 3E). The ectotympanic hook is nestled in this angular concavity on the dentary in *Sinoconodon, Morganucodon*, and *Hadrocodium*. This angular concavity is more developed in docodontans and becomes more deeply incised into the angle of the dentary, as seen in *Castorocauda, Agilodocodon, Docodon* and *Microdocodon* (Ji et al., 2006; Luo et al., 2015b; Schultz et al., 2017a; Zhou et al., 2019).

Haramiyidans do not have a pointed mandibular angle, in contrast to *Sinoconodon*, *Morganucodon*, and docodontants (Figs. 1 and 2). In the Late Triassic *Haramiyavia* – a haramiyidan - the "angular region" of the mandible is instead represented by a shallow convexing contour; the opening of the postdentary trough is narrower than those of other mammaliaforms (Fig. 3F) (Jenkins et al., 1997; Luo et al., 2015a). On comparison to the hook-like ectotympanics of advanced cynodonts (Fig. 3), it can be inferred that in the earliest representative of haramiyidans likely a similar ectotympanic would still be attached to the mandible if intact, but its hook likely is slightly separated from the angular process, in a similar arrangement as in cynodonts. *Kuehneotherium* and the morganucodontan *Megazostrodon* have a similar rounded angular region of the mandible as in *Haramiyavia*, despite being entirely different from *Haramiyavia* in molar structures (Gow, 1986; Gill et al., 2014; Kielan-Jaworowska et al., 2004). Although the middle ear bones are not preserved in these fossils, it is probable that *Kuehneotherium* and *Megazostrodon* have a short ectotympanic hook

that attached to the mandible, as seen in cynodonts and other mammaliaforms (Fig. 3). The narrow opening of the postdentary trough would correspond to a more acute angle of the ectotympanic hook in these stem mammaliaforms.

In *Vilevolodon* - a Jurassic member of haramiyidans - the anterior limb of the ectotympanic and the Meckel's elements are very short, and the corresponding postdentary trough on the mandible is also short (Fig. 3H). If *Vilevolodon* and its related *Xianshou* are still held together with *Haramiyavia* in the same haramiyidan clade (Huttenlocker et al., 2018), then there is a significant reduction in the postdentary trough, and a shortening of Meckel's sulcus, in the more derived taxa such as *Vilevolodon*, than in the basal members such as Haramiyavia, in the same haramiyidan clade. This suggests that the shortening of Meckel's element in *Vilevolodon* - the most derived member of the clade – likely evolved independently from a long Meckel's element in the more basal members of haramiyidans. This is a noteworthy evolutionary pattern - an independent size reduction in the middle ear elements among haramiyidans, in sharp contrast to the un-reduced middle ears of other mammaliaforms (Figs. 3, 4 and 5). In an alternative phylogenetic scenario, in which *Vilevolodon* and other Jurassic haramiyidans are separated into an independent clade from the Triassic *Haramiyavia* and kin (Krause et al., 2020), this parallel reduction of the middle ear elements still holds up.

There is now clear ontogenetic evidence that in stem mammaliaforms, Meckel's element and its dentary sulcus have changed in different stages of growth. The length and thickness of Meckel's element can differ with the age of individuals (Schultz et al., 2017a; Panciroli et al., 2019). In the Late Jurassic *Docodon victor*, Meckel's sulcus extends anterior to the mandibular symphyses and over the length of the jaw in younger individuals; but the sulcus (by inference the Meckel's element) has receded posteriorly and is relatively shorter to the jaw length in older adults in the growth series of *Docodon victor* (Schultz et al., 2017a). In the Middle Jurassic *Borealestes serendipitus* (Panciroli et al., 2019), the preserved Meckel's element is thread thin in younger individuals, and becomes enclosed by the growth of the dentary in older individuals (Panciroli et al., 2019). If this can be corroborated in other docodontans, this suggests that, within the docodontan clade, there is an independent reduction of Meckel's element in docodontans, as in haramiyidans. In summary, even before the cynodont-mammal lineage evolved the detached (and definitive) mammalian middle ear, the stem mammaliaforms developed disparate morphologies of the middle ear, in a series of evolutionary experiments (Figs. 2 and 3).

## 2.14.3.4 Inference of Function of Stem Mammaliaforms

A basic anatomical premise for estimating the hearing capacity of mammaliaforms is that in all stem groups of mammaliaforms, the middle ear is solidly attached to the mandible. Further, the shape of the ectotympanic and the malleus are similar to those of advanced cynodonts, although more gracile, and likely more mobile. The ectotympanic is a hook (not a ring-like) structure and the malleus in most stem mammaliaforms is triangle-shaped, without a slender manubrium to attach to the tympanic membrane.

Based on an assumption that *Morganucodon* would have a ring-like structure, Kermack and Mussett (1983) over-estimated the tympanic membrane to be about 4.5 mm<sup>2</sup> in the surface area for receiving sound. After converting this to an effective functional area (2/3 of the tympanic membrane area), the primary transformer ratio (effective area of tympanum/area of fenestra vestibuli) worked out at 10.7. When multiplied with the lever ratio of the incus of 2.7, the overall (ectotympanic + malleus + incus) transformer ratio is 28.9, which would approach the same ratio of extant echidna, and would be within the range of extant mammals as a whole (Kermack and Mussett, 1983). Given that new and better-preserved fossils unavailable in the 1970s have now shown a short hook-like ectotympanic and a malleus lacking a manubrium (contra Kermack and Mussett, 1983: Fig. 6), our functional estimate will be different from the earlier estimate of hearing function that had assumed a "ring-shaped" ectotympanic and a malleus manubrium for *Morganucodon* (Kermack and Mussett, 1983; Rosowski, 1992).

A more up-to-date estimate is that the area of the tympanic membrane would be up to 3 mm<sup>2</sup> and its effective functional area about 2 mm<sup>2</sup>. A realistic estimate of the primary transformer ratio of tympanum/fenestra vestibuli would be 7.18, multiplied by the incus transformer ratio of 2.7 would give a final transformer ratio for *Morganucodon* of 19.38, about 65% of the final ratio of the echidna at 30. This revised and more conservative estimate for *Morganucodon* is further below the estimate of monotremes by Kermack and Mussett (1983).

This ad hoc estimate of hearing capacity of *Morganucodon* remains the default interpretation (although see Rosowski, 1992 for an alternative suggestion). We suggest that, the hearing capacity are basically similar across major groups stem mammaliaforms, on the evidence that their entire middle ear is solidly attached to the postdentary trough mandible and Meckel's element is still connected to the mandible in all mammaliaforms (Kermack sand Mussett, 1983; Luo et al., 2016). This question needs further investigation by modeling other mammaliaforms in similar ways as for *Morganucodon*, by incorporating the anatomical correction from the newer fossils.

# 2.14.4 Middle Ear Evolution in Crown Mammals

# 2.14.4.1 Middle Ear Morphotypes

A basic structure of middle ears of crown mammals is the Partial Mammalian Middle Ear (PMME) (or Transitional Mammalian Middle Ear), as exemplified by two Mesozoic mammal clades, eutriconodontans ("triconodonts") and spalacotherioids ("stem therians") (Figs. 4 and 5). The PMME of crown mammals is characterized by a suite of derived characters of the mandible and the middle ear that are absent in stem mammaliaforms, including *Hadrocodium* and the Jurassic haramiyidans, all of which retain the mandibular middle ear of cynodonts (Fig. 5).

In the PMME, the posterior part of the mandible has a broad, crescent-shaped pterygoid muscle fossa bordering on a pterygoid shelf (Fig. 5C and D). These are completely different from the postdentary trough of mammaliaforms, which is bound above by

a medial ridge (Fig. 5A and B). In other words, the pterygoid fossa of mammals has "replaced" the postdentary trough as seen in stem mammaliaforms. Thus, in these two crown mammal clades and unlike in stem mammaliaforms, there is no reciprocating structure on the mandible for the ear to be attached side-by-side (Fig. 5).

It is important to note that not all characters in PMME are derived. In both eutriconodonts (Luo et al., 2007; Meng et al., 2011), and in spalacotherioids (Zhou et al. 2019, but compare Mao et al., 2020), the ear is still connected by an ossified Meckel's cartilage in continuity with the malleus and the ectotympanic. This anterior connection by itself, is a primitive feature.

A novel pattern of PMME is that the middle ear is medially displaced from the posterior part of the dentary while keeping its anterior connection via the Meckel's element to the dentary (Fig. 5). In the neonatal stage development of the middle-ear in extant monotremes, the middle ear and Meckel's cartilage are displaced in a similar pattern: the middle ear is rotated away and separated by a gap from the mandible (Zeller, 1989; Luo et al., 2007; Takechi and Kuratani, 2010). This configuration is associated with the curved external meatus in monotremes that, from the external aperture of the meatus on the lateral aspect of head, guides the sound to the tympanic membrane deep in the ventral part of the skull (Rugé, 1898). The PMME in Mesozoic mammals, which are phylogenetically intermediate between extant monotremes and extant therians, likely had a similarly curved external auditory meatus (more discussion below in Section on "External Ear").

The ectotympanic of crown mammals is more derived than those of mammaliaforms. It is slightly more curved in eutriconodont and spalacotherioid mammals than in stem mammaliaforms (Figs. 4 and 5). But basically it is still a hook-like structure (primitive). The curvature of its ventral limb is less than a 60° arc for eutriconodontans and spalacotherioids (Figs. 4 and 5) (Luo et al., 2007; Zhou et al., 2019; Mao et al., 2020). Multituberculate mammals, which are phylogenetically intermediate between eutriconodontans and spalacotherioids (Figs. 2 and 4), also have a hook-like ectotympanic, as is well documented for several taxa from the Late Jurassic and Early Cretaceous (Luo et al., 2017; Zhou et al., 2019; Wang et al., 2019). An earlier interpretation indicated that the Paleocene multituberculate *Lambdopsalis* had a ring-like ectotympanic (Miao, 1988; Meng and Wyss, 1995; Luo, 2011), but this schematic reconstruction has been shown to be inaccurate (Rougier et al., 1996a; Schultz et al., 2018). The ectotympanic of *Lambdopsalis* should be interpreted to be a hook-like structure. Taken together, the ectotympanic hook with a curvature of less than 60-degrees of arc is most probably the ancestral condition for crown Mammalia (Fig. 5C and D) (Luo et al., 2016; Anthwal et al., 2017).

In the platypus *Ornithorhynchus*, the ectotympanic ventral limb has an arc of about 90°, and 120° in the echidna *Tachyglossus*. The majority of marsupials and placentals have the ectotympanic curved more than 120°, beyond the condition of monotremes (Fleischer, 1973), which gives the bone the iconic "ring-like" morphology in living mammals (Figs. 1 and 4).

The ring-shaped morphology of the ectotympanic must have evolved in separate lineages for monotremes and forcrown therians (Fig. 2), given that the ancestral ectotympanic is curved to a much lesser degree in eutriconodonts, in multituberculates and in spalacotherioids, the three most species-rich and abundant Mesozoic clades of crown mammals. Some earlier studies (e.g., Luo, 2011; Luo et al., 2016) offered alternative hypothesis that the lesser curvature of the ectotympanic in eutriconodonts would be an atavistical reversal if the over 90 degrees of ectotympanic of extant monotremes would represent the ancestral condition of the crown Mammalia. This proposal can, however, now be rejected, with the more recent discoveries of a hook-like ectotympanic in spalacotherioids (Zhou et al. 2019; Mao et al., 2020), and in several well-preserved fossils of multituberculates (Luo et al., 2017; Zhou et al., 2019; Wang et al., 2019).

Taken together, the Mesozoic clades of mammals show quite clearly that the hook-like morphology is the ancestral pattern of the ectotympanic for crown Mammalia, not a ring (Figs. 4 and 5). This does not, however, preclude some extant mammal clades from having a wide variation in ectotympanic curvature, such as the different ectotympanic arcs in the platypus (*Ornithorhynchus*) and the echidna (*Tachyglossus*) of the monotreme clade (Fleischer, 1973; Luo, 2011).

### 2.14.4.2 Independent Evolution of DMME in Monotremes and Kin

On the best available evidence so far, we can now propose that the last common ancestor of crown Mammalia is characterized by a suite of novel characteristics of the partial mammalian middle ear PMME (Figs. 4 and 5). Within the crown Mammalia, there is significant morphological diversity of the middle and inner ears in extant mammals (Ekdale, 2016; Luo et al., 2016), and also in the extinct Mesozoic mammal clades (Zhou et al., 2019; Wang et al., 2019), which must be accounted for, in comparative studies of monotremes, marsupials and placentals (Fig. 4).

Extant monotremes (platypuses and echidnas) have a detached middle ear (DMME) like their marsupial and placental mammal relatives. However, the DMME of extant monotremes represents an independent evolution, given that the several extinct Mesozoic relatives of the monotreme clade still have the ancestral MMEC, like those of stem mammaliaforms (Fig. 4).

Two of the earliest-known fossil monotremes that have preserved the ear attachment structure of the dentary are *Steropodon* (Archer et al., 1985) and *Teinolophos* (Rich et al., 2001), both from the Early Cretaceous of Australia. *Steropodon* has a wide sulcus for Meckel's element, a feature can be reliably interpreted to have housed a Meckel's element in the same manner as in eutricono-dontans and spalacotherioids (Luo et al., 2002; Rich et al., 2016), but the more posterior part of the mandible corresponding to the postdentary trough is missing in this fossil.

*Teinolophos* was originally described to have preserved a full mandibular trough, plus a distinctive Meckel's groove similar to that containing the Meckel's element in eutriconodonts and spalacotherioids (Rich et al., 2005; 2016). The reconstruction of soft-tissue structure for the presumptive mandibular trough of this taxon has been debated. One interpretation is that the trough would contain the postdentary bones of the middle ear as in stem mammaliaforms (Rich et al., 2005; Martin and Luo, 2005). An alternative view is that this putative trough is a large groove that housed a thick mandibular nerve similar to the hypertrophied

mandibular nerve of extant Ornithorhynchus (Bever et al., 2005; Rougier et al., 2005; Rowe et al., 2008). But the interpretation that *Teinolophos* had a hypertrophied mandibular nerve has been also challenged (Phillips et al., 2009). Regardless of the debate on the trough, good evidence of several specimens supports the consensus that the Meckel's element was attached to the Meckel's sulcus in *Teinolophos* (Rich et al., 2016). In the simplest case scenario, the middle ear would still be attached to the mandible via the Meckel's element in *Teinolophos*.

In the broader phylogeny, monotremes (including their stem fossils from the Early Cretaceous) is a subclade of the australosphenidan group that has a well-documented fossil record across Mesozoic Gondwana landmasses (Luo et al., 2001a,b; Martin and Rauhut, 2005; Rougier et al., 2007; O'Meara and Thompson, 2014). The Early Jurassic fossils of this group, such as *Henosferus* and *Asfaltomylos* possess a postdentary trough and a Meckel's sulcus (Martin and Rauhut, 2005; Rougier et al., 2007). The Early Cretaceous fossils of this clade from Australia – *Ausktribosphenos* and *Bishop* – also have a postdentary trough and a Meckel's sulcus (Rich et al., 2016). Mapped on the phylogenetic tree (Rougier et al., 2007; O'Meara and Thompson, 2014), the ancestral condition of the entire australosphenidan group would be a mandibular middle ear (as inferred from the postdentary trough). The ancestral state of the middle ear for Cretaceous fossil monotremes is either a mandibular middle ear (Rich et al., 2005), or a partial mammalian middle ear (Rich et al., 2016). Thus, the detached middle ear (DMME) must be an independent acquisition by living monotremes, after their split from the Cretaceous fossil monotremes (Fig. 4).

## 2.14.4.3 Disparity of Ossified Meckel's Cartilages

A key element for deciphering how the middle ear transformed during mammal evolution comes from the comparative differences of Meckel's element between mammals and other vertebrates, and from the changes of the development of the mammalian Meckel's cartilage (Gaupp, 1913; Goodrich, 1930; De Beer, 1937). Meckel's cartilage is a thin rod and relatively uniform along its length in extant monotremes and therians. It consists of a symphyseal portion, a middle portion, and a posterior portion. The symphyseal portion is where the anterior ends of the two Meckel's cartilages are united in the mandibular symphysis (Zeller, 1989; Evans, 1995). The middle portion of Meckel's cartilage is intramandibular in late embryonic stages; this portion becomes incorporated into the mandible in later development (Zeller, 1989; Evans, 1995; Harada and Ishizaki, 1998). The posterior part the Meckel's cartilage occupies an open Meckel's sulcus in the development of the mandible and is linked to the anlagen of the malleus and incus (Evans, 1995; Harada and Ishizaki, 1998; Anthwal et al., 2017). The characteristics of these components of the Meckel's are consistent between monotremes and therians (De Beer, 1937).

The relatively uniform morphology of a thin Meckel's cartilage of extant mammals is, however, an under-representation of the evolutionary differences and ontogenetic variations of this structure in Mesozoic mammaliaforms. Fossil discoveries from recent decades have revealed some significant evolutionary differences and ontogenetic variations in all three components (the symphysal, the intramandibular, and the posterior) of Meckel's cartilage among mammaliaform groups. The structural disparity of Mesozoic mammaliaforms is far greater than could be inferred only from the relatively simple morphology of Meckel's cartilage of embryonic-neonatal stages of extant mammals. A better understanding of mammalian middle-ear evolution must take into account the variant morphologies of Meckel's elements of Mesozoic mammal fossils.

Sinoconodon, Morganucodon and Haramiyavia (at least in some specimens) have preserved evidence of the symphyseal portion of Meckel's groove, suggesting that the symphyseal portion of Meckel's cartilage was persistent into adulthood (Kermack et al. 1973; Crompton and Luo, 1993; Luo et al., 2015a). However, docodontans are different in this feature and show an ontogenetic change that has evolutionary implications. The anterior part of the Meckel's sulcus is present in juvenile individuals, but absent in adults of the same taxon, as seen in two docodontans (Schultz et al., 2017a,b; Panciroli et al., 2019). Thus the symphyseal portion of the Meckel's element was incorporated into the mandible in adults of docodontans, somewhat similar to the ontogenetic change of the symphyseal part of this cartilage in extant mammals (Zeller, 1989; Evans, 1995).

In docodontans and also in *Morganucodon*, the middle portion of the Meckel's sulcus, which corresponds to the intramandibular portion of the Meckel's in extant mammals, changes with ontogeny. It tends be straighter and wider in juveniles, but is reduced and curved to border on the ventral margin of the mandible in adults (Fig. 3). The middle portion of the ossified Meckel's cartilage ("prearticular") may become incorporated into the mandible (Kermack et al. 1973; Panciroli et al., 2019). This is similar to the ontogenetic change where the middle portion of Meckel's becomes incorporated into the dentary in extant mammals (Zeller, 1989; Evans, 1995; Harada and Ishizaki, 1998). The developmental differences of these components of Meckel's can be traced, with limited success, back into some mammaliaforms, as demonstrated in the case studies of mandibles of docodonts (Schultz et al., 2017a; Panciroli et al., 2019).

Perhaps most interesting is an ontogenetic change in the length of Meckel's element in docodontants. In older individuals of *Docodon* and *Borealestes*, there is clearly a posterior shift and concurrent shortening of Meckel's sulcus in larger mandibles of likely older individuals (Schultz et al., 2017; Panciroli et al., 2019). A similar (albeit weaker) pattern is also documented in *Morganucodon* (Kermack et al., 1973). This suggests that in some mammaliaforms, relative to the length of the mandible, the ossified Meckel's cartilage likely became shorter in older individuals.

The evolutionary reduction of Meckel's element does not show a simple linear transformation from stem mammaliaforms to the crown mammals of the Mesozoic. In *Morganucodon* and *Borealestes*, the preserved Meckel's element is very thin, about 0.1 mm in width (Kermack et al., 1973; Panciroli et al., 2019). But the ossified Meckel's cartilage of *Maotherium* is much thicker, about 0.4 mm wide and 0.2 mm deep at its thickest point. Fossilized Meckel's cartilages of eutriconodonts show a wider range of thickness, from about 0.4 mm in *Yanoconodon* and *Jeholodens* (Luo et al., 2007) to the thicker structure of this element in *Gobiconodon* 

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(Li et al. 2003). The most massive Meckel's cartilages at 3–4 mm at the widest point in *Repenomamus* (Li et al., 2001; Meng et al., 2003) is the largest example of the size variation among the gobiconodontid taxa. Relative to the mandible, the ossified Meckel's cartilage is the thickest in *Liaoconodon* and *Spinolestes* (Meng et al., 2011; Martin et al., 2015), even surpassing that of *Repenomamus*.

The stem mammaliaforms are more plesiomorphic than crown mammals in many features. But in size and thickness of the Meckel's element, *Morganucodon* and docodontans have a far slenderer Meckel's cartilage, which could be autapomorphic (uniquely derived), or even more derived than the thicker Meckel's elements (more plesiomorphic) in eutriconodontans and spalacotherioids, despite the latter groups being phylogenetically more crownward to extant therians. The more crownward clades like spalacotherioids can have a less reduced (thus more plesiomorphic) Meckel's element; this helps to show that the reduction of Meckel's cartilage, a precondition to the eventual disconnection of the middle ear, had many homoplasies, and its evolution did not follow a linear trajectory.

Fossilized Meckel's cartilage has now been found in many Mesozoic mammal specimens – but this body of new fossil evidence is totally outside the scope of the historical comparative anatomy paradigm, which has historically focused on extant vertebrates only (e.g., Maier and Ruf, 2016). These fossilized Meckel's cartilages in Mesozoic mammals are different in size and shape from those in extant mammal embryos and neonates. Naturally, the first finding of ossified Meckel's cartilage in extinct mammals (Li et al., 2001) was questioned (Rougier and Wible, 2006), until later discoveries of better fossils show the middle ear and Meckel's in intact connection (Luo et al., 2007; Meng et al., 2011), not only in eutricondontans, but also in the separate lineage of spalacotherioids (Ji et al., 2009; Zhou et al., 2019). The discovery of the ossified Meckel's cartilage and the connected middle ear *in situ* on the mandible of the spalacotherioids *Maotherium* and *Origolestes* (Figs. 4 and 5) (Zhou et al., 2019; Mao et al., 2020) indicates that Meckel's cartilage is indeed connected to the mandible in spalacotherioids, as previously interpreted for *Zhangheotherium* (Meng et al., 2003).

Maier and Ruf (2016) questioned the massive Meckel's element in the eutriconodont *Repenomamus*, and noted that it seems to be too large by comparison to the relatively small size of the embryonically transient Meckel's cartilage of extant mammals. However, the ossified Meckel's cartilage in eutriconodonts has a wide range of sizes. The thinner Meckel's element of *Yanoconodon* and *Jeholodens* represents the smaller extreme of a size variation continuum, and approximates the size range of the Meckel's in embryos and neonates of extant mammals. Meckel's element appears to be massive in all gobiconodontids such as *Repenomamus* and *Spinolestes*, and in *Liaoconodon* (Meng et al., 2011; Martin et al., 2015). But these massive Meckel's elements are a structure of adults or sub-adults, but not embryos or neonates. For example, the eutriconodont *Spinolestes* possesses both an ossified Meckel's cartilage before the completion of tooth replacement (Martin et al., 2015), and the fossil was well beyond the neonate stage. Therefore it may not be reasonable to compare the difference in absolute size of the Meckel's between neonates of extant mammals, and fully grown adult mammals of the Mesozoic.

Meckel's cartilage can still ossify in extant mammals under certain teratological conditions, as seen in the Treacher-Collins syndrome in humans (Tucker, 2017), or induced by morphogenetic experiments in model mammals (Oka et al., 2007; Anthwal et al., 2017; Urban et al., 2017). Although Meckel's cartilage does not normally ossify in extant mammals, the genetic and developmental capacity to retain a large Meckel's cartilage into adulthood is conserved from their evolutionary ancestry.

Fate-mapping studies show that Meckel's cartilage chondrocytes have the capacity for transformation into osteoblasts, fibroblasts, and osteocyte-like cells (Harada and Ishizeki, 1998; Anthwal et al., 2013; 2017). Thus, the genetic basis for ossification of Meckel's cartilage is conserved as a deep homology, at least in *Mus musculus* (laboratory mice). The enhanced signaling of *Bmp2* and *Bmp7* of the BMP family involved in chondrogenesis can "re-generate" a larger Meckel's cartilage under certain experimental conditions (Wang et al., 2013). Thus, developmental experiments support the notion that Meckel's cartilage can be retained and ossified (Anthwal et al., 2017; Urban et al., 2017). An ossified Meckel's cartilage may even become enlarged (Wang et al., 2013), enough to overlap the size range of this element as observed in some eutriconodonts.

There are now over 20 recognized clades of Mesozoic mammaliaforms (Kielan-Jaworowska et al., 2004; Close et al., 2015; Zhou et al., 2019), compared to only three extant mammal clades. Given a greater clade diversity of extinct animals, it is a matter of course that Mesozoic mammaliaforms exhibit a greater morphological disparity than the extant descendants of Mammalia. The difference between the Meckel's cartilage of Mesozoic mammaliaforms and the embryonic counterpart in extant mammals is a reflection of the great disparity exhibited by Mesozoic mammals. Overall, the weight of the fossil evidence strongly supports the idea that the eutricondont and spalacotherioid groups possessed an ossified Meckel's cartilage, and thus a PMME rather than a fully developed DMME.

### 2.14.4.4 Dual Evolution of DMME in Therians and Kin

Extant therians and the several groups that are their relatives of the Mesozoic are united under Theriimorpha, a clade defined by the common ancestor of eutriconodonts, multituberculates, spalacotherioids, and dryolestoids through living placentals and marsupials (Figs. 2 and 4: node 8) (Rowe, 1988, 1993; Ekdale, 2016b). *Fruitafossor*, a Jurassic mammal belonging to its own clade, may also be placed, putatively, in the theriimorph group (Luo and Wible, 2005). Eutriconodonts, multituberculates and spalacotherioids are the stem clades of theriimorphs, and successively closer in relationship to living marsupials and placentals. These stem "therian" groups can provide information on the ancestral or precursor characters for reconstructing how the detached middle ears evolved in modern marsupials and placentals.

The ossified Meckel's cartilage has been documented for at least seven different taxa of eutriconodonts (Martin et al., 2015; Meng and Hou, 2016), and for four taxa of spalacotherioids (Hu et al., 1997; Ji et al., 2009; Zhou et al., 2019; Mao et al., 2020). In two eutriconodonts, the connections of the middle ear to the ossified Meckel's cartilage are intact (Luo et al., 2007; Meng et al., 2011). In two specimens of spalacotherioids, the middle ear is in association with Meckel's. Although Meckel's cartilage is fractured, it is a reliable interpretation that the middle ear is fully connected by bone to Meckel's (Zhou et al., 2019).

Recently, Mao et al. (2020) suggested that, in the spalacotherioid *Origolestes*, there is a gap linked by a ligament between posterior end of the ossified Meckel's cartilage and the anterior end of the middle ear. Thus the spalacotherioids would represent an intermediate condition for disconnection of the middle ear on the one hand, and the dentary with its attached Meckel's element on the other. According to our personal observation (Luo) of the same fossil, this gap-by-ligament is a fossil fracture, and not an anatomical structure. The intact middle ear would still be fully connected to the ossified Meckel's cartilage in the same way in other spalacotherioids and in eutriconodonts.

In these stem therian groups of the Mesozoic, the ossified Meckel's cartilage is relatively thick. It is curved at mid-length, and appears to be more strongly warped in the posterior portion (Fig. 5). The curvature of Meckel's cartilage allows for the middle ear bones to be medially displaced and rotated away from the mandible while retaining a full bony connection by ossified Meckel's cartilage in its mandibular sulcus (Fig. 5C and D) (Ekdale, 2016; Luo et al., 2016). The curvature of this element is slightly variable among eutriconodonts, and is especially warped in those taxa with more massive Meckel's cartilages (e.g., Martin et al., 2015). There is now strong evidence that, before the full evolutionary disconnection of Meckel's cartilage from the mandible by resorption, the middle ear was rotated away from the pterygoid fossa by a curved Meckel's element in the Partial Mammalian Middle Ear. This is the ancestral condition for the therian line of mammalian evolution. A secondary consequence of this rotation of Meckel's cartilage and the middle ear is that the tympanic membrane between the ectotympanic and malleus would be faced ventrolaterally (Fig. 5C), making it feasible for a curved external auditory meatus to be connected to the tympanum (This will be further discussed on section on "External Ear").

The PMME could also be the ancestral condition of all crown Mammalia (common ancestor of Monotremata and Therians: Fig. 2), as the fossil monotreme *Teinolophos* is recently re-interpreted to have a similar connection of Meckel's element to Meckel's sulcus of the dentary (Rich et al., 2016). Some historical studies (e.g., Rowe, 1988) posited that the detached middle ear of extant clades could represent the ancestral condition of crown Mammalia. If so, the PMME in spalacotherioids could be an evolutionary reversal among theriiform clades (Luo, 2011: Fig. 8, hypothesis B). This hypothesis is no longer tenable and can now be rejected based on new fossil evidence. The prevailing hypothesis is that the detached middle ear was convergently gained in extant monotremes, therians, and multituberculates in three different lineages (Fig. 4) (Luo, 2011: Fig. 8 Hypothesis A; Meng et al., 2011; Luo et al., 2016; Zhou et al., 2019; Wang et al., 2019). Within the theriimorph lineage, the detached ear evolved twice in extant therians, and in multituberculates.

Multituberculata (*sensu stricto*, Kielan-Jaworowska et al., 2004) are a species-rich mammal group of the Mesozoic that first appeared in the Middle Jurassic just before 165 ma and extended to the Early Cenozoic around 34 ma (Wilson et al. 2012). Fossils of this group are abundant during the Late Jurassic and again in the Late Cretaceous (Wilson et al., 2012; Martin, 2018). Multituberculates have completely lost the Meckel's cartilage in adults, and their middle ear is disconnected from the mandible. The first multituberculate that has a disconnected middle ear is *Rugosodon*, dated to 160 ma (Yuan et al., 2013). The second oldest record of a detached mammalian middle ear are from three multituberculate species including *Sinobaatar* and *Jeholbaatar*, from the Early Cretaceous (Luo et al., 2017; Wang et al., 2019; Zhou et al., 2019). Multituberculates have retained the surangular bone in the middle ear (Wang et al., 2019), a plesiomorphic element also present in eutriconodontans and in stem mammaliaforms. But this bone is short and vertically thick with a large surangular boss that constrains the incus-malleus joint (Wang et al., 2019). The malleus has a pointed anterior process, and the ectotympanic is hook-like with an arc less than 60°, like in mammaliaforms, although slightly more curved (Zhou et al., 2019; Wang et al., 2019). The middle ear is also present, although preserved incompletely in several Late Cretaceous multituberculates (Rougier et al., 1996a). As discussed earlier in this review, the Paleocene *Lambdopsalis* is now re-interpreted to have a hook-like ectotympanic and possibly an asymmetrical bicrurate stapes (Rougier et al., 1996a; Schultz et al., 2018). The incus of multituberculates is a triangular bone with a short dorsal process (crus brevis) (Rougier et al., 1996a; Wang et al., 2019), but detailed characters of the incus are not sufficiently known.

#### 2.14.4.5 Timing of Independent Acquisitions of DMME

Multituberculates are the first group to have a disconnected middle ear in crown mammals (Fig. 2: node 1). By geological age, the earliest-known multituberculate with a fully disconnected middle ear is *Rugosodon*, dated to be 160 million years old (Yuan et al., 2013). The undisputed earliest record of multituberculates is from the late Middle Jurassic, about 165 ma (Butler and Hooker, 2005). The first acquisition of the DMME can be dated to this first appearance of multituberculates (Fig. 2: node 11), earlier than DMME in crown therians, that first occurred in the Early Cretaceous (Urban et al., 2017), and thus far earlier than the DMME of extant monotremes (Fig. 2: node 7).

Monotremes are an early-divergent clade of crown mammals and the australosphenidan-monotreme clade split from other Mesozoic mammals in late Early Jurassic around 178 ma, well before the earliest known crown therians. According to the latest molecular time estimate of monotreme evolution, however, crown Monotremata of the ornithorhynchids and tachyglossids emerged in the Cenozoic, likely before 40 ma (Phillips et al., 2009). Thus the disconnected middle ear of the platypus and the echidna was the last to evolve among crown mammals.

Multituberculates are phylogenetically placed crownward to modern therians but are the geologically oldest in possessing a detached middle ear, while monotremes as the earliest divergent mammalian clade were the last to acquire detached mammalian middle ear. This illustrates that evolutionary experiments with detached middle ear structures are contingent on the mammalian evolutionary tree, and did not occur in a simple and linear pattern.

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# 2.14.5 Inner Ear Evolution

## 2.14.5.1 Bony Housing of Inn Ear

The bony housing of the inner ears differs between mammaliaforms and cynodonts (Fig. 7). The bony housing of early cynodonts, such as *Thrinaxodon*, is a composite of several bones that are sutured in adulthood. These bones are the perotic and the postotic (collectively known as the periotic), the wing of the basisphenoid bone that overlaps the external surface of the perotic bone, the basioccipital, and the exoccipital (Fourie, 1974; Pusch et al., 2019). In most cynodonts, the cochlea is housed in a recess or



Figure 7 Transformation of the inner ear bony housing in cynodont-mammal evolution. (A). Yunnanodon (tritylodontid). Mammaliamorohs (Node 2) are more derived than other cynodonts (Node 1), in formation of a single petrosal bone by fusion of the prootic and opisthotic. The inner ear housing by the petrosal has partially displaced the basisphenoid wing from the fenestra vestibuli. By comparison, other cynodonts are characterized by separate prootic and opisthotic, the basioccipital overlapping the pars cochlearis of the petrosal, a large basisphenoid wing contributing to a composite bony housing of the cochlea and forming a part of the fenestra vestibule; all primitive characters. (B). mammaliaform Morganucodon. Mammaliaforms (Node 3) are more derived than stem cynodonts in that the pars cochlearis - bony housing of the cochlea - forms a ventral eminence known as the promontorium and the cochlea is curved similarly as in crown mammals. The perilymphatic foramen and the jugular foramen are separated wider in mammaliaforms and extant Ornithorhynchus, than in cynodonts. (C and D). monotremes Ornithorhynchus (platypus) (C) and Tachyglossus (echidna) (D) Living monotremes show the derived feature of the tractus foraminosus, a sieve-like structure with numerous fine foramina in the floor of the internal auditory meatus. The divided nerve fibers of cochlear nerve enter the inner ear cochlea through the foramina in the tractus. Mammalia (Node 3) are characterized by strongly curved cochlea (although reversed in eutriconodontans and spalacotherioids). (E). spalacotherioids ("stem therians", petrosal outline). Appmorphies of trechnotherians (node 12); formation of the canal for perilymphatic duct (= aqueductus cochleae), separated from the fenestra cochleae (the true "round window"), plus a therian-like venous vascular system from the cochlea, a prominent scala tympani impression, and possibly a secondary lamina-like structure for supporting the cochlear duct and scala tympani (Harper and Rougier, 2019). But the cochlear innervation is plesiomorphic in spalacotherioids, as in mammaliaforms. (F). stem cladotherian Dryolestes. Cladotherians as a whole show sophisticated bony structures in cochlear canal to support the organ of Corti and the cochlear ganglion: the primary bony lamina - for supporting the cochlear duct and its basilar membrane - is embedded with the bony canaliculi of habenula perforata for the radiating fibers of cochlear ganglion. The ganglion is enclosed in the Rosenthal's (ganglion) canal. The fascicles of cochlear nerve enter the cochlear through foramina in the tractus foraminosus. There is a true secondary bony lamina for supporting the basilar membrane. Cladogram nodes are the same as in Fig. 2. (A), From Luo (2001). (B), Drawing from Luo et al. (1995); CT rendering original. (D), From Schultz et al. (2017b). (E), Redrawn by Luo from Harper and Rougier (2019); Ear endocast from Luo et al. (2016). (F), From Luo et al. (2012).

a cavity formed by the prootic, with contributions from two additional bones: the basisphenoid wing and the basioccipital. The basisphenoid wing extends posteriorly to border on rim of the fenestra vestibuli. Thus the fenestra vestibuli is also a composite structure, unlike that of mammaliaforms. The margin of the fenestra is thickened and elevated for the stapedial footplate (Fourie, 1974; Luo et al., 1995). The vestibule is housed by the opisthotic, and the semicircular canals are housed mostly by the opisthotic and the exoccipital, but a part of posterior semicircular canal is enclosed by the supraoccipital (Fourie, 1974; Luo et al., 1995).

By comparison, the bony housing for the inner ear in mammaliaforms shows a much simplified, apomorphic pattern of ossification. The prootic and opisthotic are fused to become a single petrosal ("rock-like") bone, which is the hardest bone in the mammalian cranium. The petrosal encloses all parts of the inner ear (Fig. 7) (Luo, 2001; Walsh et al., 2013). The pars cochlearis - the petrosal part for the cochlea - is enlarged to form a pronounced eminence known as the promontorium on the ventral (external) aspect of the cranium. The pars cochlearis accommodates an elongate cochlear canal internally, while the promontorium is the external feature of the pars. The basisphenoid wing of cynodonts that extends to the fenestra vestibuli and covers the ventral surface of the pars cochlearis either becomes much shorter in derived cynodonts that are phylogenetically closer to mammaliaforms, or is completely lost in mammaliaforms and no longer a part of the cochlear housing (Fig. 7) (Luo et al., 1995; Ruf et al., 2013). These structural changes of mammaliaforms are key diagnostic features of the clade (Wible and Hopson, 1993; Rougier and Wible, 2006). With this significant transformation in the bony housing by a single bone, the cochlear canal could become elongate and coiled (Fig. 8), and the relatively simple perilymphatic conduit of cynodonts further differentiated the apomorphic structures in the crownward clades (Fig. 7).



**Figure 8** Phylogenetic evolution and ontogenetic patterns of cochleas across mammaliaforms. Top panel (A–H) systematic diversity of bony cochlear canals: mammaliaforms (*Morganucodon* and *Haldanodon*) have slightly curved cochlear canal ( $\geq$ 150°), a diagnostic character of the clade, and concurrently also an ancestral character for subclades in mammaliaforms. Monotremes are the earliest-divergent clade to develop a coil of the scala media (cochlear duct) beyond 180° although their Organ of Corti is less curved, to 120–150° (Schultz et al., 2017b). The cochlear canal can be curved to about 140° in *Ornithorhynchus*, or curved with a bending apex (about 280°) in *Tachyglossus* (Schultz et al., 2017b); full cochlear canals illustrated in Fig. 7). For crown mammals, the straight to slightly curved cochlear canal is the ancestral pattern (D, E and F), while the more strongly curved and coiled pattern are derived separately in monotremes, in gondwanatherians, and in cladotherians. Middle panel (I): progressive changes in curvature of cochlear duct in ontogeny of *Tachyglossus* (endocranial view of endolymphatic structure). Lower Panel (J): progressive changes in curvature and coiling of the cochlear duct in ontogeny of *Mus* (external view of the endolymphatic structure); E11.5 to E17, embryonic days 11–17; P1, post-parturition day 1. Progressive changes of curvature-to-coiling in the monotreme *Tachyglossus* and in extant therians are ontogenetic recapitulation of the monotreme clade, and of the cladotherian clade, respectively. (I), From Schultz et al. (2017b), redrawn from the original illustrations of Alexander (1904). (J), From Luo et al. (2011); ontogenetic series also illustrated by Fritzsch et al. (2015); Redrawn from originals of Bok et al. (2007).

The inner ear space inside the pars cochlearis is partitioned by a complete bony wall from the brain cavity in mammaliaforms (Fig. 9). Related to this novel ossification pattern, the internal auditory meatus forms the entrance of the vestibule-cochlear nerves (branches of cranial nerve viii) and the passage of the facial nerve (cranial nerve vii). Unlike the tubular internal auditory meatus of most extant mammals, the internal meatus of mammaliaforms is a shallow depression, but it is definitely mammal-like in having a fully ossified floor with well-defined foramina for the vestibulo-cochlear nerves. This is in contrast to most cynodonts, in which the medial wall of the periotics between the inner ear space and the brain cavity is incompletely ossified (Rowe et al., 1995; Kemp, 2007). Because the periotics are not well ossified, there is not a clearly defined internal auditory meatus to support the nerve bundle of the vestibulo-cochlear nerve as it entered the inner ear in most cynodonts (Fourie, 1974; Pusch et al., 2019), except the most derived cynodonts such as *Pseudotherium* (Wallace et al., 2019) and *Brasilitherium* (Rodrigues et al., 2013). Nonetheless, there is a well-documented convergent case of the completely ossified internal wall of the periotics, which is mammal-like, in a precynodont therapsid group, namely dicynodonts (Laaß, 2014, 2016).

In the development of the chondrocranium of the vertebrate skull (De Beer, 1937), the bony enclosure of the inner ear in the adult is ossified from the cartilaginous otic capsule of the chondrocranium of earlier ontogenetic stages. In extant mammals, the cartilaginous otic capsule is ossified into a single petrosal (Novacek, 1993; Rougier and Wible, 2006), instead of a composite housing of inner ear by several bones sutured together as in nonmammaliaform cynodonts and other extant vertebrates. The singular petrosal of mammals has a more solid structural integrity that can serve as a better acoustic insulation of the inner ear from the surrounding cranial parts (Allin, 1986; Luo et al., 1995). Building on this fundamentally more integrated structure, there is further development of interior bony structural support for the membranous labyrinth of the inner ear in extant mammals. Many of the modern mammal-like characters are now also documented in the early-divergent mammaliaforms (Luo et al., 1995; Ruf et al., 2013; Panciroli et al., 2018). Moreover, the precursor condition for some of these mammal-like characters can now be traced further into nonmammaliaform cynodonts (Figs. 7, 8 and 9) (Luo, 2001; Rodrigues et al., 2013; Wallace et al., 2019).

### 2.14.5.2 Precursor Characters of Mammaliamorphs

Mammaliamorphs are the nearest evolutionary relatives of mammaliaforms (Fig. 2: node 2) (Rowe, 1988, as modified by Liu and Olsen, 2010; Wallace et al., 2019). The main stem groups are tritylodontids, tritheledontids, and brasilodontids. These fossils show precursor character states to the mammal-like patterns in mammaliaforms.

The petrosal (or prootic) part for the cochlea of mammaliamorphs already partially resembles those of mammaliaforms (Fig. 7). For example, the medial wall of the petrosal of tritylodontids is already ossified and the internal auditory meatus bears resemblance to those of mammals. Tritylodontids and brasilodontids already have a mammal-like pars cochlearis, which contains a short and "cone-shaped" cochlear canal. The pars cochlearis is expanded to show an incipient promontorium on the ventral aspect of the cranium (Luo, 2001; Rodrigues et al., 2013; Wallace et al., 2019). The wing of the basisphenoid is partly displaced by the promontorium, and withdrawn from the fenestra vestibuli, so that the periphery of the fenestra is formed exclusively by the petrosal, all mammal-like characters (Fig. 7A) (Rodrigues et al., 2013; Wallace et al., 2019). This reduction of the basisphenoid tends to be more pronounced in small taxa than in larger taxa (Luo, 2001). The stem mammaliamorph groups of tritylodontids and brasilodontids have a thin rim of the fenestra vestibuli (Luo, 2001; Rodrigues et al., 2013). This suggests that the stapedial footplate has a tighter fit with the fenestra vestibuli than in such cynodonts as *Thrinaxodon*, that has a thick and elevated margin of the fenestra, a morphology common in cynodonts.

### 2.14.5.3 Vestibular Features of Mammaliaforms

The structural difference of the mammaliaform petrosal, as compared to the composite housing of the inner ear in cynodonts, is the underpinning for the apomorphic characters of the endocasts of the vestibule and semicircular canals of mammaliaforms. The pars canaliculus (the petrosal part for the semicircular canals), is formed exclusively by the petrosal, unlike most cynodonts, in which parts of the semicircular canals are enclosed primarily by the opisthotic, but also with participation of occipital bones (Fourie, 1974; Pusch et al., 2019).

## 2.14.5.3.1 Semicircular Canals

The semicircular canals in most mammaliaforms examined so far show a secondary crus commune at the junction of the posterior and the lateral semicircular canals (Figs. 8 and 9). This feature is present in derived cynodonts (Rodrigues et al., 2013), and some clades of crown mammals (Harper and Rougier, 2019). The secondary crus commune appears to be a plesiomorphic feature of mammaliaforms (Ruf et al., 2013; Hughes et al., 2015). In the stem mammaliaforms examined so far, the diameters of individual semicircular canals are thicker than the semicircular canals of most extant mammals (Pfaff et al., 2015; Schultz et al., 2017a,b) and of some Mesozoic mammal clades (Luo et al., 2012; Kirk et al., 2014; Harper and Rougier, 2019). The semicircular canals in stem mammaliaforms also appear to have a smaller radius of curvature (Ruf et al., 2013), than those of crown mammals (Figs. 8 and 9) (Luo et al., 2012; Harper and Rougier, 2019).

The semicircular canals serve a sensory function in body equilibrium. It has been hypothesized that behavior and agility may be correlated with variation in the curvature radii (Spoor et al., 2007; Gunz et al., 2012), and with variation in diameters of semicircular canals (Pfaff et al., 2015). Or that the variation of semicircular canals as a whole would be more pronounced in mammals

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**Figure 9** Evolutionary patterns of cochlear innervation and the interior structures of cochlear canals, as shown in endocasts of the inner ear (medial view) and schematic cross section (to the right of endocast). (A). mammaliamorph *Pseudotherium* with a short cochlear of the inner ear endocast, and (B), tritylodontid: composite bony housing of the cochlea, a more plesiomorphic structure in mammaliamorph sthan in mammaliaforms. (C). *Morganucodon*: cochlear canal is elongate (to about 2 mm), and housed by a full promontorium formed exclusively by petrosal (as in *Sinoconodon*, docodontans, and *Hadrocodium*). The cochlear canal has a large cochlear nerve foramen (plesiomorphic) indicating that a cochlear nerve entered the foramen as a bundle, and then divided into cochlear fibers to a ganglion in the cochlear space as in other amniotes (plesiomorphic). (D). Docodont *Haldanodon*: a plesiomorphic character of a single cochlear foramen into cochlear canal. *Haldanodon* is derived in having a lagenar sulcus on the cochlear canal endocast indicating a lagenar macula, as in monotremes (Ruf et al., 2013). (E). *Hadrocodium*: similar to *Morganucodon* and *Sinoconodon*. (F and G). Monotremes *Ornithorhynchus* (the platypus) (F) and *Tachyglossus* (the echidna) (G): the tractus foraminosus with foramina for divided fascicles of cochlear nerve to enter the cochlear canal via dense foramina, an apomorphy as compared to mammaliaforms. The cochlear ganglion is supported by spiral ligament, without internal bony structure in the cochlear canal of therians. Monotremes have a lagenar macula, separated by the subapical helicotrema from the organ of Corti; the lagenar nerve in a canaliculus separated from cochlear nerve fibers in the tractus

of less agile behavior than those that are more agile (Billet et al., 2012). The relatively smaller canal curvature radii and greater canal diameters (especially the lateral and the posterior semicircular canals) of the stem mammaliaforms may suggest that they lacked the same level of agility in behavior and locomotor functions (Ruf et al., 2013), as compared to extant mammals (Spoor et al., 2007; Pfaff et al., 2015, 2017). It has also been proposed that the larger radii of curvature of the semicircular canals are correlated with higher functional requirement for stabilization of eye gaze in large eyed mammals with high visual acuity, not necessarily with great locomotor agility (Kemp and Kirk, 2014). Some extant placentals are also known to show variation in the cross-sectional shape of the semicircular canals (Ekdale, 2016b), but such characters have not been examined in any of the early mammals.

#### 2.14.5.3.2 Vestibular Structures

The bony housing for the junction of the vestibule and the cochlea is better ossified in mammaliaforms than in most cynodonts. As a consequence of this developmental difference, there is a better bony separation between the utricle and the base of the cochlea, such that the utricle of the vestibule is clearly visible on surface of the inner ear endocast (Fig. 7) (Luo et al., 2012; Ruf et al., 2013; Panciroli et al., 2018). These structures are not well developed in the endocasts of cynodonts. In *Brasilitherium* and *Pseudotherium*, the cochlear structure and the vestibular structure have a gradational transition without a distinct boundary on the inner ear endocast (Fig. 9: node 1) (Rodrigues et al., 2013; Wallace et al., 2019). Accompanied by the co-ossification of the periotic bones into a single petrosal in mammaliaforms, there is an evolutionary trend toward an increased differentiation between the cochlea and the vestibule through cynodont-mammal evolution (Fig. 7) (Ekdale, 2016a; Luo et al., 2016). There is also an increasingly wider separation of the perilymphatic channel from the membranous labyrinth to the braincase, in the successively more crownward clades.

### 2.14.5.3.3 Inflated Vestibule in Mesozoic Mammals

In extant monotremes and the majority of extant therians, the vestibule is relatively small, in comparison to the size of their semicircular canals and cochleae (Ekdale, 2013; 2016a; Schultz et al., 2017b). However, the vestibules appear to be much larger in some multituberculates (Fig. 9I) (Miao, 1988; Luo and Ketten, 1991; Meng and Wyss, 1995), and in the subterranean mammal *Necrolestes* (Ladeveze et al., 2008), which is a member of the long-living dryolestid-meridiolestid clade that originated in the Jurassic and survived into the Miocene in South America (Rougier et al., 2012; Wible and Rougier, 2017).

The inflation of the vestibule has been hypothesized to be an adaptation for better sensitivity in low-frequency hearing (Miao, 1988; Luo and Ketten, 1991), by comparison to the inflated vestibules in burrowing gopher tortoise (Bramble, 1982) and in burrowing snakes adapted to a subterranean life (Olori, 2010; Yi and Norell, 2015). Among non-cynodont therapsids, the enlarged vestibule is developed in several dicynodonts (Laaß, 2014; Araujo et al. 2018) – it is extremely inflated in the dicynodont *Kawingasaurus* (Laaß, 2014). Laaß (2014) proposed that the inflated space of the vestibule increases the fluid volume and this may play an important role for lower frequency hearing by bone conduction, which is a well-known phenomenon in small subterranean placental mammals (Rado et al., 1989; Mason and Narins, 2001; Maso et al., 2010; Koyabu et al., 2017). The functional analogs of extant reptiles and placentals are informative for understanding the inflated vestibule in multituberculates.

The evolution of the inflated vestibule in multituberculates may be a functional convergence, and have evolved separately in taxa that are phylogenetically distant within multituberculates (Fox and Meng, 1997; Hurum, 1998; Ladeveze et al., 2010), or it may have a single evolutionary origin for all multituberculates. The latest quantitative analysis (Laaß, 2014) suggests that in comparison to other mammaliaforms, multituberculates as a group have larger vestibular volume after correction for their body sizes.

The subterranean mammal *Necrolestes* of the dryolestid-meridiolestid clade has an inflated vestibule. However, the Jurassic fossils of the same clade, such as *Dryolestes* and *Henkelotherium*, do not show an inflation of the vestibule. The lack of vestibular inflation represents the ancestral condition of the dryolestoids (Ruf et al., 2009; Hughes et al., 2015). Therefore, the vestibular inflation of *Necrolestes* is a separate convergence within its larger clade from the Mesozoic, likely related to subterranean adaptation of *Necrolestes* (Ladeveze et al., 2008; Wible and Rougier, 2017).

foraminosus. (H). eutriconodontans: plesiomorphic cochlear characters as in mammaliaforms. (I). the multituberculate *Meniscoessus*: plesiomorphic cochlear characters as in mammaliaforms, but an inflated vestibule (derived). (J). the gondwanatherian *Vintana*, derived condition of tractus foraminosus, complete cochlear ganglion canal, and both primary and secondary bony laminae. These complex structures evolved within allotherians, convergently to those of cladotherians. (K). The spalacotherioid *Zhangheotherium*, the perilymphatic recess is differentiated into a separate canal for perilymphatic duct – the aqueductus cochleae, and a fully formed fenestra cochleae, and the venous drainage is also modern therian-like. The cochlear endocast also shows a grove-like impression of scala tympani, and possibly a bony base of the secondary lamina. (L). *Dryolestes* (of cladotherian clade node 13): the tractus foraminosus indicating the fascicles of the cochlear nerve are divided before entering the cochlear fiber foramina (convergent to monotremes and gondwanatherians); a bony Rosenthal's canal for the cochlear ganglion in the base of primary bony lamina, a derived feature of cladotherian (including marsupials and placentals), the presence of the secondary bony lamina, all convergent to those of gondwanatherians. (M). The extant marsupial *Didelphis*. Outlines of inner ear features are original rendering on the published descriptions on respective taxa. For clade definition see **Fig. 2** caption. Abbreviations: TF, tractus foraminosus; GC, spiral ganglion canal (= Rosenthal's canal). (A), Re-drawn from Wallace et al. (2019). (B), From Luo (2001). (J), Drawing based on Hoffmann et al. (2014). (M), Cross-section schematics modified from Luo et al. (2012, 2016) and Hoffmann et al. (2014). All other drawings from Luo et al. (2016).

## 2.14.5.4 Transformation of the Perilymphatic Conduit

The perilymphatic foramen is the main conduit between the posterior end of the scala tympani in the fluid-filled inner ear space and the air space of the middle ear cavity. The perilymphatic conduit is covered by a secondary tympanic membrane that serves as a pressure release mechanism for the scala tympani at its interface to the air space of the middle ear. Mammaliaforms have a major apomorphy in that the basal part of the cochlea is tubular in shape near the perilymphatic foramen (Fig. 7: *Morganucodon*). This change led to a better differentiation between the vestibule and the base of the cochlear canal responsible for higher-frequency hearing.

Another major apomorphy is a differentiation of the perilymphatic foramen from its surrounding structures. In basal cynodonts, the perilymphatic foramen is fully confluent with the jugular foramen on the ventral (external) aspect of the cranium. The jugular foramen is the opening for cranial nerves IX – XI and a major cranial vein. By the shared opening of the jugular foramen and the perilymphatic foramen (Fourie, 1974; Pusch et al., 2019), the perilymphatic foramen is partially confluent with the endocranial space of the braincase. Thus the pressure release mechanism through the perilymphatic foramen is not directly or exclusively to the air space of the middle ear cavity in basal cynodonts. By comparison, mammaliaforms, such as *Morganucodon* (Fig. 7), have a wider bony separation of the perilymphatic foramen from the jugular foramen. Thus the scala tympani has its own opening through the perilymphatic conduit for a direct pressure release to the middle ear cavity.

As exemplified by *Morganucodon* (Fig. 7B), stem mammaliaforms have a perilymphatic foramen opening externally to a perilymphatic recess. The recess has an open sulcus for the perilymphatic duct (Wible and Hopson, 1993; Luo et al., 1995; Rougier and Wible, 2006). A similar pattern is also known for *Sinoconodon, Hadrocodium* and docodontans (Luo et al., 2001a,b; Ruf et al., 2013; Panciroli et al., 2018). It is also present in the Jurassic haramiyidans.

There is a consensus that the secondary tympanic membrane covered the perilymphatic space in mammaliaforms in the same way as in *Ornithorhynchus* (Hoffmann et al., 2014; Ekdale, 2016b; Luo et al., 2016). In the platypus, the scala tympani of the inner ear opens to the outside through the perilymphatic foramen to a perilymphatic sac housed by a bony perilymphatic recess. The secondary tympanic membrane covers the fluid-filled perilymphatic sac and separates it from the air space of the tympanic cavity (Zeller, 1989; Schultz et al., 2017b). Concurrently, the perilymphatic sac is connected by the perilymphatic duct (aqueductus cochleae) to the endocranial space (Schultz et al., 2017b). In *Ornithorhynchus*, the perilymphatic foramen for the scala tympani, the perilymphatic recess and the bony sulcus for the perilymphatic duct are not fully separated by bone from each other, although a bony separation (processus recessus) can be present, with some individual variation, in *Tachyglossus* (Wible and Hopson, 1995; Ruf et al., 2013).

When these interrelated characters of the basal cochlear canal and perilymphatic conduit are mapped onto mammaliaform phylogeny, it appears that this complex structure transformed in incremental steps. *Brasilitherium* is the first cynodont to show a bony separation of the saccular space from the utricular space on the vestibular endocast (Rodrigues et al., 2013). *Pseudotherium* and tritylodontids also show some degree of differentiation of the basal cochlear canal from the vestibule on the inner ear endocast (Luo, 2001; Wallace et al., 2019). A more fully resolved evolutionary sequence will required information on these characters in those cynodonts in intermediate phylogenetic positions (Quiroga, 1979; Allin and Hopson, 1992). Most of these cynodonts have not been fully studied by 3D visualization.

In the transformation of the perilymphatic conduit among crown mammals, an apomorphic feature is the processus recessus, an osteological structure that segregates the opening of the fenestra cochleae (fenestra rotunda or "round window") from the bony canaliculus that carries the perilymphatic duct (aqueductus cochleae) (Luo et al., 2012; Harper and Rougier, 2019). In extant marsupials and placentals, the fenestra rotunda is separated by the processus recessus from the bony canal of the perilymphatic duct (Zeller, 1985). These two structures correspond, collectively, to the confluent opening of the perilymphatic foramen, the perilymphatic recess and sulcus in *Ornithorhynchus* because *Ornithorhynchus* does not have a processus recessus to separate these structures. In *Tachyglossus*, the perilymphatic sulcus and foramen are confluent in juvenile, but can become separated by a processus recessus in most (although not all) adults (Kuhn, 1971; Ekdale, 2016; Schultz et al., 2017b).

Two Mesozoic clades of crown mammals, eutriconodonts and multituberculates, have the plesiomorphic, and mammaliaform characteristics of the perilymphatic foramen (Rougier et al., 1996b; Luo et al., 2016; Harper and Rougier, 2019), although some variations of the related characters are observed in eutriconodonts (Harper and Rougier, 2019). In therian evolution, the first step toward an extant therian-like fenestra cochleae ("round window") occurred in trechnotherians ("basal stem therians"), as evidenced by the spalacotherioid *Zhangheotherium* and other Cretaceous Hoovor petrosals that have a bony canal for the perilymphatic duct (Figs. 2 and 7: node 12) (Rougier et al., 1996b; Harper and Rougier, 2019). This derived pattern is also present in cladotherians, such as *Dryolestes* and *Vincelestes* and in living therians (Figs. 2 and 7) (Rougier et al., 1992; Luo et al., 2012; Hughes et al., 2015; Harper and Rougier, 2019). In summary, an extant therian-like fenestra cochleae for pressure release from the scala tympani evolved first in spalacotherioids in the Middle Jurassic, continuing crownward to cladotherians and extant therians (Figs. 2 and 7).

# 2.14.5.5 Cochlear Canals of Mammaliaforms

In recent years there has been rapid progress in studies of the interior structure of the petrosals in diverse stem mammaliaforms, revealing some detailed interior structures of the cochlear canal (Ruf et al., 2013; Schultz et al. 2017b; Panciroli et al., 2018; Hoffmann et al., 2018). Most cynodonts have a small cochlear recess near the fenestra vestibuli (Allin and Hopson, 1992; Luo, 2001). In tritylodontids and *Brasilitherium*, the short cochlear structure takes on a cone-like shape, and is pointed anteromedially from the

fenestra vestibuli. However, the canal is not elongate enough to give an indication of the canal curvature (Figs. 7 and 9), but by its orientation and shape, it is less similar to the cochlear recess of basal cynodonts and more similar to the cochlear canal of mammaliaforms.

The mammaliaform cochlear canals are elongate and more tubular; many are also distinctly curved (Figs. 7 and 8) (Luo et al., 1995; Ruf et al., 2013; Kirk et al., 2014; Panciroli et al., 2018). In most mammaliaforms, the ratio of cochlear canal length to body mass is higher than in cynodonts, including *Brasilitherium* (Rodrigues et al., 2013; Kirk et al., 2014). The cochlear canal is curved, and convex medially in the ventral view in most mammaliaforms. The curvature is the same as the cochlear canal of monotremes (Figs. 7 and 8), but in the opposite direction from the cochlear canal curvature of extant reptiles and birds (Hurum, 1998; Walsh et al., 2009; Luo et al., 2016).

The interior wall of the cochlear canal is uniformly simple, lacking the complex structure of extant therian mammals (Figs. 8 and 9) (Ekdale, 2016a,b), or of extinct gondwanatherians (Hoffmann et al., 2014; Krause et al., 2020). Nonetheless, recent CT studies of the cochleae in stem mammaliaforms have revealed some fine-scale osteological correlates, which would permit putative reconstruction of some of membranous labyrinth structures.

#### 2.14.5.6 Reconstruction of Membranous Structures of Cochlea

In early mammaliaforms, the cochlear nerve bundle entered the cochlear canal space through a single, large cochlear foramen in a shallow and depression-like internal auditory meatus. Once inside the cochlear space, the cochlear nerve bundle is connected to the cochlear ganglion in all stem mammaliaforms (Schultz et al., 2017b). In monotremes, the cochlear nerve bundle is divided into a branch to the lagenar ganglion that innervates the lagenar macula, and a dense pack of cochlear fibers to the cochlear ganglion that innervates the lagenar macula, and a dense pack of cochlear fibers to the cochlear ganglion that innervate the Organ of Corti (Alexander, 1904; Schultz et al., 2017b). The cochlear ganglion is embedded and suspended by soft-tissue spiral ligaments in monotremes (reviewed by Schultz et al., 2017b).

In extant reptiles and birds, the cochlear ganglion is embedded in the soft tissue of the membranous labyrinth and innervates the basilar papilla (Weston, 1939). This has been extensively documented in extant reptiles (Wever, 1978; Manley, 1990; Fischer et al., 1994) and in birds (Takasaka and Smith, 1971; Tanaka and Smith, 1978; Manley, 1990). Stem mammaliaforms are bracketed on the one hand by extant nonmammalian amniotes with the ganglion embedded in the membranous labyrinth (Weston, 1939), and by modern mammals on the other (Schultz et al., 2017a,b). Thus it is reasonable to posit that in the common ancestor of mammaliaforms, the cochlear ganglion was suspended by the primary spiral ligament (or some similar tissue) from the neural side of the membranous labyrinth, but without a primary bony lamina (Ashwell, 2013; Luo et al., 2016; Ekdale, 2016). This is the ancestral pattern in the stem mammaliaforms (Luo et al., 2016; Schultz et al., 2017b).

#### 2.14.5.6.1 Inferred Presence of Lagenar Macula

Among extant mammals, the lagenar macula is present only in monotremes (Jørgensen and Locket, 1995; Ladhams and Pickles, 1996; Schultz et al., 2017b). The lagenar macula and the Organ of Corti are two sensory areas of the membranous cochlear duct in monotremes: the lagena is restricted to the apex and the Organ of Corti is positioned more proximally along the cochlear duct and is a much longer structure (Schultz et al., 2017b). These two sensory areas of the cochlear duct are separated by a lagenar isthmus, a bending part of the cochlear duct devoid of any sensory hair cells (Pritchard 1881; Ladhams and Pickles, 1996; Schultz et al., 2017b). The lagenar macula and the Organ of Corti are clearly distinguished by the distinct histology of the sensory epithelium. The lagenar macula is similar to vestibular sensory epithelia of therians in its hair cell characteristics (Ladhams and Pickles, 1996), and its association with the otolithic membrane of the macula (Manley, 2018). In addition to the cellular differences, the lagena is innervated by its own ganglion, separately from the cochlear ganglion for the Organ of Corti (Alexander, 1904; Ladhams and Pickles, 1996; Schultz et al., 2017b). The apex of the cochlear canal is perforated by small canaliculi of the lagenar nerve fiber(s). The lagenar nerve canal is mostly distinct from the tractus foraminosus, although some local anastomotic connections have been identified (Schultz et al., 2017a,b).

The lagena and the Organ of Corti of monotremes also differ in the organization of membranous labyrinth compartments: the lagenar macula is made up of only two compartments: the scala media and the scala vestibuli, with little or no participation from the scala tympani, notwithstanding minor differences between *Ornithorhynchus* and *Tachyglossus* in this feature (Schultz et al., 2017b). The sensory epithelium of the macula is sandwiched by the scalae media and vestibuli, without the scala tympani (Schultz et al., 2017b).

By contrast, the cochlea part containing the Organ of Corti has a full complement of the scalae media, vestibuli and tympani, and the organ is positioned on the basilar membrane between the scala media and the scala tympani. The helicotrema, the connecting channel between the scala vestibuli and scala tympani, is located in the lagenar isthmus and sub-apical in monotremes (more distinctly developed in *Ornithorhynchus* than in *Tachyglossus*) (Schultz et al., 2017b: figs. 7 and 8). Constrained by these surrounding structures, the Organ of Corti does not reach beyond the helicotrema, and is much shorter than the same organ that reaches the apex of the cochlea in therians (Schultz et al., 2017b). Because the scala vestibuli – helicotrema – scala tympani forms a sound-conducting perilymphatic space connected to the fenestra vestibuli and fenestra cochleae, the sub-apical position of the helicotrema means that the lagena is by-passed by this system (Fritzsch and Elliott, 2017; Jahan et al., 2018).

It has long been recognized that the presence of a lagena in the apical region of the cochlea is a shared plesiomorphic character of reptilians and extant monotremes (Manley and Clack, 2004; Fritzsch et al., 2013; Schultz et al., 2017b). The stem mammaliaforms are phylogenetically intermediate between extant monotremes and extant reptiles. Mapping this feature on phylogeny, it is

reasonable to postulate that the lagena macula was present in stem mammaliaforms and the lagena was housed by the expanded apex of the cochlear canal, seen in several (but not all) stem mammaliaforms (Schultz et al., 2017b; Hoffmann et al., 2018). It has been further proposed that the stem mammaliaforms likely had a sub-apical helicotrema (the terminal conduit between the scala vestibuli and the scala tympani), closely associated with the formation of the lagenar macula. The latter pattern is a consistent and primitive condition of nonmammalian amniotes and monotremes (Schultz et al., 2017b).

The interpretation that the lagena macula was an ancestral mammaliaform character can be tested (and corroborated to some extent) by mapping osteological characters related to the membranous labyrinth, into stem mammaliaforms and Mesozoic mammal clades.

Among mammaliaforms, docodontans and gondwanatherians have the best available characters for inference of the lagena (Ruf et al., 2013; Krause et al., 2020). *Morganucodon* also has some indication for the presence of a lagena (Fig. 7) (Hoffmann et al., 2018). The cochlear canals of docodontans show a more pronounced apical curvature than other mammaliaforms and the curved apex of the canal is also slightly expanded. These indicate a space for the lagena macula (Ruf et al., 2013; Panciroli et al., 2018). Another informative character is that the presumptive space for a lagenar macula in the apex is connected to a distinctive lagenar sulcus on the interior wall of the canal (on the neural aspect of the cochlea) to a distinctive notch of the large cochlear foramen. These features unequivocally indicate the presence of a lagenar macula and the inference of the lagena is reliable for *Haldanodon* (Fig. 8) (Ruf et al., 2013). For the docodont *Borealestes*, the corresponding area is not preserved in the persoals that have been studied so far (Panciroli et al., 2018). More recently, it has been shown that petrosals of Early Jurassic *Morganucodon watsoni* consistently have an enlarged and curved apex on the cochlear endocast (Shahid et al., 2018; Hoffmann et al., 2013). Schultz et al., 2017a,b). *Hadrocodium* may be an exception among stem mammaliaforms, as it shows a tapering apex of the cochlea and is thus less likely to have a large lagena. If there was no lagena, then there can also be a homoplastic loss of the lagenar macula among stem mammaliaforms (Luo et al., 2016).

In the gondwanatherian *Adalatherium*, there is a well-developed lagenar nerve canal that is even better differentiated from the tractus foraminosus than in extant monotremes (Krause et al., 2020). However, *Vintana*, which is closely related to *Adalatherium* in the gondwanatherian clade, lacks an expanded apex and has no lagenar nerve canal (Kirk et al., 2014). Thus a lagena is present in at least some gondwanatherian (Figs. 2 and 9: node 10) (Krause et al., 2020). Gondwanatherians and multituberculates are parts of the allotherian clade (Krause et al., 2020). Although some earlier studies suggest multituberculates could have a lagenar macula in the cochlear apex (Hurum, 1998; Ladeveze et al., 2010), the majority of multituberculates tend to have a tapering apex of the cochlear canal. No multituberculate has a lagenar nerve canal, or a lagenar nerve sulcus on the endocast. The evidence for postulating the presence of a lagenar macula is weak for multituberculates. Taken together, the current evidence from gondwanatherians and multituberculates suggests that allotherians as whole show homoplastic variation of the lagena-related osteological characters. The well-developed lagena in *Adalatherium* is an exceptional example, in comparison to closely related *Vintana*, and most multituberculates that lack the reliable osteological correlates of this structure.

## 2.14.5.6.2 Reconstruction of the Organ of Corti

In extant monotremes, the osteological correlates of the cochlear canal are not well developed, and the evidence is weak for inferences of the Organ of Corti. With this caveat, it is nonetheless feasible to infer the well-mapped partition of the scala tympani and the scala vestibuli of monotremes, which corresponds to the impression of scala tympani (*sensu* Harper and Rougier, 2019) or "base of secondary bony lamina" (*sensu* Schultz et al., 2017b). This can be a basis for mapping osteological correlates that separate the Organ of Corti space from the presumptive space for the apical lagena in stem mammaliaforms.

The cochlear canal endocast of monotremes shows a shallow and broad groove on the convex (abneural) aspect of the cochlear canal. This is called the impression of the scala tympani by Harper and Rougier (2019), or the base of the secondary bony lamina (*sensu* Schultz et al., 2017b), as the base of the secondary bony lamina (if present) approximates to the division of the scala tympani and spiral ligament. This structure does not have a bony edge that connects the basilar membrane in monotremes, comparable to the true secondary bony lamina of therian mammals (Schultz et al., 2017b). This shallow groove of the scala tympani impression starts from between the fenestra vestibuli and the perilymphatic foramen; it extends from the perilymphatic foramen toward the distal (apical) part of the cochlea in *Tachyglossus* and in Mesozoic eutriconodonts and spalacotherioids (Figs. 7 and 8) (Schultz et al., 2017b; Harper and Rougier, 2019). The presence of this groove-like impression is variable, and can be present in some specimens, but absent in others of *Ornithorhynchus anatinus* and *Tachyglossus aculeatus* (Schultz et al., 2017a,b: Figs. 4 and 5). In *Tachyglossus* specimens in which this sulcus is well developed on the endocast, it corresponds to a soft-tissue boundary between the scala tympani (as named by Harper and Rougier, 2019) is a better anatomical term of this osteological correlate.

This sulcus of impression of the scala tympani extends along the length of the cochlear canal and would approximate the length of the scala tympani, along the convex curvature of the membranous cochlear duct (Schultz et al., 2017b). Notwithstanding the variation of this structure in monotremes, if and when present, the sulcus is a useful osteological correlate to infer the extent of the scala tympani (Schultz et al., 2017b; Harper and Rougier, 2019).

Shahid et al. (2018) took this osteological correlate from monotremes, to infer the location of the basilar membrane in *Morganucodon* by the corresponding sulcus of the presumptive secondary lamina base on the cochlear endocast of the latter. Based on this assumption, it was estimated that the basilar membrane (by inference also the organ of Corti) could extend to 70–80% of the total length of the cochlear canal (Shahid et al., 2018). This is the best available osteological correlate of extant mammals to infer the

presence of the basilar membrane and the Organ of Corti in a stem mammaliaform (Shahid et al., 2018), the two soft tissue structures assumed to be present in mammaliaforms (Manley and Clack, 2004; Luo et al., 2016).

Harper and Rougier (2019) have taken this approach one step further to interpret that the ridge corresponding to the impression of scala tympani as the secondary bony lamina (not just its base). This interpretation is based on the topographical relationship that the secondary lamina arises from this location in the cochlear canal of extant therians. In two Mesozoic mammal groups, eutriconodonts and spalacotherioids ("stem therians"), the scala tympani impression is better developed, and shows a well-defined groove along the length of cochlear canal endocasts (Harper and Rougier, 2019, synonymous with the "base of the bony secondary lamina" of Luo et al., 2016). The wall of the cochlear canal forms a bony ridge corresponding to the groove-like scala tympani impression on the endocast. In the bone of the pars cochlearis, there is a dense venous vascular network approximating the ridge (Harper and Rougier, 2019; Luo and Neander, pers. observation). The groove-like impression on the endocast, and the vascularized bony ridge that corresponds to it, provide useful evidence for estimating the length of the scala tympani in Mesozoic mammals.

## 2.14.5.6.3 Cochlear Vasculature in Mammaliaforms

In extant placentals, the membranous labyrinth of the cochlea has a vascular network for its physiological functions. The three scalae (vestibuli, media and tympani) of the cochlear membranous labyrinth correspond topographically to tracks of the vascular network (Axelsson, 1988; Nomura, 2014; Harper and Rougier, 2019). Respectively, the scala vestibuli corresponds to the radiating arterioles; the scala media corresponds to the capillaries closely associated with the Stria vascularis; and the scala tympani corresponds to collecting venules. The capillaries of the scala media supply the Stria vascularis, a structure crucial for the function of hearing in mammals (Axelsson, 1988; Wangemann, 2006; Nomura, 2014) by generating the endolymphatic potential for the scala media (Wangemann, 2006; Köppl et al., 2018; Köppl and Manley, 2019). Some of these vascular structures in mammaliaforms can to a degree be inferred by their osteological correlates. These are useful proxies to soft tissue structures of the membranous labyrinth, and can be informative for inferring cochlear evolution (Harper and Rougier, 2019).

High-resolution CT scans have made it feasible to examine very small bony structures of the inner ear in petrosals of fossil mammals (Walsh et al., 2013). Some parts of this vascular network have been examined in the petrosals of Mesozoic mammalia-forms (Panciroli et al., 2018; Harper and Rougier, 2019). On the basis of osteological correlates, Harper and Rougier (2019) proposed that the Stria vascularis and the venules of the scala tympani, and their related vasculature, were present in two key clades of Mesozoic mammals, as in extant therians, although the arrangement for the cochlear veins to exit the cochlear space is slightly different between Mesozoic and extant mammals.

In extant monotremes and therians, the cochlear artery, the primary artery for the membranous labyrinths, enters the cochlear canal space via the internal acoustic meatus (Axelsson, 1988; Schultz et al., 2017b). In therians, the arterial network in the cochlear space divides into modiolar arteries, which further split into the radiating arterioles networked with the capillaries of the Stria vascularis in therians (Axelsson, 1988; Wangemann, 2006; Nomura, 2014). This feature is present in monotremes (Smith and Takasaka, 1971). The capillaries of the Stria vascularis are connected with a network of collecting venules (Axelsson, 1988), and these venules topographically correspond to the position of scala tympani along the outer curvature of the cochlea and on the cochlear canal wall (Axelsson, 1988; Harper and Rougier, 2019). The outgoing drainages of this capillary network are connected respectively to the internal auditory meatus, and to the canal of the perilymphatic duct (Axelsson, 1988; Nomura, 2014), or in a parallel canal for the vein of the aqueductus duct (Nomura, 2014; Harper and Rougier, 2019).

The entrance of the cochlear artery into the cochlear space, and the exits of the veins from the cochlear space, can now be reconstructed for Mesozoic eutriconodonts, multituberculates and stem therians (Harper and Rougier, 2019). These groups have a single large foramen for the cochlear nerve (Luo et al., 2016; Ekdale, 2016 and literature therein). The foramen is interpreted to be the entrance for the cochlear artery into the cochlear space and possibly also the exit for one of two major veins from the inner ear space (Axelsson, 1988; Harper and Rougier, 2019). Another major exit of the venous network out of the cochlea is the vein of the cochlear aqueduct in therians. This vein is located close and parallel to the canal of the cochlear aqueduct (Axelsson, 1988; Nomura, 2014; Orliac et al., 2017). The bony canal for this vein to exit the cochlear space has been now reliably identified for the stem therians (Harper and Rougier, 2019). In the basic arrangement of the venous network exits from the cochlear space, the stem therians are similar to extant therians, with one exit through the internal auditory meatus, and another through the canal of aqueductus vein (Orliac et al., 2017; Harper and Rougier, 2019).

The vascular structures of crown mammals, as established by Harper and Rougier (2019), can be informative for reconstructing the inner-ear vasculature of stem mammaliaforms. The main conduit of the cochlear artery and vein is the single, large cochlear nerve foramen in the internal auditory meatus, presumably the same in stem mammaliaforms as in extant mammals and in therians of the Mesozoic (Schultz et al., 2017a,b; Harper and Rougier, 2019).

In two stem mammaliaforms, *Morganucodon* and *Borealestes*, there is a track of small vascular foramina on the outer wall of the cochlear canal (Panciroli et al., 2018; Luo and Neander, personal observation). These foramina form a well-defined network and are arranged in a distinct fishing-net-like pattern. Numerous tiny vessels of this network are connected internally to the cochlear canal space, and externally to the intramural hypocochlear venous channels of the circumpromontorial plexus in the docodont *Borealestes* (Panciroli et al., 2018) and in *Morganucodon* (Luo and Neander, personal observation). Most interestingly, this track of the venous vascular network in *Morganucodon* and docodonts corresponds to the position of the impression of scala tympani in monotremes. We suggest that this network of small veins is connected to the cochlear capillary and venule network, by its corresponding position to the capillary network of crown mammals (Tanaka and Smith, 1978; Axelsson, 1988; Harper and Rougier, 2019).

It cannot yet be excluded that another vein would be present and exiting the cochlear space through the large perilymphatic foramen to drain into the inferior petrosal sinus, as the perilymphatic foramen and bony recess for the perilymphatic sac could accommodate such a vessel in these stem mammaliaforms. This hypothetical perilymphatic vein would be homologous to the vein of the cochlear aqueduct in crown therians, except it did not have its own bony channel, as for the vein of the cochlear aqueduct in stem and crown therians (Harper and Rougier, 2019). The bony canal for the vein of the cochlear aqueduct (apomorphy of trechnotherians) is developed concurrently with the canal of the perilymphatic duct, as both the canal for the aqueductus vein and the perilymphatic duct itself were separated from the fenestra cochleae in stem therian evolution.

We postulate that a venous network for the Stria vascularis and the scala tympani is already developed in stem mammaliaforms, as in extant mammals, prior to the common ancestor of crown Mammalia as well documented for Mesozoic crown mammals (Fig. 2) (Harper and Rougier, 2019). This proposal is based on several inter-related osteological correlates for this venous network: the "fishing-net-like" vascular foramina correspond to the positions of the scala tympani in the cochlear space, adjacent to position of the Stria vascularis and its related capillaries in extant mammals. This network is connected to the hypocochlear venous plexus of the petrosals, which is present in mammaliaforms and in Mesozoic clades of crown mammals (Panciroli et al., 2018; Harper and Rougier, 2019).

However, this hypothetical venous network of the stem mammaliaforms has a structural difference from the venous network of extant therians. In extant therians, the collecting venules on scala tympani and the capillaries on scala media are embedded in the soft tissue of the membranous labyrinth inside the bony cochlear canal. But the fishing-net-like venous network in mammaliaforms is partly intramural and the small veins are in the bone of the pars cochlearis. Further, there is a phylogenetic difference - this fishing-net-like network of stem mammaliaforms, as seen in docodonts and *Morganucodon*, is best developed in the middle portion of the promontorium; from there the network is connected to a hypocochlear venous plexus through the pars cochlearis to the inferior petrosal sinus (Panciroli et al., 2018). By comparison, the fishing-net-like part of this network of stem mammaliaforms is reduced or completely lost in eutriconodonts and stem therians, while the hypocochlear plexus is still present in crown Mesozoic mammals. This difference suggests that parts of the vascular network for supplying the cochlea in mammaliaforms are modified, its drainage network shifted posteriorly to be associated with the canal for aqueductus cochleae, which are all derived features of therians (Harper and Rougier, 2019).

#### 2.14.5.7 Cochlear Evolution in Crown Mammalia

### 2.14.5.7.1 Cochlear Curvature and Coiling

The curvature and coiling of cochlear canals shows a wide variation across mammaliaform phylogeny (Fig. 8) (Luo et al., 2016). The fully coiled cochlea ( $\geq$ 360°) is the most distinctive feature of therians; but among living therians, there are many further variations in cochlear turns and cochlear spiral heights (reviewed by Ekdale, 2013; 2016a; Manley, 2017). Different from therian cochleas, the cochlear canal of monotremes is only curved, but does not complete a full coil (Figs. 7 and 8) (Alexander, 1904; Schultz et al., 2017b).

As a whole, mammaliaform phylogeny shows an overall pattern such that in Mesozoic mammal clades successively closer (crownward) to extant marsupials and placentals, the cochleae may be more coiled. The pattern is partly recapitulated by the morphological ontogeny of the membranous cochlear duct (Fig. 8) However, there is no linear progression from the straight to curved and to coiled cochlear canals. Rather, there are obvious evolutionary homoplasies of the straight and curved cochleas among Mesozoic mammals; more crownward clades can show less curved cochleas than the earliest-known stem mammaliaforms (Fig. 8). Furthermore, the lengths of cochleae in most Mesozoic crown clades are not further elongated beyond the range of the earliest-known mammaliaforms and this has implications for estimating hearing range (Figs. 2 and 8) (Kirk et al., 2014; Manley, 2017).

The cochlear canal of docodonts can be curved to 150–180°, with curvature being progressively more pronounced in the apical region (Ruf et al., 2013; Panciroli et al., 2018). By comparison, the cochlea is weakly curved in *Morganucodon* and *Hadrocodium* (Luo et al., 2016). In the allotherian clade (Fig. 8: node 10), the gondwanatherians have more strongly curved cochleae (Kirk et al., 2014; Krause et al., 2020), while the sister clade multituberculates have less curved or nearly straight cochleae.

From mammaliaforms through crown Mammalia, the simple, uncoiled, and tubular cochlear canal is the typical condition along the entire cladogram backbone of mammaliaform phylogeny, while a more curved cochlea evolved in monotremes, gond-wanatherians, and dryolestoids, independently in their respective clades (Figs. 2 and 8: nodes 7, 10, 13). In eutriconodonts, spalacotherioids, and some multituberculates, the cochlear canal can be completely straight (Fig. 8) (Luo et al., 2016; Harper and Rougier, 2019). The straight cochlear canals of the latter Mesozoic mammal clades are considered to be a reversal from the more curved cochleae of mammaliaforms (Fig. 8) (Harper and Rougier, 2019).

It is possible that the straight-to-curved shapes of simple cochlear canals are a labile character of a simple tubular cochlea, which is architecturally un-constrained by the complex structure of the primary lamina and the ganglion canal. This labile variation can be manifest in taxa of the same clade. For example, while many multituberculates have slightly curved cochlease (Fox and Meng, 1997; Hurum, 1998; Ladevèze et al., 2010), other multituberculates have very straight cochleae (Fig. 9: *Meniscoessus*) (Luo and Ketten, 1991). The cochlea is more curved in *Morganucodon oehleri* (Fig. 7) but less so in *Morganucodon watsoni*, two species of the same genus (Luo and Neander, personal observation).

Extant Ornithorhynchus and Tachyglossus have curved cochlear canals that are expanded for the lagenar macula at the apex. However the overall curvature is greater in Tachyglossus, as the apex is more bent in the echidna. Both monotremes lack the full coiling of the cochleae of therians. The organ of Corti of monotremes has more rows of inner hair cells and of outer hair cells than in

therians (Vater and Kössl, 2011; Fritzsch et al., 2013), and their hair cells are arranged less regularly than those of therians. Most interestingly, because the organ of Corti terminates at the lagenar isthmus and stops short of the apical part of the cochlear duct, the length of the organ of Corti is much shorter than the cochlear canal length: the organ of Corti length (estimated from the cochlear duct length along the outer curvature to the lagenar isthmus) would be about 80% of the cochlear canal length in *Tachy-glossus*, and same proportion is about 60% for *Ornithorhynchus* (Schultz et al., 2017b). By contrast, the Organ of Corti reaches into the apex of cochleae in therians.

The membranous cochlear duct (the scale media) of monotremes is not supported by the primary bony lamina as in therians. As a result, the curvature and coiling of the membranous labyrinth and the bony cochlear canal are not concordant. The cochlear duct can be more coiled that the external bony cochlear canal in monotremes (Zeller, 1989; Luo and Ketten, 1991). This has been demonstrated by reconstruction of the membranous labyrinths from histological sections (Schultz et al., 2017b). In *Tachyglossus*, the membranous cochlear duct is coiled to 310° inside a bony cochlear canal curved to 285°; in *Ornithorhynchus*, the cochlear duct is curved to about 260°, inside a bony canal less curved to 140°. Thus the membranous cochlear duct, the scala vestibuli and the scala tympani are more curved (or coiled) than the curvature of the bony cochlear canals.

The new observations in monotremes (Schultz et al., 2017b) on the discrepancy of the coiling of membranous scalae from the coiling of bony cochlear canal has implications for estimating the hearing capability from cochlear canals of extinct mammaliaforms. For Mesozoic mammals with relatively simple tubular canal canals, the length of the organ of Corti, which is the functionally relevant part for hearing, can be shorter than the cochlear duct length, and be further shorter than the canal length. The curvature of the cochlear canal may under-represent the curvature and length of the membranous cochlear duct as seen in monotremes, but it can also over-represent the length of Organ of Corti (reviewed by Kirk et al., 2014 and Schultz et al., 2017b). The relative length of the cochlear canal is very informative for estimating the hearing capability in nonmammalian vertebrates (Walsh et al., 2009). While it can be useful for studying mammaliaforms (e.g., Kirk et al., 2014), the cochlear canal length is likely an over representation for the length of the organ of Corti without accounting for the lagena, for basal mammaliaforms.

A cochlear canal curved to about  $270^{\circ}$  was developed in cladotherians such as dryolestoids and *Vincelestes* (Fig. 8: node 13) (Hughes et al., 2015). This transformation occurred no later than the Middle Jurassic, when the dryolestoid clade appeared in the fossil record, and before the first appearance of the coiling ( $\geq 360^{\circ}$ ) in crown therians (Rougier, 1993; Luo et al., 2012; Harper and Rougier, 2019). In cladotherians, and possibly also in the gondwanatherians, this greater curvature is accompanied by a fundamental change in the cochlear canal - an extant therian-like ganglion canal embedded in the base of the primary bony lamina (Luo et al., 2016; Krause et al., 2020).

### 2.14.5.7.2 Cochlear Innervation: Foramen vs Tractus Foraminosus

Abundant fossils suggest that the common ancestor of Mammalia (Figs. 2 and 8: node 5) has retained all plesiomorphic cochlear structures for innervation already present in the earliest mammaliaforms: a simple tubular cochlear canal without interior bony structures such as a ganglion canal, and a single large opening for a single (or single-bundled) cochlear nerve. The internal auditory meatus for the entrances of vestibular and cochlear nerves (also of the conduit for the facial nerve unrelated to hearing), is also an open depression (reviewed by Harper and Rougier, 2019), in contrast to a deeper and tubular internal auditory meatus in clado-therians (Luo et al., 2012), the gondwanatherians (Hoffmann et al., 2014), and the monotreme *Tachyglossus*.

Unlike the ancestral condition in which the cochlear nerve (or bundle of cochlear nerve fibers) entered the inner ear space through a single large foramen, the derived pattern of the cochlear nerve of extant mammals enters the cochlea through densely packed foramina that perforate the bony junction of the cochlear canal on the floor of the internal auditory meatus (Fox and Meng, 1997; Luo et al., 2011; Ekdale, 2016). In therian anatomy, the tract of these foramina is termed the tractus spiralis foraminosus (*sensu* Williams et al., 1989; Evans, 1995), or simplified as the tractus foraminosus, as this tract is not spiraled in monotremes (Hoffmann et al., 2014; Schultz et al., 2017b). The same structure is commonly called the cribriform plate for its sieve-like morphology as seen in the internal auditory meatus. The foramina in the tractus foraminosus are connected with a network of braided channels, each of which is a conduit of nerve fibers to connect with the cochlear ganglion in the cochlear canal. The sieve-like bony morphology and the braided network of tiny canaliculi for nerve fibers are visible on endocasts in extant and Mesozoic mammals (Figs. 7 and 8) (Schultz et al., 2017a,b; Harper and Rougier, 2019; Krause et al., 2020).

As mapped on mammaliaform phylogeny, the cribriform morphology of the tractus foraminosus evolved for the first time in the cladotherian clade from an ancestor with a singular foramen (Fig. 2: node 13: ancestor of dryolestoids and living therians) no later than the Middle Jurassic (Luo et al., 2011; Ekdale and Rowe, 2011). Similar structures appeared for a second time in gondwana-therians (Hoffmann et al., 2014; Krause et al., 2020) during the Late Cretaceous. For the third (and the last) time, the cribriform morphology evolved in living monotremes, likely in the early Cenozoic. Not only did the tractus foraminosus evolve by convergence in separate branches of the mammal evolutionary tree, this structure also appeared iteratively in different geological times (Figs. 2 and 8) (Ekdale, 2016; Luo et al., 2016). The earliest-split clade of monotremes are likely to be the last clade to evolve the tractus foraminosus in the Cenozoic (Fig. 2).

The tractus foraminosus in monotremes is differentiated from the lagenar nerve canal, although the latter canal can be anastomosed locally with canaliculi for cochlear nerve fibers (Schultz et al., 2017b). The separation of the lagenar canal from the tractus is much wider in gondwanatherians (Krause et al., 2020). The internal aspect of the tractus of monotremes is simpler than those of gondwanatherians and cladotherians, and does not have a canal to serve as structural support for the cochlear ganglion of the latter groups.

# 2.14.5.7.3 Ganglion Canal and Primary Bony Lamina

The suspension of the cochlear ganglion and cochlear duct by the primary and secondary spiral ligaments, as exemplified by monotremes (Fig. 9E and F) (Schultz et al., 2017b) is a plesiomorphic pattern of extant nonmammalian amniotes (Weston, 1939; Manley, 1990). As mentioned earlier, the suspension of the cochlear duct and ganglion by membranous tissues alone and without any companion bony structure is an ancestral condition for crown Mammalia (Ashwell, 2013; Luo et al., 2016; Schultz et al., 2017b). By comparison to this simple pattern of the ancestor of crown Mammalia (Fig. 9: node 5), cladotherians are more derived and have extensive internal bony structures for better suspension of the cochlear duct (especially the basilar membrane) and for full enclosure of cochlear ganglion (Fig. 9: node 13). Similarly derived features evolved separately for a second time in gondwanatherians (Fig. 9: node 10).

The primary and secondary bony laminae are best developed in extant therians. The primary lamina is always present on the inner wall (the neural side) of cochlear canal through to the apical part of the cochlea (Ekdale, 2016). This lamina provides a stiffer bony support for the modiolar (inner) edge of the basilar membrane along the entire length of cochlea, which is fundamental to the overall cochlear mechanics (Echteler et al., 1994). Rosenthal's ganglion canal is developed in the base of the primary bony lamina, to enclose the cell bodies of the ganglion neurons. The canal connects to the Organ of Corti through the primary lamina, which has tiny canaliculi of the habenula perforata, sandwiched in the bi-layered structure of the lamina. These canaliculi allow the radiating fibers of the ganglion neurons (afferent dendrites of neurons and efferent axons) to access hair cells on the basilar membrane. Rosenthal's canal also connects to the internal auditory meatus through the tractus foraminosus - the cochlear nerve fibers (afferent axons of the ganglion neurons) traverse from the ganglion canal to the meatus through the foramina of the tractus. The bi-layered primary lamina with canaliculi, the Rosenthal's ganglion canal, and the tractus foraminosus are an integrated complex. So far, this complex has been documented for all stem eutherians and metatherians for which the inner ears have been studied (Meng and Fox, 1995; Ekdale and Rowe, 2011; Orliac et al., 2017).

The earliest appearance of Rosenthal's canal in the primary lamina in the fossil record is in the dryolestoids of the Late Jurassic (Figs. 2 and 8: node 13) (Luo et al., 2011). In *Dryolestes*, the Rosenthal's canal and the primary bony lamina extend distally into the apical part of the cochlear canal (Luo et al., 2012). The impression of the primary lamina is also present in *Henkelotherium*, which is also a dryolestoid (Ruf et al., 2009), although the bony structure of the lamina itself is not well preserved in this fossil. As the primary bony lamina demarcates the partition boundary of the scala vestibuli and scala tympani, the apical extension of the primary lamina is from the Miocene *Necrolestes* (Ladeveze et al., 2008) which is a dryolestoid-related fossil (Wible and Rougier, 2017). Further, the lagenar macula is absent in dryolestoids, as there is no lagenar nerve canal or a lagenar nerve sulcus to indicate the presence of a lagena in the apical region of the cochlear canal of these fossils (Hughes et al., 2015). An apical helicotrema and loss of the lagena are a modern therian-like pattern, in contrast to the subapical helicotrema of monotremes and stem mammalia-forms (Schultz et al., 2017b).

The primary lamina with a ganglion canal was discovered recently in the gondwanatherians Vintana and Adalatherium, although the topographical arrangement of these structures in this clade are unique in their own right, and surprisingly different from those of therians (Fig. 2: node 10) (Hoffmann et al., 2014; Krause et al., 2020). Gondwanatherians are an endemic clade of the southern Gondwana continents during the Cretaceous. According to the latest phylogenies, this clade is more closely related to multituberculates than to therians among crown Mammalia (Krause et al., 2020), or even more stemward according other phylogenies (Huttenlocker et al., 2018). The primary bony lamina of gondwanatherians is single-layered without the habenula for radiating ganglion fibers. The radiating fibers from the hair cells to the ganglion appear to have been attached to one side of the single layered primary lamina. This structural pattern differs from extant therians and dryolestoids in which the same radiating ganglion fibers are enclosed by habenula perforata inside the "bi-layered" primary lamina. Moreover, the gondwanatherian Adalatherium (but not Vintana) had a lagenar macula, as clearly indicated by a lagenar nerve canal (Krause et al., 2020). By contrast, the lagenar structure is absent in the cochlear canal of cladotherians and lost in the membranous labyrinth of crown therians (Fritzsch et al., 2013; Schultz et al., 2017b). Taken together, the primary lamina and ganglion canal of gondwanatherians are "heterotopic" (in a different position) with regard to the ganglion neurons and their afferent dendrites as seen in living therians and Dryolestes. It is possible that ossification of the primary lamina in gondwanatherians was under-developed and did not form habenula perforata. The development of the Organ of Corti did not extend far distally enough to displace the lagena in gondwanatherians, as in cladotherians. Given these structural differences and the distant relationship of gondwanatherians from therians on the evolutionary tree (Fig. 2), the ganglion canal and primary lamina of gondwanatherians (Fig. 2: node 10) are an incomplete convergence to those of cladotherians including living marsupials and placentals (Fig. 2: node 13).

# 2.14.5.7.4 Secondary Bony Lamina

In crown therians, the secondary bony lamina of the basilar membrane is always paired to the primary lamina. Where it is present along the cochlear canal, this lamina is located on the outer (abneural) wall. Its distal (apical) extent and length can vary significantly among major clades in therians and among taxa within a clade (Ekdale, 2013; Manley, 2018). Paired with the primary lamina, the secondary lamina helps to stabilize the cochlear duct within the larger bony cochlear canal and likely stiffens the basilar membrane support (Ekdale, 2016; Harper and Rougier, 2019).

Secondary bony laminae for the basilar membrane are not universally present, and the extent of this lamina can vary widely in major therian groups (Ruf et al., 2009; Ekdale, 2013). It has been suggested that the systematic variation of the secondary lamina can be related to hearing functions among extant therians. The width of basilar membrane is reduced by the secondary lamina along the

basal turn of the cochlear canal. A narrower basilar membrane in the basal turn of the cochlea is often more sensitive to higher frequencies (Fleischer, 1976; Ketten, 1997; Ekdale, 2013). The primary and secondary bony laminae have been extensively examined in therians with exceptional hearing capabilities, such as cetaceans and bats (Pye, 1970; Fleischer, 1976; Ketten, 1997). In extant placentals with high frequency hearing, it is generally assumed that more robust and wider primary laminae and secondary laminae, together, would indicate a more rigid support for basilar membrane (Fleischer, 1976; Ketten, 1997). For Mesozoic mammals, the presence of a secondary lamina is also regarded as indicating "higher frequency hearing" (reviewed by Kirk et al., 2014; Ekdale, 2016). However, the functional significance of a secondary lamina in early mammals for higher frequency sound hearing has been questioned, since there are pronounced inconsistencies between species regarding its presence in high-frequency regions of the cochlea (Manley, 2018). In most cases, however, cochlear regions responding to frequencies above 10 kHz have a secondary lamina.

The appearance of the primary lamina (with ganglion canal) and secondary lamina with the common ancestor of cladotherians in early mammal evolution may indicate a broadening of hearing capabilities in Mesozoic stem therians more crownward to marsupials and placentals. While it is well-established that the primary lamina is fundamental to cochlear mechanics (Echteler et al., 1994), how the development of the secondary lamina can be correlated to better structural support for the Organ of Corti and the basilar membrane remains to be an elusive issue (Schultz et al., 2017b; Manley, 2018; Harper and Rougier, 2019).

The wedge-like secondary bony lamina is absent in the extant monotremes and in all stem mammaliaforms. It is also absent in most (but not all) Mesozoic mammal clades stemward of cladotherians. Notwithstanding the strong evidence that this lamina is present in gondwanatherians (Krause et al., 2020), it is parsimonious to propose that the absence of a secondary lamina is the ancestral condition of crown Mammals (Fig. 9: node 5). The subsequent evolution from this ancestral pattern of crown Mammalia to the variable development of the secondary lamina and the impression of scala tympani (= base of secondary lamina) among Mesozoic mammals would be dependent on an assessment of monotremes, and the stem therians leading to living therians (Schultz et al., 2017b; Harper and Rougier, 2019).

The wedge-like secondary lamina, as seen in extant therians, is always developed between the scala tympani and the spiral ligament (Pye, 1970; Ketten, 1997). By topographic position, this lamina corresponds to the groove-like impression of the scala tympani (*sensu* Harper and Rougier, 2019) (= base of secondary lamina of Schultz et al., 2017b) of the monotreme *Tachyglossus*. This groove-like impression is distinctive in some cochlear endocasts, but is variable and absent in others of *Tachyglossus* (Schultz et al., 2017b: figs. 4 and 5). This shallow groove on the endocast corresponds to a low ridge on the inner wall of cochlear canal. Schultz et al. (2017b: Fig. 4: f) show that the "base for the secondary lamina" in *Tachyglossus* is not a wedge-like bony lamina; neither does it connect with the basilar membrane in the histological sections. The development of this structure is also labile among individuals of *Tachyglossus*, and variable between *Tachyglossus* and *Ornithorhynchus* (Schultz et al., 2017b). Nonetheless, this osteological correlate, if present on the cochlear endocast, can be a proxy to the development of scala tympani, and an indication of the likely position of the spiral ligament. Shahid et al. (2018) extrapolate this feature to estimate the extent of the basilar membrane in mammaliaforms. This is well justified.

Harper and Rougier (2019) offer a new proposal that the "base of the secondary lamina" is a prototype for the wedge-like secondary lamina. This is based on the observation that a deeper groove in the shallower impression of the scala tympani on the cochlear endocasts of *Priacodon* (a eutriconodont), the Hoovor petrosals (stem therians). This deeper groove corresponds to a sharp ridge on the inner wall of cochlear canal that extends from between the foramen cochleae and fenestra vestibuli, in the same position as the secondary bony lamina of therians. These osteological correlates are interpreted by Harper and Rougier (2019) to suggest that the secondary bony lamina is present in *Priacodon* and the Hoovor petrosals. The groove of the scala tympani is also present, but much shallower in other eutriconodonts (e.g., *Gobiconodon*) and stem therians (e.g., *Zhangheotherium*) (Fig. 8) (Luo et al., 2016).

Eutriconodonts and spalacotherioids have no primary bony lamina and its associated ganglion canal and tractus foraminosus. By contrast, in crown therians the secondary and the primary laminae are always the paired structures, matching on the opposite sides of the basilar membrane. The proposed secondary lamina of eutriconodonts and spalacotherioids is in the right topographical position (Harper and Rougier, 2019), but it does not have any of the matching structures for the secondary bony lamina, as seen in all cladotherians. Hypothetically, if the lamina is indeed the homolog to the secondary bony lamina, then the basilar membrane (and cochlear duct as a whole) would be secured from the outer (abneural) wall of the cochlear canal, but without corresponding support by the primary bony lamina from the inner (neural side) of the cochlear canal (Harper and Rougier, 2019). If so, the structural support would be incomplete in comparison to the cochlear duct of therians.

The hypothesis by Harper and Rougier (2019) helps to extend the evolution of the secondary bony lamina (or the base of the secondary bony lamina) to the clade of theriimorphs. It is also plausible that a base of the secondary lamina was already present, and would correspond to the scala tympani impression Mesozoic mammals (stem theriimorphs) stemward of the cladotherians, as this structural correspondence is present in extant mammals. However, the wedge-like lamina was not yet developed (Figs. 2 and 8). Another related hypothesis can be that the secondary lamina, as seen in *Priacodon* and Hoovor petrosals, is an independent evolutionary experiment on osteological structure for better suspension of the cochlear duct, and it would not have any extant analog for how such structure would function. The asymmetric support by the secondary lamina in these fossils would be an incomplete convergence to the symmetrical support by both the primary and secondary laminae of crown therians (Harper and Rougier, 2019).

### 2.14.6 External Ear

## 2.14.6.1 External Auditory Meatus And Tympanum Position

The mammalian tympanic membrane, also known as the tympanum, is derived from a precursor membrane in the mandible of their cynodont ancestor. This evolutionary transformation is now supported by an overwhelmingly strong evidence from fossils of cynodonts and mammaliaforms (Fig. 1), since it was proposed four decades ago (Allin, 1975). Recent genetic studies of the morphogenesis (Kitazawa et al. 2015a,b; Tucker, 2017) have further demonstrated that the mammalian tympanic membrane is developed ventral of the primary jaw hinge of the articular (malleus) and the quadrate (incus) of amniote ancestors. The tympanum of mammals is thus a *de novo* structure and has a distinctive and fundamental genetic basis in its association with the mandible and proximal segments of the second pharyngeal arch. This is in contrast to the tympanum of the extant reptiles and birds that are developed dorsal to the primary jaw hinge (Kitazawa et al., 2015a). Further, the tympana of extant diapsids are closely associated with pharyngeal arch 2, while in mammals the tympanum is exclusively associated with Meckel's cartilage of pharyngeal arch 1 (Furutera et al., 2017). Thompson and Tucker (2013) show that an early step in the development of the middle ear space in mammals involves neural crest mesenchymal cells and formation of a neural crest-derived epithelium for the dorsal part of the middle ear space. These processes are not present in extant reptiles (Thompson and Tucker, 2013; Kemp, 2016).

For historical interest only, there were two other alternative hypotheses on the presumptive tympanic membrane in cynodonts (reviewed by Kermack and Mussett, 1983; Allin, 1986; Kemp, 2016). One hypothesis (Watson, 1953; Parrington, 1979) postulated that the tympanum of cynodonts was in the basicranium, next to the quadrate and stapes, on the assumption that the tympanum would be similar to that of extant reptiles (Goodrich, 1930; see the review by Takechi and Kuratani, 2010). Such a putative ancestral tympanum had no structural relationship to the angular and is obviously inconsistent with widely accepted homology of the ecto-tympanic membrane; it would be composed of a larger tympanum in the mandible (homologous to mammalian tympanum) and a small component stretched to the basicranium (homologous to reptilian tympanum) (Westoll, 1945; Hopson, 1966) (reviewed by Kermack and Mussett, 1983; Takechi and Kuratani, 2010).

Taken together, there is no developmental possibility for the tympanum of mammals and their cynodont predecessors to be homologous to the tympanum of extant reptiles, either in entirety (as assumed by Goodrich, 1930), or in part (as assumed by Westoll, 1945). The reflected lamina of the angular and malleus on the mandible of cynodonts is the only possible location for the precursor membrane for the mammalian tympanum (Kitazawa et al., 2015a; Furutera et al., 2017).

The tympanic membrane in the posterior part of the mandible would be necessarily near the surface of the cynodont skull. Allin and Hopson (1992), (Fig. 10) proposed that the tympanic membrane on the postdentary bones of the mandible was broadly open on the surface in a crescent-shaped depression (see also Takechi and Kuratani, 2010; Fig. 6). From this presumptive shallow position of the tympanum, the more deeply positioned tympanic membrane with a tubular external auditory meatus evolved in extant mammals in their descent from cynodonts.

The formation of the external auditory meatus, the tympanic membrane and middle-ear cavity are intricately associated in mammal ontogeny (Anthwal and Thompson, 2016; Takechi et al., 2016). The morphogenesis of the external meatus began as an invagination from an external cleft to form a tube, with the tympanic membrane as the deep end of the tube, forming the interface to the middle ear cavity (Anthwal and Thompson, 2016; Burford and Mason, 2016). The proximal part of the external meatal cartilage is developed in close juxtaposition with the malleus (from the Meckel's cartilage of pharyngeal arch I), and the dorsal part of the Reichert's cartilage (second pharyngeal arch II) (Burford and Mason, 2016; Takechi et al., 2016). In the monotremes *Tachyglossus* and *Ornithorhynchus*, the proximal part of the meatal cartilage is juxtaposed with the proximal segment of the Reichert's cartilage and the malleus (Rugé, 1898; Zeller, 1989). In all extant mammals, the connection of the external auditory meatus to the tympanum is closely associated with the proximal segment of the anterior hyoid cornu of pharyngeal arch 2 (Rugé, 1898; Burford and Mason, 2016). Therefore, even if the meatal cartilage is not ossified in fossils of mammaliaforms, the presence of segments of anterior cornu of the hyoid apparatus can be an indirect proxy to the external auditory meatus, as these structures are intricately linked in development (Rugé, 1898; Zeller, 1989).

Stem mammaliaforms, such as docodonts and haramiyidans, had already developed a modern mammal-like hyoid structure, characterized by a series of jointed segments of the anterior hyoid cornu. *Microdocodon* and *Vilevolodon* were found to have a modern mammal-like hyoid apparatus (Zhou et al., 2019). Because the proximal segments of the hyoid apparatus are closely associated with the cartilage of the external auditory meatus in extant mammals, it is conceivable that the external auditory meatus was present in docodonts, and in the extinct clades of Mesozoic mammals by inference from the segmented hyoids.

The external auditory meatus is a common feature of extant mammals. It has been suggested that the external auditory meatus canal may enhance hearing of air-bone sound in the mid-frequency range (1.5–4 kHz) by resonance and mitigating bone conduction noise transmission (Homma et al., 2010). But the external ear pinna is only present in most of extant therians, and is absent in monotremes.

### 2.14.6.2 External Pinna

A pinna has a larger funnel surface to collect sound power from the environment and helps to enhance the sense of direction of the sound source, and in steering the sound collecting surface toward the source to enhance it (Rosowski, 2002; Heffner and Heffner,

2014). In theory, by development of the pinna, therians are more sensitive and more capable of detecting directionality of the sound source than monotremes.

Several mammaliaforms are now known from skulls fossilized with fur and skin outlines (Meng et al., 2015; Luo et al., 2017), but none has been preserved with an external pinna. An earlier reconstruction of an external pinna for the Jurassic mammaliaform *Castorocauda* (Ji et al., 2006) is now considered to be inaccurate. The earliest-known case of an external ear pinna is documented in the Cretaceous *Spinolestes*, an eutriconodont on the theriimorph stem to therians, thus a pinna likely evolved in theriimorph mammals (Martin et al., 2015), after the split of theriimorphs from monotremes that lack the external pinna (Fig. 2).

# 2.14.7 Functional Evolution of Mammaliaform Ears

## 2.14.7.1 Routes of Sound Conduction to Inner Ear

In nonmammalian cynodonts, both the plate-like angular and the tympanum supported by the hook-like angular would be capable of receiving sound (Allin, 1975; Kermack and Mussett, 1983). Because the plate-like reflected lamina of the angular is a prominent structure, it likely received air-bone sound in cynodonts (e.g., Allin, 1975), and even in some pre-cynodont therapsids (Allin, 1975; Kermp, 2007). It was hypothesized that the cynodonts already had a mandibular diverticulum, an air-filled space associated with the medial aspect of the mandibular ear, which would be a homolog to the middle ear tympanic cavity of mammals (Allin and Hopson, 1992; however, see the alternative view of Kemp, 2007). The inferred presence of an air-filled mandibular diverticulum is consistent with the reconstruction of the tympanic membrane for transmission of the airborne sound.

However, the tympanic membrane and mandibular diverticulum of the mandibular ear may not be the only route for sound transmission. The reflected lamina of the angular, once having received airborne sound vibrations, would further transmit vibration via bone conduction. Implicit in this hypothesis is that the angular itself transmitted sound to the tympanic membrane through bone conduction. Bone conduction, either by direct route through the dentary to the postdentary bones (including the angular), or by indirect route through the rest of skull, is a major transmission route of sound waves to the inner ear in synapsids (Tumarkin, 1968; Kermack and Mussett, 1983; Laaß, 2016). Overall, it is still a primary mechanism conserved in most therapsids (Maier and van der Heever, 2002; Laaß, 2016), while the neomorphic tympanic membrane may have received the airborne sound in parallel.

It is also generally agreed that, with large postdentary bones, the sensitivity to higher frequencies would be more limited in basal cynodonts than in derived mammaliaforms. The attachment of the postdentary bones to the mandible is extensive and presumably stiffer in cynodonts than mammaliaforms (Kermack et al., 1973). The cranial anchoring of the quadrate and quadratojugal complex had not yet achieved the same agility as in later mammaliaforms (Luo and Crompton, 1994). This also limits hearing sensitivity thresholds. As long as the angular and its adjacent surangular are still extensively attached to the mandible, bone conduction would most likely have co-existed with the more direct reception of airborne sound by the neomorphic tympanic membrane (Fig. 10A). Most therapsids, including cynodonts, were terrestrial animals with generalized skeletal features, although some were arboreal (Fröbisch and Reisz, 2009) or even fossorial (Kemp, 2005; Fernandez et al., 2013). A mandibular route for bone conduction would convey a functional advantage for fossorial animals, and it is also feasible for hearing in terrestrial species.

The ossified Meckel's cartilage in stem mammaliaforms and some mammals (e.g., eutriconodonts and spalacotherioids) remained connected by Meckel's element to the mandible (the PMME). In that situation, airborne or substrate transmitted sound vibrations that are received by the mandible would be transmitted via the ossified Meckel's cartilage to the middle ear. Thus, sound reception via bone conduction would co-exist with the sound reception by the tympanic membrane (Fig. 10B-C). Bone conduction is reduced in successively more crown-ward mammaliaforms as the tympanic membrane gained a larger surface area for airborne sound reception, coupled with better hearing sensitivity and expanded frequency ranges (e.g., Kermack and Mussett, 1983; Rosowski, 1992). Because the middle ear bones and the ossified Meckel's cartilage, together as a unit, have a larger mass than without the Meckel's cartilage, we speculate that eutriconodonts and spalacotherioids with the PMME almost certainly did not attain the same upper frequency range as those of extant mammals. But on the other hand, the middle ear connected to the mandible by the ossified rod of Meckel's element may well compensate by enhancing the low frequency vibrations conducted from the mandible to the inner ear.

The loss of mandibular sound conduction, and the shift to a primary hearing of air-borne sound via the tympanic membrane, did not occur until the DMME evolved (Fig. 10D), independently in monotremes, in multituberculates, and in therians (Fig. 4). However, after achievement of the primary tympanic membrane sound reception, many fossorial mammals, including the monotreme *Tachyglossus* (Aitkin and Johnstone, 1972), independently evolved secondary bone conduction that correlates with their habitat preference and locomotor specialization (Mason and Narins, 2001; Laaß, 2014).

#### 2.14.7.2 Functional Interpretation of Middle-Ear Evolution

One of the most remarkable features of middle-ear evolution in mammals and their relatives is the extremely long evolutionary time during which many diverse lineages maintained a connection of the ear to the mandible, long after the neomorphic dentary-squamosal jaw hinge had relieved the malleus and incus from the loading for masticatory movement of the jaw (Fig. 2). From the Middle Jurassic to the Early Cretaceous, there were more than six clades that either retained a side-to-side attachment of the ear to the jaw (as in docodonts), or maintained an anterior connection to the mandible via the Meckel's (as in eutricondonts).



**Figure 10** Hypotheses on routes of sound conduction and transmission in cynodont-mammal evolution. (A). Cynodont *Thrinaxodon* already developed a hearing of airborne sound by the tympanic membrane suspended by the angular bone (ectotympanic) and an air-filled middle ear cavity; but it concurrently had a capacity to hear substrate-transmitted sound through the mandible to the angular and surangular, to the articular (malleus), and the quadrate (incus), then to stapes, plus a route for sound to be conducted directly from the cranium to the inner ear. (B). *Sinoconodon* has more gracile middle ear bones and a proportionately larger tympanic membrane with a great capacity to receive the airborne sound, but hearing via the substrate-transmission is still fully functioning with the mandible-attached middle ear. This is a mammaliaform pattern. (C). *Yanoconodon* with its middle ear partially displaced from the mandible (Partial Mammalian Middle Ear) could still hear the substrate-transmitted sound from the mandible through the ossified Meckel's element to the malleus, incus and stapes, while mainly relying on the tympanic membrane for hearing air-borne sound; first development of external pinna in eutricondonts, as indicated by *Spinolestes* (Martin et al., 2015). (D). *Didelphis* with DMME exclusively relying on the tympanic membrane for hearing, although various fossorial crown mammals (such as many multituberculates, and *Necrolestes*) can develop secondary a cranial route for hearing substrate-transmitted sound. From Luo et al. (2016), original work by April I. Neander of UChicago, used with permission.

and spalacotherioids), falling short of the "three ossicles separated from the lower jaw" that distinguish extant mammals from other vertebrates (Figs. 2, 3 and 4).

Perhaps the most parsimonious explanation for this is that the middle ear response was constrained by the inner ear, especially at the upper limit of frequency range (Ruggero and Temchin, 2002). In all lineages with connected middle ears, the inner ear also has a short and straight cochlea and poorly differentiated interior structures of the cochlear canal. Such under-developed cochleae, as compared to those of cladotherians (Fig. 9), were not in any position to handle sensitive, wide-frequency inputs. Therefore, the functional evolution of the middle ear was limited by a lack of more sensitive hearing capability of the inner ear. Furthermore, other

selective pressures could have worked against the changes of the mandibular middle ear configuration. The mandibular middle ear was capable of more than just receiving air-borne sound to the tympanum. It had a major function conducting sound from the lower jaw to the inner ear (Fig. 10). If sound input via the lower jaw (either directly from the substrate or from the air, or both) could convey a selective advantage for hearing, then it would have been retained in Mesozoic mammaliaforms. The ear-jaw connection is an evolutionarily ancestral feature, but this does not preclude such an ear from developing a viable (and even efficient) hearing by mandibular conduction. If stem mammals and their mammaliaform relatives had a strong complementary mechanism for sound reception, it may have downplayed, or preempted the selective advantage for airborne sound via a disconnected ear. Another possibility is that bone-conducted sound from food mastication via the jaw could have played a role in a feedback loop to fine-tuned mastication (Manley, 2017a), parallel to the well-known periodontal ligament of teeth for sensory feedback from chewing in mammaliaforms. These functional influences likely stabilized the connection of the middle ear to Meckel's cartilage, ossified or not, in early mammal evolution.

A more complex – and currently unsolvable – issue is the integration of more than one sound-input pathway (Fig. 10) (Kermack and Mussett, 1983; Luo et al., 2016). If, as seems likely, the inner ear only received low-frequency input and this arrived both through the eardrum and by bone conduction, the two inputs would have not been synchronous, as bone conduction is a faster route. It is possible that a poor correlation of inputs reduced the efficiency of the hearing organ. Nonetheless, as both inputs were important, although in different frequency ranges, they remained patent pathways to the inner ear.

During the long evolutionary time of the Mesozoic mammaliaforms, the eardrum remained relatively small and its suspension by the ossicles less extensive than in the extant mammal lineages. There is, for example, no evidence of an elongated manubrium ("long arm") of the malleus reaching the center of the tympanum in stem mammaliaforms that, in therian mammals, is the most sensitive area picking up sound vibration from the eardrum. In addition, the ectotympanic was a short hook in mammaliaforms (Fig. 3), and not ring-like as seen in therians. It is possible that this hook region was, in fact, the main sound pick-up from the eardrum the same as its precursor in cynodonts, but being short would have been inefficient and have operated only a low lever effect on the ossicular chain.

The sheer size of the middle-ear components of all early mammals with hook-like ectotympanic could also have been a hindrance to change. Small eardrums would not have been efficient while being associated with more massive bones still connected to the jaw. Instead, this suggests that the ancestral bone-conduction pathway remained the more important route, until most early mammal lineages began to transition through the evolutionary bottleneck of body size reduction (Lautenschlager et al., 2018). This size reduction bottle neck is accompanied by the evolution of the manubrium of the malleus, and the extension of the ectotympanic into a ring mostly surrounding and supporting the eardrum, which would have greatly raised the relative importance of the eardrum-linked pathway. Simultaneously, the reduction in the mass of all the bones, including size reduction and related changes of the incus and stapes, would have reduced the stimulus effectivity of low-frequency vibrations from the substrate.

The evolutionary transformation towards a middle ear purely dedicated to the reception of airborne sound required an increase in the area of the tympanum and increases in lever ratios between the ossicles that would have led to an increase in sensitivity, probably at least 20 dB at low frequencies and potentially very much more at high frequencies. These changes, however, would have also depended on the further and concomitant evolution of the cochlea, since the upper frequency limit of the middle ear is strongly influenced by the response – and therefore the impedance – of the inner ear (Ruggero and Temchin, 2002; see below).

## 2.14.7.3 Evolution of Internally Decoupled Ears in Therians

It is worth noting here that, besides the multiple evolutions of the mammalian "middle ear with three ossicles," all non-mammalian tetrapod lineages developed single-ossicle middle ears not only independently of mammals, but also of each other (reviewed in Manley, 2010; Manley and Clack, 2004). As noted above, these middle ears evolved at a higher position in the head and dorsal to the primary jaw joint (Kitazawa et al., 2015a,b; Tucker, 2017), in the region of the ancestral spiracle and with the open spiracle canal connected to the pharyngeal space. This allowed sound from either eardrum to cross the head through the mouth cavity and the two ears could interact through this air-filled space. This developed into what is now known as "internally coupled ears" in which the sound input from both ears interacts at the inner and outer face of the eardrums before either inner ear is stimulated. Such systems are also known as pressure-gradient receivers (Köppl, 2009). They provide complex, but potentially very useful, information for sound localization that precedes neural processing, and have been shown to be highly effective in ears of lizards (Christensen-Dalsgaard and Manley, 2008; Christensen-Dalsgaard, 2011). Since such middle-ear systems are "automatically" open to the outside world, they do not need special systems that equalize air pressure on both sides of the eardrum.

In mammals and their near relatives, as noted above, the middle ear is derived from the mandible and located in a lower position in the head. A middle ear connected to the jaw has a different pressure-equalization connection to the mouth cavity than those of non-mammalian tetrapods. It has been proposed that the ancestors to mammals also had the internally coupled ears (Christensen-Dalsgaard, 2011). By contrast, virtually all extant marsupials and placentals have internally decoupled ears, also known as pressure receivers. The pressure-receiver ears of therians must have evolved at the same time as the Eustachian tubes, which are narrow and not continuously open. While Eustachian tubes can equalize the pressure of the middle ear cavity when opened, the tubes are narrow and can also preclude their involvement in a pressure-gradient receiver system, when closed. The pressure-receiver system is best developed in therians with full tympanic bullae enclosing the middle ear spaces (Novacek, 1977; Ekdale, 2016a).

Nonetheless, there are some exceptions - talpid moles and golden moles have developed inter-aural airways through the highly pneumatized basicranial bones, which represents an example of independently evolved pressure-gradient system within placentals.

The monotreme *Ornithorhynchus* is different from therians in that the Eustachian tubes are patently open and connected to the mouth cavity (Zeller, 1989; Mason, 2016). In cynodonts and stem mammaliaforms, in which the mandibular middle ear excludes the possibility of an enclosed middle ear space, the two ear spaces almost certainly had a patent airway to the pharynx, as seen in *Ornithorhynchus* (Mason, 2016), or are even confluent with the pharynx. This proposal on the hypothetical connection between the ears and the pharynx is also consistent with the muscle reconstruction of the pharynx and the jaw of cynodonts (Barghusen, 1986; Allin and Hopson, 1992). Further, it is also reasonable to postulate that in Mesozoic mammal clades with connected middle ear (PMME), similar airways from ears to pharynx existed, possibly enabling a pressure gradient between the two ears, functionally analogous to those of reptiles. The question of whether any of these systems could have operated as a pressure-gradient system depends on how wide the inter-aural connections were and whether they were continuously open. But certainly the de-coupled ears only occurred in very derived therian mammals, and evolved very late in mammaliaform phylogeny.

### 2.14.7.4 Functional Interpretation of Inner-Ear Evolution

Any literature survey of mammalian inner ear characteristics and function almost always includes a general statement to characterize mammalian ears as "remarkably sensitive and selective," as compared to those of other vertebrates. This kind of statement is not only facile but in many cases incorrect. In fact a direct and detailed comparison of the sensitivity and frequency selectivity of the ears of different tetrapod groups shows that while some mammals are indeed both highly sensitive and selective, there are members of other vertebrate groups, such as birds or lizards, that are equally – or even more – sensitive and selective (Manley, 2017a). The tendency to attribute the best hearing to mammals is presumably a remnant of the "ladder" idea of functional evolution, in which mammals, especially humans, would be the pinnacle of vertebrate evolution. The current review, along with a vast literature of the last 100 years, shows clearly that mammalian evolution was anything but a single, direct lineage of groups, and that the complexity of the lineages and the timing of evolutionary events is almost mind-boggling. Our survey of middle- and inner-ear evolution has made one thing very clear: evolutionary changes tend to be a step-wise assembly of highly integrated structures (Manley, 2013), and are very complex when mapped on a fully resolved phylogenetic tree of many clades (Luo et al. 2016). The ear characteristics of each lineage, large or small, must be evaluated in its own right.

### 2.14.7.4.1 The Inner Ear of Early Mammaliaforms

It is a truism that, while it may be difficult to interpret the structures in often incomplete or distorted fossils, it is an order of magnitude more difficult to find any kind of reliable interpretation of the function of the soft-tissue organ systems from the fossilized osteological structures. Thus functional interpretations are necessarily speculative and need to be treated as such. Fortunately, soft-tissue systems often do leave tell-tale imprints and cavities in the associated bony structures, which helps enormously in reconstructing their likely configurations. It is on this basis that the following can be attempted.

The earliest mammaliaforms – like other tetrapod lineages of the Mesozoic – all had very small cochlear cavities (Figs. 8 and 9) and had inherited a small auditory papilla, perhaps 1 mm in size, containing less than ~1000 sensory cells and known as the basilar papilla. A comparative view across all inner ears of these Mesozoic tetrapods suggests that these were only low-frequency receptors, most likely limited to at most a few kHz in frequency responses (Manley, 2017a). The long evolutionary history before the final appearances of DMME, a middle ear disconnected from the lower jaw, was unlikely to have changed this well-conserved inner-ear configuration, since the sound input through the mandibular middle ears would have been mainly of low frequency, and less sensitive than through the disconnected ear.

The interpretation of Mesozoic mammaliaform ears in terms of their likely frequency response is fraught with difficulties (Kermack and Mussett, 1983; Rosowski, 1992; Luo et al., 2016). For the earliest mammaliaform *Morganucodon*, which has a middle ear broadly attached to the mandible and appears to be very stiff, some analyses (Rosowski and Graybeal, 1991; Rosowski, 1992) compared it directly to therians and suggest that it could hear high frequencies partly due to the stiffness of the middle ear. Other analyses (e.g., Kermack and Mussett, 1983), however, would consider *Morganucodon* to be inefficient in both frequency range and sensitivity, as compared to therians, because its middle ear is still bound to the mandible, and still has a relatively greater mass.

There are several difficulties with the hypothesis that *Morganucodon* had high frequency hearing: First, newer fossils of this species described since the 1990's show that it has small tympanum (about 30–40% smaller than had been assumed). Second, a stiff middle ear has also been reported from the extant monotreme *Tachyglossus* (Aitkin and Johnstone, 1972), yet this species has one of the lowest upper hearing limits of any modern mammal. Although a more stiff middle ear can be more sensitive than the less stiff middle ear among placental mammals (Fleischer, 1973; Rosowski, 1992), this is not applicable to all mammals, and especially not to monotremes. Third, the middle ear is not the only pre-requisite for high-frequency hearing and for greater sensitivity of hearing. It is also necessary to have the structure and physiology of the inner ear (e.g., endocochlear potential, tuning and sensitivity mechanisms of the hair cells) for the middle- and inner-ear system to achieve higher upper limits of the hearing frequency range and a greater sensitivity. In this regard, comparative evidence of non-mammalian land vertebrates can be instructive. *Morganucodon watsoni* had a cochlear canal length up to 1.8 mm (Luo et al., 1995; Shahid et al., 2018). Its organ of Corti likely had a length of maximally 1.5 mm (Shahid et al., 2018), and this corresponds to the longer modern lizard auditory papillae and is shorter than any bird papillae. Modern lizards and birds are, in almost all cases, confined to frequencies below 6 kHz. It is entirely



**Figure 11** Functional evolution of cochleae among Mesozoic mammaliaforms. (A). The average space devoted to one octave in many species of reptiles (blue points in a blue oval), birds (purple points in a purple oval) and placental mammals (yellow points in a yellow oval) as a function of the length of their basilar membrane, which supports the hearing organ in all cases. The red point in the yellow oval shows the value for human cochleae. If it is assumed that the Mammaliaform species of the Mesozoic conformed to a similar relationship between hearing-organ length and space per octave, then their values would fall within the dashed red oval. (B). A speculative scenario for the evolution of the upper frequency limit of hearing of Mesozoic mammal lineages, based on the dimensions of the fossil cochleae. The monophylogenetic clade of cladotherians to living therians has sophisticated interior structural support for the organ of Corti in the cochlear canals, and but less spatial constraint for elongation by coiling (yellow arrow). However, all mammaliaforms and stem Mesozoic mammals have simple cochlear canals (except for gondwanatherians) and their cochlear canal elongation reached a maximum of 6–8 mm before the extinction of all these clades (green arrow). Thus these Mesozoic mammaliaform clades never developed sensitive hearing for higher frequencies, as in cladotherians, or living therians. *Morganucodon* was an early mammaliaform, *Onychonycteris* an Eocene, non-echolocating bat (Simmons et al., 2008). The blue bar on the right covers the known range of upper frequency limits in modern placental mammals. Estimates of the upper frequency limits of hearing in fossil species are based on cochlear dimensions. The arrows indicate that the trends in different mammalian lineages were not the same. (A) Modified after Manley (2000: **fig. 9**); (B) original work.

likely that *Morganucodon* did not exceed these upper limits of reptiles and birds, and was well below the maximum of the hearing range of monotremes (Fig. 11).

Similarly, the short cochlear canals of the Late Cretaceous gondwanatherians *Vintana* (5.4 mm) and *Adalatherium* (about 6 mm) are shorter than in any extant mammal (Kirk et al., 2014; Krause et al., 2020). Kirk et al. (2014) inferred that gondwanatherians could hear high frequencies (>20 kHz) on the basis of both the primary and secondary laminae. Similar inference was also made for the Late Jurassic *Henkelotherium* and *Dryolestes* (Ruf et al., 2009; Luo et al., 2011). However, the presence of both these laminae cannot be used alone to reach conclusions about the highest frequencies that were processed (Manley, 2018). While in modern placentals, a secondary lamina is only found in cochlear areas that respond to frequencies above 10 kHz, it would not be logical to assume that the entire cochlea of *Vintana* only responded above 10 kHz (Manley, 2018), especially since for land vertebrates, low-frequency hearing is a plesiomorphic characteristic. The apex of gondwantherian cochlea certainly had a lagenar macula, and the organ of Corti did not extend the full cochlear canal length (Krause et al., 2020).

### 2.14.7.4.2 The Inner Ear of Mesozoic Crown Mammals

The disconnection of the middle ear occurred several times independently (Figs. 2 and 4). With changes in size and shape of the ectotympanic, malleus, incus and stapes, such ears would have strongly influenced hearing function. This is manifest in the subsequent evolutionary changes in the inner ear in eutriconodonts, multituberculates, monotremes, therians and their kin. It is, however, all the more noteworthy that the inner ears of these groups show remarkably different evolutionary trajectories, and many questions have yet to be answered.

Eutriconodonts, multituberculates, and spalacotherioid therians did not elongate their cochlear canals very much, and these remained either straight or only slightly curved (Fig. 8). In contrast, monotremes show a curved cochlear canal that can be more than 10 mm in length. Inside the bony cochlear canal, the soft tissues of scalae show a much stronger curvature. While eutriconodonts, multituberculates and spalacotherioids – almost without exception – show a narrow apex to the cochlear canal and no other evidence of the retention of the lagenar macula within the cochlear canal, monotremes did retain this macula, such that the basilar papilla – now known as the organ of Corti – takes up only about 70% of the cochlear length (Schultz et al., 2017a,b). Functionally, it likely that all extinct mammal clades of the Mesozoic did not exceed the small improvements in sensitivity achieved by the monotremes.

Modern monotremes have been studied sufficiently to offer a good general view of the structure and function of their auditory periphery (e.g., Aitkin and Johnstone, 1972; Ladhams and Pickles, 1996; Mills and Shepherd, 2001). The middle ear is quite stiff

(Fleischer, 1973), which is perhaps a reasonable explanation for the relatively poor sensitivity of monotreme hearing. The most interesting thing about the inner ear is that it is a mosaic of ancestral characteristics - it is not completely coiled, and has a lagena macula and few other derived features - but it shows the typical structure of an organ of Corti. This suggests that the organ of Corti-typical structure is a shared derived character of monotremes and therians, plus all Mesozoic mammal clades nested with them in phylogeny (Schultz et al., 2017b; Köppl and Manley, 2019; Harper and Rougier, 2019).

An organ of Corti has two well-defined sets of hair cells - inner and outer hair cells - and these lie on the inner and outer sides, respectively, of a "tunnel" formed by "leaning" pillar cells. In comparison to therian cochleae, monotremes have approximately an equivalent number of hair cells, but these are arranged along a shorter organ, which means that each set of hair cells has more rows than are typical for therians. Therians have one row of inner and three rows of outer hair cells; monotremes can have three to four irregular rows of inner hair cells (but decreasing to just one row near the cochlear base), and six to eight irregular rows of outer hair cells (also decreasing to two rows near the base) (Ladhams and Pickles, 1996; Vater and Kossl, 2011; Jahan et al., 2015). We do not know much about the physiology of the cochlea (e.g. whether there is a significantly large endolymphatic potential or not), and there have been no measurements of single auditory-nerve-fiber responses in monotremes. We only know that the highest response frequencies lie near 15 kHz (Aitkin and Johnstone, 1972), which is at the lower end of the range typical for therians, and that those responses are not very sensitive. It is possible that the relatively poor sensitivity and low limit on upper frequency is at least in part due to the fact that the organ of Corti of monotremes is not supported by bone within its soft tissues. Instead, the hearing organ lies as a membranous tube within a tube of bone – a very significant difference when compared to therians.

# 2.14.7.4.3 The Inner Ear of Therian Mammals

Compared to all other vertebrates, therian mammals show a number of apomorphies of the cochlea that clearly distinguish them from all other groups. Functions related to these apomorphies are often viewed as a major adaptation by therians, over the inner ears of other tetrapods. However, in most physiological respects they are no better than many inner ears of birds and even lizards (Manley, 2017a). Nevertheless, most therians have one distinguishing feature – their upper frequency limit exceeds those of other vertebrates, including monotremes. While there is sometimes no difference (e.g., the upper limit of elephants is the same as or below that of some birds and lizards), some therians such as bats and whales have limits exceeding 100 kHz (Fig. 11B). One of the most important evolutionary changes was surely cochlear elongation, the spatial constraints of which were removed by the coiling of the cochlea. Thus instead of being limited to simply one full circle of coil, the transformation to a snail-shell-like structure, with turns in a spiral, made it possible to elongate the organ of Corti through several turns (up to 4.5 are known) while still retaining the structure entirely within the petrosal bone. It is likely that this elongation, with a much reduced spatial constraint, was a key to cochlear coiling. It has been proposed the curvature changes in the coils may provide functional advantage for low frequency sound (e.g., Manoussaki et al., 2008). While such an improvement in low-frequency hearing can be regarded as a fortuitous consequence or even a side effect of that coiling, such an advantage would have been post-hoc (Manley, 2017b), and would be unlikely to have influenced their evolutionary origin, per se.

As noted above, some of the derived characteristics of therian cochleae arose in parallel in different lineages, e.g., the elongation and coiling, the bony canaliculi of the tractus foraminosus for the cochlear nerve fascicles, and the laminae supporting the basilar membrane (Fig. 9). Of course many of the functional features of the organ of Corti are not visible in fossils, in particular all hair-cell and supporting-cell functions, including the biochemical characteristics of the stria vascularis and of ion channels, etc. Their origins can only be speculated upon on the basis of limited osteological correlates in fossils, and by extrapolation by phylogenies from counterparts of extant vertebrates. However, in comparison to the long evolutionary time required for earlier changes in hearing systems, the transformation from an uncoiled cochlea driven by a less sophisticated mandibular middle ear to a coiled cochlea driven by a disconnected tympanic middle ear was relatively rapid. This coiled cochlea has bony laminae and a high endocochlear potential, and it can respond to very high frequencies. This rapid evolutionary change suggests that there was one or - only a few - critical events that precipitated a cascade of important changes during a brief period of evolution (Fig. 2).

One evolutionary change of therian cochleae, possibly decisive, was the loss of the lagenar macula (Manley, 2017a). This transformation occurred either just before or at the time when cochlear coiling was completed (Luo et al., 2012). The loss of the lagena presumably resulted from the coiling itself. It is posited that in the evolution towards extant therians, the increasing coiling would have gradually changed the relative angle of orientation of lagenar hair cells with respect to the head. The likely ancestral function of the lagena was similar to that of the saccule and utricle, namely to monitor head position. The rotation of the growing cochlear canal by coiling in therian evolution would have produced new lagenar orientations that at some point would have coincided with that of an existing and larger hair-cell epithelium of the sacculus or of the utriculus. The sensory input from the lagena and the vestibule would have been overlapping and redundant, and this could have led to the loss of the lagena, in a simple evolutionary scenario.

It has been suggested, on molecular genetic evidence, that the morphogenetic network for the lagena was conserved in all mammals. Hair-cell types of the inner ear can be strongly influenced by the morphogenetic network during their development, and lagena precursors can be induced to develop features of auditory hair cells (Fritzsch and Elliott, 2017) and vice-versa. It has been proposed that in therians, the lagena was not really lost, but became incorporated into the organ of Corti as the apical component of the latter (Fritzsch et al., 2013). One problem with this interesting idea is why a structure that almost certainly already existed (low-frequency hearing is plesiomorphic for all land vertebrates) needed to be replaced. Perhaps the apical organ of Corti and the lagena gradually merged with the eventual loss of vestibular function of the latter? The real events are not important for the purposes of the present discussion – here, it is only important to discuss the consequences of the loss of lagenar otoliths from the cochlear space.

The loss of the lagena would have had profound consequences, especially regarding calcium metabolism. As calcium is critically involved in a multiplicity of cellular functions, this event may have had a decisive influence on the further evolution of the hearing organ itself (reviewed by Manley, 2017b). It is known from fish, frogs and lizards (e.g., Manley, et al., 2004) that the endolymph surrounding otolith-bearing organs such as the lagenar macula has a high calcium concentration (at least 200 mM). This is essential for maintaining the calcareous otoliths, which would otherwise disintegrate. By contrast, the calcium concentration in the cochlear endolymph of modern therians (actually only known from eutherians) is more like 30 µM, very much lower than in any other group and lower than is possible in monotreme cochleae (although this has not been measured). Calcium ions play very important roles in hair-cell and supporting-cell metabolism and function, including the processes of sound transduction and adaptation. The transition in the cochlear endolymph from very high to extremely low calcium concentrations was likely a decisive, even revolutionary, change for therian hearing (Manley, 2017a). We do not know which functions were affected and how they were affected, but it is likely that this was not a trivial series of adjustments. Some possible influences were: a drop in sensitivity of the hair cells due to effects on channel opening probabilities, an effect on hair-cell adaptation mechanisms and their speeds, an influence of the efficiency of the stria vascularis that is a driving force for hair-cell responses, and an influence on the constitution of the tectorial membrane. During these evolutionary changes, it is likely that selection pressures were raised for those changes that would have mitigated any negative influences. One of these mitigating effects was the rapid recruitment of a new cochlear amplifier mechanism.

#### 2.14.7.4.4 Prestin, Calcium Physiology, and Cochlear Function

Therian cochlear hair cells employ two mechanisms to amplify their responses to sound (Hudspeth, 2008). One of these is very ancient and involves mechanical energy produced within the hair-cell bundle during the transduction process. The other mechanism - unique to mammals in its pattern and strength - involves the molecule prestin that occurs in very high concentration in the lateral cell membranes of outer hair cells (Dallos, 2008). Although this molecule shows evolutionary changes throughout extant mammals (Okoruwa et al., 2008), a high concentration of special forms of prestin is an apomorphic feature of therians (Fig. 2: node 14). Prestin belongs to a family of membrane-transport molecules, but in its particular form in hair cells has become a force generator. When a small electrical potential arises across the cell membrane (e.g., during a response to sounds of a particular frequency), prestin is able to change its shape very rapidly and the molecule occurs in such high concentrations that this shape change can physically elongate a hair cell, at high response levels by several percent of its length. Given that the movement of a hair-cell bundle at threshold response levels is of the order of a few nanometers, this length change even at low sound levels is large enough to drive not only the hair cell but - in concert with its neighboring hair cells - to drive the entire local organ of Corti. This effect is frequencyspecific at any given location and thus plays a critical role in local response sensitivity. The curious thing is that although this phenomenon is unique to therian mammals, they are not on average any more sensitive than some well-performing nonmammals. This suggests that the inclusion of prestin did no more than restore a sensitivity level that had been compromised by the cascade of evolutionary events following the loss of the lagena. Of course the lagena did not disappear overnight, as it were, but presumably over a time period sufficient to provide the flexibility to evolve and incorporate this new mechanism.

Therian prestins are not all identical (review in Manley, 2016) and there is evidence for parallel and rapid evolution of this motor molecule. The same change in the protein sequence occurred independently in bats (about 50 Ma) and whales (about 30 Ma) and presumably underlies the exceptional ability of these animals to hear sounds of extremely high frequencies.

Higher-frequency processing in modern therians requires cellular and physiological specializations (such as of the protein prestin). There is evidence that prestin evolution occurred in a series of steps over evolutionary time, and this occurred convergently in such lineages as bats and whales, but the echolocation-related motifs of prestin were not present in the common ancestors of placental mammals (Marcovitz et al., 2019). In bat evolution, the cochlea spiral configuration associated with echolocation occurred well after the origin of bats (ca. 50 ma) (Simmons et al., 2008). Other evidence, for example regarding the possible presence of a high endocochlear potential in the scala media, may be inferred on phylogenetic brackets for Mesozoic eutherians and metatherians (Ji, et al. 2002), or stem therians that are much closer to crown Theria (Fig. 2) (Harper and Rougier, 2019). But such soft-tissue and physiological traits may not be available for an extinct clade like gondwanatherians that is far more stemward in current phylogenies. The development of primary bony lamina with a spiral ganglion canal was related to initial evolutionary changes in ossification of the cartilaginous otic capsule to ensure the systematic distribution of radiating afferent nerve bundles between the spiral ganglion and the organ of Corti in cladotherians (Luo et al., 2011; 2012), although the structural arrangement of these fibers with the primary lamina is different in gondwanatherians (Krause et al., 2020). The basilar membrane gap between the primary and the secondary bony laminae is also relatively wide in gondwanatherians (Kirk et al., 2014) and in dryolestoids (Ruf et al., 2009; Luo et al., 2012), thus the presence of both primary and secondary laminae may not have sufficiently changed the frequency responses of the organ of Corti, in the way it does in extant therians (Manley, 2018).

During the evolution of eutherians, there is a long time lag between the earliest-known eutherian preserved with a cochlear canal of almost a full cochlear turn and a length of the hearing organ of about 4 mm on the one hand (e.g., the Early Cretaceous *Prokennalestes*) (Wible et al., 2001), and the Late Cretaceous eutherians that had achieved more than one full turn and cochlear lengths above 6–7 mm (Ekdale and Rowe, 2011) on the other. This long morphological stasis of almost 20 million years in lengthening and coiling the cochlea raises the possibility that it was the evolution of molecular and physiological changes that influenced and delayed inner ear evolution in the earliest crown therians, and not osteological characters.

It is possible that the loss of the lagenar macula had resulted in major changes in cochlear biochemistry, including fundamental mechanisms in the sensory cells (such as ion-channel properties and frequency-tuning mechanisms). During this time, the therian-like motif and distribution of the membrane protein prestin also evolved, which contributed to a new active process that raised

hearing sensitivity and likely enabled the cochlea to regain its earlier response capabilities that had been compromised by the lagenar loss. The new, prestin-based active mechanism was likely to have been supplemented by an increase in the size of the endocochlear potential (as enabled by a therian-like Stria vascularis), and a physiological capability of motor responses at very high frequencies. The Late Cretaceous crown therians may well have been equipped with the precursor structures for the Cenozoic therian mammals to raise the upper frequency responses, later, to the heights seen today in many extant mammals (Fig. 11). Among extant mammals, true high-frequency hearing evolved only in Cenozoic therians (Manley, 2018; Köppl and Manley, 2019).

Of course the variety of extant therian mammals is huge, and their body size and life style have a very large influence on their middle and inner ears and their hearing ranges and sensitivity, but these aspects are covered in other reviews.

# 2.14.8 Concluding Remarks

New information from the development of the ear in extant mammals and the paleontological record of extinct relatives has added to a growing body of evidence that the seemingly labile evolution of middle ears in Mesozoic mammals was influenced, in a major way, by developmental heterochrony and gene patterning. Extrapolating these common developmental patterns of extant mammals into the fossil record can illuminate the driving mechanism for a similar evolution to occur multiple times. On the other hand, the fossil record can provide concrete examples for morphogenetic potentials that exist in extant mammals but are rarely expressed in normal development. The absence of an ossified Meckel's cartilage in the normal adults of extant monotremes, marsupials, and placentals represents a more canalized development of the middle ear for these living lineages, in contrast to a more labile evolutionary development of the middle ear features seen in a vast range of evolutionary experimentations in diverse Mesozoic mammaliaform clades (Kielan-Jaworowska et al., 2004; Luo, 2007). This appears to be a case of development impacting and enabling a dynamic phylogenetic evolution.

From this review of functional evolution of early mammal ears, it has been quite clear that the middle and inner ears of different lineages of early mammals are not only diverse in morphological patterns, but also different in timing of evolutionary events – the ear structures of mammaliaforms as whole are a complex mosaic of features (Manley, 2013; Luo et al., 2016). The functional consequence of this evolution in a given lineage at a given time would not necessarily be directly comparable to the similar hearing function in another lineage – their functional convergence may be partial or incomplete. The broad similarity of the parallel events, however, does suggest that more-or-less the same selective pressures finally led to similar functional results in all lineages, although by no means simultaneously (Fig. 2).

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