

Monotreme middle ear is not primitive for Mammalia

Jin Meng ^{1,2,*} and Fangyuan Mao ^{1,3,4}

The study of the evolution of the mammalian middle ear has been fueled by continuous discoveries of Mesozoic fossils in the last two decades. Wang *et al.* [1] recently reported a specimen of *Vilevolodon diplomylos* [2] that adds to the increasing knowledge about the auditory apparatus of ‘haramiyidans’, an extinct Mesozoic group of mammaliaforms. The authors hypothesized that a middle ear with a monotreme-like incus and malleus and incudomalleolar articulation was primitive for mammals, which challenges the convention that the monotreme middle ear is specialized [3] or autapomorphic [4] in mammals. We raise concerns about terminology (see Supplementary Data) and identification of the incus presented by Wang *et al.* and show that their analysis does not support their preferred hypothesis but favors the alternative, which is consistent with Mao *et al.*’s hypothesis that the braced hinge joint is primitive for several lineages within Mammalia [5].

Wang *et al.* presented some valuable interpretations on previously known but still poorly understood auditory bones, such as the surangular and ectotympanic, in haramiyidans. Because these subjects have been extensively treated [2,5–8], we focus our discussion on the new evidence that Wang *et al.* provided about the incus and malleus, from which they drew their conclusion. The authors claimed that in the specimen (IMMNH-PV01699) the ossicular chain is ‘well-preserved and in near-life position’ and that the incus and incudomalleolar articulation were monotreme-like. We noted, however, that these structures differ notably from those in the holotype of

V. diplomylos, which was portrayed as not to be monotreme-like [2]. Wang *et al.* thought that the incus ‘resembles’ and ‘has a similar outline’ to those of the *Jeholbaatar* [9] and *Arboroharamiya allinhopsoni* [6–8]. The ‘incus’ of *Jeholbaatar* has been shown to be part of the malleus by new evidence [5], as noted by Wang *et al.* The only known unequivocal incus of euharamiyidans is from *Arboroharamiya*, a sister taxon of *V. diplomylos*, that has been repeatedly described as having a convex and bulbous body and a long stapedial process with a lenticular process, like that of therians [6–8]. The so-called ‘incus’ in *Qishou*, as re-interpreted by Wang *et al.* (see Extended Data Fig. 6 in ref. [1]), is a misinterpretation—it is part of the element with debatable identity (Fig. 1k–m). Why is the incus identified in IMMNH-PV01699 so different from that of the holotype and its sister taxon but similar to monotremes? The possibility that it is a non-incus structure, as those interpreted in *Jeholbaatar* and *Qishou*, cannot be ruled out. This could explain why both sets of the incus and malleus ‘were moved to that degree from their position in life and yet remain well preserved’ [1]. It is uncertain whether Wang *et al.*’s computerized tomography (CT) data with a voxel size of 32.7 μm could secure the identities of the incus and malleus; a CT-slice showing the suture between them, as we did in Fig. 1, would settle the issue. We could not verify this because the digital data were not yet available.

Based on their identification of IMMNH-PV01699, Wang *et al.* concluded that optimization of five

incudomalleolar characters in its phylogeny (Fig. 1n) ‘supports the overlapping joint as primitive for Mammalia. The partial overlapping joint is derived from the overlapping joint (and not vice versa) by the caudal shift of the incus with regard to the malleus.’ This contradicts the hypothesis that the braced hinge joint (= partial overlapping joint, POJ; Fig. 1b, i and j) is potentially primitive for mammals [5]. We noted that in non-monotreme mammals the five incudomalleolar characters were coded only in *Vilevolodon*, *Qishou* and *Arboroharamiya* [1]. However, the incus was not preserved in *Qishou* [8,10] (Fig. 1m) and the so-called malleus is subject to interpretation [1,5,10]. The two species of *Arboroharamiya* were coded as having a plate-like incus, which is factually untrue, as mentioned above. *Sinobaatar* was coded as ‘?’ for the five characters, although its well-preserved malleus and incus [5] have been illustrated in Wang *et al.*’s Fig. 3. To our knowledge, except for the purported monotreme-like incudomalleolar joint in IMMNH-PV01699, there is no convincing evidence for such a joint in any non-monotreme mammals and their relatives [1,5]. Of the five incudomalleolar characters, none showed up as a synapomorphy at any major node (clade) in the consensus tree (see Supplementary Information of ref. [1]). With an unstated method, Wang *et al.* managed to optimize the five characters and map the four types of joints (Fig. 1n) on the simplified consensus tree to show their preferred hypothesis.

Under their hypothesis (Fig. 1n), the first evolutionary step would be from the

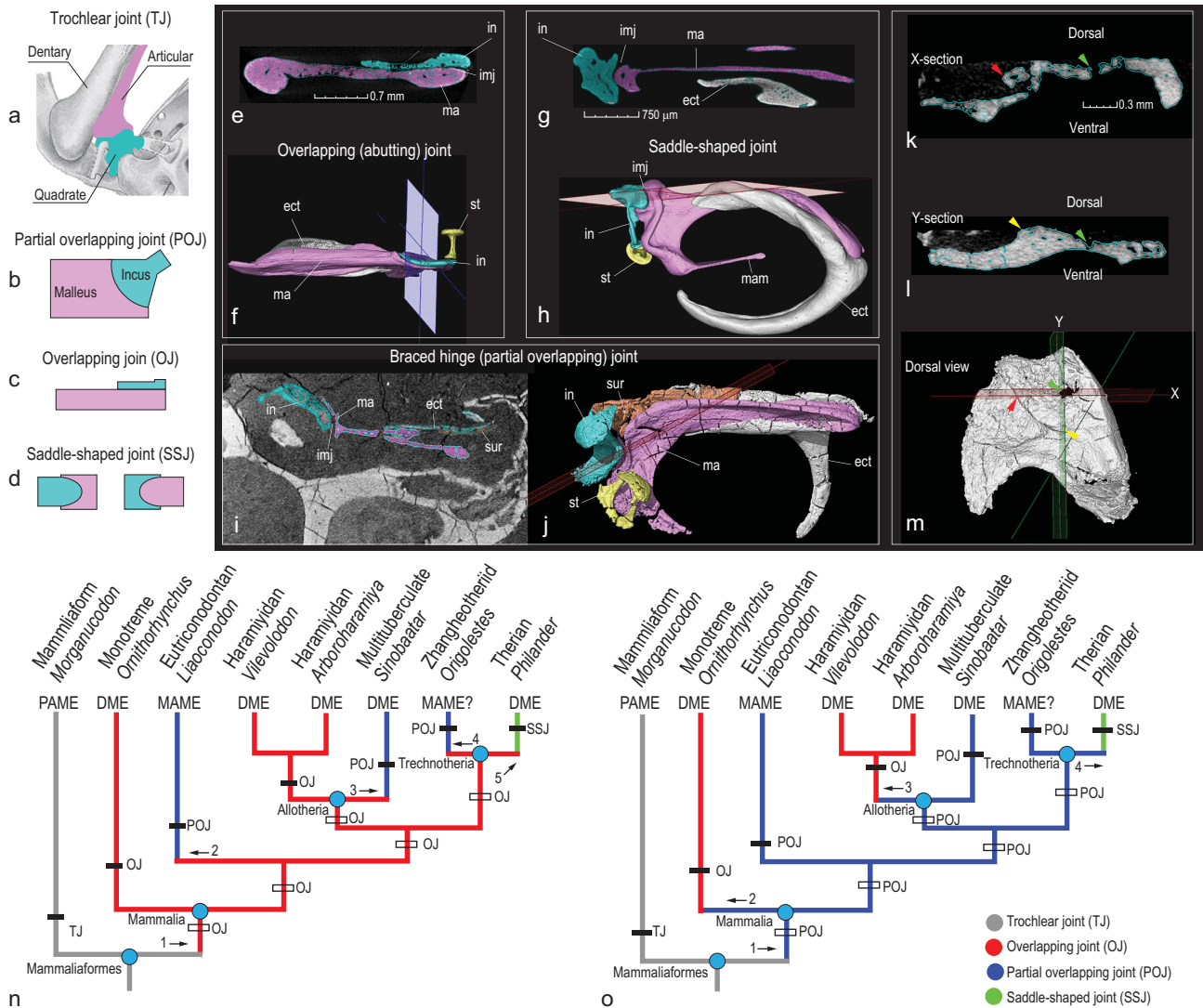


Figure 1. Types of incudomalleolar articulation and two hypotheses for the evolution of the mammalian middle ear. (a–d) Diagrams showing the trochlear, partial overlapping, overlapping and saddle-shaped incudomalleolar joints. (e–m) Computerized tomography images showing middle ear structures discussed in the text (see Supplementary Data for sources and abbreviations of the images). (n) Hypothesis preferred by Wang *et al.* [1], in which the monotreme-like overlapping incudomalleolar joint is primitive for Mammalia. (o) Alternative hypothesis overlooked by the authors, in which the braced hinge joint (= POJ) is primitive for Mammalia. Taxa, tree topology and optimized character distributions are from the original study (see Fig. 3 in ref. [1]). We added the empty bars, arrows and associated labels to visualize the evolutionary changes within the phylogeny. The comparison shows that the overlooked hypothesis (o) is more parsimonious than (n), which supports the existing hypothesis [6] but rejects the one that states that the monotreme middle ear is primitive for Mammalia.

trochlear joint (TJ) in nonmammalian cynodonts to the monotreme ‘overlapping joint’ (OJ) in Mammalia. This step requires several abrupt changes (transformation through the POJ was deemed impossible by Wang *et al.*): the incus becoming a flat platelet, complete loss of the synovial joint, and the incudomalleolar complex transforming to a nearly horizontal position with the incus shifting to the dorsal side of the malleus. As known in some develop-

mental studies, the vertical orientation of the ectotympanic in ontogeny was recognized as primitive in mammals [4] and therians [11] because the angular bone in nonmammalian cynodonts was vertically positioned. In the development of echidna the ectotympanic and malleus perform a ‘flipping’ from their original vertical position to horizontal orientation in adults [12]. The flat incus lying medial to the malleus and a horizontal ectotympanic were considered

autapomorphic for monotremes [4]. These studies do not support Wang *et al.*’s hypothesis. In addition, this evolutionary step requires direct change from the ‘postdentary attached middle ear’ to the full ‘detached middle ear’ at Mammalia and demands independent regain of the ossified Meckel’s cartilage (OMC) in adults of zhangheotheriids and eutriconodontans; this implies functional re-association of the auditory bones (hearing) with mastication at least

in eutriconodontans. These changes are supported by no fossil or developmental evidence. Within Mammalia, two evolutionary steps from the OJ to POJ took place independently at eutriconodontans and multituberculates; furthermore, the OJ at Trechnotheria would have to give rise either to POJ, which then evolved into the saddle-shaped joint (SSJ) (Fig. 1d, g and h), or to the POJ and SSJ respectively; either of the two processes involves at least two evolutionary steps. Thus, at least four evolutionary steps are required within Mammalia (Fig. 1n).

It appears that Wang *et al.* have overlooked a better supported result of their optimization: the POJ is primitive for Mammalia, as we present in Fig. 1o. Under this alternative hypothesis, the evolutionary change from the nonmammalian cynodont TJ to the mammalian POJ would be simple because the incus and malleus retain the trochlear joint, the incus is largely caudal to the malleus and the auditory bones are positioned nearly vertically. Further, this evolutionary step requires neither full detachment of the auditory bones at Mammalia nor regain of the OMC in adults of zhangheotheriids and eutriconodontans. Within Mammalia there are only three evolutionary steps: two independent evolutions of the OJ at monotremes and haramiyidans,

respectively, and one from the POJ to SSJ within Trechnotheria.

Wang *et al.* postulated their hypothesis based on the less-supported result of their analysis. Under the rule of parsimony, that hypothesis (Fig. 1n) should be falsified because it requires at least five evolutionary steps in the mammalian middle ear evolution. In contrast, their analysis corroborates the alternative (Fig. 1o) that needs only four steps, which supports the existing hypothesis [5]. Wang *et al.*'s conclusion that the monotreme-like middle ear is primitive for Mammalia is misleading.

SUPPLEMENTARY DATA

Supplementary data are available at [NSR](#) online.

Conflict of interest statement. None declared.

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National Science Review

8: nwab131, 2021

<https://doi.org/10.1093/nsr/nwab131>

Advance access publication 23 July 2021

EARTH SCIENCES

Response to ‘Monotreme middle ear is not primitive for Mammalia’

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To date, a complete auditory apparatus (with malleus, incus, stapes, ectotympanic and perhaps surangular) is known for only three Mesozoic mammals, the haramiyidan *Arboroharamiya allinhopsoni* [1], the multituberculate *Sinobaatar pani* [2] and the zhangheotheriid *Origolestes lii* [3], with subsets of these bones preserved in a handful more,

including the haramiyidan *Vilevolodon diplomylos* [4,5] discussed here. A goal of Wang *et al.* [5] was to evaluate the debate surrounding the various interpretations of these bones and to offer a cautionary tale about overinterpreting these structures. Because the new specimen of *Vilevolodon* reported by Wang *et al.* [5] preserved left and right mallei and

includes in articulation, these authors focused on the incudomalleolar joint. Wang *et al.* [5] found these two bones in *Vilevolodon* to be reminiscent of those in extant monotremes, with a relatively flat articulation between a plate-like incus and similarly thin malleus. On the strict consensus tree from their parsimony analysis, Wang *et al.* [5] optimized

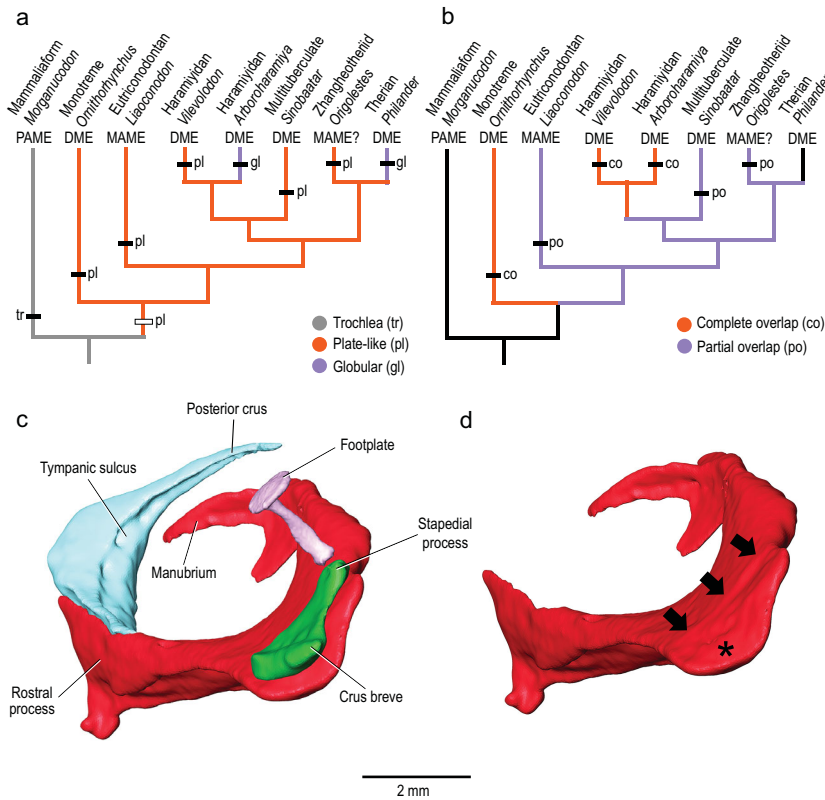


Figure 1. (a) Optimization of character 417 from Wang *et al.* [5], the shape of the incudal body, on simplified consensus tree, with plate-like identified as primitive for Mammalia. (b) Optimization of character 419 from Wang *et al.* [5], extent of overlap of malleus and incus (only applicable for taxa with the incudomalleolar articulation in the same plane as the malleolar body—character 418), on simplified consensus tree, with the primitive condition ambiguous for Mammalia (see Supplementary Data online for terminology and abbreviation). (c and d) Isosurfaces from CT scan of the extant monotreme *Ornithorhynchus anatinus*, Carnegie Museum 50815: (c) malleus (red), incus (green), stapes (purple) and ectotympanic (light blue) in oblique posterodorsal view; (d) malleus (red) with black arrows on low ridge marking the border of the incudal facet and asterisk in the concave part of the incudal facet.

five characters of the incudomalleolar articulation and reported that the character states associated with the overlapping incudomalleolar articulation of extant monotremes and *Vilevolodon* optimized as primitive for Mammalia. However, they expressed uncertainty as to whether this overlapping joint evolved convergently in haramiyidans and monotremes or was an innovation at the level of Mammalia.

Meng and Mao [6] question Wang *et al.*'s [5] identification of the incus in the new specimen of *Vilevolodon*, because it differs from that reported for the holotype [4]. Wang *et al.* [5] addressed this already, noting that the two incudes have the same shape, with the only difference being the interpretation of the

incudomalleolar joint. Meng and Mao [6] then conduct an optimization employing different criteria (their Fig. 1o) from those of Wang *et al.* [5]. Rather than optimizing the five characters individually, as had Wang *et al.* [5], Meng and Mao [6] lump the five into two broad morphologies: overlapping and partial overlapping joints (their braced hinge joint). They report that it is more parsimonious to have the partial overlapping joint as primitive for Mammalia, which they state falsifies Wang *et al.*'s hypothesis. Meng and Mao [6] illustrate what they consider to be the Wang *et al.* [5] hypothesis in their Fig. 1n, but we emphasize that this was not an analysis that was ever conducted by the latter authors.

Wang *et al.* [5] did not figure any of their five individual optimizations. We revisit them here, illustrating results for two in Fig. 1a and b. We made amendments to the protocol of Wang *et al.* [5] based on concerns raised by Meng and Mao [6]. First, we added scores for *S. pani*; Wang *et al.* [5] did not score *S. pani* for the 509 morphological characters in their taxon-character matrix as little of the anatomy of this taxon beyond the ear ossicles was reported [2]. Second, we modified scores for the haramiyidans *Arboroharamiya* (regarding the shape of the incudal body) and *Qishou* (which was changed to unknown for all incudomalleolar characters), following Meng and Mao [6]. Wang *et al.* [5] suggested that *Qishou* (based on an image in [7]) had an incus and malleus much like that in *Vilevolodon*. Meng and Mao [6] include two computerized tomography (CT) slices of *Qishou* (Fig. 1k and l) that clearly show there is only one bone present. We acknowledge the error in interpretation by Wang *et al.* [5]. However, rather than an incus preserved on the malleus in *Qishou*, based on these new cross sections, we interpret this as the malleus with a facet for the incus, still resembling the condition in *Vilevolodon*. Nevertheless, we score *Qishou* as unknown here. Third, we eliminated the optimization of character 416, the alignment of the malleus and incus, because evaluation of this character requires knowledge of the plane of the ectotympanic, which is seldom preserved in fossils.

The first illustrated optimization is of character 417 from Wang *et al.* [5] (Fig. 1a), the shape of the incudal body, the part in contact with the malleus. We scored this as a trochlea in the outgroup *Morganucodon*, plate-like in most Mesozoic mammals, and globular in the extant therian *Philander* and *Arboroharamiya*, following Meng and Mao [6]. Wang *et al.* [5] (based on movies in [2]) suggested that the incus of the zhangheotheriid *Origolestes* had a thickened body and scored it as globular. We changed their score of *Origolestes* to plate-like; Meng and Mao [6] have provided a new CT slice of *Origolestes* (their Fig. 1i), which shows an incudal body more

reminiscent of that in the monotreme *Tachyglossus* (their Fig. 1e) than in the marsupial *Didelphis* (their Fig. 1g). In our optimization (Fig. 1a), the plate-like incus is primitive for Mammalia with the globular state derived independently in *Philander* and *Arboroharamiya*. We found similar results for the amended optimizations for characters 415 and 418 (the geometry and orientation of the incudomalleolar joint, respectively), that is, the states associated with the condition in *Vilevolodon* and monotremes are primitive for Mammalia.

Character 419 concerns the extent of overlap between the malleus and incus, complete or partial, which is applicable only for taxa with the incudomalleolar articulation in the same plane as the malleolar body (character 418). Our optimization (Fig. 1b) shows that the primitive condition for Mammalia is ambiguous, which differs from the results of Wang *et al.* [5] (with complete overlap as primitive) as well as Meng and Mao [6] (with partial overlap primitive in their Fig. 1o). This change from the conclusion of Wang *et al.* [5] is a result of the addition of scores for *S. pani* and removal of *Qishou*, which highlights how fluid such analyses are, given how few taxa are known for middle ear ossicles.

A finding of Wang *et al.* [5] that we emphasize here is the similarity of the incudomalleolar joint in multiple lineages of Mesozoic mammals and monotremes. We do not see major distinctions between the overlapping and partial overlapping joints and believe the transformation from one to the other did not require massive overhauling, contra Meng and Mao [6]. We illustrate this with the condition in the monotreme *Ornithorhynchus* (Fig. 1c and d). It has an overlapping joint (Fig. 1c), but the malleus has a low ridge that marks the edge of the incudomalleolar joint surface (arrows in Fig. 1d) and a joint surface that is partially concave (asterisk in Fig. 1d), both morphologies expressed in the partial overlapping joint. Transforming the condition of the platypus into the partial overlapping joint requires a posterior shift of the incus with respect to the malleus and a more pronounced ridge marking the incudomalleolar joint surface. These morphologies represent the first steps in the transformation of the load bearing trochlear joint between the quadrate (incus) and articular (malleus), as occurs in *Morganucodon* [8], for example. No matter what the direction of the transformation of the overlapping and partial overlapping joints may have been, it likely occurred more than once in mammal evolution, as the postdentary bones detached multiple times from the lower jaw [8].

SUPPLEMENTARY DATA

Supplementary data are available at [NSR](#) online.

Conflict of interest statement. None declared.

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- National Science Review
8: nwab132, 2021
<https://doi.org/10.1093/nsr/nwab132>
Advance access publication 23 July 2021