Monotreme middle ear is not primitive for Mammalia

Jin Meng and Fangyuan Mao

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 incudomallear 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 incudomalleal 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 euhamarmiyidans is from *Arboroharamiya*, a sister taxon of *V. diplomylos*, that has been repeatedly described as having a convex and bulbous body and a long stapidal 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 incudomallear 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 incudomalleal 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 incudomalleal 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 incudomalleal 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
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 incudomallear complex transforming to a nearly horizontal position with the incus shifting to the dorsal side of the malleus. As known in some developmental 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 zangheotheriids 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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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 alinhopsoni [1], the multituberculate Sinobaatar pani [2] and the zangheotheriid 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 incudes in articulation, these authors focused on the incudomalleal 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
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 incudomallear 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, 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 incudomallear 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 incudomallear articulation and reported that the character states associated with the overlapping incudomallear 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 incudomallear 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.
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 incudomallear 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 incudomallear articulation in the same plane as the malleal 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 incudomallear 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 incudomallear 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 incudomallear 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.

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