

# Ear Ossicle Morphology of the Jurassic Euharamiyidan *Arboroharamiya* and Evolution of Mammalian Middle Ear

Jin Meng,<sup>1,2\*</sup> Shundong Bi,<sup>2,3</sup> Xiaoting Zheng,<sup>4,5</sup> and Xiaoli Wang<sup>4,5</sup>

<sup>1</sup>Division of Paleontology, American Museum of Natural History, New York City, New York

<sup>2</sup>Key Laboratory of Vertebrate Evolution and Human Origin of Chinese Academy of Sciences, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing, China

<sup>3</sup>Department of Biology, Indiana University of Pennsylvania, Indiana, Pennsylvania

<sup>4</sup>Shandong Tianyu Museum of Nature, Pingyi, Shandong, China

<sup>5</sup>Institute of Geology and Paleontology, Linyi University, Linyi, Shandong, China

**ABSTRACT** The middle ear bones of Mesozoic mammals are rarely preserved as fossils and the morphology of these ossicles in the earliest mammals remains poorly known. Here, we report the stapes and incus of the euharamiyidan *Arboroharamiya* from the lower Upper Jurassic (~160 Ma) of northern China, which represent the earliest known mammalian middle ear ossicles. Both bones are miniscule in relation to those in non-mammalian cynodonts. The skull length/stapedial footplate diameter ratio is estimated as 51.74 and the stapes length as the percentage of the skull length is 4%; both numbers fall into the stapes size ranges of mammals. The stapes is “rod-like” and has a large stapedial foramen. It is unique among mammaliaforms in having a distinct posterior process that is interpreted as for insertion of the stapedius muscle and homologized to the ossified proximal (stapedial) end of the interhyal, on which the stapedius muscle attached. The incus differs from the quadrate of non-mammalian cynodonts such as morganucodontids in having small size and a slim short process. Along with lack of the postdentary trough and Meckelian groove on the medial surface of the dentary, the ossicles suggest development of the definitive mammalian middle ear (DMME) in *Arboroharamiya*. Among various higher-level phylogenetic hypotheses of mammals, the one we preferred places “haramiyidans” within Mammalia. Given this phylogeny, development of the DMME took place once in the allotherian clade containing euharamiyidans and multituberculates, probably independent to those of monotremes and therians. Thus, the DMME has evolved at least three times independently in mammals. Alternative hypothesis that placed “haramiyidans” outside of Mammalia would require independent acquisition of the DMME in multituberculates and euharamiyidans as well as parallel evolution of numerous derived similarities in the dentition, occlusion pattern, mandibles, cranium, and postcranium between the two groups and between “haramiyidans” and other mammals. *J. Morphol.* 279:441–457, 2018.

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**KEY WORDS:** stapes; incus; Meckelian groove; postdentary trough; homology

## INTRODUCTION

Extant mammals differ from other tetrapods in having a chain of three ossicles in the middle ear, a feature that had been used to diagnose Mammalia (Simpson, 1959; Reed, 1960; MacIntyre, 1967; Kermack and Kermack, 1984). Evolution of the mammalian middle ear has been a subject that attracted numerous researches since at least Reichert (1837) and Gaupp (1908, 1913) and still remains as an active research area both in paleontology and developmental biology. During the last two decades, significant paleontological discoveries in relation to mammalian middle ear have been made from Cretaceous mammals, mostly from Asia (Hurum et al., 1996; Rougier et al., 1996a; McKenna et al., 2000; Wang et al., 2001; Li et al., 2003; Meng et al., 2003; Luo et al., 2007b; Ji et al., 2009; Meng et al., 2011; Meng and Hou, 2016). These discoveries include the ossified Meckel's cartilage (OMC), the malleus (= the articular and prearticular), incus (= quadrate) and ectotympanic (= angular) in several groups of Mesozoic mammals (multituberculates, entricodontans, “symmetrodonans,” and therians). Based on these discoveries, we now have a better understanding

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\*Correspondence to: Jin Meng; Division of Paleontology, American Museum of Natural History, New York City, NY 10024. E-mail: jmeng@amnh.org

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on how the plesiomorphic mandibular middle ear (MdME), as represented in *Morganucodon* (Kermack et al., 1973, 1981), evolved to the transitional mammalian middle ear (TMME) (Meng et al., 2011; Meng, 2014) and then to the definitive mammalian middle ear (DMME) (Allin and Hopson, 1992). During the same period of time, developmental and molecular studies focusing on the homology and gene expression of middle ear ossicles and related structures have revealed some critical mechanisms of the middle ear ossicle development (Maier, 1990; Zeller, 1993; Mallo, 1998, 2001, 2003; Sánchez-Villagra et al., 2002; Tucker et al., 2004; Wilson and Tucker, 2004; O’Gorman, 2005; Anthwal et al., 2013; Gillis et al., 2013; Kitazawa et al., 2015), as summarized in the reviews on the research history of mammalian middle ear evolution by Takechi and Kuratani (2010) and Maier and Ruf (2016).

In a recent work reporting the stapes from an early Cretaceous eutriconodontan, Meng and Hou (2016) pointed out that the aforementioned developmental and paleontological studies have focused primarily on the middle ear ossicles that are transformed jawbones of non-mammalian cynodonts, with relative minor attention on the stapes. Although the stapes has a reasonably good record in non-mammalian cynodonts, such as *Haldanodon* (Lillegraven and Krusat, 1991; Ruf et al., 2013), *Morganucodon* (Kermack et al., 1981), *Sinoconodon* (Crompton and Luo, 1993), *Brasilitherium* (Rodrigues et al., 2013) and gomphodont cynodonts (Gaetano and Abdala, 2015), it remains little known and much less investigated in early mammals. This is primarily because the fossil record of stapes was extremely rare. Among known Mesozoic mammals, there is only one nearly complete stapes known from a late Cretaceous eutherian (Archibald, 1979). Fragments of the stapes were reported from several Cretaceous species (Hurum et al., 1996; Rougier et al., 1996a; Luo et al., 2007a; Meng et al., 2011). A nearly complete stapes was recently reported from an Early Cretaceous eutriconodontan, *Chaoyangodens* (Hou and Meng, 2014), which is by far the earliest known mammalian stapes with unequivocal morphology (Meng and Hou, 2016). Although the stapes has been discovered centuries ago (Mudry, 2013) and has been known as the most conservative element of the middle ear ossicles, with its essential form and ontogeny being traceable in vertebrates (Goodrich, 1930; Eaton, 1939; Romer, 1941; Westoll, 1943; Parrington, 1949, 1955; Tumarkin, 1968; Fleischer, 1978; Lombard and Bolt, 1979; Parrington, 1979; Novacek and Wyss, 1986; Allin and Hopson, 1992; Clack and Allin, 2004), its composition and homology remains controversial today in developmental and evolutionary biology (see Meng and Hou [2016] for a brief review).

Here, we report the stapes and incus from the euharamiyidan *Arboroharamiya*, an effort to follow up the works by Zheng et al. (2013) and Meng et al. (2014). Euharamiyidans (Bi et al., 2014) are advanced members of “haramiyidans” that were often placed in Allotheria, along with Multituberculata and probably Gondwanatheria, but their taxonomic position within Mammaliaformes is highly contentious (Simpson, 1929, 1947; Hahn, 1973; Sigogneau-Russell et al., 1986; Hahn et al., 1989; Butler and MacIntyre, 1994; Jenkins et al., 1997; McKenna and Bell, 1997; Kermack et al., 1998; Butler, 2000; Kielan-Jaworowska et al., 2004; Hahn and Hahn, 2006), and the controversial interpretations of “haramiyidan” relationships are reflected in numerous phylogenetic studies (Lombard and Bolt, 1979; Luo et al., 2002; Rowe et al., 2008; Gurovich and Beck, 2009; Liu and Olsen, 2010; Averianov and Lopatin, 2011). The phylogeny of “haramiyidans” has remained enigmatic partly because most species of the group have been known from isolated teeth (Sigogneau-Russell, 1989; Butler and MacIntyre, 1994; Kermack et al., 1998; Heinrich, 1999; Butler, 2000; Heinrich, 2001; Butler and Hooker, 2005; Maisch et al., 2005; Hahn and Hahn, 2006; Martin et al., 2010; Averianov et al., 2011). *Haramiyavia clemmenseni* (Jenkins et al., 1997; Luo et al., 2015) is based on fragmentary jaws with relatively well-preserved dentition and some postcranial remains. Several species represented by associated dentitions, jaws, cranial, and postcranial remains have been discovered from the Jurassic of China (Zheng et al., 2013; Zhou et al., 2013; Bi et al., 2014; Meng et al., 2014), one of which is *Arboroharamiya*. Although the morphologies of “haramiyidans” are better known now than 5 years ago, the phylogeny of “haramiyidans” still remain controversial (Zheng et al., 2013; Zhou et al., 2013; Bi et al., 2014; Krause et al., 2014; Luo et al., 2015) (see Meng (2014) for a brief review).

The specimens reported here are the first stapes and incus known from a euharamiyidan and are the unequivocal ear ossicles from a Jurassic mammal that had been inferred having the DMME, based on lack of the postdentary trough and Meckelian groove on the dentary (Zheng et al., 2013; Meng, 2014). These ear ossicles cast new light on the study of mammalian middle ear evolution.

## MATERIALS AND METHODS

### Specimens

The middle ear ossicles are from the holotype specimen of *Arboroharamiya jenkinsi* (Zheng et al., 2013), a partial skeleton with both mandibles associated with some teeth preserved on split slabs of a laminated siltstone (STM33-9A and STM33-9B, Tianyu Museum of Nature, Shandong Province, China; Fig. 1). The general morphology of *A. jenkinsi* was reported by Zheng et al. (2013), and the detailed morphologies of the dentary and dentition were described by Meng et al. (2014).

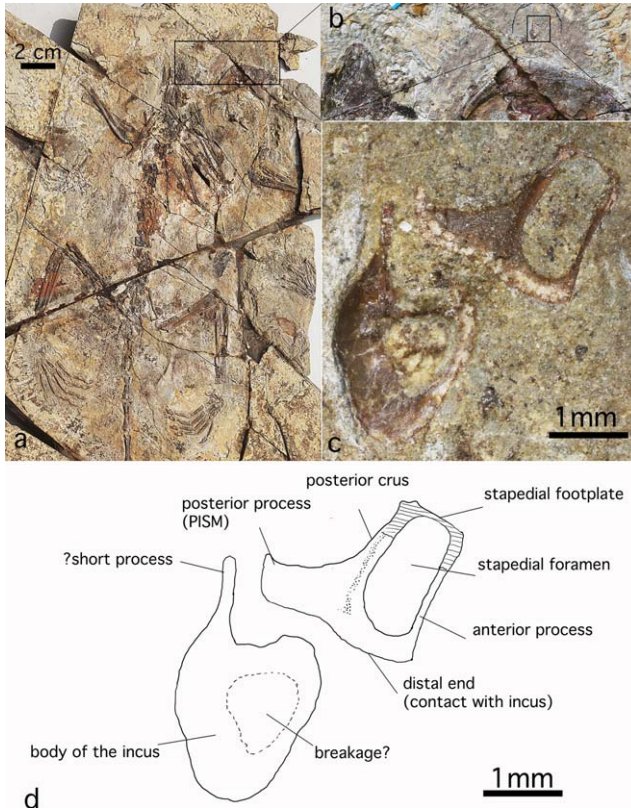


Fig. 1. Ear ossicles of *Arboroharamiya jenkinsi*, middle ear ossicles. (a) The holotype specimen of *A. jenkinsi* (STM33-9A); (b) The close-up view of the box in a; (c) The close-up view of the box in b to show the morphology of the stapes and incus; (d) line drawing illustrating the ossicle structures.

The holotype of *Arboroharamiya* (Zheng et al., 2013) was collected from the Tiaojishan Formation in the town of Mutoudeng, Qinglong County, Hebei Province (Meng, 2014; Sullivan et al., 2014). The Tiaojishan Formation is considered to range from the Middle to Late Jurassic in age (Yang and Li, 2008; Liu et al., 2012) and the fossils from the formation were regarded as part of the Yanliao (Daohugou) Biota (Meng, 2014; Sullivan et al., 2014). As Meng (2014) noted, precise chronological constrain for the Yanliao Biota, as well as the Jehol Biota, remains open. Based on available data, the Yanliao Biota was tentatively considered to be close to the Middle-Upper Jurassic boundary and the sites yielding Yanliao fossils may have spanned a time interval of roughly 9 million years from the base of the Callovian (166.1 Ma) to the end of the Oxfordian (157.3 Ma).

The two middle ear ossicles are preserved next to each other on STM33-9A. Although not fully exposed, particularly so for the incus, no serious preparation has been attempted to expose more of the specimen because the rock surrounding the ossicles is hard and coarse grained; any in-depth preparation could damage the specimen. These minuscule elements were embedded in a large rock slab, and we do not have any sophisticated facility to CT-scan the entire slab containing the specimen.

To further illustrate the mandibular morphology of euharamiyidans we present a scanning electron microscopy (SEM) image of the holotype specimen of *Xianshou linglong* (IVPP V16707). This is because the specimen has both medial and lateral sides of the mandibles exposed to show relevant features and is small enough to be placed in the SEM chamber for imaging. The uncoated specimen was imaged using a Hitachi S4700 SEM in the Key Laboratory of Vertebrate Evolution and Human Origins, Institute of Vertebrate Paleontology and Paleo-

anthropology, Chinese Academy of Sciences, Beijing, China. Measurements are made using a microscope equipped with a reticule etched with divisions of 0.01 mm (10  $\mu$ m) and double-checked with the measure tool in ImageJ 1.49v.

### Taxonomic Terminology

We follow Rowe (1988) to define Mammalia as the clade consisting of the most recent common ancestor of living monotremes and therians and all descendants of that ancestor. Mammals so defined are often referred to as crown Mammalia or crown mammals, but the descriptor “crown” is omitted for simplicity in the text. We note that a more traditional definition of Mammalia is the clade that shares a common ancestor of *Sinoconodon*, morganucodontans, docodontans, Monotremata, Marsupialia, and Placentalia, plus any extinct taxa that are shown to be nested with this clade by parsimony analyses (Kielan-Jaworowska et al., 2004). The traditional definition of Mammalia is equivalent to Mammaliaformes of Rowe (1988). In this study the term “mammaliaformes” is used to refer to animals in the taxon Mammaliaformes.

We follow Bi et al. (2014) to use Euharamiyida for the clade that does not include some taxa traditionally placed in “Haramiyida,” such as *Haramiyavia* and *Thomasia*. Krause et al. (2014) independently achieved a similar phylogenetic relationship. Haramiyida and Multituberculata were considered as two orders in Allotheria in the classification of Kielan-Jaworowska et al. (2004). However, a common view is that, if multituberculates and haramiyidans form the clade Allotheria, multituberculates would form a monophyletic group that was derived from “haramiyidans” within Allotheria; thus, “haramiyidans” form a paraphyletic group (Butler, 2000; Butler and Hooker, 2005; Hahn and Hahn, 2006; Averianov and Lopatin, 2011). Given this view and the phylogenetic relationship we preferred (Bi et al., 2014; Krause et al., 2014), we regard the traditional “haramiyidans” as a paraphyletic group and place the name between quotation marks in the text.

### Anatomical Abbreviations and Definitions

**DMME:** definitive mammalian middle ear. The DMME is defined as the configuration in which the angular, articular, prearticular, and quadrate are strictly auditory structures, fully divorced from the lower jaw (and renamed the tympanic, malleus, and incus) (Allin and Hopson, 1992). **TMME:** transitional mammalian middle ear. The TMME is defined as the configuration in which the articular, prearticular and angular lose their direct contact with the dentary (thus called as the malleus and ectotympanic) and are supported anteriorly by a persistent Meckel’s cartilage instead of cranial structures in adult; the malleo-incudal articulation is still hinge-like but lost its primary function for jaw suspension; the quadrate is freed from the skull (thus called the incus); all ear ossicles are primarily auditory structures but may not be completely free from the mandible movement; the tympanic membrane has not been fully suspended by the ectotympanic and the manubrium has not developed (Meng et al., 2011). **MdME:** mandibular middle ear. The MdME is used for “mandibular middle ear” as introduced by Kielan-Jaworowska et al. (2004). This abbreviation avoids potential confusion with the short form of “mammalian middle ear.” The typical MdME is represented by the “middle ear” of *Morganucodon* (Kermack et al., 1981) and has not been clearly defined. Kielan-Jaworowska et al. (2004: 138) wrote: “The angular bone and the prearticular-articular complex probably already functioned to transmit sound as part of the ‘mandibular middle ear’ in cynodonts and precynodont therapsids, as hypothesized by (Allin, 1975, 1986).” Based on this and the definitions of DMME and TMME, we defined MdME as the middle ear in which the articular-prearticular-angular complex is still directly attached to the dentary bone and the incus is in braced articulation with bones of the cranium; these bones have a dual-function of jaw suspension and hearing. **PISM:** process for insertion of the stapedius muscle. This abbreviation is used in Meng and Hou (2016).

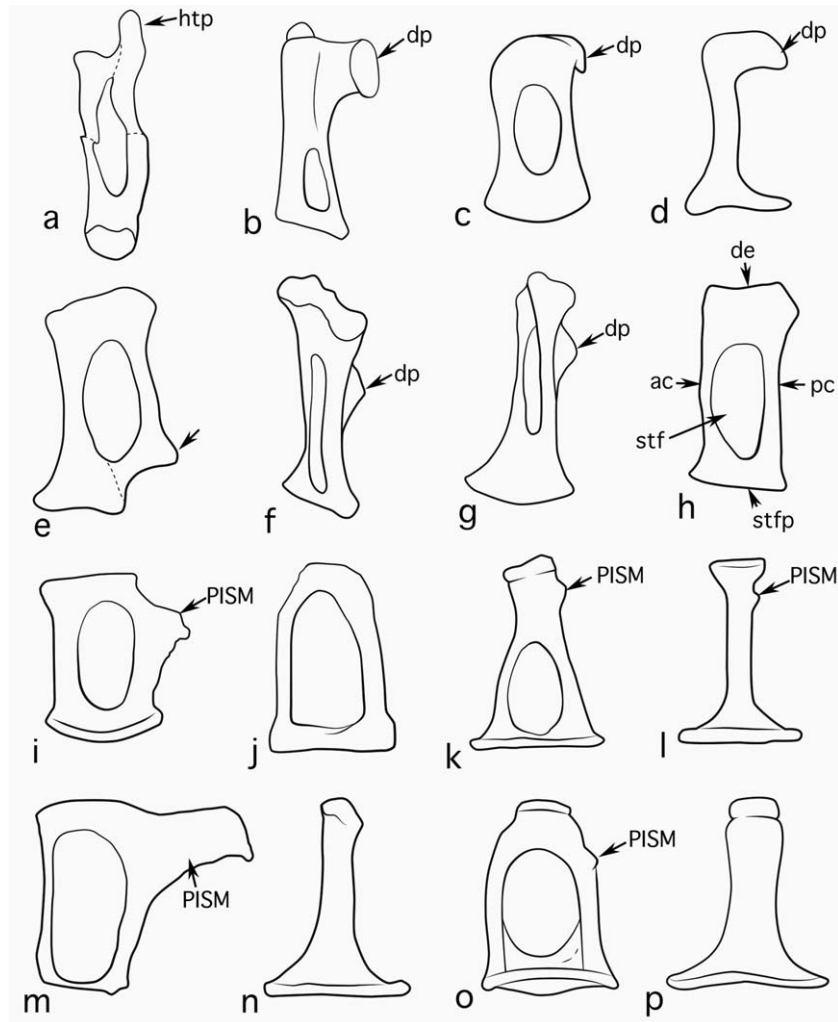


Fig. 2. Comparison of the stapes of *Arboroharamiya* with some mammals and non-mammalian cynodonts. (a) *Scalenodon*; (b) *Scylacops*; (c and d) *Thrinaxodon* (ventral and posterior views); (e) *Langbergia*; (f) *Massetognathus*; (g) *Trirachodon*; (h) *Massetognathus*; (i) *Chaoyangodens lii*; (j) Late Cretaceous “unguiculate” eutherian; (k) *Philander* (= *Metachirops*); (l) *Notoryctes*; (m) *Arboroharamiya*; (n) *Ornithorhynchus*; (o) *Orycteropus*; (p) *Manis*. Abbreviations: ac, anterior crus; de, distal end of the stapes (quadrate or incus contact area); dp, dorsal process; htp, hypothetical tympanal process or ossified extrastapes, interpreted as an artefact due to deformation (Gaetano and Abdala, 2015); pc, posterior crus; PISM, process for insertion of the stapedius muscle (Meng and Hou, 2016); stf, stapedial foramen; stfp, stapedial footplate. The figure are redrawn from the following references: a and e–h (Gaetano and Abdala, 2015), b (Watson, 1953; Novacek and Wyss, 1986), c and d (Allin, 1975), i (Meng and Hou, 2016), j (Archibald, 1979), j and k and n–p (Novacek and Wyss, 1986; also see Doran 1878; Segall 1970; Fleischer 1973). To assist comparison, some figures were flipped horizontally so that the posterior crus is toward the right side. The figures of the stapes are not on the same scale.

## Phylogenetic Analysis

To illustrate two competing hypotheses for allotherian phylogeny and map the distribution of the DMME within mammaliaforms, we use a simplified phylogeny based on Bi et al. (2014), Krause et al. (2014) and Luo et al. (2015) (Fig. 4). In addition, we reanalyzed the dataset of Luo et al. (2015), which is a modified version of the data set used by Bi et al. (2014). The data matrix of Luo et al. (2015) was downloaded from MorphoBank (see link in supplementary online material). Five mandibular characters were corrected for euharamiyidans because Luo et al. (2015) have been introduced factual errors when the authors recoded those characters for euharamiyidans. These characters are related to middle ear evolution and have played a key role in determining the phylogenetic position of “haramiyidans” and thus in interpreting the DMME evolution within mammaliaforms. To ensure objectivity in discussion, we provide illustrations

that compare different conditions for the five characters in the supplementary online material (Fig. S1–S5). After we corrected the coding for the five mandibular characters, we conducted a re-analysis of the modified dataset using Tree analysis using New Technology (TNT). The analysis was run with a traditional search with 1000 random taxon addition replicates, TBR branch-swapping and 100 trees held in each replicate. The result of phylogenetic analysis is also provided in Supporting Information.

## RESULTS

### Stapes

The length of the stapes is 1.95 mm. The maximum width at the end with the process is 2.33 mm (including the process), the width at the

midpoint of the stapes (without the process) is 1.06 mm, and the width at the end (the footplate diameter) is 0.94 mm. The stapes is bicurrate and has a large oval stapedia foramen with its length and width being 1.54 and 0.7 mm, respectively. The foramen is proportionally so large that a functional stapedia artery with intrastapedia course was likely present.

The most striking feature is the large process on the stapes, which is roughly 1.2 mm long. Such a large process has not been reported from any mammals or their close relatives so that the nature of the process is not certain. We tentatively describe it as the posterior process, a descriptive yet noncommittal term to denote this unusual process (although we homologize it to the PISM, see section "Discussion"). The posterior process can compare with two structures: the PISM in mammals (Doran, 1878; Segall, 1970; Fleischer, 1973; Henson, 1974; Parrington, 1979; Novacek and Wyss, 1986) or the dorsal process in some non-mammalian cynodonts (Hopson, 1966; Allin, 1975; Parrington, 1979; Allin and Hopson, 1992; Gaetano and Abdala, 2015). Either PISM or the dorsal process is on the posterior crus and closer to the distal end of the stapes (Fig. 2). In general, the posterior crus is thicker than the anterior one. Therefore, that the process in question is on the posterior crus appears the most likely interpretation, although we are still unable to assure whether it is a right or left stapes. From the midshaft to the distal end the posterior crus thins posteriorly to the plate-like posterior process. The process has a concave proximal edge and a convex distal edge; its proximodistal width gradually reduces toward the tip of the process. The posterior process probably serves as the insertion site of the stapedius muscle (see section "Discussion" below).

Because of the breakage on the proximal end (as marked in dashed line in Fig. 1d), the footplate and parts of the anterior and posterior crura were gone. It is unknown how much the footplate would project ventrally and dorsally. However, from the preserved proximal end, it is clear that the footplate would be thin and does not extend anteriorly or posteriorly so that the footplate is not notably wider than the space defined by the two crura. The two crura are nearly parallel, which gives the stapes body an elongate rectangular shape in dorsal (or ventral) view (Fig. 1). The stapes could be portrayed as "rod-like" (Novacek and Wyss, 1986). The slim anterior crus is slightly convex toward the foramen and has a circular cross section. The posterior crus is much thicker and column-like with a rounded cross-section for most of its proximal portion. The bone at the distal end of the stapes body is thicker than that in the footplate. The distal end is gently convex and should be in contact with the incus in life. There is a gently

concave region between the posterior process and the distal end of the stapes body.

### Incus

Identification of the other element is somewhat challenging, because it is not fully exposed and, unlike the stapes, the exposed portion of the element is not unambiguously characteristic for an incus known in mammals. Nonetheless, this element can only be either the malleus or the incus. Based on the general shape of this element, we interpret it as the incus. Whether the element is interpreted as the incus or the malleus, it shows that the middle ear ossicles of *Arboroharamiya* are small and already detached from the dentary, which is consistent with lack of the postdentary trough and Meckelian groove on the medial surface of the dentary (Zheng et al., 2013; Meng et al., 2014).

The exposed incus has a convex body and a narrow process. It measures 3.1 mm from the tip of the narrow process to the end of the body, 2.2 mm without the process, and 1.8 mm as the maximum width across the body. The body in preserved view is strawberry-shaped and has a gently convex surface. There is a small patch in light color on the body, of which the nature is unclear. It could be that the bone was worn off or broken so that the filled sediment is exposed or a concavity that is filled with matrix. The side of the body bearing the process is convex. The body smoothly converges to the narrow process, which is tentatively identified as the short process of the incus. Although the full morphology of the bone is unknown, this process is unlikely the long (stapedial) process (crus longum) because it is too small to articulate with the broad distal end of the stapes. A narrow and long stapedial process, similar to that in extant mammals (Doran, 1878; Segall, 1970; Fleischer, 1973; Henson, 1974), is unlikely developed because the broad distal end of the stapes suggests a sizable incudostapedial articulation. Nonetheless, in being proportionally small and possessing the short and slim process, the incus is significantly different in size and shape from the quadrate in non-mammaliaform cynodonts and in morganucodontids (Parrington, 1955; Kemp, 1969, 1979; Kermack et al., 1981; Luo, 1994; Luo and Crompton, 1994). The anatomical relationship of the incus and stapes are uncertain. In addition to the "concavity" mentioned above, it is also possible that the rounded area or the body represents the articulation for the malleus, which allows movement between the two bones.

### Mandible

The mandibular structures of *Arboroharamiya* has been described and illustrated by Zheng et al. (2013: Fig. S2) and Meng et al. (2014: Fig. 2);

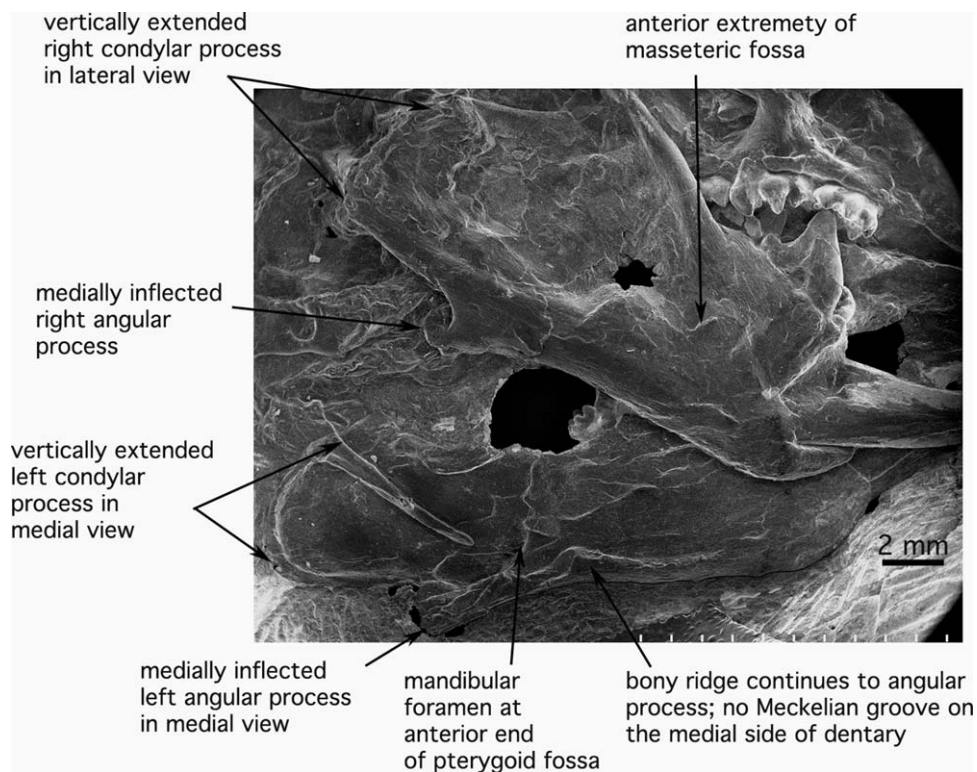


Fig. 3. *Xianshou linglong* (IVPP V16707; holotype), mandibular structures of the lower jaws. The image shows the following mandibular features: lack of the postdentary trough and complex (surangular-articularprearticular), lack of the Meckelian groove, location of the mandibular foramen at the anterior end of the pterygoid fossa, anteroventral extension of the masseteric fossa, and orientation of the dentary peduncle (condylar process) and condyle with articulation surface facing posteriorly.

thus, there is no need to duplicate the illustration here (but see supplementary online material). However, an SEM image of the mandibles of *Xianshou* (Fig. 3) is used to further illustrate the mandibular morphology of euharamiyidans. The reason for providing additional image and description is because some mandibular features pertinent to evolution of mammalian middle ear are potentially disputable for “haramiyidans,” and different interpretations of these characters have fundamentally affected mammaliaform phylogenies in which allotherians are included, as discussed below and in Supporting Information.

Figure 4 shows that the mandible of *Xianshou* is similar to that of *Arboroharamiya* and has the features common to euharamiyidans: The dentary is proportionally short and dorsoventrally deep and has a diastema between the only and enlarged incisor and the premolar. There is no postdentary trough on the medial surface of the mandible, which indicates that the postdentary bones (articular, prearticular, surangular, and angular) were fully detached from the dentary. There is no Meckelian groove on the medial surface of the mandible, suggesting lack of a persistent Meckel’s cartilage. Instead, there is a small angular process that inflects medially and continues anteriorly as a ridge along the ventral margin of the pterygoid

fossa. The pterygoid fossa is broad and at its anterior border is the mandibular foramen. On the lateral surface of the dentary the masseteric fossa is large and extends anteriorly to the point leveling with the p4-m1 junction. The mandibular condyle is transversely narrow and extends vertically for the entire depth of the posterior mandibular ramus. The mandibular condyle is confluent with the body; thus a narrowed peduncle is absent. The articular surface of the condyle is convex in lateral view and faces posteriorly.

### Phylogeny

Figure 4 is a simplified phylogenetic frame in which two competing hypotheses for “haramiyidans” and distributions of the DMME within mammaliaforms are highlighted. In one hypothesis (H-I), which is based on Bi et al. (2014) and Krause et al. (2014), euharamiyidans and multituberculates form a clade with *Haramiyavia* and *Thomasia* falling out as stem taxa of the clade. The entire clade, Allotheria, is placed in Mammalia. The other hypothesis (H-II) is based on Luo et al. (2015; Fig. 4), in which “haramiyidans” form a clade that is separated from multituberculates and placed outside of Mammalia, whereas multituberculates are nested within Mammalia.

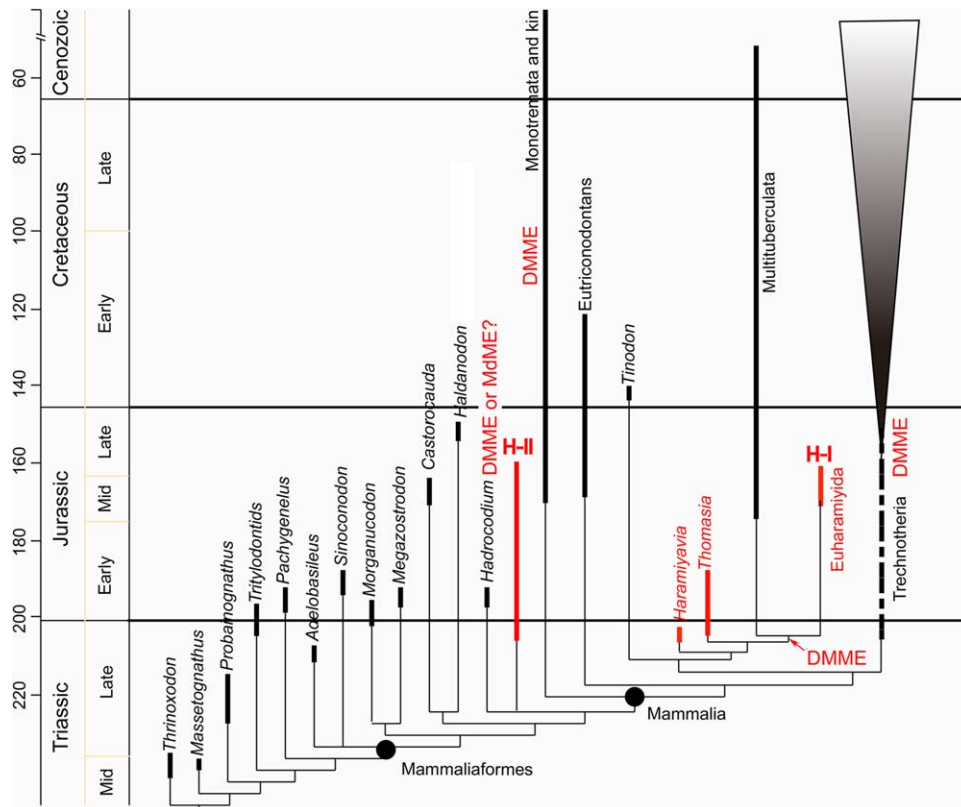


Fig. 4. A simplified phylogeny of mammaliaforms and close relatives showing evolution of the mammalian middle ear. The phylogeny is simplified primarily from Bi et al. (2014) and Krause et al. (2014), supplemented with Luo et al. (2015). H-I and H-II represent hypothesis I (Bi et al., 2014) and II (Luo et al., 2015), respectively, as discussed in the text. In H-I, the DMME evolved at least three times only within Mammalia in the lineages toward monotremes, allotherians, and therians, respectively. In H-II, the DMME in multituberculates and “haramiyidans” must have evolved independently so that at least four times of the DMME evolution took place within mammaliaforms, depending on interpretation of the middle ear of *Hadrocodium*. The question mark associated with *Hadrocodium* is explained in the text and Supporting Information. The red bold lines represent distributions and relationships of “haramiyidans” in H-I and H-II. The dashed line of Trechnotheria indicates the taxonomy that some Late Triassic mammals, such as *Kuehneotherium* and *Woutersia* (Kermack et al., 1968; Sigogneau-Russell and Hahn, 1995), were classified as “symmetrodontans” (Kielan-Jaworowska et al., 2004), which would extend the earliest trechnotherian mammals into the Late Triassic.

The strict consensus tree of the TNT analysis based on the data matrix with corrected coding of five mandibular characters is illustrated in the supplementary online material (Fig. S6). It shows that “haramiyidans” are related to multituberculates to form Allotheria; the latter are nested within Mammalia. This result is nearly identical to that of Bi et al. (2014) and Krause et al. (2014) or H-I. In Luo et al. (2015), the derived similarities that euharamiyidans share with multituberculates and other mammals were changed to plesiomorphic similarities shared with non-mammalian cynodonts. Those changes apparently played a key role in pulling “haramiyidans” out of Mammalia (Luo et al., 2015).

## DISCUSSION

### Homology of the Posterior Process

As briefly reviewed by Meng and Hou (2016), the homology of the stapes still remains controver-

sial despite of its early discovery in the mid-16th century (Mudry, 2013). Among the three middle ear ossicles the stapes is of the second branchial arch origin, whereas the malleus and incus are first arch derivatives (Goodrich, 1930; de Beer, 1937; Crompton and Parker, 1978; Kontges and Lumsden, 1996; Tucker et al., 2004; Chapman, 2011; Kitazawa et al., 2015). It remains open whether the stapes is derived from a unique source of the second branchial (hyoid) arch (Rodríguez-Vázquez, 2005, 2009; Rodríguez-Vázquez et al., 2006) or from dual sources, with the footplate being from the otic capsule and the rest of the stapes as the dorsalmost derivative of the second branchial arch (Caldwell and Anson, 1942; Anson, 1960; Masuda et al., 1978; Ars, 1989; Mallo, 1997; Nandapalan and Tos, 2000; Whittemore et al., 2013). In addition, whether development of the stapes is related to formation of the fenestra vestibuli remains disputable (Rijli et al., 1993; Mallo, 1997, 2001; Kanzler et al., 2000).

From an evolutionary perspective, it is also not crystal clear whether the mammalian stapes is homologous strictly with the “reptilian” and bird otostapes or with the proximal columella of sauropsids and other tetrapods without the extrastapedial elements (distal columella; Allin, 1975; Cambas, 1983; Presley, 1984; Novacek and Wyss, 1986; Allin and Hopson, 1992). In reporting the stapes of the eutriconodontan *Chaoyangodens*, Meng and Hou (2016) raises the issue about the homology of the PISM, which in turn is relevant to the development of the stapedius muscle that functions for protecting the inner ear from overstimulation by excessively loud sound and/or for frequency tuning in mammalian hearing (Wever and Bray, 1942; Fleischer, 1978). The basic issue is whether the PISM in mammals is homologous to the dorsal process of the sauropsid extrastapes or to the ossified proximal (stapedial) end of the interhyal.

The dorsal process is present on the stapes of some non-mammaliaform therapsids (Watson, 1953; Hopson, 1966; Allin, 1975; Parrington, 1979; Allin and Hopson, 1992; Rodrigues et al., 2013; Ruf et al., 2013; Gaetano and Abdala, 2015) and the PISM is present in extant mammals (some have it reduced) (Doran, 1878; Fleischer, 1973; Novacek and Wyss, 1986; Fig. 3). The developmental process of the stapes has been explored in many studies (de Beer, 1937; Presley, 1984; Allin and Hopson, 1992; Rodríguez-Vázquez, 2005, 2009; Rodríguez-Vázquez et al., 2006). The general pattern is that during early embryonic stages, the mesenchymal blastema of the second branchial arch has two processes, the medial and lateral, at its dorsal end. The medial process ossifies in sauropsids as the stapes proper (the otostapes) and in mammals as the entire stapes. The medial process, or stapedial anlage (Rodríguez-Vázquez, 2005), has two distinct parts: the dorsal part that forms the footplate and the ventral part forms the crura and the head of the stapes. The lateral process of the second branchial arch, part of the Reichert’s cartilage (Rodríguez-Vázquez, 2005), chondrifies as the dorsal process of the sauropsid extrastapes and as the tympanohyal (laterohyal) that fuses to the crista parotica of the otic capsule in mammals (Hanson et al., 1962; Cambas, 1983; Allin and Hopson, 1992; Rodríguez-Vázquez, 2005, 2009). In other words, the tympanohyal of mammals and the dorsal process of sauropsids are homologous, which is supported by their topographic relationships to the jugular vein and hyomandibular branch of the facial nerve, and the articulation with the crista parotica (Cambas, 1983; Rodríguez-Vázquez, 2005, 2009). During the early stage of development in mammals, the interhyal temporarily connects the embryonic stapes with the dorsal process (or the cranial end of Reichert’s cartilage); its proximal part (the stapedial portion) becomes

the tendon of the stapedius muscle, and sometimes a sesamoid cartilage (of Paauw) within this tendon, whereas the thinner distal (external) part disappears at the beginning of the fetal period and the stapedius muscle joins the interhyal at a later stage of development (Rodríguez-Vázquez, 2005).

Based on the developmental evidence (de Beer, 1937; Presley, 1984; Allin and Hopson, 1992; Rodríguez-Vázquez, 2005, 2009; Rodríguez-Vázquez et al., 2006), Meng and Hou (2016) posited that the PISM of the mammalian stapes, including the one from the eutriconodontan *Chaoyangodens*, is most probably homologous with the ossified proximal (stapedial) end of the interhyal on which the tendon of the stapedius muscle attaches, but not homologous to the dorsal process of the sauropsid extrastapes, although the PISM of *Chaoyangodens* is proportionally larger than those of extant mammals (Doran, 1878; Segall, 1970; Fleischer, 1973; Henson, 1974; Schmelzle et al., 2005).

Gaetano and Abdala (2015), however, presented an excellent documentation of the stapes in gomphodont cynodonts. According to the authors, the dorsal process is a triangular-shaped lamina pointing dorsally or dorsomedially from the dorsolateral portion of the posterior crus of the stapes in non-mammaliaform cynodonts (except for some specimens of *Thrinaxodon*) and compatible to the insertion of a small ligament or muscle such as Paauw’s cartilage or the stapedial muscle. The authors interpreted that the stapedial muscle connected the stapes, by attachment to the dorsal process, to the paroccipital process in basal cynodonts. From the developmental evidence, it seems that the dorsal process is unlikely homologous to the PISM, as discussed above; then it remains open whether the stapedial muscle attached to the dorsal process, as interpreted in gomphodont cynodonts by Gaetano and Abdala (2015), is homologous with the stapedius muscle attached to the PISM in mammals, a subject beyond the scope of this study.

The stapes of *Arboroharamiya* is considerably smaller than those of non-mammalian cynodonts in absolute size and size relative to the skull length (see below). However, the peculiar posterior process of the stapes is unique and proportionally large as part of the stapes; it compares neither with the PISM in mammals nor with the dorsal process in non-mammalian cynodonts. By far, the most robust PISM in known mammals was from the eutriconodontan *Chaoyangodens* (Meng and Hou, 2016; Fig. 2i), but it is still proportionally much smaller than the posterior process of *Arboroharamiya*. Although proportionally large, the absolute size of the posterior process is unquestionably minuscule; thus it cannot contact any cranial structure, such as the paraoccipital process as in some sauropsids and synapsids (Romer, 1956;



Allin, 1975). Similarly, the size and shape of the posterior process does not suggest any contact with the tympanic membrane as an extrastapes, a hypothesis on middle ear evolution that has been discussed in many studies (e.g., Westoll, 1943, 1944; Hopson, 1966; Allin, 1975, 1986; Parrington, 1979; Novacek, 1993). Taking the pattern of related structures into account, including the small size of the stapes and incus, lack of the post-dentary trough and Meckelian groove, and presence of the dentary-squamosal craniomandibular joint, we interpret that *Arboroharamiya* already developed the DMME and that the posterior process on the stapes functioned as the site for attachment of the stapedius muscle; thus it is homologous to the PISM in the eutriconodontan *Chaoyangodens* and other mammals (Fig. 2) and was developed from the proximal (the stapedial) portion of the interhyal.

### Size of the Stapes

The relative size of a stapes is usually obtained by comparing the footplate diameter and/or stapes length to the length of the skull, a quantitative character that has been used in phylogenetic reconstruction of mammals (Wible, 1991). Usually, the stapes is robust and proportionally large in non-mammalian cynodonts (Watson, 1953; Estes, 1961; Bonaparte, 1966; Allin, 1975; Parrington, 1979; Kermack et al., 1981; Novacek and Wyss, 1986; Rowe, 1988; Lillegraven and Krusat, 1991; Wible, 1991; Allin and Hopson, 1992; Crompton and Luo, 1993; Wible and Hopson, 1993; Rodrigues et al., 2013; Ruf et al., 2013; Gaetano and Abdala, 2015) but considerably reduced and proportionally very small in mammals (Doran, 1878; Segall, 1970; Fleischer, 1973; Henson, 1974), such that small middle ear ossicles have been regarded as an apomorphy of the mammalian crown group (Kielan-Jaworowska et al., 2004). The skull length to the footplate diameter ratio is 43 in *Morganucodon* (Kermack et al., 1981), 35.2 in *Haldanodon* (Lillegraven and Krusat, 1991) or 43.5 (Ruf et al., 2013), 21.4 in *Thrinaxodon*, 17 in *Galesaurus*, 24.8 for *Trirachodon* (based on measurements from [Parrington, 1949]), and 22.6 in *Brasilitherium* (Rodrigues et al., 2013). The same ratio is 110 in adult *Tachyglossus* (Kermack et al., 1981) and 53.5 in the eutriconodontan *Chaoyangodens* (Meng and Hou, 2016).

Similarly, the stapes length (measured or estimated) as a percentage of the skull length is usually greater in non-mammalian cynodonts than in mammals. The primitive stapes/skull length percentage ranges from 7.5 to 13% in non-mammaliaform taxa (Wible, 1991; Wible and Hopson, 1993), whereas the derived condition ranges from 3 to 5.5% in extinct mammaliaform taxa, such as *Sinoconodon*, morganucodontids, multitubercu-

lates, and *Vincelestes*, and 0.05 to 4.2% in extant mammals, although only two states were actually used for phylogenetic analysis: stapes length-greater than 7.5% (0) or less than 5.5% (1) of skull length (Wible, 1991). The same percentage is 8% for *Thrinaxodon*, 9.4% for *Galesaurus*, 12.1% for *Trirachodon*, based on measurements of Parrington (1949), and 9% for *Brasilitherium* (Rodrigues et al., 2013). In gomphodont cynodonts (*Diademodon*, *Langbergia*, *Trirachodon*, *Scalenodon*, *Luangua*, *Massetognathus*, *Menadon*, and *Exaeretodon*) the percentage ranges from 7 to 13% (Gaetano and Abdala, 2015: table 2), nearly identical to the percentage range for non-mammaliaforms recognized by Wible (1991). The same percentage is 3.3% in the multituberculate *Lambdopsalis* (Meng, 1992) and 2.97% in the eutriconodontan *Chaoyangodens* (Meng and Hou, 2016).

The skull of *Arboroharamiya* was not preserved, but both lower jaws were nearly intact. Using the lower jaw and skull length ratio from other euharamiyidans in which the cranium and lower jaws are nearly intact, we can estimate the skull length of *Arboroharamiya*. In the paratype 1 (WGMV-001) of *Shenshou lui*, the lower jaw measures 24 mm from the tip of the incisor to the distal border of the mandibular condyle (the maximum length) and the skull length is 31 mm (Bi et al., 2014); thus, the lower jaw length is 77.4% of the skull length. In the holotype of *X. linglong* (IVPP V16707, Bi et al., 2014), the lower jaw and skull length is 24.8 and 32 mm, respectively, yielding a jaw/skull length percentage 77.5%. In other mammals where the mandible and skull lengths from same individuals could be measured or reasonably estimated, this percentage is 80% in the eutherian *Acristatherium yanensis* (Hu et al., 2010), 78% in the eutriconodontan *Liaconodon* (the measured skull length is 50 mm and the dentary length is 35 mm without incisor [Meng et al., 2011] but 39 mm with the incisor), and 62–67% in *Kryptobaatar dashzevegi* (lower jaw length measured from the incisor base; these numbers would be slightly greater if the length is measured to the tip of the incisor; (Wible and Rougier, 2000: table 2).

The mandible length of *Arboroharamiya* is 37.65 (Meng et al., 2014). Using the mandible and skull length percentage (77.4%) of *Shenshou lui*, the skull length of *Arboroharamiya* is estimated as 48.64 mm. Thus, the skull length/footplate diameter ratio is estimated as 51.74 and the stapes/skull length percentage is 4% for *Arboroharamiya*. Both numbers show that the relative size of the stapes of *Arboroharamiya* is within the range of mammals. In the eutriconodontan *Chaoyangodens* the skull/footplate length ratio is 53.5 and the stapes/skull length percentage is 2.97% (Meng and Hou, 2016). These indicate that the size of the footplate, therefore the size of the fenestra vestibuli, of *Arboroharamiya* is similar to eutriconodontans

(Meng et al., 2011; Meng and Hou, 2016), but the length of the stapes in *Arboroharamiya* is proportionally longer than that of *Chaoyangodens*.

### Shape of the Stapes

The stapes of *Arboroharamiya* with nearly parallel crura and broad distal end is probably best assignable to the “rod-like” category of Novacek and Wyss (1986). The general stapes shape of *Arboroharamiya* is similar to those of non-mammalian cynodonts, such as *Thrinacodon*, *Exaeretodon*, *Probainognathus*, *Pachygenelus*, *Sinoconodon*, *Morganucodon*, and *Haldanodon* (Watson, 1953; Estes, 1961; Bonaparte, 1966; Allin, 1975; Parrington, 1979; Kermack et al., 1981; Novacek and Wyss, 1986; Allin and Hopson, 1992; Crompton and Luo, 1993; Rodrigues et al., 2013; Ruf et al., 2013; Gaetano and Abdala, 2015). Differences do exist, including that the stapedia foramen is more extensive, the crura are thinner, and there is a long posterior process in the stapes of *Arboroharamiya*. In addition, it seems that there is no distinct footplate that is wider than the margin defined by the crura, contrasting to those of *Morganucodon* and *Haldanodon* but more or less similar to the stapes of the eutriconodontan *Chaoyangodens* that also lacks a distinctive stapedia footplate (Meng and Hou, 2016).

Compared with other mammals, the stapes of *Arboroharamiya* differs considerably from that of monotremes that has a “T” shaped stapes in lateral view with a slim imperforated columelliform shaft (Doran, 1878; Fleischer, 1973; Novacek and Wyss, 1986). Many therians have a perforated stapes, but therian stapes, including that of the Late Cretaceous eutherian (Archibald, 1979), differ from the stapes of *Arboroharamiya* in having a restricted (narrow) head, a distinct footplate and a small PISM (Doran, 1878; Segall, 1970; Fleischer, 1973; Henson, 1974; Novacek and Wyss, 1986; Schmelzle et al., 2005). A restricted stapedia head is also applicable to multituberculates where the stapes is known: it can be either a stirrup shaped, as in the Late Cretaceous *Kryptobaatar* (Rougier et al., 1996b), or a columelliform, as in the Tertiary *Lambdopsalis* (Meng, 1992). As Meng (1992) noted that a restricted stapedia head conforms reduction of the incus and formation of the lenticular process for the stapes; the latter often bends at a right angle to the long process and articulates to the head of the stapes (Meng, 1992; Meng and Hou, 2016).

The stapes of *Arboroharamiya* clearly indicates that the incudostapedial articulation is sizable, compared with that of extant mammals; this suggests that the incus probably have not developed a slender long process with a restricted lenticular process. Among mammals, the stapes of *Arboroharamiya* is comparable to that of the eutricono-

odontan *Chaoyangodens* in which the stapes is also rod-like (Meng and Hou, 2016). The stapes of *Arboroharamiya* again supports the notion that the rod-like stapes with a broad end-on-end contact with the incus is a primitive condition for mammals (Novacek and Wyss, 1986; Meng, 1992; Rougier et al., 1996b). The derived condition is characterized by reduction of the distal end of the stapes to form the stapedia head, which Meng (1992) considered as the most significant modification of the stapes within a triossicular ear and is consistent with the evolutionary trend of reduction in the incus and malleus for more sensitive airborne hearing in mammals.

It is worth to note that the morphology of the stapes and incus of *Arboroharamiya* may be related to special adaptation of hearing and/or locomotion. As known from other mammals, the ear ossicles are specialized for detection of different vibrations in adaptation to different life styles, ranging from aquatic, fossorial to arboreal (Doran, 1878; Fleischer, 1973; Nummela et al., 1999; Ketten, 2000; Mason, 2001; Tubelli et al., 2014). *Arboroharamiya* and other euharamiyidans were probably tree dwellers, possibly gliders; they were specialized in having relatively short metapodials but long phalanges in both manus and pes, differing them from other arboreal mammals (Zheng et al., 2013; Bi et al., 2014). The ear ossicles may represent an adaptation for a special arboreal life that was unfamiliar to us.

### Stapedial Foramen

The perforated stapes as the primitive condition for mammals have been widely accepted (Goodrich, 1915, 1930; Kuhn, 1971; Henson, 1974; Fleischer, 1978; Novacek and Wyss, 1986; Lillegraven and Krusat, 1991; Wible, 1991; Meng, 1992; Novacek, 1993; Wible and Hopson, 1993; Gaudin et al., 1996; Rougier et al., 1996b; Sánchez-Villagra et al., 2002). This is partly because a perforated stapes is the common condition present in non-mammaliaform cynodonts and basal mammaliaforms, such as *Thrinacodon*, *Exaeretodon*, *Probainognathus*, *Pachygenelus*, *Brasilitherium*, *Sinoconodon*, *Morganucodon*, and *Haldanodon* (Watson, 1953; Estes, 1961; Bonaparte, 1966; Allin, 1975; Parrington, 1979; Kermack et al., 1981; Novacek and Wyss, 1986; Rowe, 1988; Lillegraven and Krusat, 1991; Wible, 1991; Allin and Hopson, 1992; Crompton and Luo, 1993; Wible and Hopson, 1993; Sánchez-Villagra et al., 2002; Rodrigues et al., 2013; Ruf et al., 2013; Gaetano and Abdala, 2015; Fig. 2). The stapes of *Arboroharamiya* again add additional support to the notion that the perforated stapes is primitive for mammals. The large stapedia foramen suggests presence of a functional stapedia artery in *Arboroharamiya*, although no basicranial

structure was preserved in *Arboroharamiya* to furnish additional evidence.

### Incus

In ancestral forms of extant mammals, such as monotremes and marsupials (Doran, 1878; Fleischer, 1973), the malleus usually has a long anterior process and does not have an inflated or bulbous body. This is true in the malleus of the eutriconodontan *Liaoconodon* (Meng et al., 2011). However, in advanced forms, such as human, there is a distinctive head of the malleus. The incus (or its precursor quadrate) in non-mammal cynodonts (Parrington, 1955; Kemp, 1969, 1979; Kermack et al., 1981; Luo, 1994; Luo and Crompton, 1994) is more likely to have a relative bulbous body or head; thus, we interpret the element in question as the incus.

Among Mesozoic mammals, the incus or element interpreted as the incus is known at least in two eutriconodontans, *Yanoconodon* (Luo et al., 2007a) and *Liaoconodon* (Meng et al., 2011), in the Late Cretaceous multituberculate *Chulsanbaatar vulgaris* (Hurum et al., 1996), and possibly in the Cretaceous eutherian *Daulestes* (McKenna et al., 2000). Among Mesozoic mammals, only the incus of *Liaoconodon* has preserved an unequivocal shape and relationship with other middle ear bones; it has a hinge-like articulation with the malleus and does not have an elongate long process with a lenticular process (Meng et al., 2011). This morphology is consistent with the shape of the stapes reported in another eutriconodontan, *Chaoyangodens* (Hou and Meng, 2014; Meng and Hou, 2016), which has a broad distal end to articulate with the incus. In the multituberculate *Chulsanbaatar*, the incus is a flat bone (Hurum et al., 1996), generally similar to the inferred incus in the Tertiary multituberculate *Lambdopsalis* (Miao and Lillegraven, 1986; Meng and Wyss, 1995). In addition, the preserved part of the incus of *Chulsanbaatar* is roughly A-shaped with the short process (*crus breve*) being longer and more robust than the more gracile but incomplete long process (*crus longum*), similar to that of *Liaoconodon* (Meng et al., 2011). Compared with the multituberculate *Chaoyangodens* and *Lambdopsalis* and the eutriconodontan *Liaoconodon*, the exposed portion of the incus of *Arboroharamiya* is distinctive in at least two aspects: it has a bulbous body or head and a slim process that is interpreted as the short process of the incus.

In contrast, the precursor of the incus, the quadrate, has been known in several non-mammalian cynodonts. The quadrate is proportionally large, has a simple dorsal plate and is suspended by multiple bones or in articulation with the petrosal (Parrington, 1955; Kemp, 1969, 1979; Kermack et al., 1981; Luo, 1994; Luo and Crompton, 1994; Kielan-Jaworowska et al., 2004; Luo et al., 2011).

The small size and the exposed bulbous body or head show that the incus of *Arboroharamiya* does not seem to have a strong dorsal plate that inserts into the deep fossa in the petrosal, as in *Morganucodon* (Kermack et al., 1981; Luo and Crompton, 1994). This is supported by a completely developed and longitudinally orientated glenoid fossa, similar to that of multituberculates, in *Shenshou* and *Xianshou*, that implies an exclusive dentary-squamosal craniomandibular joint (Bi et al., 2014). The greatly reduced incus was most probably moored to the cranium via a ligament as in extant mammals, although it differs from the incus of extant mammals in lacking a narrow and long stapedial process. In other words, the incus was freed from the cranium and functioned solely as part of the auditory apparatus, meaning again that euharamiyidans already acquired the DMME as early as in the early Middle Jurassic.

### Mandibular Characters

In addition to the middle ear ossicles, some mandibular structures also provide important information for evolution of the mammalian middle ear and, as critical osteological characters, contribute to phylogenetic reconstruction of mammals, which in turn affects interpretation of the middle ear evolution. The general morphology of the mandible is similar among euharamiyidans (Zheng et al., 2013; Bi et al., 2014; Fig. 3 and Figs. S1–S5) and to those of multituberculates (Kielan-Jaworowska and Hurum, 1997; Kielan-Jaworowska et al., 2004). In both groups, the mandible is proportionally short and dorsoventrally deep and has a distinct diastema between the sole incisor and the mesial premolar. In both groups, the postdentary trough and Meckelian groove are absent. In addition, the masseteric fossa is large and extends anteriorly, and the mandibular condyle is vertically orientated. The mandible of euharamiyidans differs from that of multituberculates in having a small angular process that inflects medially. Based on the mandibular characters, presence of the DMME was inferred for euharamiyidans (Zheng et al., 2013; Bi et al., 2014; Meng et al., 2014), which is confirmed by the small ear ossicles reported here.

In a recent phylogenetic analysis, Luo et al. (2015) used one of the data matrixes of Bi et al. (2014) but modified codings of over 50 characters for euharamiyidans, *Arboroharamiya* included. The outcome of the phylogenetic analysis with recoded characters fundamentally altered the phylogenetic position of “haramiyidans” and thus, interpretation of the middle ear evolution within mammaliaforms. The character re-coding for euharamiyidans was not justified, but we found it unusual that the original codings of over 50 characters for euharamiyidans were changed either to

“?” or, for most characters, to “0.” These changes ought to reduce the similarities between “haramiyidans” and multituberculates and other mammals but increase similarities of “haramiyidans” to basal members of mammaliaforms. In this study, we examined five mandibular characters re-coded by Luo et al. (2015) because these characters are relevant to interpretations of mammalian middle ear evolution; other recoded characters will be revisited in separate studies.

A comparative discussion and illustrations about the five mandibular characters in euharamiyidans are provided in the supplementary online material (Figs. S1–S5). The way Luo et al. (2015) re-coded those mandibular characters indicates that these authors believe that *Arboroharamiya*, *Shenshou*, and *Xianshou* have the Meckelian groove and the postdentary complex (for *Shenshou* and *Xianshou*), lack the anterior extension of the masseteric fossa, and do not have a vertically orientated mandibular condyle. We disagree with those changes made by Luo et al. (2015).

Presence of the Meckelian groove suggests a persistent Meckel’s cartilage or OMC in adult individuals (Wang et al., 2001; Meng et al., 2003) and presence of the TMME (Meng et al., 2011). A persistent Meckel’s cartilage or OMC have been observed in several Mesozoic mammals, such as eutriconodontans (*Repenomamus*, *Gobiconodon*, *Yanoconodon*, *Liaconodon*, and *Chaoyangodens*) (Wang et al., 2001; Li et al., 2003; Meng et al., 2003, 2011; Luo et al., 2007a; Meng and Hou, 2016) and “symmetrodontans” (*Zhangheotherium* and *Maotherium*) (Meng et al., 2003; Ji et al., 2009). A persistent Meckel’s cartilage or OMC was also inferred for various taxa in which a distinctive Meckelian groove was present on the medial surface of the dentary (Meng et al., 2003, 2011). The Meckelian groove is absent in euharamiyidans, as illustrated in Fig. 3 (see also Figs. S1–S5). Moreover, the TMME was by far only inferred from mammals without a palinal (posterior) jaw movement, such as eutriconodontans and “symmetrodontans.” In contrast, euharamiyidans probably had a palinal jaw movement during mastication, as shown by their tooth morphology, wear facets and striations on teeth, shape of the mandibular condyle and shape of the longitudinally orientated glenoid fossa that lacks the postglenoid process (Butler and MacIntyre, 1994; Butler, 2000; Butler and Hooker, 2005; Zheng et al., 2013; Bi et al., 2014; Meng et al., 2014; Fig. 3). Absence of the Meckelian groove, and thus lack of the TMME, is consistent with the inferred jaw movement of euharamiyidans. Otherwise, when the lower jaw moved backward, how the middle ear functioned remains a challenging issue, if possible at all.

Presence of the postdentary complex (surangular, articular, prearticular, and angular) implies presence of the MdME and a compound jaw joint,

which is typically shown in *Morganucodon* (Kermack et al., 1973, 1981; Figs. S1b, S3b–S5b). Figure 3 shows clearly that, similar to *Arboroharamiya* (Figs. S1–S5), the jaw suspension is exclusively between the dentary and the squamosal in *Shenshou* and *Xianshou*. The postdentary complex and trough are absolutely absent in euharamiyidans.

The anterior extension of the masseteric fossa in *Arboroharamiya* and other euharamiyans is obvious and cannot be the same as in *Morganucodon* (Fig. S4b). The condition of euharamiyidans is similar to that of multituberculates in which the masseteric fossa is large and anteriorly extended, usually ending below the junction of p4 and m1 on the lateral surface of the dentary (Gambaryan and Kielan-Jaworowska, 1995; Kielan-Jaworowska et al., 2004). In *Morganucodon*, the masseteric fossa is relatively small and its anterior extremity does not extend anterior to m3. The anterior extension of the masseteric fossa is associated with the enlarged lower incisor, morphology of the mandibular condyle and the jaw movement during mastication. There is no reason to score euharamiyidans as having the same condition of the masseteric fossa as in *Morganucodon*.

The mandibular condyle and the condyle process of *Arboroharamiya* and other euharamiyans are also similar to those of multituberculates (Kielan-Jaworowska and Hurum, 1997; Kielan-Jaworowska et al., 2004) and differ significantly from those of *Morganucodon* (Fig. S5b) and most Mesozoic mammals. The vertically orientated condyle, along with other mandibular features, is probably related to the palinal move in mastication in euharamiyidans, as in multituberculates (Krause, 1982; Gambaryan and Kielan-Jaworowska, 1995).

### Evolution of DMME

Small middle ear ossicles have been regarded as an apomorphy of the mammalian crown group (Kielan-Jaworowska et al., 2004: table 3.4). The greatly reduced stapes and incus of *Arboroharamiya* are thus highly mammalian. Coupled with absence of the postdentary trough and Meckelian groove, as well as the shape of the mandibular condyle and glenoid fossa in euharamiyidans (Zheng et al., 2013; Bi et al., 2014; Meng et al., 2014; Fig. 3), it is clear that euharamiyidans have evolved the DMME. However, the significance of the middle ear ossicles of *Arboroharamiya* to the evolution of the mammalian middle ear depends on the higher-level phylogeny of mammals. Unfortunately, the phylogenetic relationship of “haramiyidans” has long been controversial (Simpson, 1929, 1947; Hahn, 1973; Sigogneau-Russell et al., 1986; McKenna, 1987; Hahn et al., 1989; Butler and MacIntyre, 1994; Jenkins et al., 1997;

McKenna and Bell, 1997; Kermack et al., 1998; Butler, 2000; Kielan-Jaworowska et al., 2004; Hahn and Hahn, 2006). There remain at least two fundamental issues: whether “haramiyidans” are closely related to multituberculates to form the clade Allotheria and whether allotherians, regardless the content of the group, are phylogenetically in or outside of Mammalia. Most phylogenetic analyses concurred the notion that allotherians include “haramiyidans” (commonly represented by *Haramiyavia*) and multituberculates and that allotherians are nested within Mammalia (Luo et al., 2002, 2007a,b, 2011; Luo and Wible, 2005; Rowe et al., 2008; Ji et al., 2009; Meng et al., 2011; Zheng et al., 2013; Bi et al., 2014; Krause et al., 2014), but alternative hypotheses do exist (Averianov et al., 2011; Luo et al., 2015). Although the hypotheses are diverse (see Meng [2014] for a brief review), two are commonly mentioned in literatures, as illustrated in Figure 4.

Assuming the interpretation that *Haramiyavia* has the postdentary trough (Luo et al., 2015, but see Averianov et al., 2011), then H-I predicts that there is one evolutionary step from the condition of *Haramiyavia* to give rise to the DMME in the common ancestor of euharamiyidans and multituberculates. In H-II, however, the DMME in euharamiyidans and multituberculates must have evolved independently. Our evidence shows that H-II is weakly supported. This is not only because H-II requires convergent evolution of the DMME in multituberculates and euharamiyidans but also requires parallel evolution of numerous derived similarities in the dentition, occlusion pattern, mandibles, cranium, and postcranium between euharamiyidans and multituberculates as well as between euharamiyidans and other mammals (Bi et al., 2014). More importantly, after we corrected the coding of the five mandibular characters in the data matrix of Luo et al. (2015), the phylogenetic result became nearly identical to that of Bi et al. (2014; supplementary online material). This indicates that the modified coding of the five mandibular characters played a key role in pulling “haramiyidans” out of Mammalia in Luo et al.’s (2015) analysis. Once only the five codings are corrected (there are more than 40 character codings to be examined), the phylogenetic result of Luo et al. (2015) no longer holds. Thus, based on available evidence, we regard H-I as the working hypothesis in interpreting evolution of the mammalian middle ear. Based on H-I, the DMME evolved at least three times independently in Mammalia. These events took place in the lineages leading toward allotherians, monotremes and therians, respectively.

Interpretation of the DMME evolution depends not only on phylogenetic hypotheses but also on interpretation of the middle ear of *Hadrocodium* (Luo et al., 2001). *Hadrocodium* was originally

considered as having the DMME because its dentary bone was interpreted as lacking the postdentary trough and its brain as so expanded as the mechanism to pull the postdentary bones (middle ear ossicles) off the dentary (Luo et al., 2001). Luo (2011: 363) considered *Hadrocodium* “the earliest form in which both the postdentary trough and Meckel’s groove for holding the middle ear are completely lost” and the inferred separation of the middle ear from the dentary represents an independent acquisition of the DMME either in *Hadrocodium* or in the common ancestor of *Hadrocodium* and crown Mammalia. Whether brain-expansion is the mechanism for the detachment of the middle ear ossicles has been controversial (Wang et al., 2001; Meng et al., 2003, 2011), but a new interpretation about the middle ear of *Hadrocodium* reversed the original interpretation. In the new interpretation, the postdentary trough and bones were considered as present in *Hadrocodium* (see Bi et al., 2014; supplementary online material). Because both the postdentary trough and Meckelian groove were still coded as absent in *Hadrocodium* (Luo et al., 2015), a confusing message has been signaled about the middle ear of *Hadrocodium*. At present, we can only entertain two possibilities for *Hadrocodium*, presence of the DMME or MdME, as shown in Figure 4. If the former is true, *Hadrocodium* represents the earliest known member of mammaliaforms that evolved the DMME in the Early Jurassic, independent that of mammals.

## CONCLUSIONS

The stapes and incus of the late Middle or early Late Jurassic (the Callovian to Oxfordian) euharamiyidan, *Arboroharamiya*, from northern China are described. Some characters of the stapes were discussed, including homology of the posterior process, size and shape of the stapes, the stapedia foramen and the shape of the incus. The stapes is further compared with those of mammals and non-mammalian cynodonts. The general result is that the ear ossicles of *Arboroharamiya* were reduced to the range of extant mammals, but the stapes has a broad distal end in articulation with the incus, similar to that of the eutriconodontan *Chaoyangodens*, and a prominent posterior process, which is interpreted as the PISM but is larger than that of *Chaoyangodens*. Some mandibular characters that are related to the middle ear and have played a critical role in phylogenetic analyses are discussed. The mandibular structures show no sign of the postdentary trough, postdentary bones or Meckelian groove, indicating that the middle ear ossicles have completely detached from the dentary and functioned exclusively for hearing. The middle ear and mandibular features support the phylogenetic relationship that

euharamiyidans are closely related to multituberculates and the clade consisting of the two groups is nested within Mammalia. This phylogenetic relationship implies that the detachment of the middle ear ossicles from the dentary to form the DMME happened once in allotherians (containing euharamiyidans and multituberculates) and many similarities in dentition, occlusion pattern, mandibular structures, cranial and postcranial skeleton between euharamiyidans and multituberculates are probably due to a common ancestor. However, the acquisition of the DMME in allotherians is probably independent to those of monotremes and therians, and the DMME has evolved at least three times independently in mammals.

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