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Comparative anatomy of the middle ear ossicles of extant hominids – Introducing a geometric morphometric protocol

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ABSTRACT

The presence of three interconnected auditory ossicles in the middle ear is a defining characteristic of mammals, and aspects of ossicle morphology are related to hearing sensitivity. However, analysis and comparison of ossicles are complicated by their minute size and complex three-dimensional shapes. Here we introduce a geometric morphometric measurement protocol for 3D shape analysis based on landmarks and semilandmarks obtained from μCT images and apply it to ossicles of extant hominids (great apes and humans). We show that the protocol is reliable and reproducible over a range of voxel resolutions, and captures even subtle shape differences. Using this approach it is possible to distinguish the hominid taxa by mean shapes of their malleus and incus ($p < 0.01$). The stapes appears less diagnostic, although this may in part be related to the small sample size available. Using ancestral state estimation, we show that, within hominids, *Homo sapiens* is derived with respect to its malleus (short manubrium, long corpus, head anterior-posterior flattened, articular facet shape), incus (wide intercrural curvature, long incudal processes, articular facet shape) and stapes (high stapes with kidney-shaped footplate). *H. sapiens* also shows a number of plesiomorphic shape traits whereas *Gorilla* and *Pan* possess a number of autapomorphic characteristics. The *Pongo* ossicles appear to be close to the plesiomorphic hominid condition. The malleus shows little difference in size among hominids, and allometry is thus of little importance. In contrast, the incus and stapes are more variable in size, and their shape is more strongly related to size differences. Although the form-function relationships in the middle ear are not fully understood, some aspects of ossicle morphology suggest that interspecific differences in hearing capacities are present among hominids. Finally, the results of this study provide a comparative framework for morphometric studies analyzing ossicles of extinct hominids, with a bearing on taxonomy, phylogeny and auditory function.

1. Introduction

The presence in the middle ear of three interconnected auditory ossicles – malleus, incus, and stapes – is a defining character of mammals. The ossicular chain connects the tympanic membrane and the inner ear, and plays an important role in amplifying and regulating sound waves. It thus helps to overcome the impedance mismatch between air and the fluid of the inner ear, transferring high-frequency sound better than the single ossicle system of non-mammalian tetrapods (Zwislocki, 1965; Coleman and Ross, 2004; Puria and Steele, 2010; Schmidt et al., 2011). As such, the evolution of three auditory ossicles is a crucial adaptation allowing mammals to hear high-frequency sound (Rosowski, 2013). The transmission of sound energy from the tympanic membrane to the oval window involves two lever arms: one formed by the manubrium of the malleus and the other by the long crus of the incus. Furthermore, mass of the ossicles, moments of inertia, the morphology of the connecting joint and the resulting differences in rotational motion are important factors influencing the nature of sound transfer through the middle ear (Puria and Steele, 2010). Indeed, metrics expressing such morphological differences correlate with variation in frequency range of hearing across mammals (Hemilä et al., 1995; Coleman and Ross, 2004; Coleman and Colbert, 2010; Rosowski, 2013).

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Ossicle morphology shows a wide range of variation within and between groups of mammals (Hyrtl, 1845; Doran, 1878; Fleischer, 1973; Hemilä et al., 1995; Nummela, 1995; Schmelzle et al., 2005; Masali and Cremasco, 2006; Mason, 2013; Quam et al., 2014), and can thus be used for taxonomic discrimination of extant and fossil species in, for example, primates (Masali et al., 1992; Quam and Rak, 2008; Quam et al., 2013a,b; 2014). However, studying ossicles is methodologically challenging because of their small size and highly complex three-dimensional shape. Analyses of ossicle morphology have therefore focused on their mass or two-dimensional measurements, often to address questions related to middle ear physiology (Hemilä et al., 1995; Nummela, 1995; Coleman and Ross, 2004; Coleman and Colbert, 2010), although some quantify ossicle shape in more detail (Arensburg et al., 1981; Siori and Masali, 1983; Masali et al., 1992; Quam and Rak, 2008; Quam et al., 2013a,b; 2014). Such studies frequently employed size-calibrated photographs to obtain measurements, a method potentially prone to inaccuracies because of parallax errors and inter-observer differences in specimen orientation (Flohr et al., 2010). Furthermore, complex shape characteristics, such as manubrial curvature and head shape of the malleus and articular facets, cannot be represented in two dimensions (Schmidt et al., 2011).

Computed tomography with a spatial resolution well below 100 μm (micro CT) yields three-dimensional (3D) image data sets that provide completely new opportunities for the comparative and functional study of the auditory ossicles. Detailed CT-based surface reconstructions can be quantified accurately in 3D using landmarks (Schmidt et al., 2011). Subsequently, geometric morphometrics (GM) can be used for a full 3D analysis of size and shape (Bookstein, 1991; Slice, 2007; Mitteroecker and Gunz, 2009).

The purpose of this paper is twofold. First, we introduce a measurement protocol, based on 3D landmarks and semilandmarks, which comprehensively describes the size and shape of the ossicles, including functionally important aspects. Second, using the newly introduced methods we quantitatively describe and compare the ossicular chain of extant *Homo*, *Pan*, *Gorilla* and *Pongo*. To assess the phylogenetic polarity of morphological changes within hominids we also included the ossicles of *Symphalangus syndactylus* – the largest hylobatid species – and apply phylogenetic comparative methods. In addition to providing a detailed exploration of extant hominid ossicle morphology, this study also aims to provide a framework for the comparative and functional interpretation of the fossil record of hominid ossicles (Angel, 1972; Arensburg and Nathan, 1972; Rak and Clarke, 1979; Heim, 1982; Arensburg and Tillier, 1983; Arensburg et al., 1996; Moggi-Cecchi and Collard, 2002; Spoor, 2002; Martínez et al., 2004; Lisonek and Trinkaus, 2006; Crevecoeur, 2007; Quam and Rak, 2008; Quam et al., 2013a,b).

Previous comparative work on hominid ossicle morphology has particularly highlighted how modern humans and extant great apes differ (Masali, 1968; Siori and Masali, 1983; Masali and Cremasco, 2006; Quam, 2006; Quam et al., 2013a,b; 2014), with some reference to differences among the latter. Great ape species show large differences in their vocal behavior and repertoire, even between closely related species like *Gorilla gorilla* and *Gorilla beringei* (Hohmann and Fruth, 1995; Hedwig et al., 2014), and they inhabit a large range of environments. Hence, given the correlations between hearing sensitivity and ossicle morphology (Hemilä et al., 1995; Coleman and Ross, 2004; Coleman and Colbert, 2010), distinct differences in ossicle shape may be expected between all hominid species.

2. Materials and methods

2.1. Sample and imaging the ossicles

The sample used in this study comprised mallei ($n = 93$), incudes ($n = 89$) and stapes ($n = 45$) of *Homo sapiens*, *Pan troglodytes*, *Pan paniscus*, *G. beringei*, *G. gorilla*, *Pongo* sp. and *S. syndactylus*. We pooled both species of *Pongo* because the provenance (Borneo or Sumatra) of these specimens was not known. Table 1 summarizes the information about provenance, sex, and age at death; these data (also including image spatial resolution) are provided for each specimen in Supplementary Online Material [SOM] Table S1. Whenever possible, ossicles were extracted from the temporal bone and subsequently CT scanned with a spatial resolution in the range of 0.010–0.020 mm. In those cases where ossicles could not be removed without the risk of damage, or if the specimen was from a soft tissue collection, the temporal bone or, in some cases the entire skull, was CT scanned with the highest spatial resolution possible (0.022–0.091 mm). Subsequently, the ossicles were segmented manually (see below). Whenever possible, specimens were selected based on the presence of at least the malleus and incus. The majority of the modern human ossicle sample comes from the University of Leipzig anatomy collection (Germany), removed during cadaver dissections and from a collection of human skeletal remains from an early medieval cemetery at Greding, Germany, dated to the late 7th and early 8th century (Flohr et al., 2010). The majority of the non-human samples are wild specimens obtained from museum collections. CT images of specimens housed in the American Museum of Natural History were kindly provided by Rolf. M. Quam. All other specimens were scanned with the BIR ACTIS 225/300 or the Skyscan 1173 housed at MPI-EVA in Leipzig. Whenever possible, the right ossicles were analyzed, but when left ones were used, these were treated as right ones by mirror-imaging the image stack. Avizo 7.1 (Visualization Science Group) was used to create 3D digital surface models of the ossicles and place the landmarks and semilandmarks. In the case of isolated ossicles, Avizo's Isosurface module was employed using a single threshold value. In order to test for accuracy of threshold values, half maximum height levels were calculated periodically (Spoor and Zonneveld, 1995). Ossicles scanned inside the temporal bone were isolated and visualized using the Segmentation Editor. Surface models were saved in PLY format. Landmark coordinates were exported from Avizo 7.1 and analyzed using Mathematica 8 (Wolfram Research, Inc.), with software routines developed by Gunz and Mitteroecker (2013).

2.2. Measurement protocol

The measurement protocol was designed so that the landmarks and semilandmarks represent the overall size and shape of the ossicles and quantify features known to be important functionally (Fig. 1). Several of the anatomical landmarks follow the protocol of Schmidt et al. (2011).

2.2.1. Malleus Four landmarks were placed on the surface of the malleus (Fig. 1). Landmark 1 represents the apex of the manubrium, positioned furthest away from the corpus of the malleus (Schmidt et al., 2011), and landmark 2 the center of the apex of the lateral process (*processus lateralis*). Landmark 3 was placed on the deepest point of the posteriorly facing part of the articular facet of the malleus (Schmidt et al., 2011). Although easily recognized on an isolated malleus, the placement of the landmark can be aided by calculating a best–fit plane through landmarks placed on the margin of the posteriorly facing surface ('Slice' module in