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Phylogenetic relationship of a fossil macaque (*Macaca* cf. *robusta*) from the Korean Peninsula to extant species of macaques based on zygomaxillary morphology

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ABSTRACT

Little is known about the biogeographical and evolutionary histories of macaques (*Macaca* spp.) in East Asia because the phylogenetic positions of fossil species remain unclear. Here we examined the zygomaxillary remains of a fossil macaque (*M.* cf. *robusta*) from the Durubong Cave Complex, South Korea, that dates back to the late Middle to Late Pleistocene, to infer its phylogenetic relationship to extant species. We took 195 fixed- and semi-landmarks from the zygomaxillary regions of the fossil specimen and from 147 specimens belonging to 14 extant species. We then conducted a generalized Procrustes analysis followed by a multivariate statistical analysis to evaluate the phenetic affinities of the fossil to the extant species and reconstructed the most parsimonious phylogenetic tree using a phylogenetic morphometric approach. We found that the fossil was most similar to *Macaca fuscata* (Japanese macaque) in the zygomaxillary morphospace although it was at the limit of the range of variation for this species. The second closest in the morphospace was the continental *Macaca mulatta* (rhesus macaque). Parsimonious reconstruction confirmed that the fossil was most closely related to *M. fuscata*, even after controlling for the effects of allometry. These findings suggest that in the late Middle to Late Pleistocene, close relatives of *M. fuscata* that looked like the extant species were distributed on the Korean Peninsula, where no species of macaques are found today. Thus, some morphological characteristics of *M. fuscata* may have developed before its ancestor dispersed into the Japanese archipelago.

1. Introduction

The genus *Macaca* consists of approximately 20 extant species and occupies a wider range of climates and habitats than any other genus of nonhuman primates (Fleagle, 2013). Consequently, macaques have attracted much attention as an analogy to understand how humans left tropical regions and adapted to various other environments (Rae et al., 2003; Márquez and Laitman, 2008; Hanya et al., 2011; Karen, 2011; Tsuji et al., 2013; Ito et al., 2015). Furthermore, some East Asian species, particularly *Macaca mulatta* (rhesus macaque) and *Macaca fuscata* (Japanese macaque or snow monkey), are often used in biological laboratory studies (Sibal and Samson, 2001). To better understand these cold-adapted and well-studied species, we need to understand their biogeographic and evolutionary histories, particularly at the northern limit of their distribution in East Asia.

The phylogeny of extant species of macaques has been well studied, with most molecular phylogenetic studies (Tosi et al., 2000; Li et al., 2009; Jiang et al., 2016), except mitochondrial studies (Morales and Melnick, 1998; Liedigk et al., 2014), supporting the classification of Delson (1980). Delson's classification subdivides macaques into four phylogenetic groups: *fascicularis*, *sinica*, *silenus*, and *sylvanus* groups. A molecular phylogenetic study by Jiang et al. (2016) suggested that the African *sylvanus* group first diverged ca. 5.5 million years ago (Mya), the *silenus* group diverged ca. 4.5 Mya, and the *fascicularis* and *sinica* groups diverged ca. 3.5 Mya.

The most recently diverged groups (i.e., *fascicularis* and *sinica* groups) are distributed in East Asia and partly overlap each other (Fooden, 1988, 2006). The *fascicularis* group consists of four extant species, including *M. fuscata* and *M. mulatta*, which are found in temperate regions: *M. fuscata* is distributed in the Japanese

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archipelago, which is at the northernmost limit of extant nonhuman primates (Fooden and Aimi, 2005), while *M. mulatta* is widely distributed from Afghanistan to southern China (Fooden, 2000). The *sinica* group consists of at least five (Sinha et al., 2005; possibly six or seven; Chakraborty et al., 2007; Li et al., 2015; Fan et al., 2016) extant species, with some of the large-bodied species, such as *Macaca thibetana*, *Macaca assamensis*, and *Macaca leucogenys*, distributed in high-altitude areas in southern China (Fooden, 1982, 1983; Li et al., 2015). Thus, both phylogenetic groups inhabit relatively cold environments in East Asia. However, no species currently inhabit northern China and the Korean Peninsula, isolating *M. fuscata* from the other continental species.

However, some excavations of Pleistocene fossil macaques have been reported from northern China and the Korean Peninsula (Schlosser, 1924; Young, 1934; Zhang et al., 1986; Pan and Jablonski, 1987; Park and Lee, 1998; Takai et al., 2008). The first record of a fossil macaque from East Asia was *Macaca anderssoni*, which was described from a nearly complete face excavated from the Early Pleistocene sediment of Mianchi, Henan Province, China (Schlosser, 1924). A decade later, another species, *Macaca robusta*, was described from a partial maxilla excavated from the Middle Pleistocene sediment of Choukoutien, Beijing, China (Young, 1934). Since then, nearly all of the fossil specimens that have been discovered in northern China or the Korean Peninsula from the Early to Middle Pleistocene have been referred to as one of these two fossil species. Although *M. anderssoni* has a larger dental size than *M. robusta*, it is sometimes considered a junior synonym of the latter (Simons, 1970; Delson, 1980; but see; Fooden, 1990). These fossils are believed to fill the biogeographical gap in the current distribution of macaques, which will potentially elucidate their evolutionary histories. However, the phylogenetic relationships of these fossils and extant species remain controversial (Delson, 1977, 1980; Jablonski and Pan, 1988; Fooden, 1990; Pan and Yanzhang, 1995; Park and Lee, 1998; Ito et al., 2014b), particularly with regard to whether they are phylogenetically related to members of the *sinica* group or the *fascicularis* group.

The fossil records from the Korean Peninsula is key to understanding the evolution of *M. fuscata* in particular. Some fossil macaques have been reported from the Middle and Late Pleistocene sediments of several localities in the Korean Peninsula (Park and Lee, 1998; Fooden and Aimi, 2005; Lee and Woo, 2005; Lee, 2006; Lee and Takai, 2012; Lee et al., 2013). Most of these fossils are isolated teeth or partial fragments of skeletons, but the fossil specimen excavated by the Chungbuk National University Museum Team from the Durubong (=Turupong) Cave Complex, Cheongju City, Chungbuk Province, South Korea, is a well-preserved zygomaxillary region (Fig. 1). This was identified as *M.* cf. *robusta* by Park and Lee (1998), who reported that its morphology was intermediate between *M. robusta* and *M. fuscata* but more similar to *M. robusta* with regard to tooth size. Lee and Takai (2012) stated that the upper molars of the Korean fossils were relatively large, making them comparable with those of *M. anderssoni*, while the lower molars were relatively small compared with other fossil and extant macaques. Furthermore, they also stated that most of the Korean fossils retain some accessory cusps (distoconules, interconulus, 6th cusp, and 7th cusp), which are frequently found in the molars of *M. fuscata* (Lee and Takai, 2012). Fooden and Aimi (2005) demonstrated that measurements of the Korean fossil molars were within the range of variation shown by *M. fuscata*. Together, these findings imply that the Korean fossils may be related to the ancestors of *M. fuscata* or the other fossil species, namely *M. anderssoni* and/or *M. robusta*; and that more than one lineage may have been distributed in the Korean Peninsula during the Middle to Late Pleistocene. However, most previous studies have based their conclusions on tooth morphology alone, and no phylogenetic assessment of the fossil species in comparison with the various extant species of macaques has been conducted to date.

In this study, we compared the morphology of the zygomaxillary region in the Korean fossil specimen that was discovered in the Durubong Cave Complex with that of extant species of macaques. This is the most well-preserved fossil macaque specimen in Korea. Zygomaxillary morphology is likely to be informative for the reconstruction of phylogenetic relationships, because zygomaxillary shape can be partially differentiated among the four phylogenetic groups in macaques (Ito et al., 2014a) and has some phylogenetic signal (Ito et al., 2014a). First, we evaluated the pattern of zygomaxillary variation in extant species and estimated the evolutionary and phylogenetic significance of this variation. We then assigned the fossil to the morphospace of the extant species and performed a cladistic analysis to infer the evolutionary and phylogenetic relationships of this fossil to the extant species. To do this, we used semi-landmark-based geometric morphometrics to capture the surface topography of the zygomaxillary region, because anatomically defined landmarks were limited on the fossil. Moreover, we examined the nasal cavity morphology using computed tomography (CT), because it has been reported that this reflects phylogenetic relationships (Nishimura et al., 2014; Ito et al., 2014b; Ito and Nishimura, 2016). Based on the findings of these analyses, we discuss the biogeographic and evolutionary histories of East Asian macaques.

2. Materials and methods

2.1. Molecular phylogeny

The phylogeny that was used for the phylogenetic comparative analyses was estimated using 11 mitochondrial and 53 nuclear DNA sequences (Supplementary Online Material [SOM] Table S1). The DNA sequences were obtained from the 10KTrees webserver (Arnold et al., 2010; http://10ktrees.fas.harvard.edu/Primates/) and Perelman et al. (2011). The sequences were aligned using MAFFT version 7 (Katoh and Standley, 2013) and then concatenated using SequenceMatrix software (http://gaurav.github.io/taxondna/). BEAST 2 software (Bouckaert et al., 2014) was used for phylogenetic inference using a site model that was estimated by the Bayesian approach with the bModelTest package (Bouckaert, 2015). Markov Chain Monte Carlo simulations were performed for 10,000,000,000 generations with a sampling frequency of 100,000. Five species belonging to the subtribe Papionina (*Cercocebus torquatus*, *Lophocebus aterrimus*, *Mandrillus sphinx*, *Papio hamadryas*, and *Theropithecus gelada*) were used as an outgroup. The maximum clade credibility tree was chosen after removing a 10% burn-in using TreeAnnotator version 2.4.7 (SOM Fig. S1).

2.2. Cranial sample

For the fossil specimen, we used the zygomaxillary specimen of a fossil macaque stored at the Chungbuk Natural History Museum, Chungbuk, South Korea (2ㅜㄷ-12-2169; Fig. 1), which has been partially broken and repaired. The fully erupted third molars and relatively large canines indicate that this individual was an adult male. This specimen originated from the Durubong (=Turupong) Cave Complex, Cheongju City, Chungbuk Province, South Korea. The faunal composition of Durubong Cave Complex (*Ursus arctosi*, *Crocuta ultima*, *Dicerorhinus* cf. *choukoutiensis*, and *M.* cf. *robusta*) suggests that this specimen dates back to the late Middle Pleistocene to the Late Pleistocene period (Park and Lee, 1998), while the faunal complex together with the results of pollen analyses suggests that this was a warm (interglacial or interstadial) period (Lee and Woo, 2005).