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Phylogenetic relationships and genetic diversity of badgers from the Korean Peninsula: Implications for the taxonomic status of the Korean badger

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ABSTRACT

Accurate taxonomic classification of wildlife species is crucial for guiding biological research and for developing effective management and conservation programs. The taxonomic status of Eurasian badgers from South Korea remains poorly resolved. Here we assessed the phylogenetic relationships and genetic variation of Eurasia badgers using partial mitochondrial fragments to elucidate the evolutionary history and taxonomic status of badgers from the Korean Peninsula. Forty-eight unique haplotypes from 125 individuals were observed. Phylogenetic reconstructions and reduced median networks indicate that Eurasian badgers consisted of four geographic clades (Japan, Eastern Eurasia, Western Eurasia, and Caucasus) with a relatively weak split observed within Eastern Eurasia. Estimated divergence time between the Japanese and Eastern Eurasian clades, including the Korean population, was 467,100 years (69,200–1,085,500 years). The results of this study support the hypothesis that the Japanese badger migrated from the Eurasian continent over the Korea-Japan land bridge and that the Korean Peninsula was an important refugia during the Pleistocene. Our study confirmed that the South Korean badger, *Meles meles*, belongs to the Eastern Eurasian clade. Based on these results and those of previous studies, we recommend that the scientific name of the Korean badger be changed from *M. meles* to *Meles leucurus* (Asian badger).

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1. Introduction

Correct taxonomic classification of species is critical and is the foundation of comparative biology, including ecological, biogeographic, and conservation genetic research. Furthermore, a valid assessment of taxonomic divisions prevents the waste of limited resources (Daugherty et al., 1990; Frankham et al., 2002), facilitates species identification, and provides a formal framework through which to compare species across geographical space and time (Kociolek and Stoermer, 2001). For example, within the Korean Peninsula, recent studies on the phylogenetic relationships of the extinct Korean tiger (*Panthera tigris coreensis*) and Asiatic black bear (*Ursus thibetanus*) helped clarify the subspecific status of the extinct Korean tiger (Lee et al., 2012) and have provided the basis for the reintroduction of the Asiatic black bear in South Korea (Kim et al., 2011). However, there remains the urgent need to update the taxonomic status of several South Korean mammals for which there is limited molecular phylogenic and population genetic information.

The badger is one of the South Korean mammals in urgent need of modern taxonomic assessment. Currently, two scientific names, *Meles meles* and *Meles leucurus*, are used without the support of explicit scientific evidence. Although *M. meles* is commonly used within Korea (Won and Smith, 1999; Yoon et al., 2004; Kim et al., 2012), *M. leucurus* is generally used by international authors. Proper taxonomic assessment has been hindered by the relatively small number of specimens that have been studied. For example, only a few specimens from the Korean Peninsula were utilized in a seminal study comparing morphological features such as head color and cheek teeth patterns (Abramov, 2003; Baryshnikov et al., 2003).

Phylogeographic assessment of eastern Eurasian mammals, including the Korean Peninsula, have revealed complex evolutionary patterns of eastern Asia (Kim et al., 2013; Choi et al., 2014) that have demonstrated the varying effects of glaciation in each region (Lee et al., 2008; Sakka et al., 2010; Kim et al., 2014). More studies, including samples from the Korean Peninsula, are necessary to reconstruct the complete evolutionary history in East Asia (Kim et al., 2013), and to improve our understanding on the role of the Korean Peninsula as a landbridge or refuge during the last ice age.

In Korea, the Eurasian badger inhabits most of the mountain regions, except for islands and some urban parks in South Korea (Yoon et al., 2004), and is widely distributed in North Korea (Won, 1968). Won and Smith (1999) summarized historical changes in population size of Eurasian badgers in South Korea. The Eurasian badger, which was once abundant throughout the mountainous areas of Korea (Allen and Andrews, 1913), suffered declining numbers and reduced distributions during the 1950s because of excessive trapping for the fur trade and folk medicine (Won and Smith, 1999). However, currently populations are increasing or stable because of improved habitat quality and reduced illegal poaching (Woo, DG personal communication).

More broadly, Eurasian badgers are widely distributed throughout the Palearctic region from Ireland and Spain in the west to China, Korea, and Japan in the east (Nowak, 1999). The phylogeographic patterns and evolutionary history of badgers in Eurasia have been assessed using molecular phylogenetic approaches (Kurose et al., 2001; Marmi et al., 2006; Tashima et al., 2011), Marmi et al. (2006) demonstrated a strong phylogeographic division (Europe, Southwest Asia, North and East Asia, and Japan) that was produced by cold Pleistocene and geographical barriers. Tashima et al. (2011) extended these results using two mitochondrial DNA (mtDNA) and Y chromosomes, However, badgers from the Korean Peninsula were not included in these broad molecular phylogenetic studies.

In the present study, we investigated the genetic variation and phylogenetic relationships of badgers from the Korean Peninsula using the previously published sequences of the mtDNA control region. This region has been successfully used to assess patterns of badger phylogeography and genetic variation (Marmi et al., 2006; Tashima et al., 2011). Together with previously reported findings, the results of the present study elucidate the taxonomic status of badgers from the Korean Peninsula and improve our understanding of the biogeographic role of the Korean Peninsula during the Pleistocene.

2. Materials and methods

2.1. Sample and data collection

The samples used in this study were obtained from the Conservation Genome Resource Bank for Korean wildlife (CGRB). Fourteen individuals came from the Korean Peninsula and Far East Russia, eight from South Korea, two from North Korea (Yeontan-gun and Shinwon-gun, Hwanghae Do), and six from Primorsky Krai, Russia (Fig. 1; Table 1). The specimens were collected from 2004 to 2013. To evaluate phylogenetic relationships and genetic variation, sequences of mtDNA control region of 39 previously published haplotype sequences of Eurasian badgers were retrieved from GenBank (Tashima et al., 2011), including five sequences from eastern Eurasia, four from western Eurasia, two from Armenia, and twenty-seven from Japan (Table 2).

2.2. DNA extraction, PCR amplification, and sequencing

DNA was extracted from muscle, hair, and blood using the Blood and Tissue kit (Qiagen, USA). We amplified the complete control region using the primer pair L15775 and H651 (Uchiyama, 1998). Amplification reactions were carried out in a total volume of 30 μL using a PCR machine (TaKaRa, Japan). Each 30 μL amplification reaction contained 20–60 ng of template DNA, 50 mM KCI, 10 mM Tris-HCI, 1.5 mM $MgCl_2$, 10 p mol of each primer, and 0.2 units of *Taq* polymerase (iNtRON Biotechnology, Korea). The cycling profile consisted of an initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 44 °C