

Intraspecific phylogeny of the house shrews, *Suncus murinus*-*S. montanus* species complex, based on the mitochondrial cytochrome *b* gene

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Abstract. A phylogenetic tree was reconstructed based on the mitochondrial cytochrome *b* gene nucleotide sequences of 169 individuals of house shrews (*Suncus murinus* and *S. montanus*) from 44 localities in East Asia, Southeast Asia, West Asia, and islands in the western Indian Ocean. Shrews from China (Zhejiang), Japan (Okinawa), Vietnam, and Indonesia (Java) formed a monophyletic group with less genetic variation. Therefore, the shrews of these regions appeared to have originated from one or a few localities. Contrary to this, shrews from Sri Lanka, Myanmar, and Pakistan consisted of several haplogroups. This finding suggests immigration movements to these areas. Fascinating findings were also obtained concerning the islands in the western Indian Ocean. First, shrews on Zanzibar Island (Tanzania) had almost the same haplotype as those in southwestern Iran. Therefore, the house shrew in Zanzibar may have immigrated from Iran (or vice versa). Second, shrews from Madagascar and Grande Comore Island shared the same haplotype, whereas the shrews on Réunion Island were clearly different from those of Madagascar and Comoros. Thus, there appears to have been several immigration routes to the islands of the western Indian Ocean.

Key words: human introduction, immigrations, Indian Ocean, *Suncus montanus*, *Suncus murinus*.

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The house shrew or musk shrew, *Suncus murinus* (Linnaeus, 1766) is widely distributed across southern Japan, southern China, Southeast Asia, the Indian sub-continent including Sri Lanka, the coastal region of the Arabic Peninsula and Iran, Madagascar, the coastal region of eastern Africa, and across many small islands in the Indian Ocean (Hutterer 2005; Motokawa 2015). *Suncus murinus* has been found on the island of Guam in the Pacific Ocean (Peterson 1956; Wiewel et al. 2009), but it now seems to be nearly or completely extinct there (Richard Yanagihara, personal communication). In some regions, it is estimated that house shrews were unintentionally introduced by humans, transported by trade ships (Hutterer and Trainier 1990; Yamagata et al. 1995; Kurachi et al. 2007a, 2007b). It is reported that *S. montanus* (Kelaart, 1850), which is regarded as a distinct species from *S. murinus*, is distributed in Sri Lanka (Phillips 1980; Meegaskumbura et al. 2010). However, because the phylogenetic relationships between *S. montanus* and *S. murinus* have not been fully investigated, we have treated them as a *Suncus murinus*-*S. montanus* species complex and treated them conventionally as one taxon in the present paper.

Intraspecific phylogeny of *S. murinus* has been partially reported based on the mitochondrial DNA or protein polymorphisms (Yamagata et al. 2004; Yamagata and

Kurachi 2005; Kurachi et al. 2007a, 2007b) and on karyotype (Yosida 1982). However, these previous studies mainly focused on shrews from the eastern part of the range. In the present paper, we briefly report the phylogenetic relationship of the *S. murinus*-*S. montanus* complex, including shrews from the western parts of the range, based on mitochondrial cytochrome *b* gene (*cytb*). The main purpose is to gain a basic information of the phylogenetic relationship based on the *cytb* among house shrews to infer the routes of immigration of the shrews.

Materials and methods

Sampling

Nucleotide sequences of the mitochondrial cytochrome *b* gene for 149 individuals of the *S. murinus*-*S. montanus* complex from 33 localities ranging from East Asia, via Southeast Asia, to islands in the western Indian Ocean were determined by ourselves. In addition to these, we cited the sequence data for 20 individuals of the *S. murinus*-*S. montanus* complex from 11 localities (including *S. murinus murinus*, *S. murinus kandianus*, *S. murinus caerulescens*, and *S. montanus* in Sri Lanka) from DNA databases to reconstruct a phylogenetic tree. In total, 169 individuals from 44 localities were analyzed (Appendix 1, Fig. 1). As outgroups, *Suncus etruscus* (Savi, 1822),

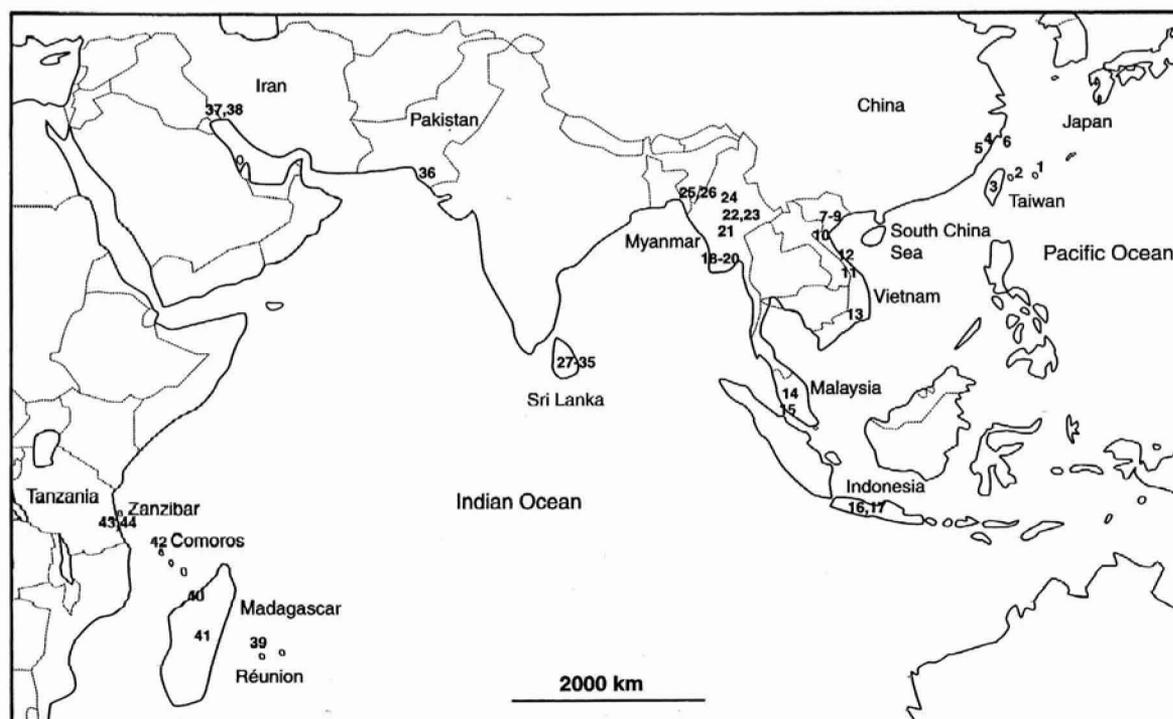


Fig. 1. Sample localities of the *Suncus murinus*-*S. montanus* complex. The numbers correspond with those of Appendix 1.

S. madagascariensis (Coquerel, 1848) (probably, a synonym of *S. etruscus*; Meeegaskumbura et al., 2012a), *S. stoliczkanus* (Anderson, 1877), *S. fellowesgordoni* Phillips, 1932, *Crocidura rapax* G. Allen, 1923, and *C. dsinezumi* (Temminck, 1842) were used (Appendix 1).

DNA sequencing and phylogenetic analysis

The nucleotide sequence of the mitochondrial cytochrome *b* gene (1,140 bp) was determined following the method and procedure (primer set, chemicals, PCR conditions, etc.) of the previous investigations (Ohdachi et al. 2004; Dubey et al. 2007; Arai et al. 2012).

To infer the phylogenetic tree, a Bayesian phylogenetic tree was calculated, using BEAST version 2.3.0 (Bouckaert et al. 2014) with an exponential population coalescent as the tree prior. The dataset was analyzed in TN93+G+I model of nucleotide substitution, which was selected with the lowest Bayesian information criterion scores using a best fit model selection as implemented in MEGA version 5.05 (Tamura et al. 2011). Although the main purpose of the present paper is to gain a topology of the phylogenetic relationship, we show the phylogenetic tree of a dating analysis under a strict clock model with a conventional clock rate of a 3.1%/site/million years (Suzuki et al. 2015) to roughly estimate divergence time among lineages.

Results and discussion

All of 149 samples of the *S. murinus*-*S. montanus* complex and one specimen of *S. etruscus* were successfully sequenced and no indels were found. The monophyly of the *S. murinus*-*S. montanus* complex was strongly supported based on the *cytb* sequences, and *S. stoliczkanus* was a sister species to them (Fig. 2). In the phylogenetic tree (Fig. 2), there are 14 main clades in the *S. murinus*-*S. montanus* complex.

Shrews from Japan (Okinawa Prefecture, Loc. # 1–2), China (Zhejiang Province, Loc. # 4–6), Vietnam (Loc. # 7–13), and Indonesia (Java Island, Loc. # 16–17) formed a monophyletic group with less genetic variation (Fig. 2). Okinawa (the Ryukyu Arc) and Java are islands which finally separated from the Asian continent approximately 200,000 years ago (Kimura 2002) and approximately 11,000 years ago (Biswas 1973; Voris 2000; Bintanja et al. 2005), respectively. If the shrews in Okinawa and Java were originally distributed there, they would have more genetic differentiation from Vietnam and southern China. Therefore, it seems most natural

that the shrews of these islands immigrated from the Asian continent (Vietnam, southern China, or the nearby area) after the geologic separation. However, the result of the present investigation cannot determine the direction of immigration, and the immigration from Okinawa (the Ryukyu Arc) and Java to the continent might be possible. To determine the direction of immigration, remain (or semi-fossil) records of the house shrew are necessary. However to date, so far, only a single report has been published discussing the remain record of house shrews in the Ryukyu Arc (Nishioka et al. 2016). After an intensive survey of fossils in these areas (Japan, China, and South-east Asia) has been done, we will conclude the direction of the immigration in this region.

Contrary to this, shrews from Sri Lanka and Myanmar consisted of at least four different haplogroups (including *S. montanus*) each (Fig. 2). Especially in Sri Lanka, the genetic diversity was very high. For instance, KDSL2/KDLS3 and KDSL1/KDSL4/KDSK5 were captured in the same location in Kandy, Sri Lanka (Loc. # 29), but their haplotypes of *cytb* differed significantly (Fig. 2). *Suncus montanus*, *S. murinus kandianus*, and some house shrews from Sri Lanka were grouped into one clade (*S. montanus*-*S. mu. kandianus* complex = [Sri Lanka 3 + Sri Lanka 4]). According to the divergence time analysis under a strict clock model with 3.1%/site/million years, estimation of the divergence time between the *S. montanus*-*S. mu. kandianus* complex and the other haplogroups of house shrews (including Sri Lanka 1 & Taiwan and Sri Lanka 2) was approximately 1.36 million years ago (MYA) (Fig. 2). This divergence degree could be regarded as a distinct species level. The divergence time between Sri Lanka 1 & Taiwan and Sri Lanka 2 was 0.42 MYA and that between Sri Lanka 3 and Sri Lanka 4 was 0.10 MYA. For Myanmar shrews, the divergence time was estimated to be 0.28 MYA between Myanmar 2 and Myanmar 3 and 0.88 MYA between Myanmar 4 and Myanmar 2 + Myanmar 3 (Fig. 2). Moreover, the divergence time between Myanmar 1 & Malaysia and Myanmar Main Clade (Myanmar 2–4) was 0.93 MYA. Thus, Myanmar 1 (& Malaysia) and Sri Lanka 1 (& Taiwan) were phylogenetically quite distant from other shrews in Myanmar and Sri Lanka, respectively. This finding suggests the populations in Sri Lanka and Myanmar consist of several species/or lineages of house shrews.

In addition, it is also found that hybridizations among distinct lineages occurred in Myanmar and Sri Lanka, based on karyological investigation (Yosida 1982),

suggesting immigration from other regions occurred in Sri Lanka and Myanmar. Furthermore, our phylogenetic analysis based on mitochondrial DNA demonstrated that *S. montanus* and *S. mu. kandianus* were included in a monophyletic group (Fig. 2), although they have different morphotypes (Meegaskumbura et al. 2010) and habitats (Phillips 1980; Yapa and Ratnavira 2013) from each other. The discrepancy between phylogeny and morphology/ecology in the *S. montanus*-*S. mu. kandianus* complex could be caused by hybridization of several species or lineages. Further investigation of the hybridization of the house shrews in Sri Lanka might resolve this problem.

Haplotypes of the shrews from Karachi, Pakistan (Loc. # 36) were divided into two clades which are significantly differentiated from each other (Fig. 2). In the present study, we cannot conclude whether or not the shrews in Pakistan immigrated from another region.

For the islands in the western Indian Ocean, some interesting results were found. First, shrews on Zanzibar Island, Tanzania (Loc. # 43–44) had almost the same haplotype as those in southwestern Iran (Loc # 37–38) (Fig. 2), despite the two regions being far from each other (Fig. 1). Therefore, the house shrews in Zanzibar probably immigrated from Iran (or vice versa). Second, shrews from Madagascar and Grande Comore (Ngazidja) Island had almost the same haplotype (Fig. 2). However, the phylogenetic position of the shrews on Réunion Island (an overseas department of France, Loc. # 39) was clearly different from those of Madagascar and Comoros (Fig. 2) although these regions are close to each other (Fig. 1). Since the shrews of Réunion are phylogenetically close to those from Sri Lanka, East Asia, and Southeast Asia, they might have immigrated from these regions.

Further collection of shrew samples, especially from the Arabic Peninsula and India, are greatly needed to complete our understanding of the emigration-immigration process and hybridization among the *Suncus murinus*-*S. montanus* complex, and further genetic information, such as nuclear genes, microsatellite DNA, and karyotype, and morphological relationship should be investigated in the future.

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