Re-evaluation of the Phylogeny based on mitochondrial cytochrome b gene in the house shrew, *Suncus murinus*-*S. montanus* species complex, with special reference to Yemen and Myanmar populations


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Abstract

The house shrew (*Suncus murinus*-*S. montanus* species complex) is considered to have been unintentionally introduced by humans from their original range to other regions around the Indian Ocean and neighboring seas, but this has yet not fully been investigated. A phylogenetic tree and haplotype network were reconstructed based on the mitochondrial cytochrome b gene nucleotide sequences (1140 bp) of 179 individuals of house shrews from 46 localities in southern East Asia, Southeast Asia, West Asia, and islands in the western Indian Ocean. There was small genetic variation among shrews in Japan (Okinawa), southern China, Vietnam, and insular Southeast Asia. However, the shrew populations in Myanmar and Sri Lanka showed of a variety of different haplotypes. In the region of the western Indian Ocean, three interesting findings were obtained. First, the shrews on Zanzibar Island (Tanzania) shared same haplotype as those in southwestern Iran, and the haplotype was close to a group in Pakistan, despite these three regions being distantly located. Second, inferring from the haplotype network, it was suggests that the shrews in Yemen might have derived from Madagascar/Comoros populations. Third, the shrews on Réunion Island were genetically different from other populations around the western Indian Ocean but closer to Malaysia and Myanmar populations. Thus, the present study demonstrates that there have been dynamic immigration/emigration processes in the house shrews, especially for those around the western Indian Ocean. In addition, the house shrews in Myanmar may include several different species.

Keywords: Musk shrew, Indian Ocean, Human introduction, Immigrations, Phylogeny

Introduction

The house shrew or musk shrew, *S. murinus* (Linnaeus, 1766) is widely distributed across
southern East Asia, Southeast Asia, South Asia, West Asia, the coastal region of eastern Africa, and many islands in the Indian Ocean (Hutterer 2005, Motokawa 2015). House shrews were often unintentionally introduced by humans to non-native regions (Hutterer and Trainier 1990, Yamagata et al. 1995, Kurachi et al. 2007, Ohdachi et al. 2016). In addition, Ohdachi et al. (2016) revealed based on mitochondrial cytochrome b gene (cyt b) that it is difficult to separate S. murinus from S. montanus (Kelaart 1850), although S. montanus is regarded as an independent species in Sri Lanka (Phillips 1980, Meegaskumbura et al. 2010). Thus, “house shrew” refers to the “Suncus murinus-S. montanus species complex” in the present paper.

Intraspecific phylogeny of the S. murinus-S. montanus complex has been reported based on the mitochondrial cyt b sequences by Ohdachi et al. (2016), and unexpected distribution of haplogroups were found between distant regions. Since the previous report (Ohdachi et al. 2016), further samples of house shrews were obtained from Yemen and Myanmar. Hence, these individuals were added to the phylogenetic analysis, and the median-joining network was also drawn to extract more information from the sequence data. The main purpose of the present study is to reveal new insights into the phylogenetic relationships in the S. murinus-S. montanus complex and to infer the routes of human introduction.

Material and methods

Study area

Nucleotide sequences of the mitochondrial cytochrome b gene (1140 bp) for 10 individuals of the S. murinus from Yemen and Myanmar were obtained, following previous investigations (Ohdachi et al. 2004, Dubey et al. 2007, Arai et al. 2012). In addition to them, we cited the sequence data for 169 individuals of the S. murinus-S. montanus complex (including S. murinus murinus, S. murinus kandianus, S. murinus caerulescens, and S. montanus) from 44 localities (Ohdachi et al. 2006, Meegaskumbura et al. 2007, 2010, 2012, Lin et al. 2014, Ohdachi et al. 2016) to reconstruct a phylogenetic tree and a median-joining network. In total, 179 individuals from 46 localities were analyzed (Table S.1, Fig. 1). Two individuals of Suncus etruscus (Savi 1822) were used as outgroups.

To infer the phylogenetic relationships among house shrews, the maximum likelihood (ML) tree was reconstructed using RAxML ver. 8.2.10 (Stamatakis 2014). The confidence of tree topology was evaluated by the rapid bootstrap method with 1,000 replications. A Bayesian (BI) phylogenetic tree was also reconstructed by MrBayes ver. 3.2 (Ronquist et al. 2012). The settings of the Metropolis Coupled Markov Chain Monte Carlo (MCMCMC) are as follows: 10,000 trees were samples for every 1,000 generations under two sets of the four simultaneous chains. The first 2,500 trees were discarded as the burn-in. Convergences of each parameter were confirmed by checking that all effective sample sizes (ESSs) were larger than 200 with Tracer 1.6 (http://tree.bio.ed.ac.uk/software/tracer/).

The dataset was analyzed for both ML and BI methods using GTR+Γ 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).
Taking account of the different tempos and modes of the nucleotide substitutions, the codon positions were distinguished by the partition model. The phylogenetic relationships among the three main groups in the ML tree obtained (see Fig. 2 in results) were further examined in detail by using the CODEML program implemented in the PAML ver. 4.9 (Yang 2007). For this analysis, the codon substitution (CS) + Γ model (Yang et al. 1998) was applied due to the better fitness of the CS + Γ model to our own sequence data than the standard nucleotide substitution model (e.g., GTR + Γ model): AICCS+Γ = 8006.9 and AICGTR+Γ = 8107.0. Because CS model is computationally too expensive, it is not practical to apply this model for a heuristic search. Therefore, the topologies within each of the three main groups were ready fixed in accordance with the result of the RAxML, and only three topologies among these three groups were examined. A median-joining network was reconstructed using the program Network ver. 5.0.0.1 (Bandelt et al. 1999) to infer evolutionary relationships of haplotypes and/or haplogroups.

Results
Since the topologies of ML and BI trees were fundamentally the same, the BI tree was shown in Supplemental materials (Fig. S.2). The posterior probabilities of BI method were indicated near the corresponding nodes of the ML method (Fig. S.1). In the ML tree, three main groups (clades) were identified with high confidence values. The first was the “Suncus murinus” group, which included the majority of the house shrews. This group included two subspecies, S. mu. murinus and S. mu. caerulescens in Sri Lanka, whose subspecific ranks has been given in the previous studies (Meegaskumbara et al. 2007, Meegaskumbara et al. 2010). The second clade was the Suncus montanus-S. murinus kandianus species complex, which included S. montanus, S. mu. kandianus and the other house shrews (identified as S. murinus) in Kandy region. The third clade was the Nay Pyi Taw group, which consisted of two individuals from Nay Pyi Taw region, Myanmar (Loc. # 22, Fig.1). The “Suncus murinus” group was divided in several subgroups (Fig. S.1).

Among them, the Yemen and Madagascar and Comoros groups made a monophyletic group with high confidence (Fig. S.1). Shrews from Sri Lanka, Myanmar, and Pakistan appeared at several distant positions in the ML tree. On the contrary, shrews from Iran and Zanzibar (Tanzania) shared a single haplotype (Fig. S.1) despite distant sampling locations (Fig. 1). Shrews from Japan, China, Vietnam, and Indonesia (Java Island) formed a monophyletic subgroup. Those from central Myanmar and peninsular Malaysia formed another group, although it was statistically non-significant (Fig. S.1). Shrews from Réunion Island (an overseas department of France) in the western Indian Ocean (Loc. # 41, Fig.1) were included in a monophyletic subgroup with those from East Asia, Southeast Asia, and Sri Lanka (Fig. S.1).

Among the three main groups of the ML tree (Fig. S.1), a detailed comparison was conducted between the three possible topologies of these groups (Table 1). The topology of [("S. murinus" group, S. montanus-S. mu. kandianus complex), Nay Pyi Taw group)] was selected as the most likely one, although the other two topologies could not be statistically rejected. The branching pattern of the median-joining network (Fig. 2) was similar to the ML tree (Fig. S.1). The Suncus montanus-S. mu. kandianus complex and the Nay Pyi Taw group in the ML tree (Fig. S.1) were also clearly clustered in the network (Fig. 2). The network demonstrated that Yemen shrews were branched from Madagascar and Comoros shrews (Fig. 2). Shrews in Japan, China, and Indonesia were branched from the main
haplotype that includes almost all individuals in Vietnam and some of the former regions with only a few mutations (1-3 sites) (Fig. 2). Shrews in peninsular Malaysia were branched from **Figure 2.** Median-joining network of the house shrews (*Suncus murinus*-*S. montanus* species complex), based on the mitochondrial cytochrome *b* gene sequences. Pie area approximately corresponds with the number of individual OTUs. Arabic numerals near branches denote the number of mutated sites. No number listed indicates a single mutation. Coloring is based on locality and is similar to those in Table S.1 and Fig S.1 A small open circle denotes a hypothetical taxonomic unit (HTU).

**Table 1.** Comparison of the tree topologies among three groups using the CS+Γ model. lnL, log-likelihood; Dli, difference of log-likelihood from the maximum likelihood tree (Tree 1), ±SE, standard deviation of the difference of log-likelihood from Tree 1; pKH, p-values of Kishino-Hasegawa test; pSH, p-values of Shimodaira-Hasegawa test; pRELL, bootstrap probabilities.

<table>
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Tree1 = ("*S. murinus*" group, *S. montanus-S. mu. kandianus* complex), Nay Pyi Taw group)

Tree2 = (Nay Pyi Taw group, *S. montanus-S. mu. kandianus* complex), "*S. murinus*" group)

Tree3 = (Nay Pyi Taw group, "*S. murinus*" group), *S. montanus-S. mu. kandianus* complex)

See Fig. S.1 for the names of haplogroups.
those in central Myanmar. In addition, the shrews of Réunion were closely located to those in central Myanmar and Malaysia (Fig. 2).

**Discussion**

Ohdachi *et al.* (2016) suggested that house shrews in Japan (Okinawa), southern China and Indonesia (Java) were likely immigrated or emigrated by humans, but could not determine the direction of immigration (either from Vietnam to those regions or vice versa). In the present study, the median-joining network (Fig. 2) suggested that shrews of the former regions were probably immigrated from Vietnam since some individuals of those regions were located in peripheral positions around the main haplotype that includes most individuals from Vietnam and some other regions (Fig. 2), although we should carefully interpret the result of median-joining network for evolutionary event (Kong *et al.* 2016). From around the 10th-17th century, the sea trade between southern East Asia and Southeast Asia flourished and many Chinese and Japanese merchants formed settlements in Southeast Asia (e.g., Sakamaki 1964, Ogura 1989, Ikehata 1999, Ishii and Sakurai 1999, Schottenhammer 2007). Thus, it is thought that the house shrews moved with these trade ships.

Shrews of southwestern Iran and Zanzibar Island (Tanzania) had the same haplotype (Fig. S.1), indicating they moved between these two distant regions. Myths of Swahili people of maritime East Africa, including Zanzibar, tell that their ancestors immigrated from Persia (De Vere Allen 1993, Iejima 1993, Tominaga 2001). Therefore, house shrews may have been transported by Persians to Zanzibar. To verify this hypothesis, shrew samples from the eastern part of the Arabian Peninsula should be surveyed. The house shrews in Madagascar and Grande Comoro Island had very similar haplotype (Fig. S.1). Ancestors of the main ethnic groups in Madagascar are Austronesians originating from insular Southeast Asia that gradually mixed with Bantu peoples from East Africa (Iida *et al.* 2013, Pierron *et al.* 2017), while some Austronesians came to Madagascar via Comoros after the 6th century (Chagnoux and Haribou 1990). Therefore, it is plausible that the house shrews of Madagascar and Comoros might have immigrated with Austronesians or proto-Malagasies. However, the present study could not infer when and from where house shrews came to Madagascar and Comoros because the sampling are insufficient (Fig. S.1). In particular, samples from India were lacking. Thus, these are still open questions.

Shrews in the Yemen and Madagascar/Comoros subgroups are genetically close to each other (Fig. S.1). Furthermore, the median-joining network (Fig. 2) suggests that the house shrews in Yemen might have been derived from the population in Madagascar or Comoros, although the interpretation of median-joining network should be discreet (Kong *et al.* 2016). A similar genetic relationship between Yemen and Madagascar has been reported for the house mouse *Mus musculus* (Duplantier *et al.* 2002, Sakuma *et al.* 2016). However, in contrast to the house shrew, the direction of anthropological transportation of the house mouse is considered to have been from Yemen to Madagascar. In addition, Hadhramis, an ethnic group of Arabic people in southern Yemen, have undertaken commercial activity between Yemen and many regions around the Indian Ocean from East Africa including Madagascar to Southeast Asia (De Vere Allen 1993, Horton and Middleton 2001, Sherrif 2010, Alpers 2013). Other groups of Arab merchants have also been active in northern Madagascar (Vérin 1986). Thus, Hadhramis or other Arabs are likely to have transported the house shrew and the house mouse between Yemen and Madagascar/Comoros. Furthermore, the house shrew is called “voalavo arabo” in Malagasy language in Mahajanga, northwestern
Madagascar, which means “Arabian mouse” (Hideo Fukazawa, personal communication). This also suggests the transportation of house shrews by Hadhramis or Arab merchants between Yemen and Madagascar. For the islands in the western Indian Ocean, the phylogenetic position of the shrews on Réunion Island (Fig. S.1) was obviously different from Madagascar, Comoros, Iran, Zanzibar subgroups, and the shrews of Réunion are phylogenetically close to those from central Myanmar and peninsular Malaysia (Fig. S.1 and 2). Many Chinese workers and merchants immigrated to Réunion from Malaya after the 19th century (Yu-Sion 2003, Alpers 2013, Kang 2015). Thus, house shrews might have immigrated to Réunion with Chinese immigrants from Malaya. Finally, we mentioned the taxonomic status of the *S. murinus*-*S. montanus* complex. As clearly indicated in the phylogenetic analysis (Fig. S.1, Table 1), Nay Pyi Taw group has a unique phylogenetic position. In addition, *S. murinus kandianus* and some shrews from Kandy, Sri Lanka are included in a single clade (*S. montanus*-*S. mu. kandianus* species complex). If *S. montanus* is a valid species, *S. mu. kandianus* and some other shrews in Kandy should be included in it, and the Nay Pyi Taw group should be considered a new independent species. Otherwise, all of the *S. murinus*-*S. montanus* species complex should be referred to as a single species, *Suncus murinus*. Thus, house shrews seemed to have been moved throughout maritime regions of the Indian Ocean and its neighboring seas by a variety of people and routes. Furthermore, an intensive re-examination of the taxonomic status of the *S. murinus*-*S. montanus* complex needs to be done. Additional sampling of the shrews, especially from India and eastern Arabian Peninsula, and analysis based on other sources of genetic information (especially nuclear genes) are necessary to develop the discussion of the present study.

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**References**


