Short Communication

Phylogenetic position of *Suncus fellowesgordoni* with pigmy shrews from Madagascar and Southeast Asia inferred from cytochrome-*b*

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ABSTRACT

This study evaluates the taxonomic and phylogenetic position of *S. fellowesgordoni* with *S. malayanus*, *S. etruscus* and *S. 'madagascariensis*'. We reconfirm that *S. fellowesgordoni* is a distinct species, which constitutes the sister species to *S. malayanus* from Southeast Asia. The pairwise uncorrected genetic distance of 9.1–9.7% between the two species suggests a prolonged independent evolution of each and they may constitute relict species of formally widely distributed group of shrews from South Asia to Southeast Asia. *Suncus etruscus* from Sri Lanka and India fall as the sister taxon to *S. 'madagascariensis'*, suggesting a recent introduction of *S. etruscus* from South Asia to Madagascar and their low genetic distance of 1.2–1.4%, confirm that the two populations constitute a single species. *Suncus etruscus* being a widely distributed species shows a substantial genetic divergence among populations. Individuals from Italy show a 7.3–7.5% genetic distance to those from South Asia and Madagascar, which can be explained by geographic distance. However, additional data from many other populations of *S. etruscus* is needed to evaluate the status of this species in extreme locations of its geographic range.

Key words: Sri Lanka, Suncus etruscus, Suncus malayanus, Suncus madagascariensis, taxonomy

INTRODUCTION

Several species of pigmy shrews belonging to the genus Suncus were recognized by Hutterer (2005). However, their species status have been re-evaluated by different authors, some agreeing on their full species status, some placing them as subspecies of S. etruscus or under the name S. etruscus. Two recent publications evaluated the species status and the phylogenetic position of four of these taxa, S. etruscus and S. fellowesgordoni (Meegaskumbura et al., 2012) and S. etruscus, S. madagascariensis and S. malayanus (Omar et al., 2012). Using both morphological and molecular data Meegaskumbura et al. (2012) confirmed S. fellowesgordoni to be a distinct species from S. etruscus. Although with limited taxon sampling, they also showed that these two are sister species.

Suncus etruscus, a denizen of dry lowland habitats, was described from Italy in 1920, and is known to be widespread throughout the Old World (Hutterer, 2005). Omar *et al.* (2012) revealed that the Sri Lankan population of *S. etruscus* is genetically very close to *S.*

'madagascariensis', a presumed endemic from Madagascar, and hence they synonymised this species under *S. etruscus*. The close genetic distance of 1.4-1.5% between the two populations is suggested to be the result of a recent human transfer of *S. etruscus* to Madagascar from South Asia. In contrast, these authors showed that *S. malayanus* is a distinct species with a genetic distance of 6.6 - 8.5% to its sister species, *S. etruscus* (and *S. 'madagascariensis'*).

The taxonomic and phylogenetic position of *S. fellowesgordoni*, a species restricted to montane forests of Sri Lanka, in relation to *S. malayanus* and *S. 'madagascariensis'* remains untested. Here, we present an updated phylogeny including all these species together with *S. etruscus* and evaluate their taxonomy.

MATERIALS AND METHODS

All sequences used in this study, with the exception of two *Myosorex* species, *M. geata* and *M. kihaulei*, are from Meegaskumbura *et al.* (2012) and Omar *et al.* (2012), though some sequences were originally published elsewhere.

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Species	Country	GenBank Accession Number	
Crocidura attenuata	Vietnam	EU122211	
Crocidura fuliginosa	Vietnam	EU122212	
Crocidura horsfeildii	Sri Lanka	EU122213	
Crocidura miya	Sri Lanka	EU122216	
Crocidura hikmiya	Sri Lanka	EU122217	
Suncus montanus	Sri Lanka	GQ290368	
Suncus murinus	Sri Lanka	EU122224	
Suncus murinus	Philippines	GQ290365	
Suncus 'montanus'	India	GQ290374	
Suncus stoliczkanus	Nepal	AB175076	
Suncus dayi	India	DQ630389	
Suncus fellowesgordoni	Sri Lanka	JF914980	
		JF914981	
Suncus malayanus	Malaysia	JF817391	
	-	JF817392	
		JF817393	
Suncus etruscus	Sri Lanka	FJ716836	
Suncus etruscus	South India	JN556043	
Suncus 'madagascariensis'	Madagascar	JF817394	
		JF817395	
Suncus etruscus	France	DQ630396	
		JF817396	
Suncus etruscus	Italy	DQ630397	
Suncus remyi	Gabon	DQ630399	
Suncus varilla	South Africa	DQ630434	
Myosorex geata	Tanzania	JQ433888	
Myosorex kihaulei	Tanzania	JQ433889	
Myosorex sclateri	South Africa	FJ814043	
Myosorex cafer	South Africa	FJ814024	

Table 1. Country and GenBank accession numbers of gene sequences of taxa included in the study.

Phylogenetic analysis

Complete or partial sequence of coding gene cytochrome-b was used in the phylogenetic analysis. Bayesian inference as implemented in the program MrBayes v3.0b4 (Huelsenbeck and Ronquist, 2001) was used to estimate evolutionary relationships. We used the GTR+I+G model of sequence evolution, which was determined as the best-fit model of the 56 models examined by the hierarchical likelihood ratio test as implemented in Modeltest 3.06 (Posada and Crandall, 1998). To search parameter space and determine the posterior probabilities (PP) of phylogenetic trees, we ran four Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) chains for 1,000,000 generations. The posterior probabilities were calculated after excluding the first 250,000 generations as burn-in. The data were also analyzed under a maximum parsimony (MP) criterion in PAUP*v.4.0b10 (Swofford, 2000) with all characters unordered and weighted

equally. Bootstrap (BS) values were also determined. The percent pair-wise uncorrected distances between the species were calculated using PAUP* v.4.0b10. Nine hundred and ninety eight base pairs were used in calculating the genetic distances after excluding 129 base pairs from the 5' and 13 base pairs from the 3' end, as they were missing for some taxa.

RESULTS

All pigmy shrews fall in a single clade with 100% PP and BS support (Fig. 1). Suncus fellowesgordoni forms the sister taxon to S. malayanus from Malaysia, with 100% PP and 65% BS values. These two taxa are distinct species and are separated by a genetic distance of 9.1–9.7%. Suncus etruscus population from India falls with that of Sri Lanka, together forming the sister taxon to S.

'*madagascariensis*' from Madagascar. This clade has 100% PP and 98% BS support. The genetic distance between *S.* '*madagascariensis*' and *S. etruscus* populations from Sri Lanka and India is small (1.2–1.4%). *Suncus etruscus* from Italy is separate and genetically distinct from all

of the other taxa in the clade, with a genetic distance of 7.3–7.5% from South Asian and Malagasy populations (Table 2). The genetic distance between the two European populations is 4.9%.

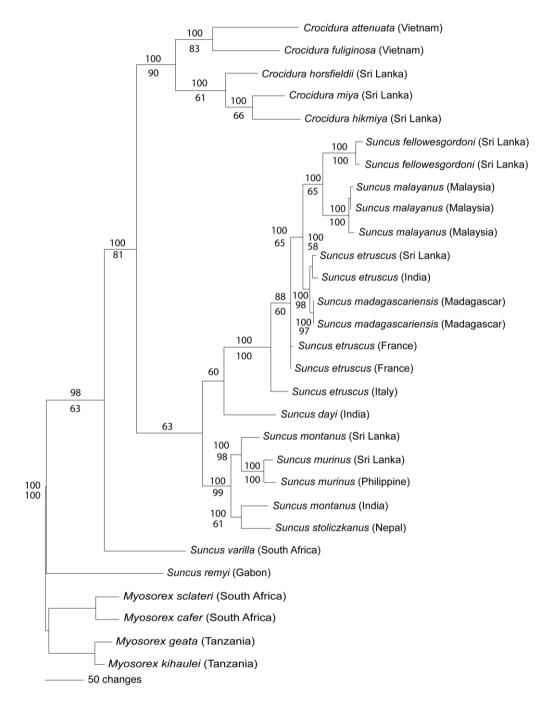


Figure 1. Phylogram inferred from Bayesian analysis of cytochrome-*b* gene under model GTR+I+G. Over 50% posterior probability and bootstrap values (expressed as percentages) are given above and below branches, respectively.

	S. malayanus (Malaysia)	S. 'madagascariensis' (Madagascar)	S. etruscus (Sri Lanka+India)	S. etruscus (France)	S. etruscus (Italy)
S. fellowesgordoni	9.1–9.7	8.2	8.3	8.7	10.8–11.1
S. malayanus	_	7.2–7.8	7.0-8.0	6.2–7.3	8.7–9.4
S. 'madagascariensis'	_	-	1.2-1.4	2.9	7.3
S. etruscus (SL+I)	_	_	_	3.3–3.5	7.3–7.5
S. etruscus (France)	_	_	_	_	4.9

Table 2. Percent pair-wise uncorrected genetic distances between Suncus species included in the study.

DISCUSSION

Suncus fellowesgordoni and S. malayanus constitute two distinct species. They are genetically divergent from S. etruscus (including S. 'madagascariensis') and each other. Both are regarded as distinct species by several taxonomists (Corbet and Hill, 1992; Hutterer, The genetic divergence 2005). of S. fellowesgordoni and S. malayanus is considerable (9.1-9.7%), and these two taxa from etruscus/ madagascariensis' (8.2 - 11.1% and 7.2 - 9.4%, respectively). Though defining species solely on the basis of mtDNA sequencedifferences is problematic, we consider the genetic divergence among S. fellowesgordoni, S. malayanus and S. etruscus to indicate independent evolutionary histories of mtDNA lineages for a prolonged period. Suncus fellowesgordoni is a species restricted to montane forests of Sri Lanka, while S. malayanus is found in lowland rainforests of One possible Thailand and Malaysia. explanation for this disjunct distribution is that these species are relict species of a formally continuously distributed taxon. Much of South and SE Asia was once covered with a continuous belt of wet forest (Meher-Homji, 1983), which has subsided with glaciations, leaving patches of relict forests that contain endemic organisms (Eudey, 1980). Some cases of disjunct distribution of species are explained by convergence, where closely related species are from the adjoining dry zones in the same region (Bossuyt and Milinkovitch, 2000). However, as we have included lowland dry zone dwelling S. etruscus from Sri Lanka and India in the analysis, this scenario may not be true for this case. But, inclusion of a lowland S. etruscus from SE Asia will further clarify this.

Since the genetic distance is low between *S. etruscus* from South Asia and *S. 'madagascariensis'* from Madagascar, we are in agreement with Omar *et al.* (2012) that they constitute a single species. Given that *S.* "madagascariensis" is genetically more closely related to South Asian population of *S. etruscus*, this corroborates the conclusion of Omar *et al.* (2012) to place *S. "madagascariensis*" as a junior synonym of *S. etruscus* from this region. However, *S. etruscus* population from Italy is more divergent from populations of *S. etruscus* from other regions (Genetic distance of 7.3– 7.5% with South Asian and Malagasy) and may even constitute a distinct species. However, since *S. etruscus* is a widespread species, it will be worthwhile to study the divergence among the populations from different regions and analyze along with their morphology prior to making further taxonomic recommendations.

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