

New data on distribution and taxonomy of the genus *Suncus* (Mammalia: Soricidae) in Iran; molecular evidence

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We studied 1140 base pairs of mitochondrial cytochrome *b* (*cytb*) gene of three samples of small shrews, genus *Suncus* Ehrenberg, 1832, from Iran. Using previous published data for the genus, phylogenetic trees showed affiliation of the Iranian samples to the Madagascan Pygmy Shrew, *Suncus madagascariensis* which is the first report of the species for the Iranian Plateau. This result questioning the presence of *Suncus etruscus* for the country. Kimura 2 parameter genetic divergence of 5.4% between the two nominative geographic clades, 'etruscus' and 'madagascariensis' (the Western and Eastern clades, respectively) is located beyond of intraspecific divergence for mammalian cytochrome *b* (2.4%). This finding reflects the importance of molecular data to discriminate groups, when traditional approaches are not adequate, as well as it sheds more light on the current geographic range of small shrews.

Key words: morphology, molecular phylogeny, *Suncus etruscus*, *Suncus madagascariensis*, Iran.

INTRODUCTION

Heretofore, nine species of the subfamily Crocidurinae Milne-Edwards, 1872, were recognized for the fauna of Iran, of which five species assumed to be endemic for the country (sensu, Karami et al. 2008). Although, few studies were recently established on the Iranian shrews, there is however large ignorance in view of distribution and taxonomic status of this poorly known group (Hutterer, 2005b; Dubey et al., 2007b; Esmaili et al., 2008a,b; Mohammadi et al., 2013). Our ignorance mainly originated from the difficulties which one may confront for capturing of this very small size mammal (Nowak 1990). On the other hand, high morphological resemblance besides lacking of molecular data have always been the source of long debate over true identification of the closely related species in mammals (Pečnikar and Buzan 2013). This is more common for the Middle East, and especially for the Iranian Plateau (Dianat et al. 2013; Moshtaghi et al. 2016, Haddad et al. 2016). Etruscan shrew, *Suncus etruscus* (Savi, 1822) species complex is the most suitable candidate of such uncertainty in the region, with long dispute over its taxonomic scope (Omar et al. 2011). The authors applied genetic distance criterion to delimit *Suncus* species, suggested that *Suncus madagascariensis* (Coquerel, 1848) is not a distinct species on its own, rather should be assigned as a new established population of *S. etruscus* by human intervention. *Suncus etruscus* is the only member of the genus in Iran which are currently known from northern and southern Iran (Goodwin, 1940; Lay, 1967; Esmaili et al., 2008a). The authors entirely based their studies on morphology (Goodwin,

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1940; Lay, 1967; Esmacili et al., 2008a). Molecular studies have discovered the existence of many biological species without relevant morphological disparities. In addition to this, species delimitation upon on morphology is always challenging task which may sometimes led to misidentification of an organism (Pečnikar and Buzan 2013). Therefore, in present study we provided *cytb* sequences of three samples from southern and northeastern Iran to explore the relations of the Iranian haplotypes within genus *Suncus*. Phylogenetic trees represent close affiliation of the Iranian samples to that of '*madagascariensis*' population.

MATERIAL AND METHODS

We captured three individuals of *Suncus* from two widely scattered localities in Iran (see Fig. 1): an individual from Iranian-Afghanistan border, Sarakhs suburb (36°15' N/ 61°11' E), and two specimens from Persian Gulf, Qeshm Island (26°57' N/ 15°19' E). The samples are deposited in Zoological Museum of Ferdowsi University of Mashhad (ZMFUM). DNA was obtained from muscle preserved in 80% ethanol using the Qiagen method (DNeasy tissue kit, Qiagen, Hilden, Germany). Complete mitochondrial cytochrome *b* (*cytb*) sequence was amplified using the trans-mammalian primers L14724-SP and H15149-SP (Jaarola & Searle, 2002). Cycling conditions followed Jaarola et al. (2004). To understand phylogenetic relationships of the genus *Suncus*, we obtained 20 additional *cytb* sequences belonging to the genus as complementary dataset from GenBank (Table 1). *Myosorex gata* was used as an out-group. Appropriate model of sequence evolution (GTR+G+I) was chosen based on Akaike Information Criterion (AIC) as implemented in jModeltest 0.1.1 (Posada, 2008). Maximum Likelihood heuristic tree search with ten random addition sequence replicates was carried out to construct Maximum Likelihood (ML) tree using PAUP 4.0B10 (Swofford, 2003). The branch support of the ML tree, assessed as bootstrap value (BP) with 100 replicates. The Neighbor-joining (NJ) tree was constructed using MEGA 6 (Tamura et al., 2013) based on the Kimura two-parameter (K2P) distance matrix and uniform rates across sites. Nodal robustness of the NJ tree were estimated with 1000 replications to compute 50% majority rule consensus trees for node confidence limits (Felstenstein, 1985). Inter and intra-specific genetic divergence for *S. madagascariensis* and *S. etruscus* using K2P model (Kimura, 1980) were calculated by MEGA6 (Tamura et al., 2013).

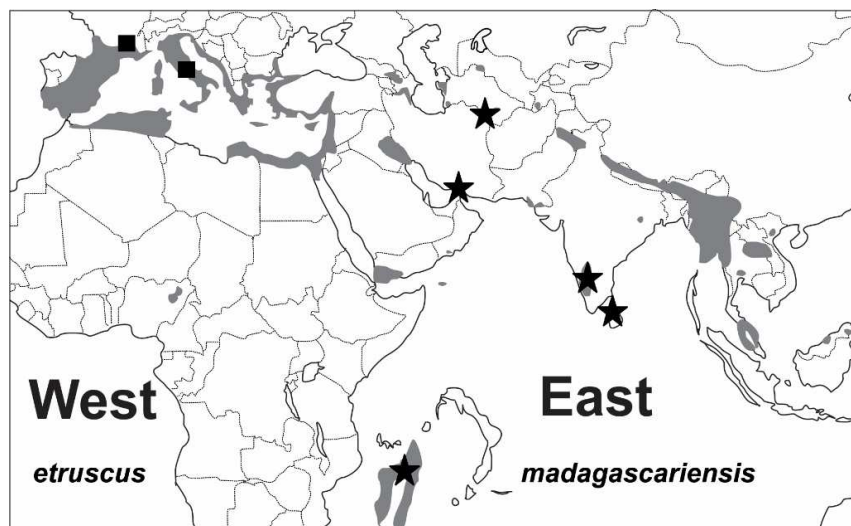


FIGURE 1. Map showing the collection sites of the specimens either classified as *S. etruscus* (rectangles) or *S. madagascariensis* (stars) as west and east clades (See the text for more information). [Range (shaded grey) is modified from Aulagnier et al., 2008 and Vololomboahangy & Goodman, 2008).

TABLE 1. Locality and GenBank accession number of *Suncus* species and *Myosorex* (out-group) that were used in this study.

Species	Locality	GenBank Accession No.	Reference
<i>Suncus madagascariensis</i>	Qeshm, Iran	MH673727	Present study
<i>S. madagascariensis</i>	Qeshm, Iran	MH673728	Present study
<i>S. madagascariensis</i>	Sarakhs, Iran	MH673729	Present study
<i>S. madagascariensis</i>	Madagascar	JF817395	Omar et al., 2011
<i>S. madagascariensis</i>	Madagascar	JF817394	Omar et al., 2011
<i>S. madagascariensis</i>	-	EU426973	Willows-Munro and Matthee, 2011
<i>S. fellowesgordoni</i>	Sri Lanka	JF914983	Meegaskumbura et al., 2014
<i>S. stoliczkanus</i>	Nepal	AB175077	Ohdachi et al., 2004
<i>S. murinus</i>	Sri Lanka	GQ290366	Meegaskumbura et al., 2010
<i>S. etruscus</i>	France	DQ630396	Dubey et al., 2007a
<i>S. etruscus</i>	Italy	DQ630397	Dubey et al., 2007a
<i>S. etruscus</i>	India	JN556043	Meegaskumbura et al., 2014
<i>S. etruscus</i>	France	JF817396	Omar et al., 2011
<i>S. etruscus</i>	Sri Lanka	FJ716836	Meegaskumbura and Schneider, 2008
<i>S. hututsi</i>	Rwanda	KF876417	Demos et al., 2014
<i>S. varilla</i>	South Africa	DQ630434	Dubey et al., 2007a
<i>S. remyi</i>	Gabon	DQ630399	Dubey et al., 2007a
<i>S. dayi</i>	Indonesia	DQ630432	Dubey et al., 2007a
<i>S. montanus</i>	Indonesia	DQ630388	Dubey et al., 2007a
<i>S. murinus</i>	Sri Lanka	GQ290372	Meegaskumbura et al., 2010
<i>S. malayanus</i>	Malaysia	JF817393	Omar et al., 2011
<i>S. malayanus</i>	Malaysia	JF817392	Omar et al., 2011
<i>S. malayanus</i>	Malaysia	JF817391	Omar et al., 2011
<i>Myosorex geata</i>	Tanzania	JQ433901	Meegaskumbura et al., 2014

RESULTS

Body coloration was not uniform for the collected samples; the sample from Sarakhs, and one sample from Qeshm Island were brownish gray, whereas another sample from Qeshm Island was slightly yellowish (Fig. 2). Small body size ranges from 40-56 mm (mean=51.02 mm). Iranian haplotypes showed the highest identity (> 97%) with *S. madagascariensis* haplotypes (JF817395 - JF817394), rather than with other species of the genus. Phylogenetic relationships within genus *Suncus* were almost accorded with previous works. *Suncus madagascariensis* and the Iranian haplotypes were clustered together as a highly supported monophyletic clade (94/100), which subsequently formed the sister group to the *S. etruscus* from Europe (99/98) (Figure 3). Corrected (K2P) pairwise genetic distance between *S. madagascariensis* and *S. etruscus* was $5.4 \pm 0.7\%$. While, the intraspecific divergence ranged from 1.8% and 2% for *S. madagascariensis* and *S. etruscus*, respectively.

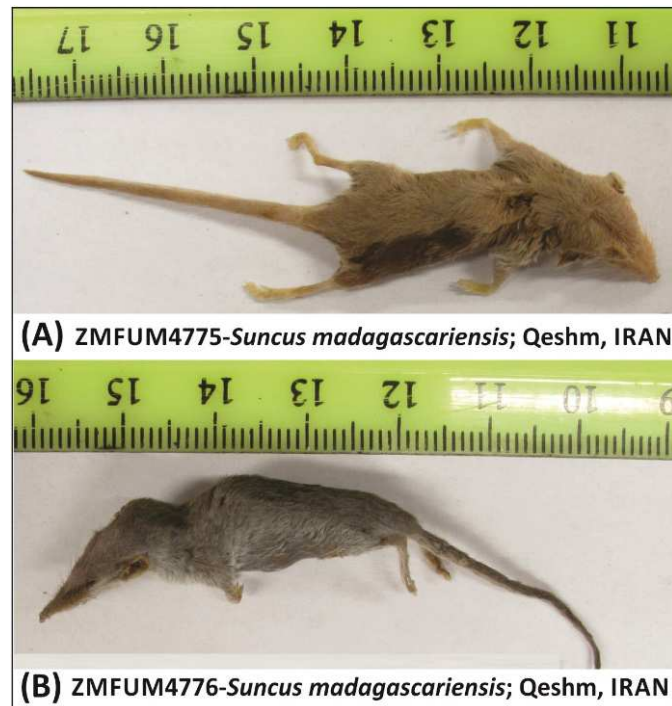


FIGURE 2. (A) and (B): Madagascan Pgmy Shrew, *S. madagascariensis* from Qeshm Island (Persian Gulf), southern Iran.

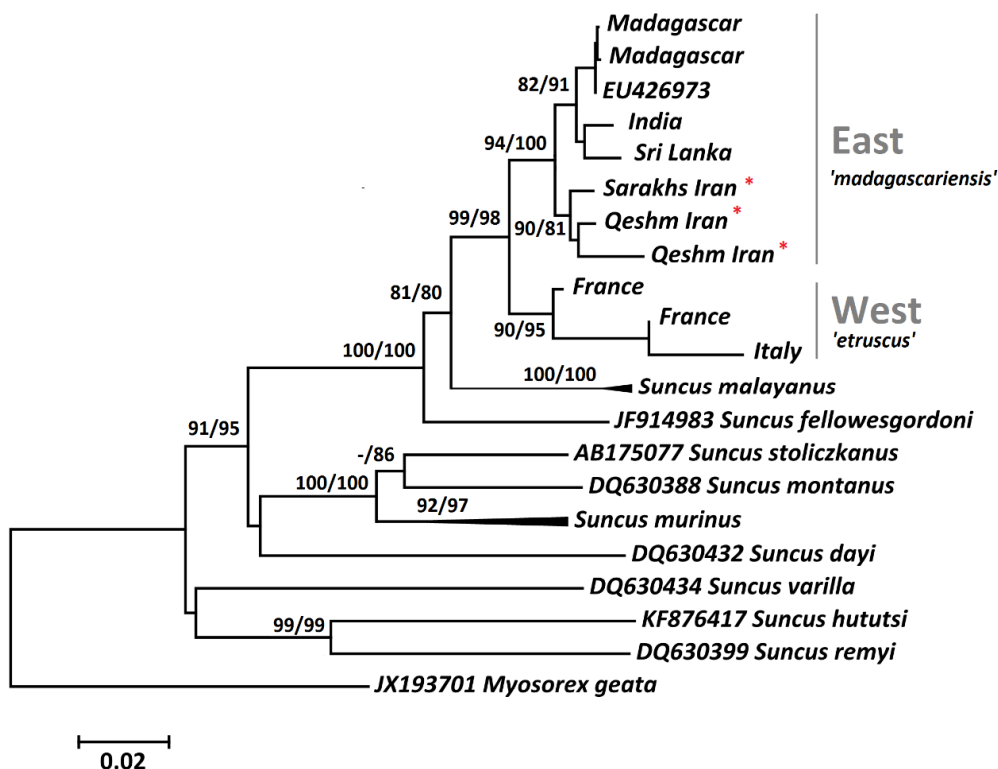


FIGURE 3. Phylogenetic tree obtained from *cytb* sequences, numbers on branches are indices of nodal robustness for Maximum Likelihood (ML) and distance (NJ) analyses, respectively. Iranian samples are shown by red star.

DISCUSSION

In a geographic frame, *S. etruscus* is a fairly well distributed shrew through most parts of Palearctic, mainly confined to the Mediterranean lowlands, also has been reported from Africa (Aulagnier et al., 2008), whereas *S. madagascariensis* is a native to Comoros; Madagascar (Vololomboahangy & Goodman, 2008) showing relatively confined distribution to southeastern Africa and Yemen. From the genetic point of view, *S. etruscus* and *S. madagascariensis* were appeared as two sister groups with strong geographic association; Western and Eastern clades, respectively (see Fig. 1). Of these two nominative species, *S. etruscus* has already been listed to the fauna of Iran by morphology (Goodwin, 1940; Lay, 1967; Esmaceli et al., 2008a). However, owing to morphologic resemblance in shrews, attempts to species discerning based only upon morphologic parameters seems to be suspicious very often. Then, considering individuals to a species is difficult, as it was the case in the previous reports from Iran. Our result showed that misidentification probably was happened in samples taken from Sri Lanka (FJ716836; Meegaskumbura & Schneider, 2008) and India (JN556043; Meegaskumbura et al., 2014), caused shallow interpretation in their taxonomy (Omar et al. 2011). Aforementioned samples were ascribed to *S. etruscus* by morphology, while *cytb* sequences were clustered them in a clade containing the reference sequences from Madagascar (=Eastern clade, corresponding to *S. madagascariensis*). Molecular marker has been shown to be very useful in species delimitation in shrews (Ohdachi et al., 2004; Dubey et al., 2007b; Bannikova et al., 2006; Omar et al., 2011; Meegaskumbura et al., 2014), especially in the case of closely related species with quite conserved morphology (Omar et al. 2011). Iranian haplotypes placed within the Eastern clade, *S. madagascariensis* separated by 5.4% K2P genetic distance from Western clade (= *S. etruscus*). Such amount of divergence is beyond the scope of intraspecific variation ($\geq 2.4\%$) for *cytb* of mammals (Bradley & Baker, 2001). Furthermore, considering them as two valid species on their own is not peculiar, or alternatively, *S. madagascariensis* could be demoted and synonymized with *S. etruscus* as two divergent phylogenetic lineages of one species. At present we do not advocate either of these solutions, but available body of evidence allow us to conclude that speciation is an ongoing process within *Suncus*, expanding the known geographic range for Madagascan pygmy shrew, as well as questioning the presence of *S. etruscus* in the country, especially for northeastern Iran (Sarakhs), where previously reported in *S. etruscus* range (see, Karami et al., 2008). The magnitude of our ignorance depends, mostly on the very little study from region. Therefore to have a clear documentation for distribution, genetic variation and taxonomic status of *Suncus* species in the region, dense sampling and further accumulation of relevant biological data are unavoidable.

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