

Original Article

Simultaneous Morphological and Molecular Characterization of *Tatera indica* in Southwestern Iran

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Abstract

Background: Interest in *Tatera indica* rodent arises mostly because it is believed that this species is survived among four subspecies reported from Iran, two of which exist in Khuzestan Province. In addition, it might has a role as reservoir hosts of zoonotic cutaneous leishmaniasis in the transmission of *Leishmania major* in some of the widespread Asian foci including southwestern Iran.

Methods: Diagnostic morphological and molecular markers for *T. indica* were sought by characterizing from individual specimens, such as some taxonomic features and mitochondrial cytochrome *b* gene that had previously proven useful for the taxonomy of rodents. Wild rodents were caught using live wooden and wire traps. The specimens were identified morphologically using external criteria and molecularly by sequencing of Cyt *b* gene and phylogenetic analyses.

Results: Forty one *T. indica* were collected and identified morphologically in Khuzestan Province, Iran. Two morphotypes of *T. indica* were found and classified but sequencing and phylogenetic analyses of mitochondrial Cyt *b* gene did not support any subspecies between two morphotypes of *T. indica*. Because all 21 sequences of both morphotypes of *T. indica* had no variation with only one common and novel haplotype (GenBank accession No KP001566).

Conclusion: This is the first time that *T. indica* was characterized molecularly in Iran. There is no molecular evidence for *T. indica* morphotypes or subspecies, and so a population genetics approach using several polymorphic genes might be employed using species-specific molecular markers. In addition, more specimens of *T. indica* species in large geographical locations should be tested.

Keywords: *Tatera indica*, Molecular characterization, Reservoir host, Zoonotic cutaneous leishmaniasis, Iran

Introduction

The Indian Gerbil, *Tatera indica* (Hardwicke) is believed to be the main reservoir host of zoonotic cutaneous leishmaniasis (ZCL) in Khuzestan Province, in border of Iran and Iraq, southwest of Iran. Khuzestan Province is an important focus of ZCL in Iran and the reports shows that the occurrence of the disease has increased through the recent years (Javadian et al. 1998, Kia et al. 2010, Shirzadi 2012, Vazirianzadeh et al. 2013). The female *Phlebotomus papatasi* (Scopoli) (Diptera: Psychodidae) sandfly is the proven vector of the parasitic protozoan *L. major* (Yakimoff and Schokhor) (Kinetoplastida:

Trypanosomatidae), the causative agent of ZCL in Iran (Parvizi and Ready 2008, Bordbar and Parvizi 2014).

Tatera indica is distributed through Iran, Afghanistan, Pakistan, India, Sri Lanka, Turkey, Iraq and Syria (Harrison and Bates 1991, Agrawal 2000, Yigit et al. 2001).

The genus *Tatera* Lataste, (Rodentia: Gerbillinae) are widespread in the Africa, Near East, Middle East and Pakistan sub-continent (Colangelo et al. 2005).

At the morphological taxonomic level, 12 species of genus *Tatera* were identified of which 11 are present in Africa (*T. afra*, *T.*

brantsii, *T. bohemi*, *T. guineae*, *T. inclusa*, *T. vicina*, *T. kempi*, *T. leucogaster*, *T. nigricauda*, *T. phillipsi* and *T. robusta*) and only one species (*T. indica*) lives in Asia (Corbet 1978, Corbet and Hill 1991).

Based on morphological characters, *T. indica* can be considered as *Tatera sensu stricto* and consequently all 11 African *Tatera* species were placed in one genus of *Gerbilliscus* Thomas. Molecular characterizations of genomic DNA data showed that the genus of *Tatera* is a polyphyletic taxon (Chevert and Dobigny 2005).

Of several subspecies of *T. indica*, four subspecies are present in Iran. *T. indica persica* exists in Sistan, *T. indica scansa* in Kerman; and finally *T. indica monticola* and *T. indica bailwardi* in Khuzestan (Ellerman 1948, Missone 1959, Mirshamsi et al. 2007).

Studies on *Tatera* genus have highlighted a complex situation by karyological studies in Africa (Colangelo and Civitelli 2001).

The main objective of this research was to characterize the *T. indica* populations based on taxonomic knowledge by using and comparing morphological, genetic and molecular criteria concurrently from a specific geographical location of Iran where this rodent is predominant. In addition, phylogenetic analyses were employed to get the better understanding of *T. indica* subspecies in case of any homology at their nucleotide scales. Accurate and firm identification of animal reservoir hosts of ZCL are essential, requires any epidemiological research, and are crucial for predicting species-specific population, and developing control strategies.

Materials and Methods

Study sites, collections and identification of rodents

The active colonies of rodents were identified firstly from ten locations of Khuzestan Province, southern Iran with an altitude of 18 meters above sea level and geographical

coordinates as 31° 32' 73" N and 48° 69' 40" E, and then the rodents were trapped alive in various parts of these areas using wooden and wiry live traps (Fig. 1).

Sampling was conducted from 10 cities in Khuzestan Province. However, only from 6 of them (Table. 1) including 15 villages (Fig. 1) *T. indica* rodents were presented and captured.

The collection of samples was done from the colonies of rodents' burrows located around the villages where ZCL were endemic using 50 live wooden and wirytraps for each location.

Sampling was conducted from 10 cities in Khuzestan Province, however in only 6 of them *T. indica* rodents were present and captured.

The collection of samples were done from the colonies of rodents burrows located around the villages where ZCL were endemic using 50 live wooden and wire traps for each collection. Rodents were caught by baiting cucumbers and dates in 2012–2013. The traps were set up early in the morning and evening then the trapped rodents were transferred to Pasteur Institute of Iran, Tehran, and maintained for morphological and molecular testing (Mehrabani 2011). The morphological identification of collected specimens were employed using external criteria such as color, head and body length, body measurements, ears length, tail length, hind foot length, cranial and dental measurements and cranium. The standard external characters of body and tail were measured using a ruler and/or digital calipers. The sizes of dental characters were measured using Nikon measuring microscope MM-40 (Table 1, 2) (Etemad 1978).

DNA extraction and PCR amplification of Cyt b gene from rodents

All the captured rodents first identified morphologically then genomic DNA of *T. indica* was extracted using Genet Bio kit,

Phenol-Chloroform and ISH-Horovize methods (Parvizi and Ready 2008). After comparing these three DNA extraction methods, ISH-Horovize method was found to be more sensitive than others were and selected as main DNA extractions method with minor modifications.

Mitochondrial Cytochrome *b* (Cyt *b*) gene, which is a maternally inherited marker were used to characterize this rodent. For molecular characterization of rodents, extracted DNA from the tissue and blood of each rodent was used to amplify a fragment of Cyt *b* with the forward primer UNFOR403 (5'-TGAGGACAAATATCATTCTGAGG-3') and revers primer UNREV1025 (5'-GGTTGTCCTCCAATTCATGTTA-3') from single rodent by PCR (Kent and Norris 2005). The PCR products were directly sequenced, and the sequences edited and aligned using Sequencher™ v. 4.4 to permit the identification of haplotypes (= unique sequences) and their phylogenetic analysis using MEGA5.05 and PAUP* softwares (Tamura et al. 2011).

Results

Collection and Morphological identification of *Tatera indica*

Of all rodents caught during this study a total of 41 *T. indica* were collected and identified morphologically from 6 different locations in Khuzestan Province (Fig. 1). The most *T. indica* were captured from Behbahan district with 24 out of 41, then Ahvaz (9/41), Dezful (4/41), Shush (2/41), Shushtar and Ramhormoz each with one samples.

Morphological characterizations of *T. indica* showed that the rodents were in two color groups, buff black (21/41) and buff brown (20/41), total rodent length was about 33–39 cm, body length 16–20 cm, ears length 2.1–2.5 cm, tail length 16–19 cm and hind foot length 3.8–4.5 cm (Table 1) (Etemad 1978).

Three morphological characters of total

rodent length, hind foot (HF), head and body (HB), ear length (EL) and tail length (TL) were considered for separating *T. indica* from other rodent species in the region. The details of morphological characters of captured *T. indica* in Khuzestan are summarized and shown in Table 1 and 2 (Fig. 2).

Based on the development degree of the supraorbital crests and dental wear especially upper molars, three ages group among our *T. indica* samples were classified (Etemad 1978). The incisor teeth of *T. indica* are formed with a groove and upper molars (Fig. 2b).

By considering the colors of rodent body, two types of morphological characters of *T. indica* were found. The buff- black group was larger with wider snout and they had a semi wide white halo above their eyes. However, the rodents of buff- brown group were smaller, with longer snout and a narrow white halo above their eyes (Table 2, Fig. 3). On bone formation of *T. indica*, zygomatic bones and pulled Bsyarh is longer than other rodents (Fig. 2a).

Molecular characterization and identification of *Tatera indica*

Cyt *b* gene of extracted DNA from all 41 *T. indica* was screened and amplified by PCR. The PCR products of 21 out of 41 were sequenced, aligned and analyzed via molecular softwares (Sequencher™ v. 4.4, MEGA 5.05). This is the first time that a molecular study is performed on Iranian *T. indica*, which provided us with novel findings that all the sequences are similar and no variation was found among the nucleotides; therefore, all the samples from one haplotype of *T. indica*. Cyt *b* sequences were identified among 21 sequences obtained from individual *T. indica*. Only haplotype KHT01 (GenBank accession no. KP001566) predominated and was common in Khuzestan Province. After comparing our sequences with only one submitted sequence in GeneBank from France (GenBank accession no: AJ430563.1) some

similarities and few differences were observed.

Based on the results of our sequences along with the sequences of different rodents

submitted in GeneBank, a phylogenetic tree was constructed (Fig. 4).



Fig. 1. Location of villages and cities in Khuzestan Province, Iran where *Tatera indica* was sampled

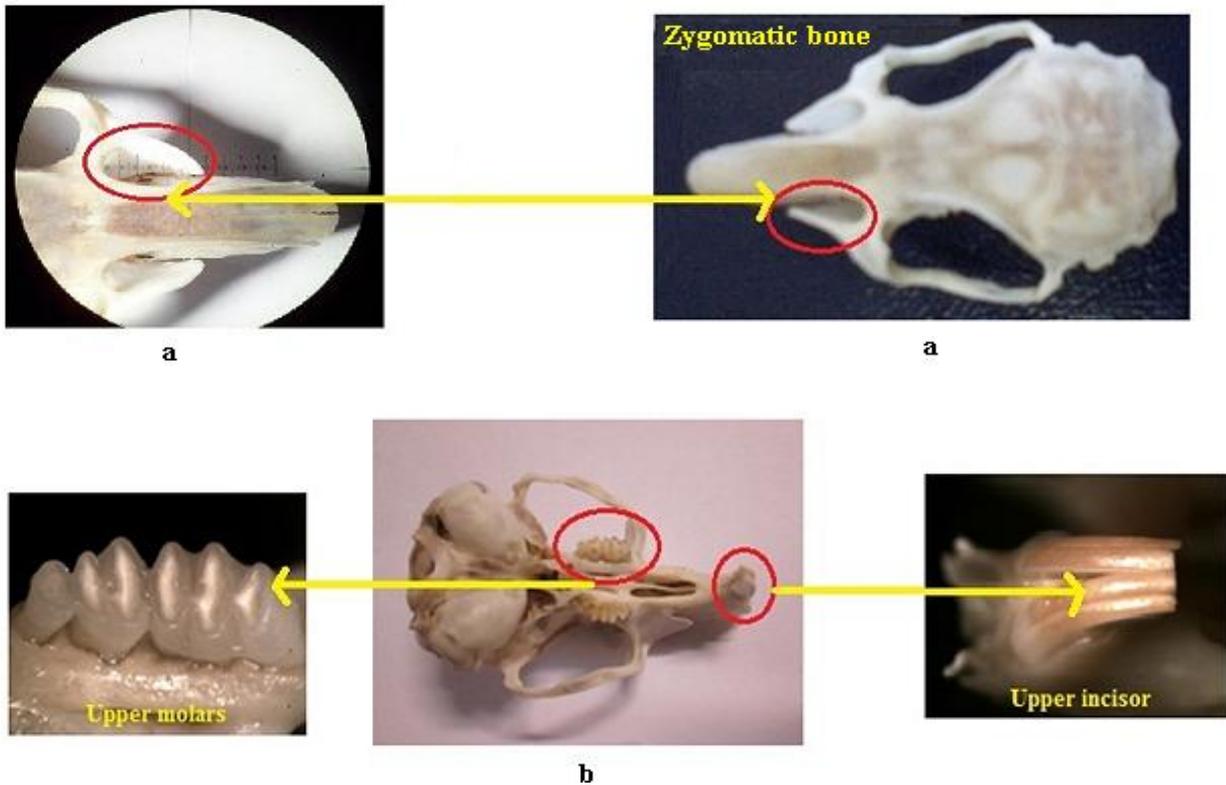


Fig. 2. *Tatera indica* Skull: (a): Zygomatic plate, (b): Upper molars and Upper incisors

Table 1. Morphological characters of *Tatera indica* which were collected in Khuzestan Province, Iran (TB: Total rodent length, HB: Head and body, TL: Tail length, HF: Hind foot, EL: Ear length)

Morphometric characters		TB									HB					TL						HF						EL					Color		Total	
		33-39									16-20					16-19						3.8-4.5						2.1-2.5					Black	Brown		
Size (Cm)		33	33.5	34.5	35	36.5	37.5	38	38.5	39	16	17.5	18	19.5	20	16	16.5	17	17.5	18	18.5	19	3.8	3.9	4	4.2	4.3	4.5	2.1	2.2	2.3	2.4			2.5	Black
Locations and the Numbers of <i>T. indica</i>	Ramhormoz	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	
	Shushtar	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	
	Shush	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	2	1	1	
	Dezful	0	0	1	0	2	0	1	0	0	0	0	3	0	1	0	1	0	0	1	2	0	1	2	1	0	0	0	0	2	1	1	0	2	2	
	Behbahan Ahvaz	0	0	4	0	7	9	4	0	0	0	0	12	0	13	0	5	0	9	4	7	0	9	10	4	1	0	0	2	3	5	4	10	11	13	
Total		1	1	8	1	10	10	8	1	1	1	1	19	1	19	1	8	2	10	7	12	1	11	15	9	4	1	1	6	6	6	8	15	21	20	41



a



b

Fig. 3. Buff brown (1) and buff black (2) *Tatera indica* rodents (b) along with their skull image (a) casing the morphologic differences of two groups

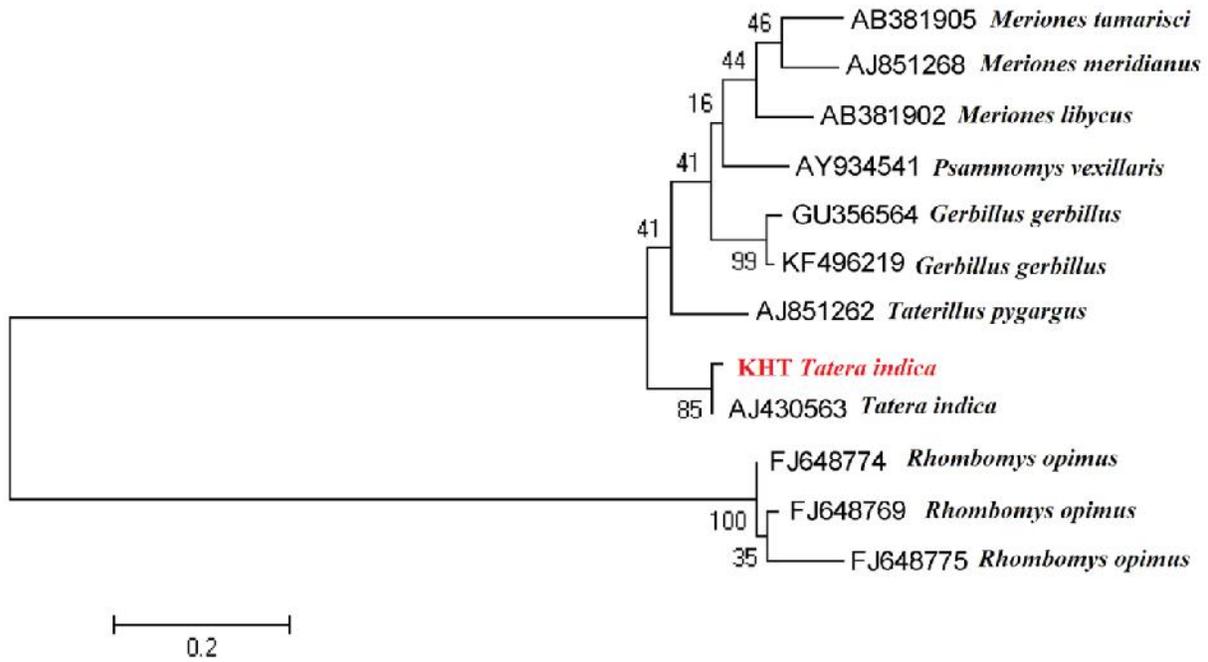


Fig. 4. Unrooted maximum-likelihood bootstrap tree showing the relationships of different genus and species of rodents by employing Cyt *b* gene fragment, including rodents submitted in GenBank and our *Tatera indica* sample using MEGA5 software. (KHT sample showed in the phylogenetic tree is our *Tatera indica* sample captured from Khuzestan Province, southern Iran)

Table 2. Morphological classification of *Tatera indica* based on their colors, Khuzestan Province, Iran

Morphological Characters	<i>T. indica</i>	
	Buff Black (cm)	Buff Brown (cm)
Width of rostrum	0.55-0.57	0.42-0.47
Occipitonasal length	4.75-4.88	4.26-4.47
Condylbasal length	4.38-4.51	3.91-4.14
Zygomatic width	2.64-2.67	2.18-2.41
Least interorbital width	0.73-0.78	0.60-0.76
Cranial width	1.68-1.79	1.53-1.56
Length of nasal	2.13-2.11	1.93-1.95
Length of diastema	1.33-1.36	1.17-1.26
Length of anterior palatine foramina	0.91-0.93	0.75-0.76
Length of tympanic bullae	1.28-1.29	1.14-1.19
Width of tympanic bullae	0.76-0.79	0.61-0.66
Upper cheekteeth	0.67-0.75	0.61-0.64
Lower cheekteeth	0.63-0.71	0.55-0.61
Height of skull	1.98-1.99	1.71-1.87
Length of mandible	2.58-2.65	2.41-2.51

Discussion

Bates (1988) drew attention to the importance of systematic and zoogeography of

genus *Tatera* of northeast Africa and Asia. The genus *Tatera* has an extensive geograph-

ical distribution in Africa and Asia (Agrawal 2000, Yigit et al. 2001, Mirshamsi et al. 2007). There are reports of numerous studies on morphological and karyological taxonomy of genus *Tetera*, however only few molecular characterizations are available worldwide (Mirshamsi et al. 2007).

T. indica was considered the main important reservoir host of ZCL in Khuzestan (Mohebbali and Javadian 2004, Mehrabani et al. 2007, Hajjaran et al. 2013). Principally *P. papatasi* may have been acquiring *L. major* from *T. indica* and other rodent reservoir hosts living peridomestically (Yaghoobi-Ershadi et al. 2013). Although different rodent species were found but they were limited and located in only one location. Only *T. indica* is considered as prominent reservoir host, which has a significant role in maintaining *Leishmania* parasites in different locations of ZCL in Khuzestan Province and this, highlights the importance of current survey on *T. indica*.

In this survey, *T. indica* was investigated more precisely in case of morphological features and molecular methods for the first time in Iran (Khuzestan Province) as the foremost and dominant reservoir in this focal region of leishmaniasis (Table 1, 2, Fig. 2, 3).

The comprehensive study in broad-spectrum ranges and distribution of *T. indica* was done in Khuzestan Province but this rodent was restricted to only six locations.

The *Cyt b* gene was employed for molecular typing and characterizing of *T. indica* for the first time in Iran and only one *Cyt b* gene sequence of *T. indica* is available from France in GenBank (GenBank accession no: AJ430563.1) and this indicates that sufficient molecular analyzing has not been carried out yet. Previous investigation of *T. indica* in Iran were only based on morphological identification and/or on *Leishmania* infection of this reservoir (Etemad 1978, Mehrabani et al. 2011, Hajjaran et al. 2013) and there was not any study on determining

the principle reservoir host, morphological features along with molecular systematics analyzing simultaneously.

The interesting finding of this investigation was that two types of morphological features (phenotypes) of *T. indica* was found in Khuzestan however after using *Cyt b* gene, only one haplotype from all 21 sequences (11 buff- black and 10 buff-brown) was identified (Table 2, Fig. 4)

It is not clear how authors identified four subspecies of *T. indica* in Iran and classified four subspecies (Mirshamsi et al. 2007). They did not give any definitive morphological or molecular taxonomic characterization for separating the *T. indica* subspecies.

A phylogenetic analysis of the new sequence, and those previously reported for *T. indica* and other rodents, found no support for recognizing more than one species or subspecies (*T. indica*) in Khuzestan Province, Iran (Fig. 4).

Mitochondrial *Cyt b* demonstrated an absence of any subspecies between two morphotypes of *T. indica*, which classified previously and this study in Khuzestan Province (Mirshamsi et al. 2007, Oshaghi et al. 2011). All four taxa (*T. indica persica* in Sistan, *T. indica scansa* in Kerman, *T. indica monticola* and *T. indica bailwardi* in Khuzestan) might be good biological species and did not characterize molecularly but could showed mitochondrial introgression caused by occasional inter-breeding the same as conclusions for some sandfly sibling species are reported (Testa et al. 2002, Pesson et al. 2004, Parvizi et al. 2010). For resolving sibling *T. indica* species, a population genetics approach using several polymorphic genes might be tested by considering carefully for species-specific molecular markers and more specimens of *T. indica* species in large geographical locations. We only could conclude if two subspecies in Khuzestan Province or four subspecies of *T. indica* exist in Iran, might have good evidence for associating specific taxa with phe-

notypes of epidemiological importance. There is no such evidence for *T. indica* subspecies, and so there is no reason to give priority to resolving the species status of four siblings *T. indica* species.

Conclusion

Tatera indica was characterized by both Cyt b molecular marker and morphological features for first time in Iran. There is no any morphotypes or subspecies of *T. indica* was found by molecular tools. We only could conclude if two subspecies in Khuzestan Province or four subspecies of *T. indica* exist in Iran, might have good evidence for associating specific taxa with phenotypes of epidemiological importance. There is no such evidence for *T. indica* subspecies, and so there is no reason to give priority to resolving the species status of four siblings *T. indica* species.

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