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The Karaman vole *Microtus irani karamani* is a new record for Iran (Arvicolinae; *Microtus*)

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We studied 1038bp of cytochrome *b* gene for social voles from three localities in Iran. The new sequences were compared with the previous published data correspond to eight species of social voles. Our results indicated that new material from west of Iran belong to the two species; *Microtus socialis* and *M. irani karamani*. This finding led to prove more knowledge about the Iranian vole distribution rang in Iran, and showed that west part of Iran is occupied with three social voles; *M. socialis*, *M. qazvinensis* that already have been documented, and *M. irani karamani* that is a new addition record for Iran.

Key words: cytochrome *b*; *Microtus irani*; social voles; Iran.

INTRODUCTION

The Iranian vole *M. irani* is the most enigmatic species within social voles that was described by Thomas, 1921 from the southern border of *Microtus* species range (Shiraz- Iran) (Musser & Carleton, 2005). Later studies on Iran, Syria, Turkey, Iraq, and Turkmenistan specimens ascribed their intermediate voles as *M. irani* (e.g., Kock et al., 1972; Morlok, 1978; Kockand Nader, 1983; Nadachowski et al., 1990; Colak et al., 1997). To date, therefore, five different cytotypes (2n= 46, 54, 60, 62, 64) were reported for this species that made this species as the most notorious example of unstable taxonomy steaming from ignorance over the exact chromosomal properties (Zima et al., 2013).Our recent study on chromosomal data of *Microtus* species from Iran have revealed two different cytotypes (2n=48, 64) from the type locality (Mahmoudi et al., in prep). Kryštufek & Kefelioglu (2001), based on morphological comparison of the social voles from Iran, Turkey, Lebanon and Syria stated that *M. irani* is a valid species with a restricted distribution range to its type locality. An integrative study based on molecular, karyological, and morphological approaches on the social voles in southern of Turkey, have documented a new northern distribution range for the Iranian vole in Turkey, as subspecies namely *Microtus irani karamani* (Kryštufeket al., 2009, 2010). In recent molecular studies the distribution range of Karaman vole was extended to Lebanon (Kryštufeket al., 2013).

Lacking of comprehensive data on the social voles in Iran, the Iranian vole scope has been remained unknown in the region. In this study, collected social voles from west part of Iran subjected to molecular analyses using mitochondrial *cytb* gene. We showed a new distribution record for the

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Karaman vole along with other already recorded distributions for *M. socialis* and *M. qazvinensis*. (e.g., Golenishchev et al., 2003; Musser & Carleton, 2005).

MATERIAL AND METHODS

The whole genomic DNA of six collected samples from west of Iran was extracted using standard salt extraction method (Bruford et al., 1992). 1038bp mitochondrial cytochrome *b* fragment was amplified using following primers; L7 and H6 (Montgelard et al. 2002). The partial *cytb* gene was sequenced using dye-labeled dideoxy terminator cycle sequencing with Big Dye V.3.1 (Applied Biosystems, Inc). Sequences were aligned with Clustal W algorithm (Thompson et al., 1997) using BioEdit 7.0.5 (Hall, 1999). Ambiguous bases were resolved by eye using CodonCode Aligner software (CodonCode Corporation).

TABLE 1. Accession numbers of mitochondrial cytochrome *b* sequences included in this study.

Species	GenBank No.	Reference
M. i. karamani	KM269337	this study
	KM269338	this study
	KM269339	this study
M. socialis	KM269334	this study
	KM269335	this study
	KM269336	this study
M. i. karamani	FJ767748- FJ767750	Kryštufek et al., 2009
	KC953617- KC953619	Kryštufek et al., 2013
M. anatolicus	FJ767740-FJ767742, GU187363	Kryštufek et al., 2009, Buzan et al., 2010
M. dogramacii	AY513793- AY513795	Jaarola et al., 2004
M. hartingi	AY513804,FJ767744-FJ767747,FJ767751, FJ767752	Jaarola et al., 2004, Kryštufek et al., 2009
M. guentheri	AY513805-AY513807, FJ767743,KC953620, KC953621	Jaarola et al., 2004, Kryštufek et al., 2009, 2013
M. socialis	AY513829-AY513831,GQ352468, GU987118	Jaarola et al., 2004; Banikova et al., 2010; Fink et al., 2010
M. paradoxus	KC953622- KC953624	Kryštufek et al., 2012

Phylogenetic analysis were performed on an assembled file include of six generated sequences from this study and 40 complementary sequences belong to eight species of *Microtus* that retrieved from GenBank (see Table 1), except those belong to *M. qazvinensis* that have not been submitted to GenBank (Mahmoudi et al., in prep). Nucleotide composition and genetic distances were analyzed assuming Kimura 2 parameter (K2P) model in MEGA v5 (Taumar et al., 2011). Phylogenetic analyses were conducted with the Bayesian inference (BI), using MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), and Maximum Likelihood (ML) as implemented in the PAUP 4.0b10 (Swofford, 2002).

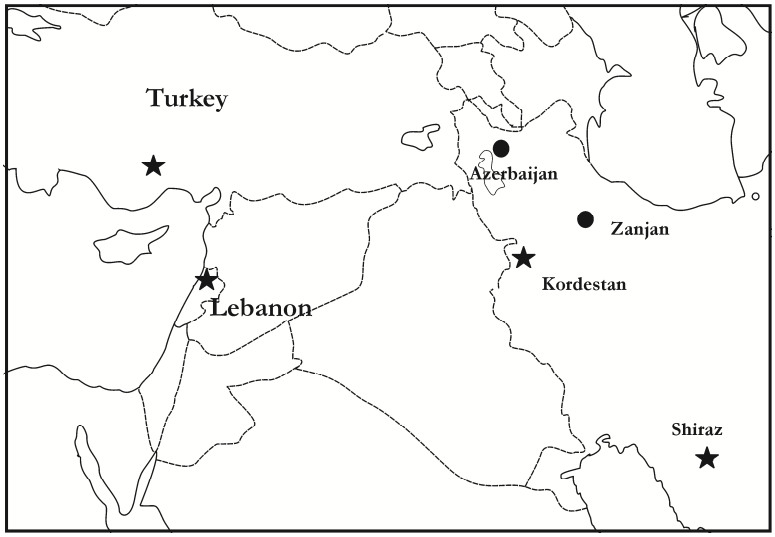

FIGURE 1. New location for *M. irani karamani* in west part of Iran (Kordestan), shown by star sign. Black circles correspond to the new haplotypes for *M. socialis* in Iran (Zanjan and Azerbaijan).

The best fitting models of sequence evolution were determined for both BI and ML methods using jModeltest 0.1.1 (Posada, 2008).The GTR model + gamma distribution (G) + proportion of invariable sites (I) (G=0.4697 and I=0.4678) based on Bayesian Information Criterion (BIC) were used to construct the BI tree. Four Monte Carlo Markov chains were run simultaneously for 2,000,000 generations. The trees were sampled every 100th generation after removing the first 5000 trees as the burn-in stage, and branch support was assessed as Bayesian posterior probabilities (BPP). The ML tree reconstruction was performed based on the Akaike Information Criterion (AIC), with GTR+I+ G model. The branch support of the ML tree, assessed as bootstrap value (BP) with 100 replicates. We considered a BPP≥ 0.95 as 'good' and BPP=0.90–0.95 as 'moderate' supports, while BP > 90% as 'good' support, and BP = 80–90% as 'moderate' support, in line with other authors (e.g., Krystufek et al., 2009). The phylogenetic trees were rooted by three species of the genus *Microtus*: *M. kirgisorum*, *M. arvalis*, and *M. agrestis* (Jaarola et al., 2004).

RESULTS

Altogether, our study provides five new haplotypes of social voles corresponds to two separate species. The analyzed sequences file showed 168 polymorphic positions after excluding missing sites, that 142 of them were parsimony informative. Phylogenetic relationships obtained using the two influential methods (BI and ML), with the same topology showed high PP and BPP supports for the all terminal nodes. In the same line with previous studies, the trees showed two strongly supported sister lineages encompass of '*socialis'* and '*guentheri'*.

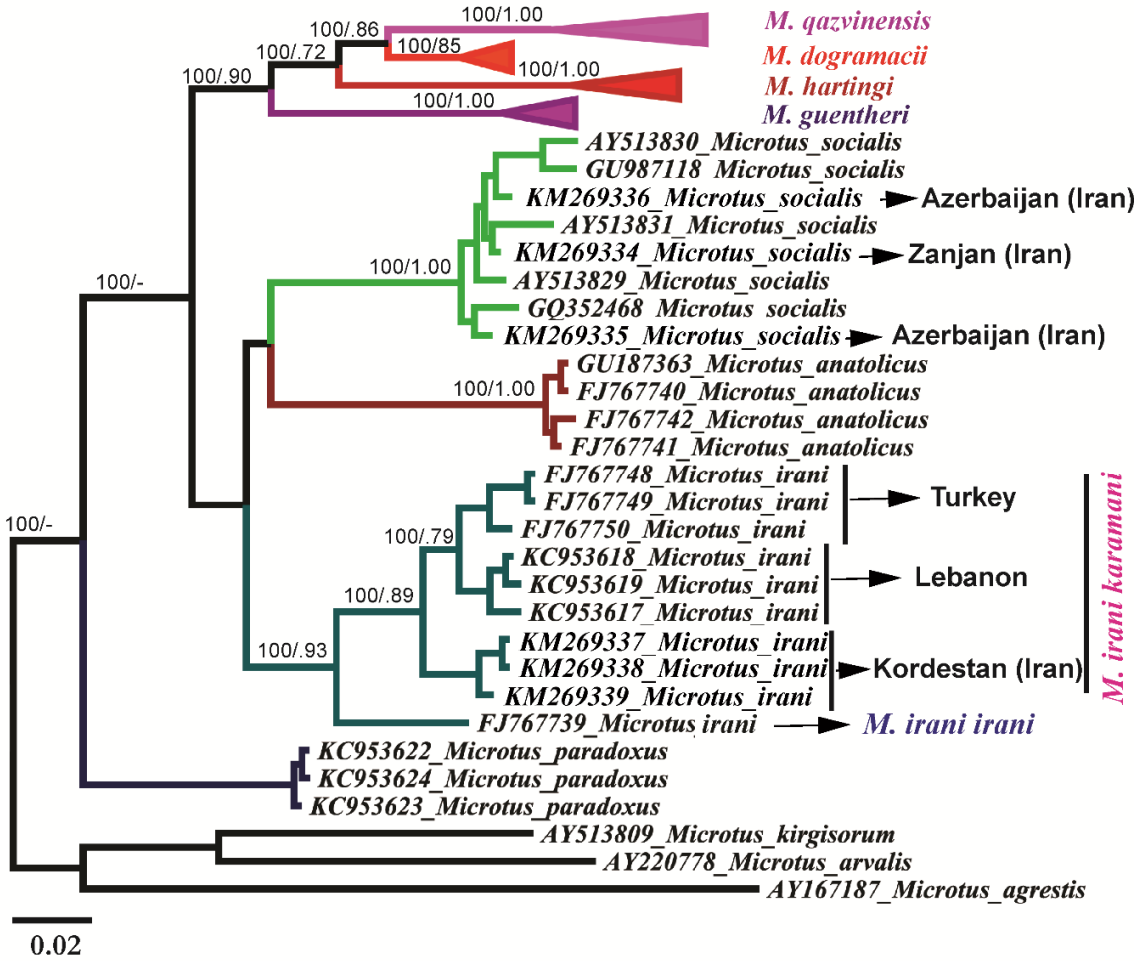

FIGURE 2. Bayesian inference tree reconstructed from *cytb* sequences of social voles. The trees were rooted using *M. agrestis*, *M. kirgisorum*, and *M. arvalis*. The first and second numbers on the branches correspond to posterior probability and bootstrap values in the BI and ML analyses, respectively.

All relationships within *guentheri* lineage were supported with high BP and BPP values. Whereas the internal nodes of *socialis* lineage did not obtain good support (Fig. 2). So, in congruent earlier studies (Kryštufek et al., 2009, 2012, 2013), we observed polytomy within *socialis* lineage. Surprisingly, our BI tree showed the major incompatibility with conventional hypothesis about the *M. paradoxus* position within *socialis* lineage. *Microtus paradoxus* take placed a basal position as a sister taxon against all other social voles (BPP: 1.00). This inconsistency did not support in ML tree. The new sequences in the phylogenetic trees were clustered with two different groups; Zanjan and Azerbaijan samples belonging to *M. socialis* that are not unexpected, as already it has been reported (e.g., Musser and Carleton, 2005). Samples from Kordestan are clustered with those from Turkey and Lebanon belong to *M. i. karamani* with low genetic distance (1.6±0.04% and 1.6±0.03% for mean and p-distances, respectively). This relationship obtained high support both in BI and ML trees.

DISCUSSION

From the geographical respect, *M. i. karamani* distribution were reported more westerly than present. This finding also further confirms the limited scope of *M. irani irani* just to the type locality (Shiraz), and showed more extended range of *M. i. karamani* between Iran, Lebanon and Turkey. Social voles

of Iran (especially W Iran) excepting *M. socialis* have already been classified as either *M. qazvinensis* (Shenbrot and Krasnov, 2005), or *M.*cfr. *irani* (Kryštufek & Kefelioglu, 2001). According to the present results, western of Iran is occupied with three distinct taxa of social voles (*M. qazvinensis*, *M. socialis*, and *M. i. karamani*) that highlight species richness of the area. However, further sampling is necessary to resolve the distribution of Iranian vole *M. irani irani*, and also identifying the true taxonomic affiliation of the social vole complex that resident western of Iran.
Microtus is a young and rapidly evolving genus among arvicoline rodents (DeWoody & Triant, 2006), that have been proposed simultaneous allopatric speciation model in refugial area for this group (Kryštufeket al., 2012). The star-like radiation such as observed in our phylogenetic tree, could be correspond to rapid evolving and simultaneous radiation of allopatric populations. Such cases are shown as polytomy in molecular tree, like those present in *socialis* lineage.

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