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# A new and highly-divergent mitochondrial lineage in the Small Five-toed Jerboa, *Allactaga elater*, from Iran

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**A new and highly-divergent mitochondrial lineage in the Small Five-toed Jerboa, *Allactaga elater*, from Iran**

The Small Five-toed jerboa, *Allactaga elater*, is a small rodent adapted to desert and semi-arid habitats with a widespread distribution around the Caucasus. Previous studies have suggested the occurrence of subspecific variation within the species but, except for a recent phylogeny of the genus *Allactaga*, most of the work done on the taxonomy of the group relies on morphological data only. To contribute to the current understanding of patterns of genetic diversity of *A. elater* we analysed one mitochondrial locus,

cytochrome b, from 13 Iranian specimens. Comparing to a recent phylogeny, our results suggest the existence of two additional mitochondrial lineages, one that clusters within previously described lineages and a new and highly divergent one. The two novel mitochondrial lineages occur in the north and form two highly divergent monophyletic groups (Dxy = 14%), which likely separated during the Pleistocene.

Keywords: *Allactaga elater*; Iran; cytochrome b; Pleistocene divergence

## Introduction

The number of species of *Allactaga* is controversial and the genus has been the subject of many systematic studies in recent decades (e.g. Tarahomi *et al.* 2010; Zhang *et al*. 2013; Lebedev *et al.* 2013; Kryštufek *et al*., 2013) with some authors classifying these species as belonging to genus *Paralactaga*, Young, 1927 (Lebedev *et al*., 2013; Hamidi *et al*., 2016). However, with the exception of a few studies on the taxonomy of rodent species (e.g. Montgelard *et al.* 2002) and a recently published molecular phylogeny (Dianat *et al.* 2013), the genetic differentiation within the genus has not been studied in detail. The Small Five-toed jerboa, *A. elater*, is a desert rodent species distributed in most regions of South-East Europe to Central Asia, including the Middle East, Russia and the Caucasus (Holden and Musser 2005; Shenbrot *et al.*, 2008b); in Iran it is found across the desert and semi-arid regions (Karami *et al*. 2008; Figure 1). This species is readily distinguishable from other species of *Allactaga* by its smaller size and external measurements (Womochel 1978). The latest version of subspecific systematics of Small Five-toed jerboas (Shenbrot *et al.* 1999) showed the existence of seven well differentiated subspecies that fall into two groups, *elater* and *indica*. Prior analyses in the *elater* and *indica* groups revealed the existence of five subspecies in Iran: *A. e. elater*, *A. e. caucasicus* in *elater* group and *A. e. indica*, *A. e. aralychensis* and *A. e. turkmeni* in *indica* group (Shenbrot *et al*. 2008a). However, the existence of these subspecies in *A. elater* is based on mostly only morphologic and/or ecologic data and thus the taxonomic status of *A. elater* subspecies may be subject to revision. In fact, a recent work, using two mitochondrial DNA loci, further highlighted the existence of three divergent clades within *A. elater* related to subspecies *A. e. turkmeni, A. e. elater and A. e. indica* (Dianat *et al.* 2013), arguing in favour of a more thorough analysis of the genetic diversity of this species. In this study we aim at contributing to

the current understanding of patterns of genetic diversity of Iranian populations of *A. elater* using mitochondrial cytochrome b gene data.

## Materials and Methods

### *Sampling*

Fourteen specimens of *A. elater* from Qusheh, Damghan, in Semnan Province, Iran, were captured in 2009 in a Pistachio orchard. The sampling was opportunistic, since the animals were killed by the owners of the orchard and the studied specimens were collected after dead. Qusheh is a desert habitat covered by *Artemisia siberi* and *Zygophyllum* sp. Species identification was performed according to morphology (Darvish *et al.* 2008). Figure 1 presents sampling sites for all samples used in this study.

### *Molecular study*

Genomic DNA was extracted from muscle. Partial mitochondrial cytochrome b gene (Cyt b) was amplified and sequenced using primers designed based on *A. elater* sequence AJ389534: AeCytB_F - 5' CGA CAC CAC AAC AGC CTT C 3‘and AeCytB_R2 - 5' GGG TGC TCC ACT GGT TGT 3'. PCR products were sequenced in both directions on an ABI PRISM 310 Genetic Analyser (PE Applied Biosystems).

Sequences were aligned using the ClustalW option on BioEdit v7.1.3.0 (Hall 1999) and manually corrected. Since two highly differentiated mitochondrial lineages (named mtA and mtB) were observed in the data (details in the Result section), the divergence between the two groups was calculated by the average number of nucleotide substitutions per site (Dxy), the number of net nucleotide substitutions per site and with K2P distance using Arlequin v3.11 (Excoffier *et al.* 2005), DnaSP v5 (Librado and Rozas 2009) and MEGA v4 (Tamura *et al.* 2007). For phylogenetic inference, the dataset was composed by specimens sequenced in this study and by *A. elater* Cyt b sequences available on GenBank (accession numbers AJ389534, JQ954927-JQ954932 and JQ954934-JQ954937), which caused a reduction in the total alignment length (756 bp to 675 bp). Since a previous phylogeny of the genus showed that *A. toussi* grouped within *A. elater* (Dianat *et al*. 2013), samples from *A. toussi* (GenBank accession numbers JQ954933, JQ954938, JQ954954-JQ954959) were also included. One *A. williamsi* (GenBank accession number KC465441) and one *Jaculus jaculus* sequence (GenBank Accession Number JN214545) were used as outgroup. Models of Cyt b

sequence evolution were evaluated using jModelTest (Posada 2008) and the Akaike Information Criterion (AIC) and the TIM + I + G model was selected. Maximum likelihood (ML) and Bayesian (BI) inferences of the phylogenetic relationships within *A. elater*, *A. toussi* and *A. williamsi* were performed using Garli v1.0 (Zwickl 2006) and BEAST v.1.7.4 (Drummond and Rambaut 2007) and were conducted applying the optimal mutation (ML) and the GTR + I + G model (BI), respectively. A Yule prior on branching rates and a random local clock model (Drummond and Suchard 2010) were used. Tracer v1.5 (Rambaut and Drummond 2007) was used to determine the burn-in portion and assess convergence between Markov Chain Monte Carlo runs and their effective sample sizes. The time of the most recent common ancestor (TMRCA )was determined also using BEAST v.1.7.4, and calibrated with a mutation rate of 0.176 substitutions/third codon position/My rate calculated for rodents (Nabholz *et al.* 2008). The BI procedure described was applied, using the HKY+I mutation model. Divergence time between the two new lineages was additionally inferred using the assumption that under a neutral model of evolution $k = 2\mu t + \pi$, where k is Dxy, $\mu$ is the neutral mutation rate and $\pi$ the average nucleotide polymorphism in each mtDNA lineage.

**Results**

We sequenced 756bp of Cyt b gene from 13 *A. elater* individuals (one sample failed to amplify; GenBank accession numbers KX018295 - KX018307). Six distinct haplotypes were observed and two groups of sequences could be distinguished based on 104 fixed substitutions. ML and BI phylogenies were congruent and confirmed the identification of these two well supported reciprocally monophyletic mtDNA lineages (Figure 2), named mtA and mtB. One previously sequenced *A. elater* specimen (Allactaga_elater_AJ389534; Figure 2), from Turbat Jam (Figure 1), and all *A. toussi* samples clustered within mtB group. When considering all sequences, five clusters can be identified: the three previously described (Dianat *et al.* 2013) and the two identified in this work (Figure 2). The sample from *J. jaculus* was basal to all *Allactaga* samples included in this analysis; *A. williamsi* was basal to clades 4, 6 and 7 (from Dianat *et al.* 2013) and the newly described mtB lineage but not to mtA lineage (Figure 2). Independently of the model used to reconstruct the phylogenies, the same topology was recovered.

Given that the inclusion of sequences from other studies caused the reduction of the alignment length, we used only the new sequences to determine the TMRCA between the two mtDNA lineages observed (but note that these lineages also represent the two most divergent groups: mtA and mtB plus clades 4, 6 and 7 from Dianat *et al.* 2013). Using the pairwise third codon difference between both lineages as a proxy to a neutral evolution rate (Nabholz *et al.* 2008) and assuming that $k = 2\mu t + \pi$, these mitochondrial lineages were estimated to have diverged at about 1.06 Myr ago. Results from the BI are concordant with this estimate: using a relaxed clock model (very strong support for this model was obtained using the Bayes Factor, following Kass and Raftery 1995; 2ln(B10) = 17.228) both lineages diverged 1.328 My (95% CI: 0.648 - 2.096) ago. Thus, both methods place the time of divergence in the Pleistocene.

**Discussion**

We analysed the mitochondrial diversity in one north Iranian population of *A. elater* and identified two highly divergent lineages: mtA and mtB (Figure 2). MtB clade is closely related to clade 4 from Dianat *et al.* (2013), which corresponds to samples from Western Iran and was identified as subspecies *A. e. turkmeni*, and these two clades are closely related to clades 6 and 7 (Dianat *et al.* 2013), including samples from Eastern Iran and corresponds to a form related to subspecies *A. e. elater* and *A. e. turkmeni* and subspecie *A. e. indica*, respectively. MtA clade forms an independent monophyletic lineage highly divergent from the rest of mitochondrial lineages from the "*elater*" group. The mean genetic distance between the two mitochondrial lineages (Dxy = 14%; K2P=15.7%) was higher than the overall value found for rodent sister species (K2P=9.55%, 4 - 11%; Bradley and Baker 2001), also based on Cyt b variation. This indicates a complex evolutionary history within the species that is probably related to a scenario of deep population divergence in the central part of Iranian plateau. Further work including a thorough geographic and genomic sampling of this taxon is needed and may help clarifying the history of divergence among these evolutionary units, including testing the hypothesis of mitochondrial introgression (a scenario reported in different taxa; see e.g. Melo-Ferreira *et al.* 2012), the level of reproductive isolation between them, and the taxonomic status of these entities. Still, our results clearly show the existence of two well-differentiated mtDNA lineages co-existing in a single location and that diverged during the Pleistocene.

Intriguingly, all samples from Toussi jerboa (*A. toussi*, Darvish *et al.*, 2008) clustered within the group formed by *A. elater* clades 4, 6 and 7 (from Dianat *et al.* 2013) and the newly described mtB lineage. This species was recently described based solely on morphology (Darvish and Rastegar-Pouyani 2012; Tarahomi *et al.* 2010). Thus, given that results from a nuclear marker confirms the clustering of these samples within *A. elater* clades (Dianat *et al.* 2013), it seems plausible to hypothesise that *A. toussi* might not be a true species but instead a morphological variant of *A. elater*. Again, the analyses of more specimens and genetic markers may help to elucidate this question. On the other hand, the clear separation of the mtA lineage from the remaining specimens may suggest the occurrence of a new *Allactaga* species but only a more detailed investigation on the morphological characteristics and genetic composition of samples carrying this mitochondrial lineage can confirm this.

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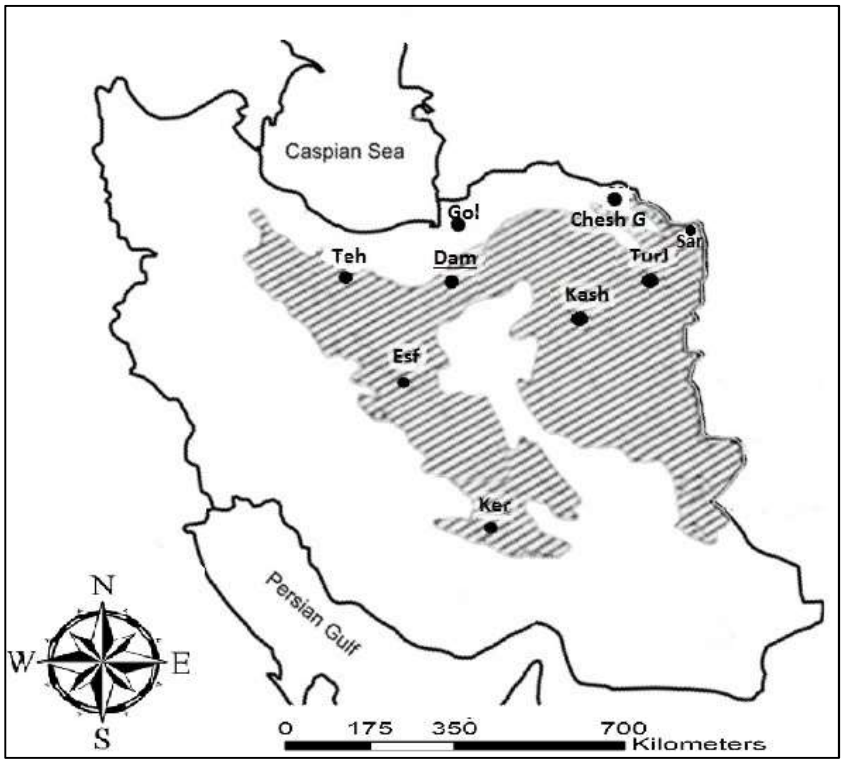


Figure 1. Map of Iran showing the geographical distribution of the Small Five-toed jerboa, *Allactaga elater* (Shenbrot *et al*., 2008b), and the location of the sampling area (Dam: Damghan, Semnan Province; underlined). The sampling location from Montgelard *et al*., 2002 (TurJ - Turbat Jam) and the six sampling locations from Dianat *et al*., 2013 (*A. elater*: Gol - Golestan; Kas - Kashmar; Esf - Esfahan (Mirabad); Teh - Tehran; *A. tousse*i: Chesh G - Cheshmeh Gilas; Sar - Sarakhs) are also indicated.

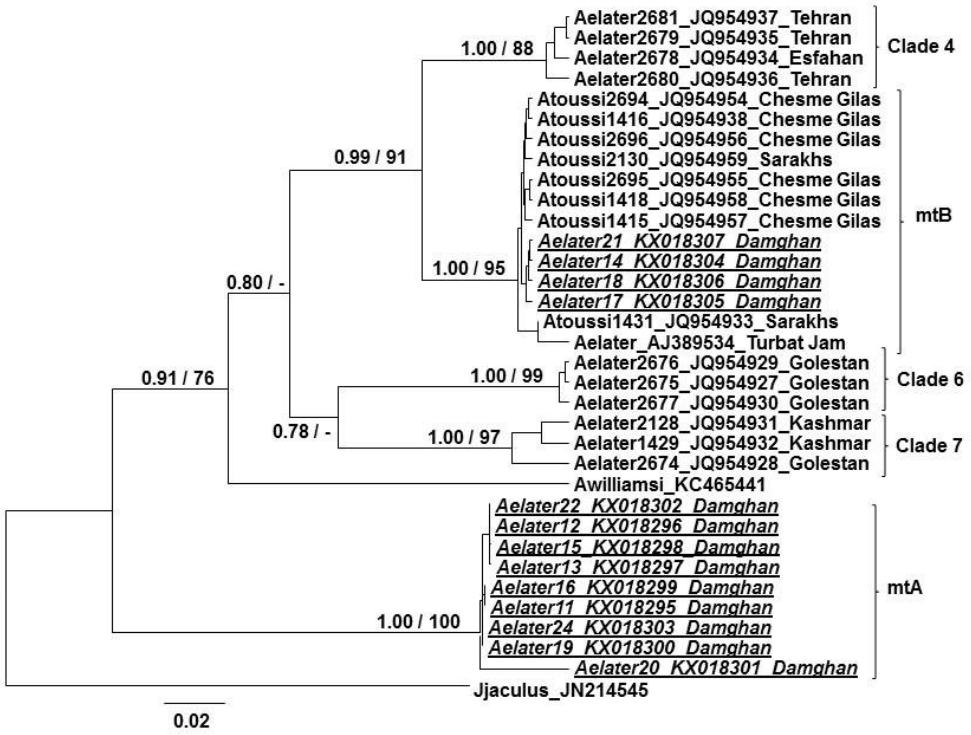


Figure 2. Bayesian Inference tree with estimated branch lengths reconstructed from the CytB data obtained in this study (indicated with italics underlined) and from Dianat *et al.*, 2013. Clades 4, 6 and 7 are according to this latter study. Posterior probabilities, if higher than 0.95, and bootstrap support values, if higher than 95%, from the Maximum Likelihood analysis are provided at the nodes.