



Cryptic species diversity in the genus *Allactaga* (Rodentia: Dipodidae) at the edge of its distribution range

Authors: Moshtaghi, Samira, Darvish, Jamshid, Mirshamsi, Omid, and Mahmoudi, Ahmad

Source: *Folia Zoologica*, 65(2) : 142-147

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/fozo.v65.i2.a9.2016>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Cryptic species diversity in the genus *Allactaga* (Rodentia: Dipodidae) at the edge of its distribution range

Samira MOSHTAGHI¹, Jamshid DARVISH^{1,2}, Omid MIRSHAMSI^{1,3} and Ahmad MAHMOUDI^{1*}

¹ Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran; e-mail: a.mahmoudi.bio@gmail.com

² Rodentology Research Department, Institute of Applied Zoology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

³ Zoological Innovations Research Department, Institute of Applied Zoology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

Received 2 January 2016; Accepted 29 April 2016

Abstract. Present study aimed to address molecular diversities of the small five-toed jerboa *Allactaga elater* Lichtenstein, 1825, and the Toussi jerboa *Allactaga toussi* Darvish et al., 2008, in marginal geographic distribution of the genus in Iran. The study involved 35 individuals of *A. elater* and *A. toussi*, from the east and central parts of Iranian plateau. The two probabilistic phylogenetic algorithms, Maximum Likelihood (ML) and Bayesian Inference (BI), applied on 68 sequences of the two mitochondrial genes (34 cytochrome *b* and 34 cytochrome *c* oxidase subunit 1), retrieved reciprocal monophyly of the two species. Independent species status of *A. elater* and *A. toussi* is further evident from their sympatry in eastern Iran. Each of these species was further subdivided into two deeply divergent phylogeographic lineages within Iranian plateau, showing high level of genetic divergence ranging from 7-10.7 % for *cytb* and 7.8-12.4 % for *cox1* genes. Such values exceed the intraspecific level of variation in rodents.

Key words: *Allactaga elater*, *Allactaga toussi*, *cox1*, *cytb*, molecular phylogeny

Introduction

Five-toed jerboas are typical inhabitants of arid areas in Asia and north-eastern Africa. Twelve species were recognized in Wilson & Reeder (2005) and classified as a genus *Allactaga*. The diploid number of chromosomes is stable across the genus and molecular data are available only for few regions and species groups (Arslan et al. 2012, Dianat et al. 2013, Kryštufek et al. 2013). Taxonomy has therefore been entirely based on morphology although it is known that characters used in species delimitation (e.g. size, colour, and shape of glans penis) are subjected to the intraspecific variations (Shenbrot 2009). Recent phylogenetic reconstructions, based on molecular markers, questioned the validity of established taxonomy at the level of species and of genera. Lebedev et al. (2013) proposed split into up to five genera and other analyses (Dianat et al. 2013, Kryštufek et al. 2013) showed that number of species may be higher than that of conservative 12 species in Wilson & Reeder (2005).

We address in this paper the small five-toed jerboa *A. elater* (Lichtenstein, 1825) which was recently split in Iran into two species. The new species *A. toussi* Darvish et al., 2008 was recognized on morphological ground but its distinctness from *A. elater* received support in molecular reconstruction (Dianat et al. 2013). Molecular tools have the potential to facilitate both the identification of known species and the discovery of unrecognized cryptic diversity (Hebert et al. 2003, Jaarola et al. 2004). Herewith we provide evidence on deep divergence within *A. elater* and *A. toussi*, which may be indicative of further cryptic species in this group.

Material and Methods

Our study utilized 35 specimens of small five-toed jerboas from the eastern and central regions of Iran (Fig. 1 and Table 1). Specimens were classified either as *A. elater* or as *A. toussi* on the basis of morphological characteristics provided by Darvish et al. (2008); body mass, external (HBL, TL, EL and

* Corresponding Author

Table 1. Details of the sample localities for *Allactaga* were included in phylogenetic analysis. Museum vouchers are deposited in Zoological Museum of Ferdowsi University of Mashhad (ZMFUM).

Species	Locality	Museum voucher No.	Cytb accession no.	Cox1 accession no.	Reference	
<i>A. elater</i> 1	Golestan	2749	KX219811	KX219845	Present study	
		2674	JQ954928	JQ954893	Dianat et al. 2013	
	Gonabad	3524	KX219808	KX219842	Present study	
		1905	KX219809	KX219843	Present study	
	Bojnord	2842	KX219812	KX219846	Present study	
		2862	KX219813	KX219847	Present study	
	Kashmar	2897	KX219815	KX219849	Present study	
		1412	KX219805	KX219839	Present study	
		1377	KX219804	KX219838	Present study	
		1374	KX219814	KX219848	Present study	
		2084	KX219806	KX219840	Present study	
		2128	JQ954931	JQ954902	Dianat et al. 2013	
		1429	JQ954932	JQ954900	Dianat et al. 2013	
		2704	KX219810	KX219844	Present study	
		1740	KX219807	KX219841	Present study	
	<i>A. elater</i> 2	Torbate Heydarieh	2738	KX219817	KX219851	Present study
		Golestan	2751	KX219818	KX219852	Present study
2732			KX219816	KX219850	Present study	
		2675	JQ954927	JQ954894	Dianat et al. 2013	
		2676	JQ954929	JQ954895	Dianat et al. 2013	
		2677	JQ954930	JQ954896	Dianat et al. 2013	
		Sarakhs	3547	KX219819	KX219853	Present study
3548			KX219820	KX219854	Present study	
3554			KX219821	KX219855	Present study	
Torbate Jam		3601	KX219822	KX219856	Present study	
		3602	KX219823	KX219857	Present study	
		3603	KX219824	KX219858	Present study	
		3604	KX219825	KX219859	Present study	
		3609	KX219826	KX219860	Present study	
		3613	KX219827	KX219861	Present study	
		<i>A. toussi</i> West	Tehran	2745	-	KX219871
2680				KX219836	KX219870	Present study
	2679		JQ954935	JQ954898	Dianat et al. 2013	
	2680		JQ954936	JQ954899	Dianat et al. 2013	
	2678		JQ954934	JQ954897	Dianat et al. 2013	
Esfahan	4503		KX219837	-	Present study	
	<i>A. toussi</i> East		Sarakhs	3558	KX219830	KX219864
3553		KX219831		KX219865	Present study	
3557		KX219829	KX219863	Present study		
Torbate Jam		3614	KX219835	KX219869	Present study	
Mashhad		T1045	AJ389534	-	Dianat et al. 2013	
		2694	JQ954954	JQ954918	Dianat et al. 2013	
		2695	JQ954955	JQ954919	Dianat et al. 2013	
		2696	JQ954956	JQ954920	Dianat et al. 2013	
		1415	JQ954957	JQ954921	Dianat et al. 2013	
		1416	JQ954938	-	Dianat et al. 2013	
	1418	JQ954958	JQ954922	Dianat et al. 2013		
	Sarakhs	2130	JQ954959	JQ954923	Dianat et al. 2013	
1431		JQ954933	JQ954901	Dianat et al. 2013		
Sabzevar	1438	KX219834	KX219868	Present study		
Tabas	1434	KX219833	KX219867	Present study		
Bojnord	2864	KX219828	KX219862	Present study		
	2875	KX219832	KX219866	Present study		

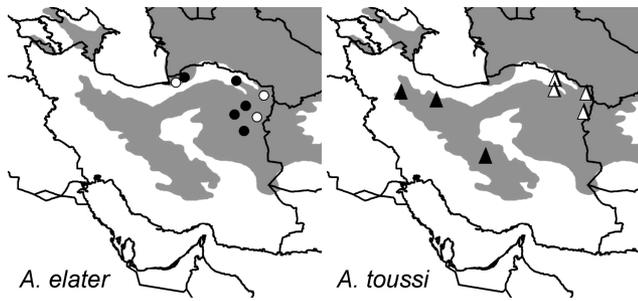


Fig. 1. Map showing collection sites of the specimens used in this study. Different symbols refer to phylogenetic sub-lineages of *A. elater*, and *A. toussi*. (The approximate distribution of small five-toed jerboa (grey shadow) follows IUCN database, Shenbrot et al. 2008b; <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T853A13083932.en>).

FL) and cranial measurements are larger in *A. toussi* than *A. elater*. Moreover, external characteristics are obviously discernable in these two species; inner

surface of ear is dark in *A. toussi*, while it is light in *A. elater*, hind sole margin is naked in *A. toussi*, while it covers with black dense setae in *A. elater* (for more details see Darvish et al. 2008). Museum vouchers are deposited in the Zoological Museum of Ferdowsi University of Mashhad (ZMFUM), Iran. Whole genomic DNA of *Allactaga* was extracted from 96 % ethanol-preserved tissues using salt standard extraction method (Bruford et al. 1992). Polymerase Chain Reactions were performed using L7 and H6 primers for *cytb* gene (Montgelard et al. 2002), and VF1d and VR1d for *cox1* gene (Ivanova et al. 2006). Purified PCR products were sequenced commercially by Macrogen Company, Republic of South Korea. 34 specimens were successfully sequenced. Sequences were edited manually using CodonCode aligner software (CodonCode Corporation) and aligned with Clustal W (Thompson et al. 1997) algorithm, using

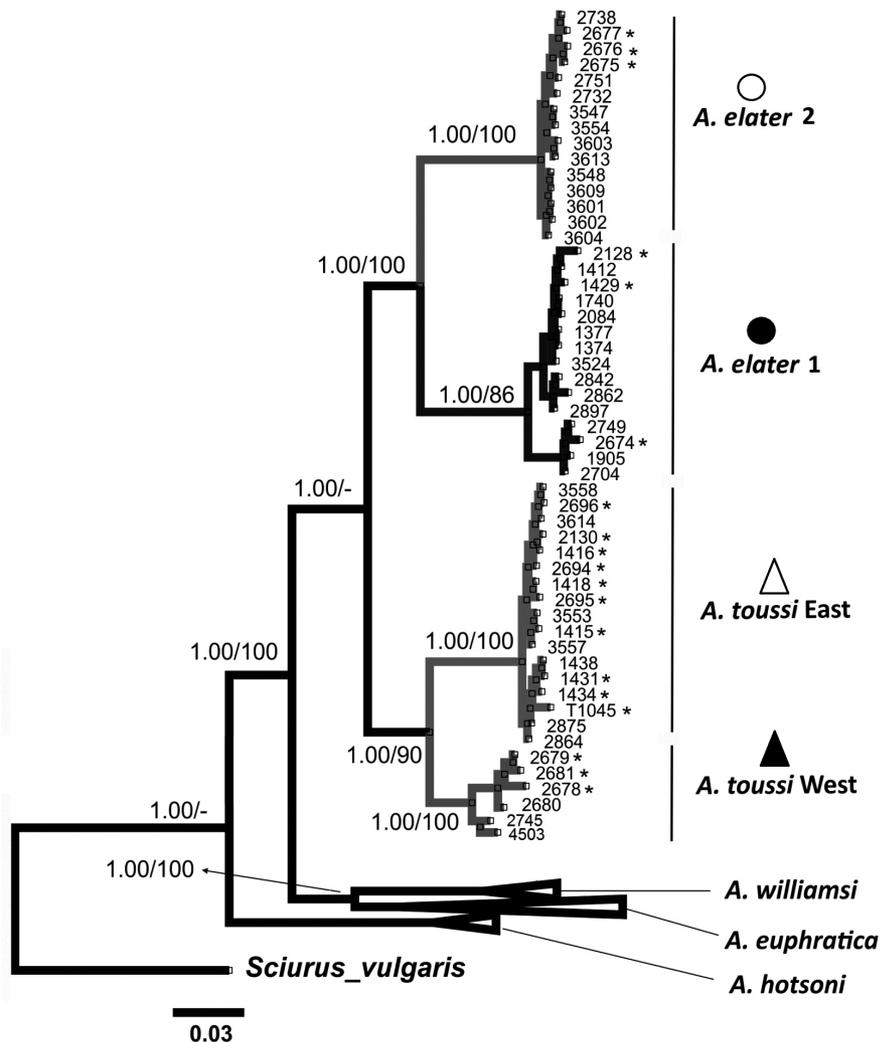


Fig. 2. Phylogenetic relationships among five species of *Allactaga* obtained from concatenated dataset (*cytb* and *cox1*). Bayesian Inference and Maximum Likelihood analyses pose an identical topology. The first and second numbers on the branches correspond to posterior probability (BPP) and bootstrap (BP) values in the BI and ML tree analyses, respectively. (*Sequences were downloaded from GenBank; published by Dianat et al. 2013). The symbols correspond to those in Figure 1.

Table 2. The K2P (mean \pm SD) estimates of intraspecific and interspecific *cytb* and *cox1* divergences in five species of *Allactaga*.

Description of node	Cytb: K2P (\pm SD)	Cox1: K2P (\pm SD)
<i>A. elater</i> / <i>A. toussi</i>	12.6 \pm 0.9	13.5 \pm 1.3
<i>A. elater</i> / <i>A. hotsoni</i>	15.9 \pm 1.2	18.1 \pm 1.4
<i>A. elater</i> / <i>A. euphratica</i>	16.2 \pm 1.0	20.2 \pm 1.8
<i>A. elater</i> / <i>A. williamsi</i>	15.4 \pm 1.1	18.4 \pm 1.6
<i>A. elater</i> / <i>A. sibirica</i>	18.6 \pm 1.4	18.9 \pm 1.7
<i>A. toussi</i> / <i>A. hotsoni</i>	16.5 \pm 1.2	13.4 \pm 1.2
<i>A. toussi</i> / <i>A. euphratica</i>	16.4 \pm 1.1	18.5 \pm 1.8
<i>A. toussi</i> / <i>A. williamsi</i>	15.5 \pm 1.3	17.1 \pm 1.5
<i>A. toussi</i> / <i>A. sibirica</i>	17.3 \pm 1.3	19.1 \pm 1.9
<i>A. hotsoni</i> / <i>A. euphratica</i>	18.3 \pm 1.2	20.9 \pm 1.8
<i>A. hotsoni</i> / <i>A. williamsi</i>	17.9 \pm 1.4	18.3 \pm 1.5
<i>A. hotsoni</i> / <i>A. sibirica</i>	18.4 \pm 1.5	19.9 \pm 1.7
<i>A. euphratica</i> / <i>A. williamsi</i>	15.0 \pm 1.0	14.6 \pm 1.6
<i>A. euphratica</i> / <i>A. sibirica</i>	20.7 \pm 1.4	19.6 \pm 1.9
<i>A. williamsi</i> / <i>A. sibirica</i>	18.7 \pm 1.5	19.5 \pm 1.9
<i>A. toussi</i> : <i>toussi</i> the east/ <i>toussi</i> the west	7.0 \pm 0.8	7.8 \pm 1.1
<i>A. elater</i> : <i>elater</i> 1/ <i>elater</i> 2	10.7 \pm 1.1	12.4 \pm 1.4

BioEdit 7.0.5 (Hall 1999). Genetic distances were analyzed assuming Kimura 2 parameter (K2P) model with 10000 bootstraps in Mega v6 (Tamura et al. 2013).

Phylogenetic analyses were performed on a combined multiple sequence alignment including 68 sequences obtained in this study (34 *cox1*, and 34 *cytb*), and 75 sequences (34 *cox1*, and 41 *cytb*) belonging to five species of *Allactaga* which were retrieved from GenBank (Dianat et al. 2013, Kryštufek et al. 2013). The best fitting model of nucleotide substitution was estimated, using jModeltest 0.1.1 (Posada 2008). Bayesian Inference (BI), was performed under the General Time Reversible model (GTR) with a proportion of invariable sites (I) and a gamma distribution (G) using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Four Monte Carlo Markov chains were run simultaneously for 4000000 generations. The trees were sampled every 100th generation after removing the first 5000 trees as the burn-in stage. Branch support was assessed as Bayesian Posterior Probability (BPP). Maximum Likelihood analysis was performed based on the Akaike Information Criterion (AIC), with the assumed model. The branch support of the ML tree was assessed as bootstrap value (BP) with 200 replicates. We considered a BPP \geq 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. Kryštufek et al. 2009). The phylogenetic

trees were rooted with red squirrel, *Sciurus vulgaris* (AJ238588) as an out-group (Reyes et al. 2000).

Results

Together with data reported in Dianat et al. (2013), 40 *cytb* and 34 *cox1* haplotypes are known for lesser five-toed jerboas. Of the 911 bp-long *cytb* sequence, 218 polymorphic sites (23.92 %) were found, 182 (83.48 %) of which were parsimony informative. Of the 620 bp-long *cox1* sequence, 241 (38.87 %) and 183 (75.93 %) sites were polymorphic and parsimony informative sites, respectively. No stop codons, insertions or deletions were observed in the alignments.

We constructed BI and ML trees for the two mitochondrial genes independently (*cytb*, *cox1*) and for the concatenated sequence. Because of congruence between the outputs only the BI tree drawn for combined sequences is shown (Fig. 2). Seven highly supported (BPP = 1.00) lineages are eminent. *Allactaga hotsoni* was basal in the tree and *williamsi* + *euphratica* were in a sister position against the small five-toed jerboas. All specimens of small five-toed jerboas were in two lineages which matched their morphological classification. In north-eastern Iran ranges of these two species overlapped and in Bojnord, Torbat Jam, and Sarakhs, they were sympatric. Each of the two small five-toed jerboas' species was further structured into two sub-lineages. Sub-lineages were strictly allopatric in *A. toussi*, being confined to north-eastern Iran (*A. toussi* the east) and

to the area between the Zagros and Elbruz Mountain ranges (*A. toussi* the west). The two sub-lineages of *A. elater* were allopatric in the extreme north-eastern Iran, but were found in sympatry in Golestan. K2P genetic distances between the species of five-toed jerboas ranged between 12.6 and 20.7 % for *cytb* and between 13.4 and 20.9 % for *cox1*. Corresponding values for the sub-lineages of *A. elater* and *A. toussi* were 10.7 and 7.0 % for *cytb*, and 12.4 and 7.8 % for *cox1* (Table 2).

Discussion

Our results confirmed association between the morphotype and the molecular profile in small five-toed jerboas classified as *A. elater* and *A. toussi*. *Cytb* divergence between them (12.6 %) was above the values reported for intraspecific divergences in rodents (Baker & Bradley 2006). Also importantly, these two jerboas were broadly sympatric in a large area of north-eastern Iran. It is therefore beyond doubt that *elater* and *toussi* are two distinct species.

Of no lesser interest are divergences within both, *A. toussi* and *A. elater*. In both species, the intraspecific *cytb* distances (7.0 and 10.7 % for *toussi* and *elater*, respectively) exceeded the intraspecific divergences in rodents (≤ 4.7 %; Baker & Bradley 2006), being well within the range of K2P distances found between species. Since the two lineages of *A. elater* were sampled from the same place in Golestan, one may assume for them to be at least parapatric. Although we believe that cryptic species are most likely involved in both cases, we refrain at this stage from taxonomic conclusions. First of all, nuclear genetic markers have to be included into analyses, sampling in the zone of overlap has to be intensified, and

morphological variation of glans penis is to be taken into consideration for these highly divergent sub-lineages. Following recent reports on cryptic species richness in five-toed jerboas (*Paralactaga*; Kryštufek et al. 2013), our results further emphasize the extent of undetected species richness in dipodids. It seems that the phenomenon is widespread, if not overwhelming and that cryptic species will follow to be reported along with progress of molecular screening in the family. Small five-toed jerboas seem a particularly good candidate, considering large range, high number of subspecies and categorical divergence evidenced by the morphology of glans penis. Noteworthy, significant portion of variation takes place outside Iran since only one of the two groups of subspecies discerned from glans penis was reported for the country (Shenbrot et al. 2008a).

Allactaga vinogradovi as the closest relative to *A. elater* (Lebedev et al. 2013) has not been sequenced so far. Information on nucleotide sequences in *A. vinogradovi* is essential to get an unbiased insight into the phylogenetic structuring of the entire group of small five-toed jerboas (tentatively classified as *Microalactaga*; Lebedev et al. 2013) and particularly the relationships between *A. vinogradovi* and *A. toussi*. It came as a surprise that *A. toussi* is more widespread in Iran than is *A. elater*.

Acknowledgements

We would like to thank Prof. Boris Kryštufek for his valuable comments, and two anonymous reviewers whose comments and suggestions greatly improved the paper. This study was funded by grant number 1.19727 for studying fauna of North Khorasan Province to JD.

Literature

- Arslan A., Toyulmaz T., Toyran K. et al. 2012: C-banding and Ag-NOR distribution patterns in Euphrates jerboa, *Allactaga euphratica* (Mammalia: Rodentia), from Turkey. *Mammalia* 76: 435–439.
- Baker R.J. & Bradley R.D. 2006: Speciation in mammals and the genetic species concept. *J. Mammal.* 87: 643–662.
- Bruford M.W., Hanotte O., Brookfield J.F. et al. 1992: Single-locus and multilocus DNA fingerprinting. In: Hoelzel A.R. (ed.), *Molecular genetic analysis of populations: a practical approach*. Oxford University Press, Oxford, U.K.: 225–269.
- Darvish J., Hajjar T., Matin M.M. et al. 2008: New species of five-toed jerboa (Rodentia: Dipodidae, Allactaginae) from North-East Iran. *J. Sci. I. R. Iran* 19: 103–109.
- Dianat M., Aliabadian M., Darvish J. & Akbarirad S. 2013: Molecular phylogeny of the Iranian Plateau five-toed jerboa, *Allactaga* (Dipodidae: Rodentia), inferred from mtDNA. *Mammalia* 77: 95–103.
- Hall T.A. 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41: 95–98.
- Hebert P.D., Ratnasingham S. & de Waard J.R. 2003: Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B* 270 (Suppl. 1): S96–S99.
- Ivanova N.V., Dewaard J.R. & Hebert P.D.N. 2006: An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Mol. Ecol. Notes* 6: 998–1002.
- Jaarola M., Martínková N., Gündüz I. et al. 2004: Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 33: 647–663.
- Kryštufek B., Arslan A., Shehab A. et al. 2013: Mitochondrial sequences point on a cryptic species in five-toed jerboas, subgenus *Paralactaga*. *Mammalia* 77: 433–438.

- Kryštufek B., Bužan V.E., Vohralík V. et al. 2009: Mitochondrial cytochrome *b* sequence yields new insight into the speciation of social voles in south-west Asia. *Biol. J. Linn. Soc.* 98: 121–128.
- Lebedev V.S., Bannikova A.A., Pisano J. et al. 2013: Molecular phylogeny and systematics of Dipodoidea: a test of morphology-based hypotheses. *Zool. Scr.* 42: 231–249.
- Montgelard C., Bentz S., Tirard C. et al. 2002: Molecular systematics of Sciurognathi (Rodentia): the mitochondrial cytochrome *b* and 12S rRNA genes support the Anomaluroidea (Pedetidae and Anomaluridae). *Mol. Phylogenet. Evol.* 22: 220–233.
- Posada D. 2008: jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25: 1253–1256.
- Reyes A., Gissi C., Pesole G. et al. 2000: Where do rodents fit? Evidence from the complete mitochondrial genome of *Sciurus vulgaris*. *Mol. Biol. Evol.* 17: 979–983.
- Ronquist F. & Huelsenbeck J.P. 2003: MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Shenbrot G.I. 2009: On the conspecificity of *Allactaga hotsoni* Thomas, 1920 and *Allactaga frouzi* Womochel, 1978 (Rodentia: Dipodoidea). *Mammalia* 73: 231–237.
- Shenbrot G.I., Sokolov V.E., Heptner V.G. & Kowalskaya Y.M. 2008a: Jerboas: mammals of Russia and adjacent regions. *Science Publishers Inc., Enfield, New Hampshire, U.K.*: 507–512.
- Shenbrot G.I., Tsytsulina K., Batsaikhan N. et al. 2008b: *Allactaga elater*. The IUCN Red List of Threatened Species. <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T853A13083932.en>
- Tamura K., Stecher G., Peterson D. et al. 2013: MEGA6: Molecular Evolutionary Genetics Analysis, version 6.0. *Mol. Biol. Evol.* 30: 2725–2729.
- Thompson J.D., Gibson T.J., Plewniak F. et al. 1997: The CLUSTAL – windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25: 4876–4882.
- Wilson D.E. & Reeder D.M. 2005: Mammal species of the world: a taxonomic and geographic reference, 3rd ed. *Johns Hopkins University Press, Baltimore*.