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Chromosomal diversity in the genus *Microtus* at its southern distributional margin in Iran

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Abstract. We karyotyped six species of *Microtus* voles collected along the southern edge of their range in northern and western Iran. Diploid and fundamental numbers were as follows: *M. socialis* and *M. paradoxus* 2n = 62, FNa = 60, *M. qazvinensis* 2n = 54, FNa = 54, *M. transcaspicus* 2n = 52, FNa = 52, and *M. mystacinus* (= *M. rossiaemeridionalis*) 2n = 54, FNa = 54. Two cytotypes were retrieved in *M. irani* from its type locality: 2n = 48, FNa = 46 and 2n = 64, FNa = 62. While our results confirmed an early report of 2n = 64 for this vole, the 2n = 48 cytotype remains unexplained. Karyological variability is relatively low in social voles and chromosomal data contribute little to individual species recognition. We argue that *Arvicola mystacinus* De Filippi, 1865, described from Lar Valley (north-east of Tehran) is the oldest available name for 2n = 54 voles with the following synonyms: *M. subarvalis* Meyer, Orlov & Skholl, 1969, *M. epiroticus* Ondrias, 1966, and *M. rossiaemeridionalis* Ognev, 1924.

Key words: karyotype, *Microtus irani*, *Microtus mystacinus,* zoological nomenclature

Introduction

Latitudinal diversity gradient with species richness decreasing from the equator towards the poles is one of the most general patterns in biogeography (Brown 1995). Voles and lemmings (subfamily Arvicolinae) contradict the generality of this rule by attaining peak numbers of species per area in temperate and boreal latitudes (Shenbrot & Krasnov 2005). The genus *Microtus*, which accounts for about one half of arvicoline species, appears to have ongoing speciation (Jaarola et al. 2004) and the centres of diversification in two of its lineages coincide with south-western Palaearctic. Pine voles (subgenus *Terricola*) speciated in the Mediterranean glacial refugia and the Caucasus area (Jaarola et al. 2004) and the social voles (subgenus *Sumeriomys*) diversified in south-western Asia (Kryštufek et al. 2009). Both groups contain evolutionary young species, therefore taxonomic uncertainties aggregate along the southern margin of the range of arvicolines (Musser & Carleton 2005). Delimitation of species in *Microtus* is traditionally based on dental morphology (Hinton 1926, Gromov & Polyakov 1992). Because cryptic species are omnipresent in the group, the understanding of taxonomic relationships benefited tremendously from karyological studies (Zima & Král 1984) and utilization of molecular markers (Jaarola et al. 2004). In this paper we provide new evidence on karyotypes of six out of nine *Microtus* species reported so far for Iran (Karami et al. 2008). The investigated species belong to two lineages, which are either classified as subgenera (*Microtus* and *Sumeriomys*; Gromov & Polyakov 1992, Shenbrot & Krasnov 2005) or species groups (*arvalis* and *socialis* species groups; Jaarola et al. 2004, Martínková & Moravec 2012).

Although karyological data on voles have been reported for south-western Asia (e.g. Matthey 1952, 1954, Golenishchev et al. 2002, 2003, Arslan & Zima 2014) many uncertainties are left open. Firstly, several species still need to be defined chromosomally and the most notable example is the enigmatic *Microtus irani* Thomas, 1912 (Zima et al. 2013). Secondly,

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more information is needed from southern range borders for widespread voles to define their taxonomic and geographic scope.

Material and Methods

Twelve *Microtus* voles were collected in 2010-2013 from ten localities in Iran (Fig. 1, Table 1). Specimens were classified to a species on the basis of traditional morphological characteristics (Corbet 1978, Kryštufek & Vohralík 2005, Darvish et al. 2006). Vouchers were prepared as standard museum specimens and deposited in the Zoological Museum of Ferdowsi, University of Mashhad, Iran (ZMFUM). Chromosomes were prepared directly from the bone marrow of colchicine-treated animals following a slightly modified method by Ford & Hamerton (1956).

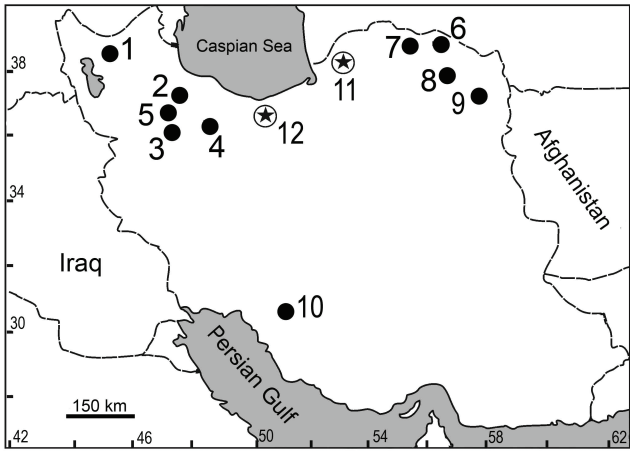


Fig. 1. Localities of the *Microtus* species karyotyped in present study (dots). For numbers see Table 1. Stars denote the type locality for *Microtus mystacinus* (12) and the eastern-most occurrence for this vole (11).

Five slides were prepared from each individual and at least ten well spread metaphase plates were photographed to determine the diploid chromosome number (2n) and the autosomal fundamental number (NFa). The karyograms were prepared by the Chromosome Image Processing (CIP) software created at the ZMFUM.

Results

In a sample of 12 males which have been karyotyped, we identified six morphological species with six distinct chromosomal complements. The results are summarized in Table 1 and described subsequently.

Four morphological species were from the lineage of social voles. Two species, the smaller-bodied *Microtus socialis* (Pallas, 1773) and the larger-bodied *M. paradoxus* Ognev & Heptner, 1928, displayed an identical conventionally stained karyotypes of 62 acrocentric chromosomes of gradually decreasing size (Fig. 2a, b). Both heterosomes were acrocentric. Karyotypes of two individuals from north-western Iran were identical to that of *M. qazvinensis* Golenishchev, Malikov, Nazari, Vaziri, Sablina & Polyakov, 2003 (2n = 54, NF = 56), which poses no surprise given that one of our individuals originated from Qazvin-Bui'n Zahra, the type locality for the species. All autosomes were acrocentric and both heterosomes were bi-armed (Fig. 2e). Two individuals which we karyotyped from the type locality of *M. irani* Thomas, 1912, at Shiraz (Baghe-razi, vicinity of Mamasani) appeared morphologically identical but displayed different complements of all-acrocentric chromosomes. Diploid number in one specimen was among the lowest (2n = 48, FNa = 46) reported for social voles, while

Table 1. Karyotypic characteristics of *Microtus* species from Iran. For details on localities see Fig. 1. N – number of individuals karyotyped (all were males); 2n – diploid number of chromosomes; NFa – number of autosomal arms; A – number of acrocentric chromosomes; A and SM/M – acrocentric and submetacentric/metacentric heterosomes (X and Y), respectively.

Species	N	No on map	Latitude	Longitude	Locality	2n	FNa	A	X	Y
M. socialis	1	1	36°47′04″	48°31′26″	Zanjan	62	60	62	A	A
	1	2	38°21′07″	45°47′46″	Tabriz	62	60	62	A	A
M. qazvinensis	1	3	35°46′20″	48°29′57″	Qeydar	54	54	52	SM	M
	1	4	35°49′30″	50°09′01″	Qazvin-Bui'n Zahra	54	54	52	SM	M
M. mystacinus	2	5	36°22′46″	48°44′11″	Zanjan-Soltanieh	54	54	51	SM	A
M. paradoxus	1	6	37°23′41″	58°52′32″	Tandoreh	62	60	62	A	A
	1	7	37°38′04″	57°46′25″	Shirvan	62	60	62	A	A
M. transcapicus	1	8	36°38′36″	59°6′57″	Chenaran	52	52	54	A	A
	1	9	35°95′77″	60°1′38″	Fariman	52	52	54	A	A
M. irani	1	10	29°58′18″	51°46′40″	Shiraz	48	46	48	A	A
	1						62	64	A	A

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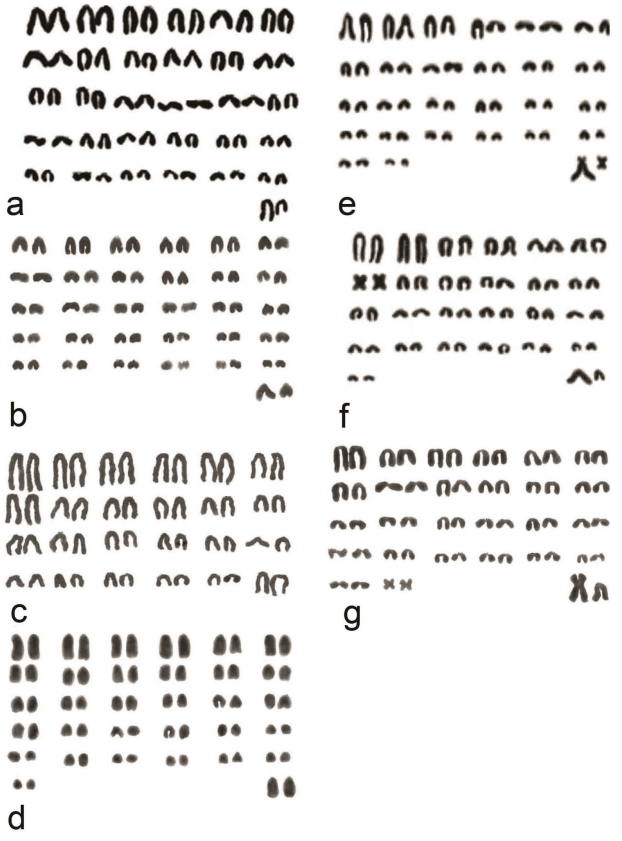


Fig. 2. Karyograms of *Microtus* species from Iran and analysed in this study: a – *M. socialis*, b – *M. paradoxus*, c, d – *M. irani*, e – *M. qazvinensis*, f – *M. transcaspicus*, g – *M. mystacinus*.

the other individual displayed the highest diploid number (2n = 64, NFa = 62; Fig. 2c, d) recorded so far in this group of voles (cf. Zima et al. 2013).

From the lineage of *Microtus* sensu stricto we studied two species. *Microtus transcaspicus* Satunin, 1905, was easily recognizable in our material by its large size and by a unique diploid number of 52 chromosomes. The autosomal complement comprised single metacentric pair of medium size and 24 acrocentric pair. Both heterosomes were acrocentric (Fig. 2f). On the other hand, voles from Zanjan province showed a karyotype of 54 chromosomes. With the exception of a small metacentric pair, all the autosomes were acrocentric (Fig. 2g). Such a karyotype is typical of *M. rossiaemeridionalis* Ognev, 1924. Heterosomes were dissimilar in our sample with a large sub-metacentric X chromosome and a medium-sized acrocentric Y chromosome.

Discussion

Karyotypes of all six species of the genus *Microtus* examined in this study have already been published (Mohammadi et al. 2013, Zima et al. 2013, Arslan & Zima 2014) and our results were congruent with the available information in the majority of cases. This is particularly true for *M. qazvinensis* which was karyotyped by Golenishchev et al. (2003). *Microtus qazvinensis*, known so far only from Iran, has an identical diploid count as *M. guentheri* (Danford & Alston, 1880) (Zima et al. 2013, Arslan & Zima 2014), a vole which is unknown from Iran (Karami et al. 2008). Despite this resemblance, recent molecular work placed *M. qazvinensis* close to *M. dogramacii* Kefelioğlu & Kryštufek, 1999, an Anatolian endemic with 48 chromosomes (Golenishchev & Abramson 2011, Mahmoudi et al. 2015). In line with published data, *M. socialis* and *M. paradoxus* displayed identical standard karyotypes. The former has been karyotyped in various parts of its extensive range, including Iran (see review in Zima et al. 2013), and chromosomal data for the latter were provided by Zykov & Zagorodnyuk (1988), Golenishchev et al. (2002) and Mohammadi et al. (2013). Because of relatively low karyological variability in social voles, the data on conventional chromosomes often contribute little to individual species recognition (Zima et al. 2013). Evidence on species distinction of *M. socialis* and *M. paradoxus* stems from morphology (Zykov & Zagorodnyuk 1988) and from cytochrome *b* nucleotide sequences (Kryštufek et al. 2012). These two voles however produced fertile F1 hybrids under laboratory condition (Golenishchev & Abramson 2011, Golenishchev & Malikov 2011).

Karyotyping two individuals from the type locality of *M. irani* provided very different standard karyotypes (2n = 48 and 64). The two karyotyped individuals were indistinguishable morphologically and also genetically (our unpublished results). At least five distinct cytotypes have so far been ascribed to *M. irani*, ranging in numbers between 46 and 64 (reviewed in Zima et al. 2013) and the taxonomic scope of *M. irani* remains puzzling. *Microtus irani karamani* Kryštufek, Vohralík, Zima, Koubínová & Bužan, 2010, from Turkey, Lebanon and Iran (Kryštufek et al. 2010, 2013, Mahmoudi et al. 2014) displays different diploid count (2n = 60; Kryštufek et al. 2010) and it is possibly closely related to or identical with *M. schidlovskii* Argyropulo, 1933 (Golenishchev & Abramson 2011, Golenishchev & Malikov 2011, Zorenko et al. 2014). Next, the 62-chromosome voles from Shiraz, reported by Golenishchev et al. (1999), possibly present *M. socialis* which is known from Shiraz (Kryštufek & Kefelioğlu 2001). Our results on the one hand confirm an early report of 2n = 64 for *M. irani* (Matthey 1954), the reliability of which has always been

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under doubt. The lower number (2n = 48) is much more puzzling. Chromosomal polymorphism in *M. irani* is not likely explanation because of profound differences in fundamental numbers. Karyotype of 2n = 48 is unique to *M. dogramacii*, but this vole is not closely related to *M. irani* (Kryštufek et al. 2009). In lack of banded chromosomes, we cannot draw any conclusions on putative homologies between the two divergent karyotypes. Clearly, further karyotyping of social voles from Shiraz is indispensable for final answer on a long dispute of arguments over the chromosomal identity of *M. irani*.

The karyotype of *M. transcaspicus*, which we examined from the southern edge of its distribution range, was congruent with previous studies (Meyer et al. 1985, Mazurok et al. 1996). Chromosomal complement of *M. rossiaemeridionalis* was reported under different taxonomic names (*subarvalis*, *epiroticus*) from various parts of its extensive range (reviewed in Zima & Král 1984, Arslan & Zima 2014), including Iran (Mohammadi et al. 2013). Our material differed from published evidence in a submetacentric X chromosome (normally acrocentric; Arslan & Zima 2014). Besides, Mohammadi et al. (2013) report lower fundamental number of autosomal arms (NFa = 52) which may be a genuine property of the local population, but can equally well reflect differences in classifying chromosomes as either acrocentric or bi-armed.

Our material of *M. rossiaemeridionalis* originates 260 km west of the type locality for *Arvicola mystacinus* De Filippi, 1865, and Mohammadi et al. (2013) identified additional 2n = 54 voles from the easternmost Elbruz Mts. (locality 11 in Fig. 1). Sufficient amount of information is therefore available to disentangle a long-disputed issue on the nomenclature of a 54-chromosome cryptic species which since its discovery (Meyer et al. 1969) has been reported under at least four different names: *M. subarvalis* Meyer, Orlov & Skholl, 1969, *M. epiroticus* Ondrias, 1966, *M. rossiaemeridionalis* Ognev, 1924, and *M. levis* Miller, 1908. Of these names, *M. subarvalis* Meyer, Orlov & Skholl, 1969, is preoccupied by *M. subarvalis* Heller, 1933, from the Pleistocene of Germany and is therefore *nomen preoccupatum* (Corbet 1978). The identity of *M. levis* is not indisputably solved. While some authors (e.g. Musser & Carleton 2005) believe it to represent the 2n = 54 cryptic species, others (Kryštufek & Vohralík 2005, Pavlinov & Lissovsky 2012) retain *M. levis* within the scope of *M. arvalis*.

Already Petrov & Ružić (1982) argued that *mystacinus* might be the oldest available name for this species. Two vouchers from "Lar Valley" are preserved in the Natural History Museum of London (formerly British Museum of Natural History) and reported as "Co-types" of *mystacinus* in Ellerman & Morrison-Scott (1951). Material was exchanged with the "Turin Museum" (evidently the Museo regionale di scienze naturali in Turin where De Filippi served as a Director), consisting of carcasses in alcohol with skulls extracted. Both crania are badly damaged and lack mandibles. Ellerman & Morrison-Scott (1951) synonymized *mystacinus* with *M. arvalis* (Pallas, 1778) (as understood in their time) evidently on the basis of the two vouchers from Turin. We examined these specimens and concur with the taxonomic conclusion by Ellerman & Morrison-Scott (1951) about the species group *arvalis*. Relying on the taxonomy of Musser & Carleton (2005), *mystacinus* can represent either *M. arvalis* or *M. levis* (*M. obscurus* and *M. rossiaemeridionallis* sensu Kryštufek & Vohralík 2005). *Microtus arvalis* (Pallas, 1778) is restricted only to the extreme north-western provinces of Iran while the range of *M. rossiaemeridionalis* extends further east along the southern shore of the Caspian Sea (Shenbrot & Krasnov 2005, Mohammadi et al. 2013) and therefore encompasses also the type locality of *mystacinus*. Under the Principle of Priority, the valid name of a taxon is the oldest available name applied to it (Article 23 of the International Code of Zoological Nomenclature, 4th edition). Thus, the valid name for the 54-chromosome voles is *Microtus mystacinus* (De Filippi, 1865), which clearly predates all the above names (*subarvalis*, *epiroticus*, *rossiaemeridionalis*, and *levis*). The list of synonyms of *M. mystacinus* is available, under *M. levis*, in Musser & Carleton (2005). The type locality of *M. mystacinus* is "Lar Valley, Northern Persia" (= Iran) (Ellerman & Morrison-Scott 1951) "about 64 km northeast of Tehran" (Lay 1967). For the species range see, under *M. rossiaemeridionalis*, Shenbrot & Krasnov (2005). We stress however that karyotyping of animals from the type locality of *A. mystacinus* will provide the definitive answer on the nomenclature of 54-chromosome voles.

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