ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg., 72 (2), June 2020: 193-198

Published online 30 June 2020
http://www.acta-zoologica-bulgarica.eu/002334

Systematics and Phylogenetics
Research Article

Cytogenetic Characteristics of Four *Meriones* spp. (Rodentia: Gerbillinae) from Turkey and Iran

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This article is dedicated to the memory of our colleague Professor Jan Zima, who passed away shortly before the finalisation of the present work

Abstract: The karyotypes of four species of jirds of the genus *Meriones* Illiger, 1811 from Turkey and Iran (*M. tristrami, M. persicus, M. vinogradovi* and *M. libycus*) were studied using C-banding and AgNOR staining. The obtained basic karyotype characteristics of the studied species are in agreement with previously published data. Intra-specific variation in the number of autosomal arms was recorded in *M. tristrami*. Extensive variations were recorded in the number or localisation of active NOR sites between species and within species. The cytogenetic markers, such as the positive C-bands or NORs, seem to have only limited importance in inter-specific differentiation but they may be applied in studies of the relationships between geographic populations within species.

Key words: Karyotype, jirds, C-banding, Ag-NOR staining, Middle East

Introduction

Gerbils and jirds are currently classified as the subfamily Gerbillinae within the speciose rodent family Muridae (Wilson et al. 2017, Burgin et al. 2018). The molecular phylogeny of these rodents has been recently discussed by Alhajeri et al. (2015). The species of the subfamily Gerbillinae are widespread in steppe and semi-arid areas of the Palaearctic and have been successfully used in various research areas as laboratory models (e.g. Radtke-Schuller et al. 2016, Quintar et al. 2017). Gerbils have also been frequently considered in cytogenetic studies (e.g. de la Fuente et al. 2014, Iwasa et al. 2016). Chromosomal variation appears to be an important marker for phylogenetic studies within this group (e.g. Viegas-Péquinot et al. 1982, Qumsiyeh 1988, Wahrman et al. 1988).

We describe the karyotypes of four species of jirds of the genus *Meriones* from Turkey and Iran: *M. tristrami* Thomas, 1892, *M. persicus* (Blanford, 1875), *M. vinogradovi* Heptner, 1931 and *M. liby-*

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cus Lichtenstein, 1823. The jirds of *Meriones* may be amongst the major natural reservoirs of plague and other infections in the Middle East and are distributed in both endemic and non-endemic disease areas in Iran (Mostafavi et al. 2017, Assmar et al. 2018). This important role in epidemiological threats enhances the necessity of gaining knowledge of the systematics and phylogenetics of these rodents in the respective region. Several studies using morphometric and allozymic or molecular variation have been conducted in this respect recently (Yazdi & Adriens 2013, Darvish 2011, Yiğit et al. 2013, 2016, Dianat et al. 2017). A useful marker in systematic studies is the characteristics of the chromosomal complement. There are numerous karyotypic studies on the abovementioned four species and they have revealed substantial variation between species and populations (for review see Arslan & Zima 2014).

The aim of the present study is to explore and precise the distinct pattern of karyotypic variation between and within the studied species using C-banding and AgNOR staining, which is the first study for Iranian species of *Meriones*.

Materials and Methods

Altogether, we examined 14 specimens of four species of jirds collected in Turkey and Iran during 2017–2018. The number of analysed specimens and the location of the collection sites are shown in Fig. 1 and Table 1. The specimens were caught using live traps. Standard voucher specimens (skins, skulls and tissues) are deposited in the Mohammad Hanifi's Health Museum, Akanlu, Hamadan in Iran (MHHM) and in the Zoological Museum of Selçuk University, Turkey (ZMSU). Karyotype preparations were obtained from bone marrow of animals treated with colchicine (Ford & Hamerton 1956). From each specimen, 10 to 20 slides were prepared and at least 20 well-spread metaphase plates were analysed. After preparation of chromosome slides, conventional Giemsa-staining was carried out. Constitutive heterochromatin and

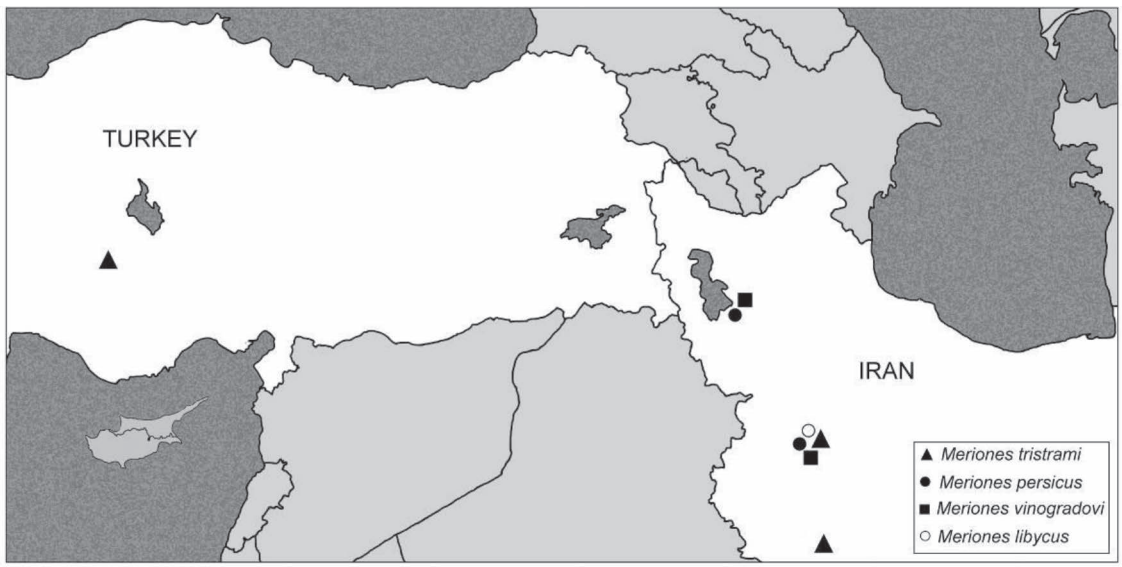

Fig. 1. Collecting sites of species of *Meriones* in Turkey and Iran. The symbol of sampling localities corresponds to data in Table 1.

Table 1. Species of the genus *Meriones* and localities studied in Turkey and Iran. The symbol (designation) of the sampling sites corresponds to those in Fig. 1. Abbreviations: Sm – submetacentric, M – metacentric, A – acrocentric.

Symbol	Species	Locality and province	Coordinates	♂	♀	2n	NFa	NF	X	Y
▲	*Meriones tristrami*	Selçuklu, Konya,Turkey	38°02'N/32°27'E	3	3	72	80	84	Sm	m
▲	*Meriones tristrami*	Akanlu, Hamedan, Iran Dorod, Lorestan, Iran	35°38'N/48°06'E 33°28'N/49°10'E	-	1	72	84	88	Sm	-
■	*Meriones vinogradovi*	Akanlu, Hamedan, Iran Maragha, E Azerbaijan, Iran	35°38'N/48°06'E 37°20'N/46°20'E	-	2	44	74	78	M	-
○	*Meriones libycus*	Akanlu, Hamedan, Iran	35°38'N/48°06'E	1	1	44	72	76	A	A
●	*Meriones persicus*	Akanlu, Hamedan, Iran Bonab, E Azerbaijan, Iran	35°38'N/48°06'E 37°24'N/46°01'E	1	2	42	74	78	Sm	Sm

nucleolus organizer regions (NORs) were detected in individual autosomal and sex chromosome pairs via C-banding (Sumner 1972) and Ag-NOR staining (Howell & Black 1980), respectively. We applied the accepted (conventional) system of classification of chromosomes according to the centromere position after Hsu & Benirschke (1967–1977). The fundamental number of autosomal arms (NFa) and the number of all chromosomal arms in the female complement (NF) were calculated.

All procedures performed in this study, involving capturing and euthanizing of the animals, were in accordance with international ethical standards. The institutional animal and human ethical committee of the Pasteur Institute of Iran approved the project based on the national and international standards (Code: IR.PII.REC.1395.9).

Results

Meriones tristrami

We found a diploid number of 72 chromosomes in all examined individuals. The autosomes were either bi-armed or acrocentric in respect of the centromere position, while the proportion of both morphological types varied between the specimens examined in Turkey and Iran. The complement of the Turkish specimens comprised two metacentric and three submetacentric autosomal pairs, whereas one metacentric, two submetacentric and four subtelocentric were found in the complement of the Iranian specimens from both study sites. The fundamental number of autosomal arms varied correspondingly (NFa=80 in Turkey, NFa=84 in Iran). No variation was observed in the morphology of the sex chromosomes. The X chromosome was submetacentric and the largest in the set. The Y chromosome was metacentric and its size was comparable to that of the second or third bi-armed autosomal pairs (Fig. 2).

C-banding revealed distinct positive blocks of C-heterochromatin in all pairs of bi-armed autosomes. These blocks were in the pericentromeric areas and/or they formed the whole C-heterochromatic arms. Except for some acrocentric autosomes which had not heterochromatin blocks, in most of them only slight centromeric dark C-bands were observed. The positive C-banding was indistinct in the sex chromosomes (Fig. 3).

The presence of the NOR signals was recorded in the telomeres of four bi-armed pairs in the complement of the Turkish specimens, while five more AgNOR positive sites were recorded in the complement of the Iranian specimens. The additional AgNOR sites were observed in one of the bi-armed

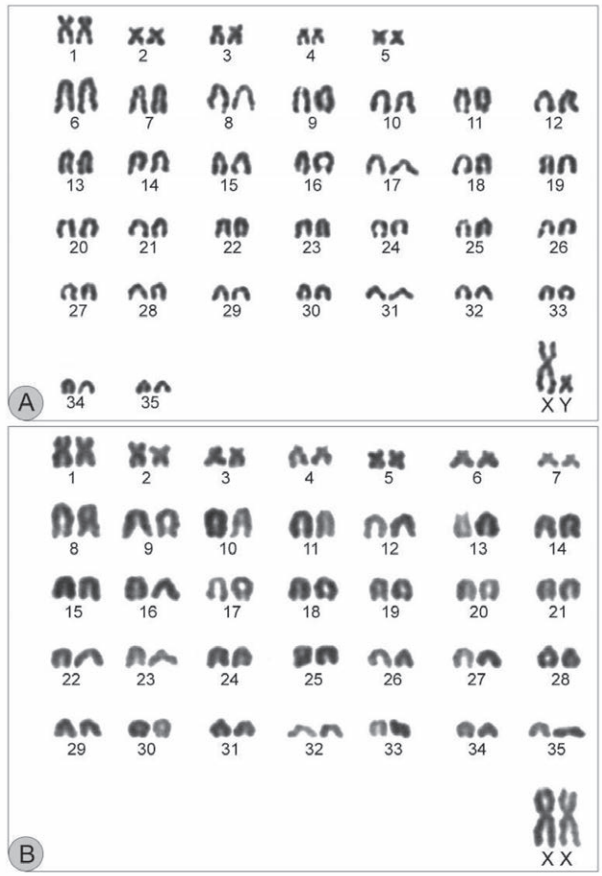

Fig. 2. Standard karyotype of *Meriones tristrami* from Turkey (A) and Iran (B).

autosomal pairs and in four acrocentric autosomal pairs. Altogether, nine AgNOR positive sites were recorded in the Iranian populations (Fig. 4).

Meriones vinogradovi

The chromosome diploid number found in the complement of the two females from Iran was 2n=44. The karyotype comprised 16 bi-armed and five acrocentric autosomal pairs (NFa=74). The X chromosome was identified tentatively as the largest bi-armed autosome in the set (Fig. 5A).

C-positive heterochromatin occurred in the pericentromeric areas of most autosomes and the short arms of all the acrocentric autosomes seemed to be completely C-heterochromatic, except for chromosome number 3 and 13. The C-banding pattern was only indistinct in the X chromosomes (Fig. 6A).

The positive AgNORs signals were revealed in the telomeric area of the short arm of two submetacentric (nos. 4, 7 and 16) and all the acrocentric autosomal pairs (except for 21 chromosomal pair; Fig. 7A).

Meriones libycus

The complement of both examined specimens included 44 chromosomes that could be differentiated

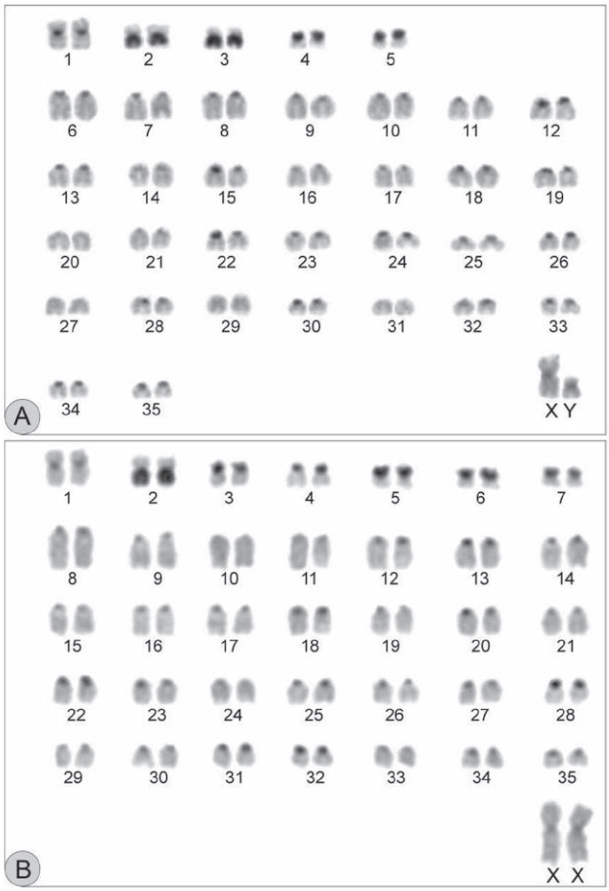

Fig. 3. C-banded karyotype of *Meriones tristrami* from Turkey (A) and Iran (B).

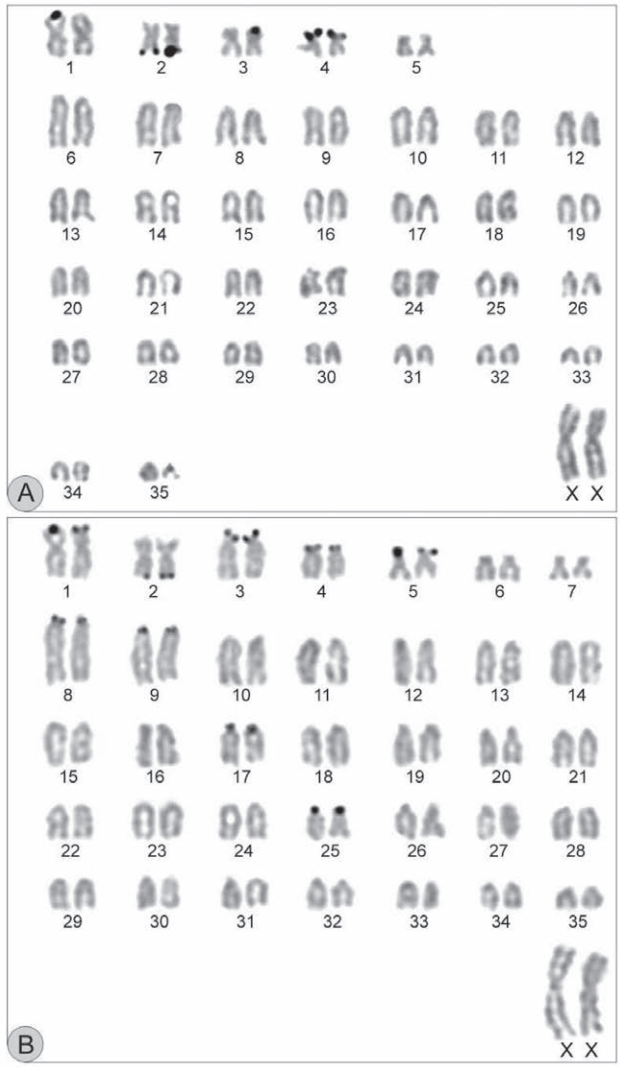

Fig. 4. Silver stained karyotype of *Meriones tristrami* from Turkey (A) and Iran (B).

into 15 bi-armed and six acrocentric autosomal pairs (NFa=72). The X chromosome was a medium-sized acrocentric with distinct short arms and its size was comparable to the autosomes nos. 2-4. The Y chromosome was a small acrocentric (Fig. 5B).

Distinct dark C-bands were found in the centromeric regions of all bi-armed autosomes and the acrocentric autosomes possessed C-heterochromatic short arms. The chromosome pair 14 was entirely heterochromatic. The centromeric area and the short arms of the X chromosome were stained positively in C-banding, whereas the whole Y chromosome was C-positive (Fig. 6B).

The AgNOR staining regions were observed in the short arms on five acrocentric autosomal pairs. The only acrocentric pair lacking the positive AgNOR staining was the no. 19. A positive AgNOR signal was only detected on bi-armed 4th autosome (Fig. 7B).

Meriones persicus

The complement of the specimens from both sites examined in Iran comprised 42 chromosomes. In the autosomal set, 17 pairs were bi-armed and three pairs acrocentric (NFa=74). The X chromosome was large submetacentric, while the Y chromosome was small submetacentric (Fig. 5C).

C-positive blocks of heterochromatin were detected in the centromeric areas of most autosomal pairs but no C-heterochromatic whole arms were recorded. The positive C-banding was only indistinct in the sex chromosomes (Fig. 6C).

The AgNOR positive sites were observed in the short arm of the three acrocentric autosomal pairs and in the telomeric area of the short arm of the bi-armed autosome no. 14 (Fig. 7C).

Discussion

The karyotype characteristics established in the individual species we studied are in agreement with hitherto published data (Kaya & Coskun 2012, for

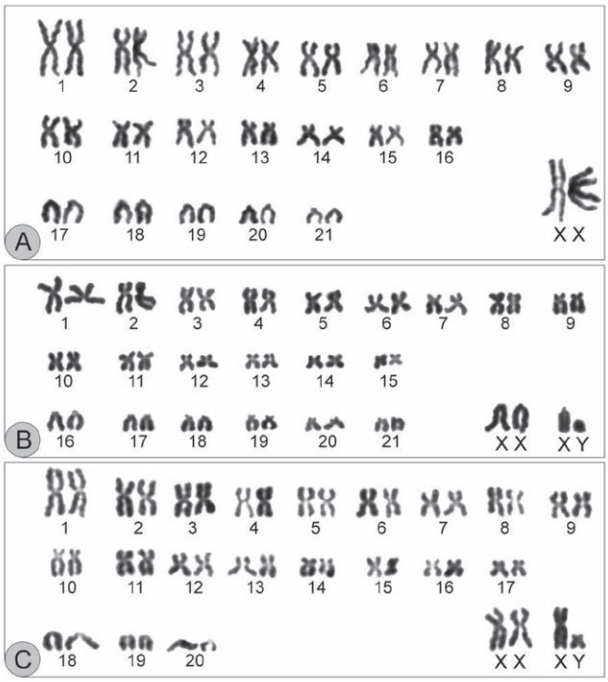

Fig. 5. Standard karyotype of *Meriones vinogrdovi* (A), *Meriones libycus* (B) *Meriones persicus* (C) from Iran.

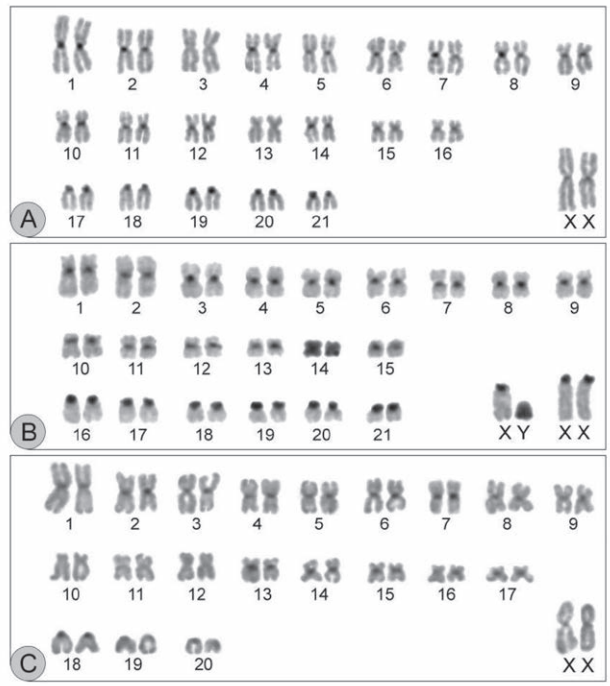

Fig. 6. C-banded karyotype of *Meriones vinogrdovi* (A), *Meriones libycus* (B) *Meriones persicus* (C) from Iran.

review see Arslan & Zima 2014). Intra-specific variation in the number of autosomal arms was previously reported in *M. tristrami* (NFa=76-82) but the number of bi-armed autosomes observed in the specimens from Iran (NFa=84) seems to be the highest ever reported.

Recent molecular studies of the four species of *Meriones* under study (Alhajeri et al. 2015) suggested that *M. persicus*, the species classified in a separate subgenus (Pavlinov 2008), might represent a basal lineage, whereas *M. libycus* and *M. tristrami* appeared as derived ones. Recently, this separation has been confirmed by molecular and geometric morphometrics studies, which clearly showed the distinct position for *M. persicus* in comparison to other species of *Meriones* (Dianat 2017). *Meriones vinogradovi* is considered closer phylogenetically to *M. libycus* and *M. tristrami* than to *M. persicus* (Pavlinov 2008, but see Yiğit et al. 2013) However, Dianat (2017) has recently placed it into a sister clade of the clade comprising *M. shawi*, *M. grandis*, *M. tristrami*, *M. crasus*, *M. rex* and *M. libycus*. This topology suggests that chromosome divergence between the four examined species proceeded in the direction of increasing the diploid number, possibly through chromosomal fissions. A slight increase in the number of autosomal arms could also be assumed. The acrocentric X chromosome in the complement of *M. libycus* could be considered as an autapomorphic feature of this species.

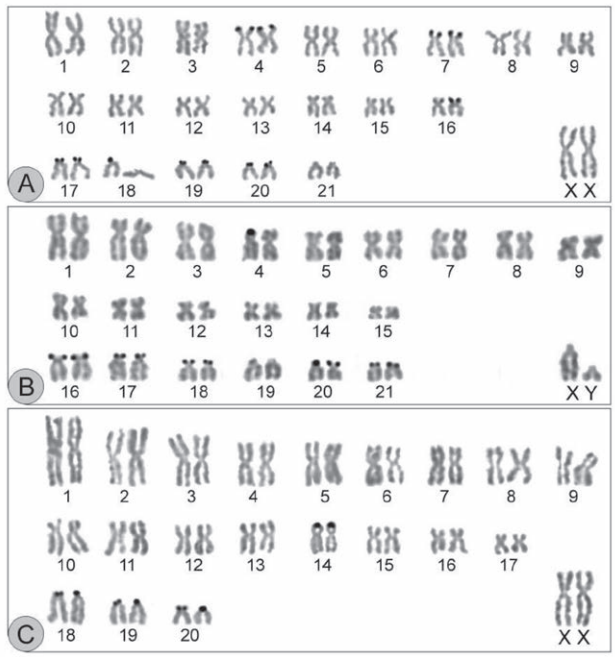

Fig. 7. Silver stained karyotype of *Meriones vinogrdovi* (A), *Meriones libycus* (B) *Meriones persicus* (C) from Iran

Variable number of C-positive blocks of heterochromatin was previously reported in *M. tristrami* from Transcaucasia (Korobitsyna & Korablev 1980) and our results confirmed that this variation is widespread over the species range. Similar variation may occur also in other jird species (Chelomina et al. 2010) and it has been confirmed by polytypy re-

corded in *M. libycus* in this study. The number of NOR sites seems to be highly variable in the jirds of the genus *Meriones*. Aşan et al. (2010) reported in *M. tristrami* from Turkey that NORs occurred in telomeric areas of three metacentric and seven acrocentric autosomal pairs. Extensive variation in the number and localisation of the NORs prevents the use of these markers in inter-specific comparisons but they may be used in studies of the relationships between geographical populations within a species.

Acknowledgements: We are grateful for the financial support of the Pasteur Institute of Iran and Centre for Disease Control of the Iranian Ministry of Health and Medical Education (Grant no. 810).

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Received: 12.01.2019
Accepted: 02.03.2019