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A New Cytotype of *Meriones tristrami* Thomas, 1892 (Rodentia: Gerbillinae) and its Nucleolar Organizer Regions (NORs) from Batman, South-eastern Turkey

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Abstract: The karyotype and Ag-NORs of *Meriones tristrami* Thomas, 1892 from Batman, South-eastern Anatolia, are described in order to reveal chromosomal differences in these specimens and to compare them with other cytotypes (NF=76, 78, 80, 82, 84 and 86). The results showed a diploid chromosome set (2*n*) consisting of 72 chromosomes. The chromosome types (metacentric, submetacentric and acrocentric) were 5, 4 and 27, respectively. Thus, the present study reveals the highest number of biarmed chromosome pairs recorded from Turkey. Active NORs were found on five autosomal pairs (nos. 1, 2, 4, 28 and 29) in specimens from Batman. In some cells, only one homologue of the pairs 4, 28 and 29 exhibit a positive silver signal. The karyotypes of *M. tristrami* specimens from the region of Batman are different from those of other populations from Anatolia.

Key words: karyotype, chromosome, morphology, distribution, Anatolia

Introduction

The genus *Meriones* Illiger, 1811 is adapted to the semiarid steppe in the Palaearctic region; in Turkey, it is represented by five species (Yiğit et al. 1997, 1998, 2006, 2018, Yiğit & Çolak 1998, Wilson & Reeder 2005, Kryštufek & Vohralik 2009). Four species from Turkey, i.e. *M. tristrami* Thomas, 1892, *M. dahli* Shidlovsky, 1962, *M. crassus* Sundevall, 1842 and *M. vinogradovi* Heptner, 1931, belong to the subgenus *Parameriones* Heptner, 1937. The fifth species, *M. persicus* (Blanford, 1875), is a member of the subgenus *Pallasiomys* Heptner, 1933. Considering their distribution in Anatolia, the most widespread species occurring in different habitats is *M. tristrami* (Yiğit et al. 2018). In addition to Turkey, the Tristram's jird *M. tristrami* occurs in the Middle East and Transcaucasia (Musser & Carleton 2005). Its karyotype was found to be 2n=72 in all the regions of its distribution (Neuhauser 1936, Benezzou et al. 1982, Qumsiyeh et al. 1986, Wahrman et al. 1988, Kefelioğlu 1997, Yiğit et al. 1997, 1998, 2006, 2018, Yiğit & Çolak 1998, Coşkun 1999, Kryštufek & Vohralik 2009, Aşan et al. 2010, Kaya & Coşkun 2012, Mahmoudi et al. 2020). *Meriones tristrami* is represented by six subspecies occurring in Turkey: *M. t. blackleri*, *M. t. intraponticus*, *M. t. lycaon*, *M. t. kilisensis*, *M. t. bodenheimeri* and *M. t. bogdanovi* (Yiğit et al. 1998, 2006). Results on their morphology, karyology and distribution have been reported by previous publications (Neuhauser 1936, Kefelioğlu 1997, Yiğit et al. 1997, 1998, 2006, 2018, Yiğit & Çolak 1998, Coşkun 1999, Aşan et al. 2010, Kaya & Coşkun 2012). While the diploid metaphase complement is 72 for *M. tristrami* in Turkey, karyological analyses have differentiated seven chromosomal forms from Turkey, with NF=76–86 (Yiğit 1995, Kefelioğlu 1997, Yiğit et al. 1998, 2006, Yiğit & Çolak 1998, Ulutürk 2002, Aşan et al. 2010, Kaya

& Coşkun 2012, Mahmoudi et al. 2020). Yiğit & Çolak (1998) proposed *M. t. kilisensis* as a new subspecies, which has a karyotype of 2n=72, NF=78 and NFa=74. Yiğit et al. (2006) reported that the Doğubeyazıt population has a karyotype of 2n=72, NF=84, and the sex chromosomes were acrocentric, thus differing from other chromosomal forms, which had metacentric sex chromosomes. The most widespread NF-value in Turkey is 84 (Kryštufek & Vohralik 2009). The Ag-NORs localisation of this chromosomal form (NF=84) has been studied by Aşan et al. (2010) in Central Anatolia. Yiğit et al. (1998) provided the distribution map of the subspecies of *M. tristrami* in Turkey and reported the fundamental numbers of the three subspecies (*M. t. blackleri*, *M. t. lycaon* and *M. t. intraponticus*) as 72, 76, and 82, respectively. Kaya & Coşkun (2012) reported a new chromosomal form of *M. tristrami* from Southeast Anatolia with NF=86; this population was also reported to have the highest number of biarmed chromosome pairs recorded in Turkey.

The present cytogenetic study on *M. tristrami* species provides a description of the karyotype of the species based on conventional Giemsa and Ag-NOR banding. These data will increase the cytogenetic information, which can be used as a basis for a comprehensive examination of the taxonomy and evolutionary relationships of *M. tristrami* and other gerbils distributed throughout Anatolia.

Materials and Methods

Four animals were captured from two localities of Batman, i.e. İkiköprü Village (1♂ and 1♀; 37S 704986 4205496) and West Raman Province (2♀, 37S 682402 4183115) (Fig. 1). Species identification was made according to Wilson & Reeder (2005), Kryštufek & Vohralik (2009) and Yiğit et al. (1998). The karyotype and nucleolar organizer regions (NORs) localization analyses were performed in all specimens. After being injected intramuscularly with 0.4 % colchicine solution, the specimens were karyotyped using bone marrow as described by Lee & Elder (1980). The preparations were stained conventionally with Giemsa. The diploid number (2n), fundamental number (NF) and autosomal number (NFa) along with the shapes of autosomes and sex chromosomes were also determined. Definition of the chromosome shapes was established according to Levan et al. (1964). Ag-NORs localizations were subjected by the method of Howell & Black (1980). From each specimen, 10 to 20 slides were prepared and at least 10 well-spread metaphase plates were analysed for conventional karyotypes and the assessment of NOR-bearing chromosomes. The stuffed skins and metaphase slides of the examined specimens are deposited at the Batman University, Department of Biology.

Fig. 1. Sampling localities of *Meriones tristrami* from Batman, Turkey. 1. İkiköprü Village (1♂, 1♀; 37S 704986 4205496), 2. West Raman Province (2♀, 37S 682402 4183115).

Results

In the karyotype of *M. tristrami* from Batman specimens, the diploid number of chromosomes (2n) is 72, the fundamental number (NF) is 90 and the number of autosomal arms (NFa) is 86 in all the individuals studied (Fig. 2). The diploid metaphase complement contained 72 chromosomes in both male and female specimens comprising 5 pairs of metacentric (nos. 1-4, X and Y), 4 pairs of submetacentric (nos. 5-8) and 27 pairs of acrocentric (nos. 9-35) chromosomes. While X is the largest metacentric, Y is the smallest metacentric of the chromosome set. Chromosome morphology of *M. tristrami* was determined (Table 1). The active NORs were found on three metacentric (nos. 1, 2 and 4) and two acrocentric (nos. 28 and 29) autosomal pairs in Batman specimens; in some cells, only one homologue of the pairs 4, 28 and 29 bore the positive Ag-NOR signal (Fig. 3). While the AgNORs were observed in the telomeric regions of the short arms of the autosomes 1 and 2, it is detected in the centromeric region of the autosome 4.

Discussion

M. tristrami, including six subspecies occurring in Anatolia, has a constant diploid chromosome number 2n=72 in all areas where it is distributed. Although the diploid chromosome number of this spe-

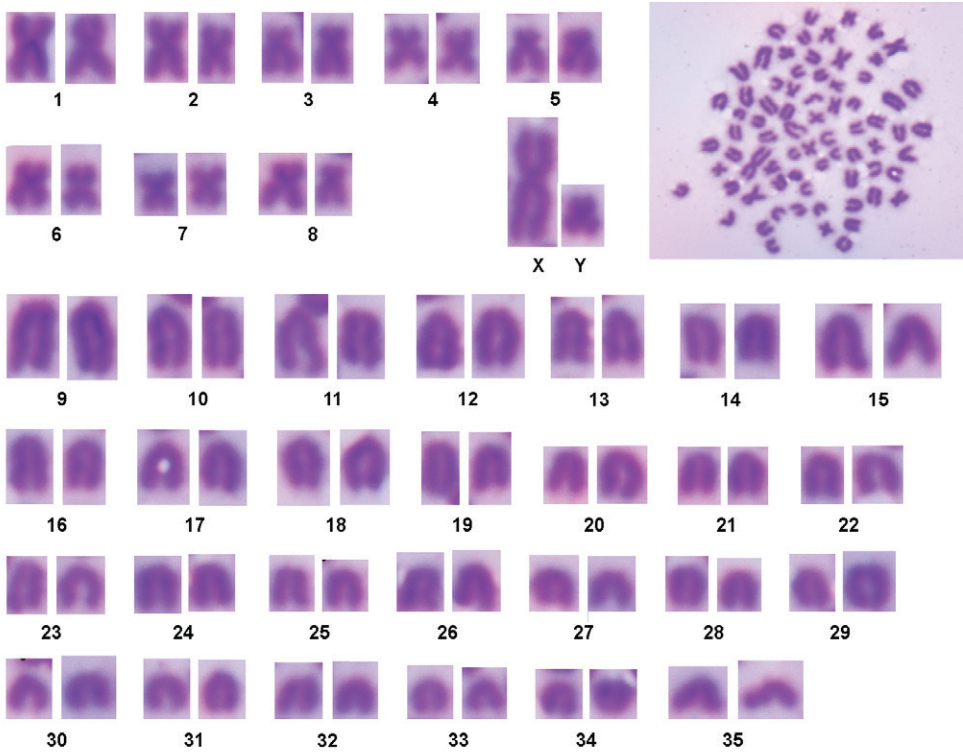

Fig. 2. Metaphase plate and karyotype of *Meriones tristrami* from Batman, Turkey.

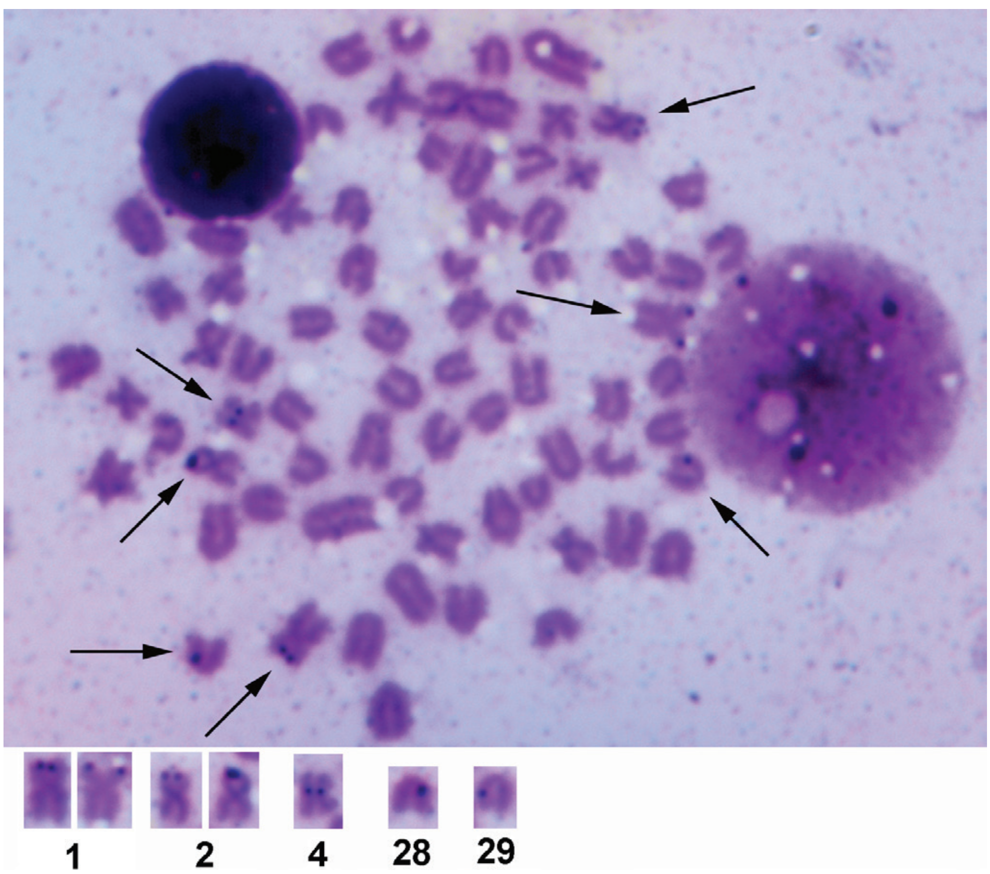

Fig. 3. Silver-stained karyotype of *Meriones tristrami* from Batman, Turkey.

Table 1. Chromosome classification (µm) of *Meriones tristrami* from Batman, Turkey (m: metacentric, sm: submetacentric, a: acrocentric).

No	Short arm (p)	Long arm (q)	Total length	Arm ratio (q/p)	Relative length	Centromere index	Centromeric position
1	2.75	3.30	6.05	1.20	3.05	2.20	m
2	2.39	2.92	5.31	1.22	2.68	2.22	m
3	1.72	2.20	3.91	1.28	1.97	2.28	m
4	3.00	4.46	7.46	1.49	3.76	2.49	m
5	2.07	4.06	6.13	1.96	3.09	2.96	sm
6	1.98	3.50	5.48	1.77	2.77	2.77	sm
7	1.84	3.66	5.50	1.99	2.78	2.99	sm
8	1.65	2.79	4.44	1.69	2.24	2.69	sm
9	-	8.61	8.61	-	4.34	-	a
10	-	6.66	6.66	-	3.36	-	a
11	-	6.39	6.39	-	3.22	-	a
12	-	6.29	6.29	-	3.17	-	a
13	-	6.24	6.24	-	3.15	-	a
14	-	6.20	6.20	-	3.13	-	a
15	-	6.09	6.09	-	3.07	-	a
16	-	5.87	5.87	-	2.96	-	a
17	-	5.58	5.58	-	2.81	-	a
18	-	5.49	5.49	-	2.77	-	a
19	-	5.42	5.42	-	2.73	-	a
20	-	5.29	5.29	-	2.67	-	a
21	-	5.04	5.04	-	2.54	-	a
22	-	4.86	4.86	-	2.45	-	a
23	-	4.80	4.80	-	2.42	-	a
24	-	4.72	4.72	-	2.38	-	a
25	-	4.66	4.66	-	2.35	-	a
26	-	4.63	4.63	-	2.34	-	a
27	-	4.61	4.61	-	2.32	-	a
28	-	4.43	4.43	-	2.24	-	a
29	-	4.32	4.32	-	2.18	-	a
30	-	4.12	4.12	-	2.08	-	a
31	-	3.97	3.97	-	2.00	-	a
32	-	3.87	3.87	-	1.95	-	a
33	-	3.68	3.68	-	1.86	-	a
34	-	3.56	3.56	-	1.79	-	a
35	-	3.26	3.26	-	1.65	-	a
X	5.43	5.67	11.09	1.04	5.60	2.04	m
Y	1.90	1.99	3.89	1.05	1.96	2.05	m

cies is constant, there are differences in the number of chromosomal arms (NF) that ranged between 76 and 86 (Yiğit 1995, Kefelioğlu 1997, Yiğit et al. 1998, 2006, 2018, Yiğit & Çolak 1998, Ulutürk 2002, Aşan et al. 2010, Kaya & Coşkun 2012, Mahmoudi et al. 2020). To date, seven chromosomal races of *M. tristrami* were defined in Turkey (Table 2). In the present study, NF and NFa values (90 and 86) differ from the other chromosomal races but the diploid chromosome number and morphology of the sex chromosomes show similarities with the previous findings except for Doğubeyazıt specimens, in which the sex chromosomes were defined as acrocentric (Yiğit et al. 2006). Kaya & Coşkun (2012) reported that the specimens of *M. tristrami* collected by them from the Southeast Anatolia had the highest number of biarmed chromosome pairs recorded in Turkey (2n=72, NF=86, and NFa=82). However, NF and NFa values found by our study are higher than those from the South-eastern Anatolia.

Karyological studies on the subspecies of *M. tristrami* distributed in Anatolia found the numbers of fundamental and autosomal chromosome arms as follows: 82-78 in *M. t. lycaon*, *M. t. bodenhaimeri* and *M. t. intraponticus*, 78-74 in *M. t. kilisensis* and 76-72 in *M. t. blackleri* (Yiğit et al. 1998, 2006, 2018, Yiğit & Çolak 1998). No data on the karyotype of *M. t. bogdonavi* have been published. As seen from these three subspecies (*M. t. lycaon*, *M. t. bodenhaimeri* and *M. t. intraponticus*) with identical karyotypes, it would not be justified to differentiate subspecies only based on their chromosome morphology. According to the distribution map provided by Yiğit et al. (1998), Batman specimens are from an area within the distribution boundaries of *M. t. bodenhaimeri*; however, the number of chromosomal arms is different from that in *M. t. bodenhaimeri*. Some researchers have stated that the main trend in the evolution of chromosomal variations in rodents is the process of Robertsonian rearrangements, which is associated with changes in the autosome arm numbers explained by pericentric inversions or centromeric shifts (Yüksel 1984, Gülkaç & Yüksel 1989, Nevo et al. 1995, Ivanitskaya et al. 1997, Ivanitskaya & Nevo 1998). By the results of the present study, it can be concluded that NF and NFa variations can be a result of Robertsonian rearrangements. The chromosomal variations in small mammals are a major problem, making it difficult to determine the status of such taxa (Zima 2000, Veyrunes et al. 2004). Arslan et al. (2014) used chromosomal rearrangements as characters to hypothesise on the phylogenetic relationships among chromosomal races in a species. In addition, Dobigny et al. (2004) indicated that chromosomal data have not been used sufficiently in phylogenetic studies and chromosomal changes could be considered as a character. As Zima (2000) has pointed out, such taxa are perfectly suited for the study of general problems of speciation and evolution. Considering that cytogenetic features play a major role in the speciation, we can conclude that revealing chromosomal variations can be used to reconstruct the evolutionary history of these species.

Some researchers believed that Ag-staining cannot be used as a reliable method to identify NORs (Sanchez et al. 1995, Dobigny et al. 2002). Nevertheless, Ag-staining is a widely used technique for assessing the locations of active NORs in mammalian genomes (Dobigny et al. 2002). In our study, the number and location of NOR signals differed from the results of Aşan et al. (2010) and Mahmoudi et al. (2020) based on specimens of *M. tristrami* from Turkey. While Aşan et al. (2010) described that the NOR signals were located in the telomeric regions of three metacentric and seven acrocentric autosomal pairs, Mahmoudi et al. (2020) recorded that Ag-NORs were observed in the telomeres of four biarmed pairs. The fact that it was observed in three metacentric and two acrocentric autosome pairs in our study shows that the numbers and localisations of the active Ag-NORs in *M. tristrami* from Turkey is highly variable. According to Mahmoudi et al. (2020), these variations prevent the use of these markers in inter-specific comparisons but they may be used in studies on relationships between geographical populations within a species. In line with this opinion, we believe that Ag-NOR studies can be used to reveal the relationships between the cytotypes of Turkish *M. tristrami* species.

Table 2. The karyological records of *M. tristrami* defined in Turkey.

2n	NF	NFa	X	Y	References
72	90	86	m	m	Present study
	86	82	m	m	Ulutürk (2002), Kaya & Coşkun (2012)
	84	80	Sm	Sm	Kefelioğlu (1997)
					Aşan et al. (2010)
				m	Mahmoudi et al. (2020)
		82	a	a	Yiğit et al. (2006)
	82	78	Sm	Sm	Yiğit (1998)
	80	76	m	m	Kefelioğlu (1997)
	78	74	Sm	Sm	Yiğit & *Çolak* (1998)
	76	72	m	m	Yiğit (1998)

Finally, it seems that Batman specimens (NFa=86, NF=90) have the highest number of biarmed chromosome pairs in Turkish *M. tristrami* chromosomal forms. Furthermore, Ag-NORs bearing chromosomes of our specimens are differing from those in the previous studies. Because of the higher chromosomal variations found within the Turkish *M. tristrami* populations, it is difficult to relate directly these differences to considerations of their taxonomic significance. However, the present results will contribute to further cytotaxonomic studies on *M. tristrami*. Therefore, the taxonomical problems and distribution borders of these chromosomal races might be solved in the future based on additional studies.

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