Research Article

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Karyotypic Characterization of the Harvest Mouse *Micromys minutus* (Pallas, 1771) (Rodentia: Muridae) from Upper Thracian Valley, South Bulgaria

Tsenka G. Chassovnikarova[1,2*], Nasko I. Atanasov[1], Hristo A. Dimitrov[2] & Vesela I. Mitkovska[2]

[1]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1Tzar Osvoboditel Blvd., 1000 Sofia, Bulgaria
[2]Department of Zoology, Faculty of Biology, Plovdiv University Paisii Hilendarski, 24 Tzar Asen Street, 4000 Plovdiv, Bulgaria

Abstract: This study presents the first cytogenetical studies of the harvest mouse *Micromys minutus* (Pallas, 1771) in Bulgaria. A population of harvest mice from the rice fields near Plovdiv in the Bulgarian part of the Thracian Valley was examined. Five individuals (3 males and 2 females) were cytogenetically analyzed. The diploid chromosome number was found to be 2n = 68. Autosomes consisted of 1 pair of large and 1 pair of small metacentric chromosomes, 2 pairs of middle-sized submetacentric chromosomes, 28 pairs of subtelocentric chromosomes and 1 pair of acrocentric chromosomes. The X chromosome was the largest subtelocetric chromosome, whereas the Y chromosome was found to be the smallest acrocentric chromosome. The FN of the chromosomes was estimated to be 133, as the NFa was 130. All chromosomes, except the largest metacentric pair, exhibited well-expressed heterochromatin blocks in the cetromeric regions, which also extended into the short arms of the most bi-armed chromosomes. Block of centromeric heterochromatin was also found on the X chromosome, while the Y chromosome appeared to be entirely heterochromatic.

Key words: Micromys minutus, karyotype, C-banding

Introduction

The harvest mouse *Micromys minutus* (Pallas, 1771) belongs to the monotypic genus *Micromys*. It is among the smallest known species of mammals, with a size approximately half that of the house mouse (Jiang & Lin 2009). The geographical range of the species is relatively wide, extending from northwest Spain through central Europe to Siberia, Tibet, Assam, Taiwan and Japan (Trout 1978). In Bulgaria, the harvest mouse is often found in wet habitats at early succession stages. The species inhabits aquatic biotopes, near the banks of rivers, lakes and marshes and on cattail-like plants, but also in wet meadows, rice fields and sparse forests. Remarkably, the species adheres to unstable, azonal or intrazonal, anthropogenically-influenced habitats (Popov 2000).

Mitchell-Jones *et al*. (1999) reported 16 subspecies in Europe. Atanassov & Peshev (1963) and Mitev (1967) included the Bulgarian populations from the Thracian Valley to the range of *Micromys minutus pratensis* Ockskay, 1831, while Niethammer & Krapp (1982) assigned the populations from the Turkish part of the Thracian Valley to the range of *Micromys minutus brauneri* Martino, 1930.

Chromosomal analyses of some European and Asian harvest mouse populations have been reported. The diploid number (2N = 68) was first reported by Makino (1944) and subsequently confirmed in several studies (Tsuchiya 1979, Jüdes 1981, Zima 1983, Schmid *et al*. 1984, Özkan *et al*. 2003, Nakamura *et al*. 2007). The chromosomal morphology and the fundamental numbers of chromosomes and autosomes have been described with varying results. In such a widely distributed species, it is possible that an independent chromosomal evolution among populations could have arisen as a result of geographic isolation (Liang-Kong Lin *et al*. 2013).

Since there are no karyotype observations of the Bulgarian populations of the harvest mouse, the aim of this study was to characterize the karyotype and C-banding pattern of the species.

Materials and Methods

Five individuals (3 males and 2 females) of the harvest mouse, *Micromys minutus*, from the rice fields in the region of Plovdiv (N 42.183333°, E 24.566667°) were studied. Morphological criteria were used in determining the species (Peshev *et al*. 2004). The karyotype of bone marrow cells was analyzed according to the standard method (Rothfels & Siminovitch 1958). The chromosomes in the karyotype were arranged according to their morphology and decreasing size. Differential C-banding was performed by standard methods (Sumner 1972, Goodpasture & Bloom 1975). A total of 80 metaphases were analyzed in all five individuals.

Results and Discussion

All specimens studied exhibited a diploid chromosome number 2N = 68. Autosomes consisted of 1 pair of large and 1 pair of small metacentric chromosomes, 2 pairs of middle-sized submetacentric chromosomes, 28 pairs of subtelocentric chromosomes and 1 pair of acrocentric chromosomes. The X chromosome was the largest subtelocetric chromosome and the Y chromosome was the smallest acrocentric chromosome (Fig. 1). The fundamental number (FN) of the chromosomes was estimated to be 133, as the NFa was 130. Furthermore, no intraspecies variation in 2N or FN was noted.

The diploid number estimated in the present study was consistent with the previous studies on this species (Makino 1944, Tsuchiya 1979, Jüdes 1981, Zima 1983, Schmid *et al*. 1984, Özkan *et al*. 2003, Nakamura *et al*. 2007, Liang-Kong Lin *et al*. 2013).

However, this diploid number is unusually high for the subfamily Murinae. The average diploid number in Murinae is 42, and only 4% of the species have a diploid number exceeding 60 (Schmid *et al*. 1984). There is some controversy regarding the fundamental number of chromosomes. The FN varies from 70 in the Japanese *Micromys minutus* (Nakamura *et al*. 2007), through to 72 in the German and Taiwan populations (Schmid *et al*. 1984, Liang-Kong Lin *et al*. 2013), to 136 in the Turkish populations of the species (Özkan *et al*. 2003). The controversy about FN originated from the fact that different researchers used different methods of counting chromosomal arms. Differences in chromosome morphology are usually a result of subjective evaluation of the centromeric position in some chromosomes. The classification of the individual chromosomes as subtelocentric (bi-armed) or acrocentric (one-armed) seems to be the reason for these variable results.

In the studies of German and Taiwan populations, the subtelocentric chromosomes were classified as one-armed and the FN was defined as 72 (Schmid *et al*.1984, Liang-Kong Lin *et al*. 2013). The karyotype of a Turkish population consistsed of 1 pair of large and 3 pairs of small metacentric chromosomes and of 29 pairs of subtelocentric or telocentric chromosomes. The authors defined the FN of this species as 136, indicating that they considered all chromosomes to be bi-armed (Özkan *et al*. 2003). Zima (1983) reported that the karyotype of the species in the Danube Delta consists of one large metacentric pair and 2-4 pairs of small metacentric and submetacentric chromosomes and 28-30 pairs of

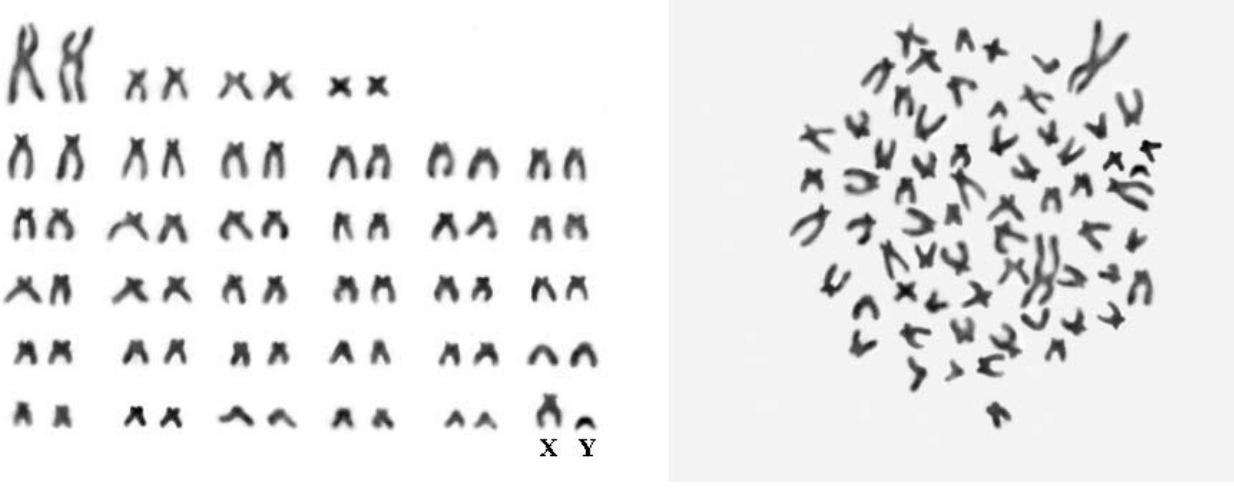

Fig. 1. Male metaphase plate and karyogram of *Micromys minutus* from South Bulgaria (Plovdiv region)

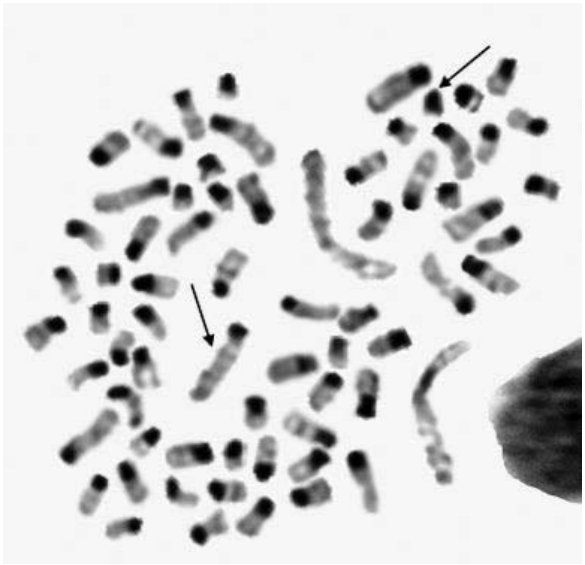

Fig. 2. C-stained male metaphase plate of *Micromys minutus*. The sex chromosomes are indicated by arrows

acrocentrics or subtelocentrics chromosomes. In our study, we decided to classify metacentrics, submetacentrics and subtelocentrics as bi-armed, and acrocentrics as one-armed chromosomes. Our findings concerning the karyotypes of Bulgarian specimens are consistent with the data of Özkan *et al*. (2003) and Zima (1983).

All chromosomes except the largest metacentric pair exhibited well-expressed heterochromatin blocks in the cetromeric regions, which also extended into the short arms of the most bi-armed chromosomes. C-banding detected only a small amount of constitutive heterochromatin in the largest metacentric pair located in the centromeric region. A block of centromeric heterochromatin was also found on the X chromosome, while the Y chromosome appeared to be entirely heterochromatic (Fig. 2).

According to Jüdes (1981), Zima (1983), Schmid *et al*. (1984) and Liang-Kong Lin *et al*. (2013), dark centromeric C-bands could be observed in all chromosomes, and mostly in the region of the short arms of bi-armed autosomal chromosomes. Our findings regarding C-banding patterns in the harvest mouse were in concordance with these previous studies. Liang-Kong Lin *et al*. (2013) suggested that the large amount of centromeric heterochromatin could be associated with a high probability of evolutionary changes. Evans *et al*. (1973) suggested that fusions, instead of fissions, may be more likely in the evolutionary history of the first metacentric pair, which was probably derived from the centric fusion of two acrocentric chromosomes derived from their ancestor. Similar evolutionary events were observed in other rodents such as in the genus *Rattus* (Miklos *et al*. 1980).

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