

# Cytogenetic Investigations in *Sciurus anomalus* from Turkish Thrace (Rodentia: Sciuridae)

Atilla Arslan<sup>1</sup>, Jan Zima<sup>2</sup>

<sup>1</sup> Department of Biology, Faculty of Science, Selçuk University, 42031 Konya, Turkey; E-mail: aarslan@selcuk.edu.tr

<sup>2</sup> Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, 60365 Brno, Czech Republic;

E-mail: jzima@brno.cas.cz

**Abstract:** In this study, cytogenetic characteristics of Caucasian squirrel (*Sciurus anomalus*) from Thrace province of European Turkey were investigated. Conventional chromosome staining, Ag-NOR staining and C-banding analysis were carried out in the karyotype of two female specimens studied. The karyotype included 40 banded chromosomes (FN=80). Most of chromosomes had distinct positive C-heterochromatic regions in the centromeric areas. The short arms of two submetacentric autosomes appeared to be entirely C-heterochromatic, and a small submetacentric autosome possessed a dark C-band in the terminal region of the long arm. The NORs were localized in the telomeric areas of the C-positive short arms of two pairs of submetacentric autosomes possessing secondary constrictions. The distribution of C-heterochromatin regions and the active NORs differentiates the individuals from Thrace from the previously studied Caucasian squirrels from Anatolia.

**Key words:** karyotype, C-banding, Ag-NOR staining, Caucasian squirrel, Turkey

## Introduction

The family Sciuridae includes arboreal rodents widely distributed over parts of Europe, Africa, Asia, and both Americas. Among the 28 extant recognized species of the genus *Sciurus* LINNAEUS, 1758, only three are native to the Palearctic region and two of them occur in Turkey (MUSSEY, CARLETON 2005). The Turkish species of tree squirrels are classified in two subgenera (CORBET 1978, KRYŠTUFEK, VOHRALÍK 2005); the nominate *Sciurus* (red squirrel, *S. vulgaris*) and *Tenetes* THOMAS, 1909 (Caucasian squirrel, *S. anomalus*).

Caucasian squirrel (*Sciurus anomalus* GMELIN, 1778) is restricted in its distribution to the extreme southwest Asia: Anatolia, Caucasus, Western Syria, Lebanon, Northern Jordan, Israel, Northern Iraq, and the Zagros Mountains in Iran (HARRISON, BATES 1991, MUSSEY, CARLETON 2005). In Turkey, Caucasian squirrel is widespread in all the coastal regions of Black Sea and Mediterranean Sea in Anatolia (KRYŠTUFEK,

VOHRALÍK 2005, YIĞİT *et al.* 2006). The species is also common on two islands off the west Anatolian coasts, LESVOS (ONDRIAS 1966) and Gökçeada (ÖZKAN 1999). MURSALOĞLU (1973) reported that *S. vulgaris* occurs only in Kırklareli province in Turkish Thrace and the uplands of Artvin and Erzurum provinces in Eastern Anatolia. OSBORN (1964), MITCHELL-JONES *et al.* (1999), and KRYŠTUFEK, VOHRALÍK (2005) assumed that Caucasian squirrel was introduced in Belgrade Forest near Istanbul in Thrace in 1964. This introduction apparently initiated the establishment of this species on the European side of Bosphorus, because YIĞİT *et al.* (2003) recorded Caucasian squirrel from Velika in Istranca Mountains.

The conventionally stained karyotype of *S. anomalus* was described by L'APUNOVA, ZHOLNEROVSKAYA (1969) from Armenia and by NADLER, HOFFMANN (1970) from Iran. In Turkey,

ÖZKURT *et al.* (1999) studied the conventionally stained karyotype of this species from Balıkesir and TEZ *et al.* (2006) from Kayseri and Adana. ARSLAN *et al.* (2008) described G- and C-banded and Ag-NOR stained karyotype of this species from Aydın, Balıkesir, Çorum, Elazığ and Konya. However, information on differentially stained chromosomes and detailed structure of the karyotype of populations from the European part of Turkey is still lacking.

Chromosomal studies are an important part of recording and describing biological diversity (ZIMA 2000). In this respect, the aim of this paper is to perform a chromosomal banding analysis of the karyotype in Thrace specimens with the use of C-banding and Ag-NOR staining and to compare the findings with those obtained in a previous study of Anatolia specimens (ARSLAN *et al.* 2008).

## Materials and Methods

Cytogenetic analyses were performed in two female specimens collected in Tekirdağ - Çerkezköy in Thrace, European Turkey (Fig. 1). The species was identified according to standard diagnostic characters, particularly the upper tooth row and the pad at hind foot (Fig. 2; KRUŠTUFEK, VOHRALIK 2005). Karyotype preparations were obtained in the field from bone marrow after colchicine treatment (FORD, HAMERTON 1956). Air-dried preparations were stained conventionally by Giemsa. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected by the techniques of C-banding (SUMNER 1972) and Ag-NOR staining (HOWELL, BLACK 1980), respectively. From each specimen, 10 to 20 slides were prepared, and at least 20 well-spread metaphase plates were analysed. The chromosomal pairs were arranged in the karyotype according to their centromeric position and size. The sex chromosomes were identified only tentatively, with respect to the previously published male karyotype of the same species studied by G-banding (ARSLAN *et al.* 2008). Standard voucher specimens (skins and skulls) are deposited at Selçuk University, Biology Department, Faculty of Science, Konya, Turkey.

## Results

Both specimens studied had the diploid number ( $2n$ ) of 40 and the fundamental number of chromosomal arms (FN) of 80. The autosomal complement com-

prised 6 pairs of metacentrics (nos. 1-6) and 13 pairs submetacentrics or subtelocentrics (nos. 7-19). The X chromosomes were tentatively identified as a medium-sized submetacentric pair. Secondary constrictions were observed in telomeric regions of the short arms of autosomal pairs 11 and 16 (Fig. 3).

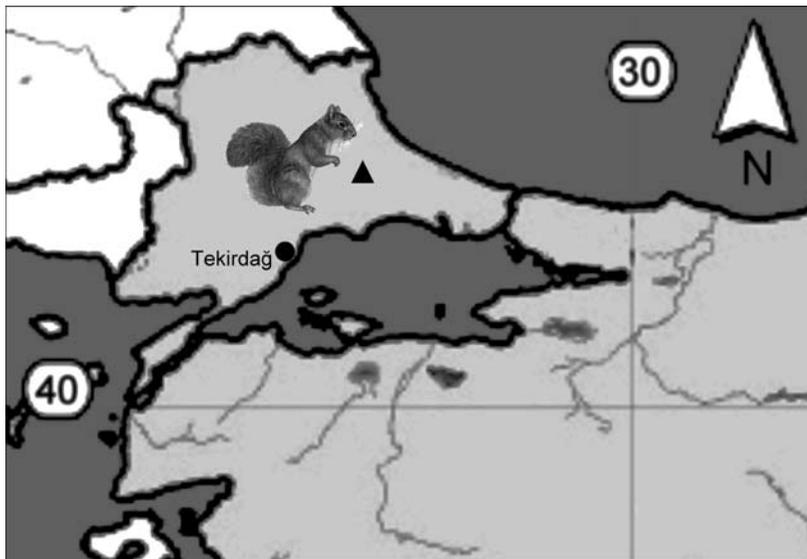
Most of the autosomes possessed distinct C-positive bands. C-heterochromatic short arms were recorded in two autosomal pairs (nos. 11, 16) and a dark C-band was observed in the telomeric region of the long arm of the autosomal pair no. 18. The only pair lacking any C-positive staining was the autosome no. 19 (Fig. 4).

By using silver-nitrate staining, NORs were localized in the secondary constrictions situated in the short arms of the submetacentric pairs no. 11 and 16. The arms containing the NORs stained positively during the C-banding procedure. All the observed NORs were homomorphic and occurred in both the homologues (Fig. 5).

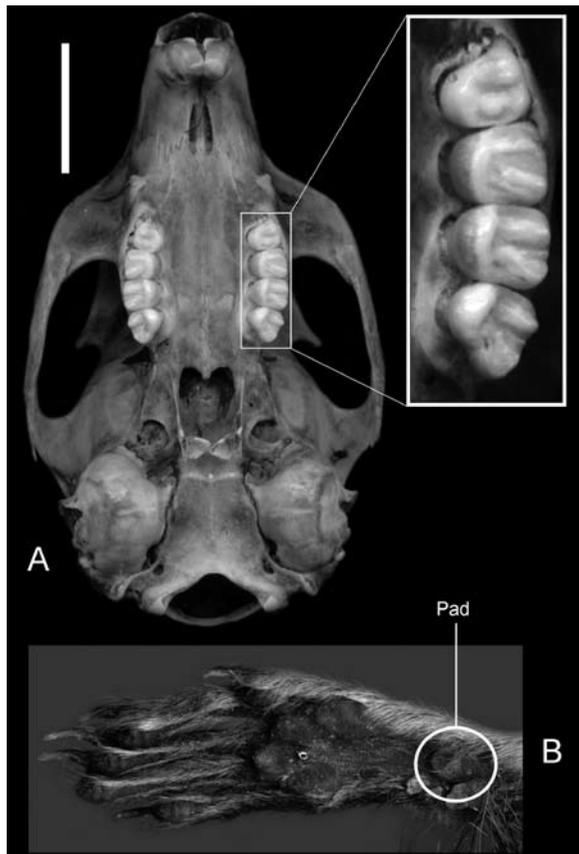
## Discussion

The basic chromosomal characteristics ( $2N$ ,  $NF$ ) of the specimens studied were similar to those reported previously from Armenia (L'APUNOVA, ZHOLNEROVSKAYA 1969), Iran (NADLER, HOFFMANN 1970), and Anatolia (ÖZKURT *et al.* 1999, TEZ *et al.* 2006, ARSLAN *et al.* 2008). The karyotype of the red squirrel (*S. vulgaris*) has the same diploid chromosome number but differs from that of Caucasian squirrel by the presence of two or three autosomal acrocentric pairs of medium or small size with resulting  $FN = 74-76$  (L'APUNOVA, ZHOLNEROVSKAYA 1969, ZIMA 1987). This difference can be tentatively explained by additions or deletions of heterochromatic short arms.

All the autosomes of specimens investigated in Anatolia carried centromeric constitutive heterochromatin, although the dark staining was only slightly manifested in two pairs of autosomes. However, no entirely C-heterochromatic arms and/or dark telomeric C-bands were recorded in the karyotype of the specimens from Anatolia (ARSLAN *et al.* 2008). In the C-banded karyotypes of *S. vulgaris* and *S. lis*, five and four large heterochromatin blocks were recognised, respectively. All long arms of three autosomes appeared to be entirely heterochromatic in both the species. In addition, large blocks of the heterochromatin occurred near the centromere in one autosome (OSHIDA, YOSHIDA 1997).



**Fig 1.** The collection locality of Caucasian squirrel (*Sciurus anomalus*): Tekirdağ -Çerkezköy in Turkish Thrace (▲).

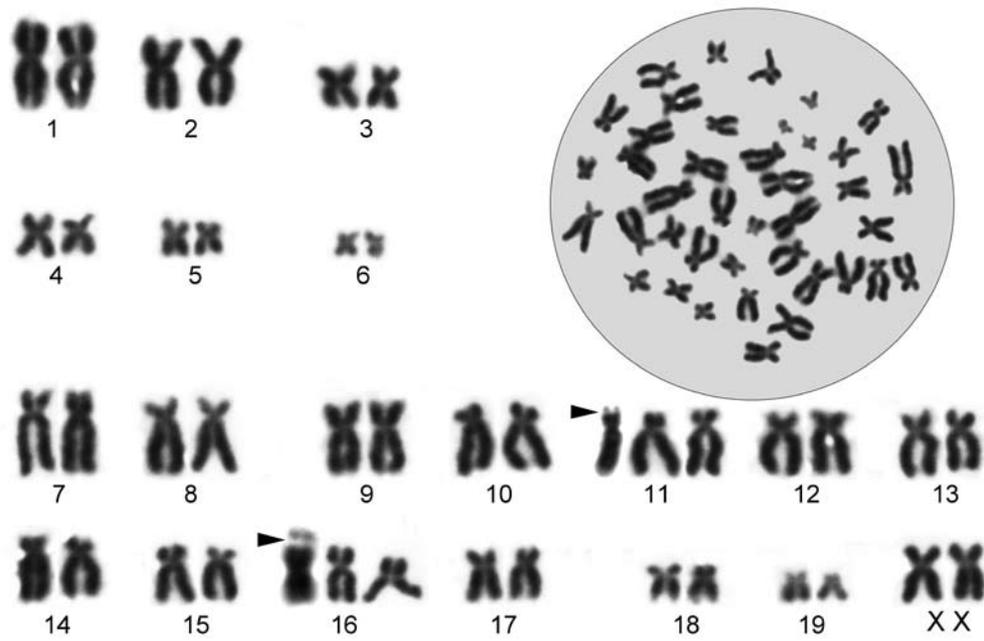


**Fig. 2.** The ventral view of skull with the upper tooth row (B) and the pad at the hind foot (B) of the specimen from Tekirdağ, Turkish Thrace. The bar is 10 mm.

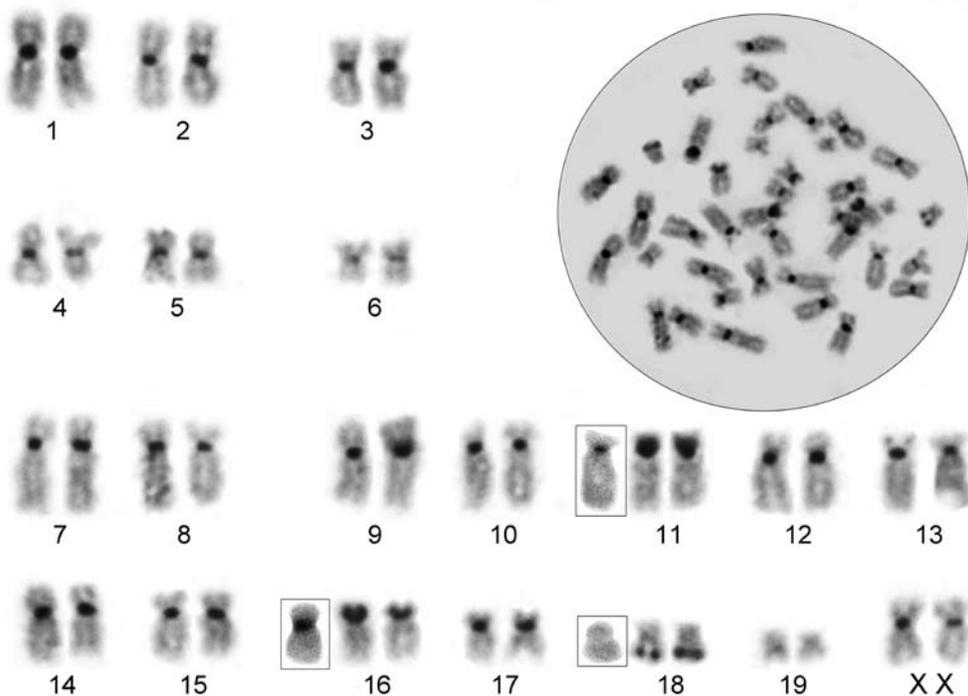
In specimens of *S. anomalus* from Anatolia, only single Ag-NOR site was detected in the terminal arm region of a medium-sized metacentric or submetacentric autosome (ARSLAN *et al.* 2008). In *S. vulgaris*, Ag-NORs were located in telomeric regions of long arms

of four autosomal pairs (including two metacentric pairs and two submetacentric or subtelocentric pairs), and in *S. lis*, Ag-NORs are detected in telomeric regions of long arms of three pairs, including two metacentric pairs and a subtelocentric pair (OSHIDA, YOSHIDA 1997). Therefore, the karyotypes of these Eurasian species, as well as those of Anatolia and Thrace populations of *S. anomalus* vary in the number of Ag-NORs. Additionally, NADLER, SUTTON (1967) described NORs in two chromosomal pairs of *S. griseus* from North America and concluded that the number of secondary constrictions is generally low in karyotypes of Sciuridae. A secondary constriction was recorded on long arm of one pair in the karyotype of South American species *S. alphonsei* and *S. spadiceus* (LIMA, LANGGUTH 2002), and on long arms of four pairs of *S. aestuans ingrami* (FAGUNDES *et al.* 2003).

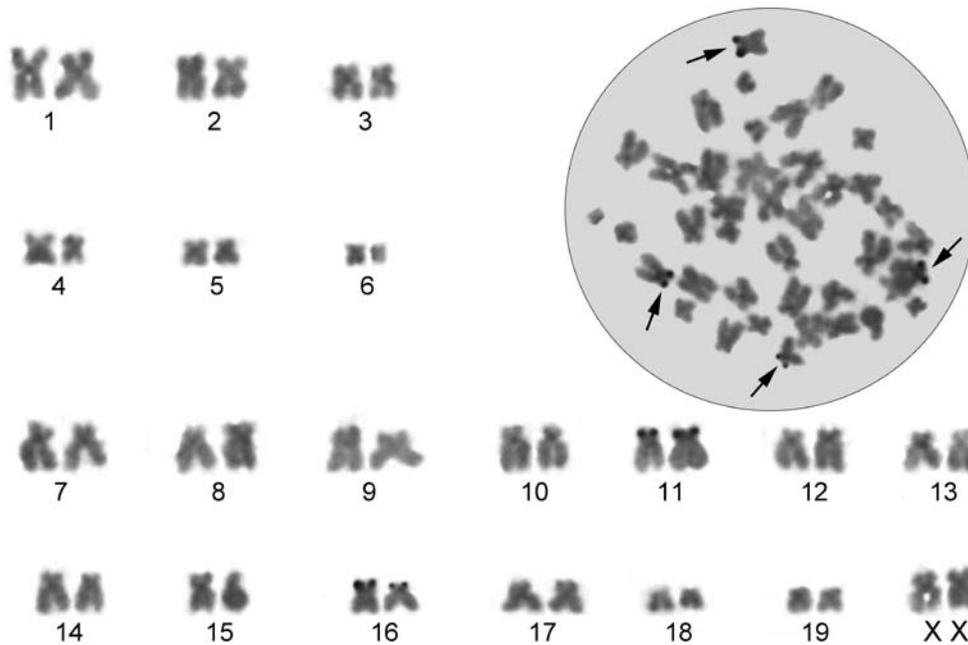
There is evidently variation in the distribution and amount of C-heterochromatin and the number and position of NORs between individual species of the genus *Sciurus* and between geographic populations of the Caucasian squirrel. The differences recorded between the populations of Caucasian squirrel from Thrace and Anatolia are particularly difficult to be interpreted because of the origin of the European population after introduction. Nevertheless, our findings indicate distinct variation of chromosomal characteristics within this species which may be of taxonomic significance. It would be useful to study other populations of *S. anomalus* within its native range, as well as to compare the banding pattern between the red and Caucasian squirrels.



**Fig. 3.** Metaphase spread and karyotype of *Sciurus anomalus* from Tekirdağ, Turkish Thrace. Arrows indicate the apparent secondary constrictions in chromosomes from another cell of the same specimen.



**Fig. 4.** Metaphase spread and C-banded karyotype of *Sciurus anomalus* from Tekirdağ, Turkish Thrace. The chromosomes in the frames belong to Anatolia specimens of this species (ARSLAN *et al.* 2008).



**Fig. 5.** Silver-stained metaphase spread and Ag-NOR positive chromosomes of *Sciurus anomalus* from Tekirdağ, Turkish Thrace. Arrows indicate the NOR-bearing chromosomes.

## References

- ARSLAN A., İ. ALBAYRAK, T. OSHIDA 2008. Banded karyotypes of Persian squirrel *Sciurus anomalus* from Turkey. – *Caryologia*, **61**: 139-143.
- CORBET G. B. 1978. The mammals of the Palaearctic region: a taxonomic review. British Museum (Nat. Hist.), London, 314 p.
- FAGUNDES V., A. U. CHRISTOFF, R. C. AMARO-GHILARD, D. R. SCHEIBLER, Y. YONENAGA-YASSUDA 2003. Multiple interstitial ribosomal sites (NORs) in the Brazilian squirrel *Sciurus aestuans ingrami* (Rodentia, Sciuridae) with  $2n=40$ . An overview of *Sciurus* cytogenetics. – *Genetics and Molecular Biology*, **26**: 253-257.
- FORD C. E., J. L. HAMERTON 1956. 'A colchicine-hypotonic-citrate' squash sequence for mammalian chromosomes. – *Stain Technology*, **31**: 247-251.
- HARRISON D. J., J. J. BATES 1991. The mammals of Arabia. 2nd ed. Sevenoaks, Kent, Harrison Zoological Museum, England, 345 p.
- HOWELL W. M., D. A. BLACK 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. – *Experientia*, **36**: 1014-1015.
- KRYŠTUFEK B., V. VOHRALÍK 2005. Mammals of Turkey and Cyprus. Rodentia I. Sciuridae, Dipodidae, Gliridae, Arvicolinae. Knjižnica Annales Majora, Koper, 292 p.
- L'APUNOVA E.A., J. I. ZHOLNEROVSKAYA 1969. The chromosome complements of some species Sciuridae. – In: VORONCOV N. N. (Ed.): Proceedings of the 2nd Soviet Mammal Research Symposium, Novosibirsk, 57-59. (In Russian).
- LIMA J. F. S., A. LANGGUTH 2002. Karyotypes of Brazilian squirrels: *Sciurus spadiceus* and *Sciurus alphonsei* (Rodentia, Sciuridae). – *Folia Zoologica*, **51**: 201-204.
- MITCHELL-JONES A. J., G. AMORI, W. BOGDANOWICZ, B. KRYŠTUFEK, P. J. H. REIJNDERS, F. SPITZENBERGER, M. STUBBE, J. B. M. THISSEN, V. VOHRALÍK, J. ZIMA 1999. Atlas of European mammals. Poyser Natural History. Academic Press, London, 496 p.
- MURSALOĞLU B. 1973. New records for Turkish rodents (Mammalia). – *Communications de la Faculté des Sciences de l'Université d'Ankara, Série C*, **17**: 213-219.
- MUSSER G. G., M. D. CARLETON 2005. Superfamily Muroidea. In: WILSON D. E., D. A. M. REEDER (Eds.): Mammal species of the world. A taxonomic and geographic reference. Vol. 2, 3rd ed. John Hopkins University Press, Baltimore, 894-1531.
- NADLER C. F., R. S. HOFFMANN 1970. Chromosomes and some Asian and South American squirrels (Rodentia: Sciuridae). – *Experientia*, **26**: 1383-1386.
- NADLER C. F., D. A. SUTTON 1967. Chromosomes of some squirrels (Mammalia, Sciuridae) from the genera *Sciurus* and *Glaucomys*. – *Experientia*, **23**: 249-251.
- ONDRIAS J. C. 1966. The taxonomy and geographical distribution of the rodents of Greece. – *Säugetierkundliche Mitteilungen*, **14**: 1-134.
- OSBORN D. J. 1964. The hare, porcupine, beaver, squirrels, jerboas, and dormice from Turkey. – *Mammalia*, **28**: 573-592.
- OSHIDA T., M. C. YOSHIDA 1997. Comparison of banded karyotypes between the Eurasian red squirrel *Sciurus vulgaris* and the Japanese squirrel *Sciurus lis*. – *Chromosome Science*, **1**: 17-20.
- ÖZKAN B. 1999. Rodent fauna of Imbros and Tenedos (Mammalia: Rodentia). – *Turkish Journal of Zoology*, **23**: 133-147.
- ÖZKURT S., M. SÖZEN, N. YİĞİT, E. ÇOLAK, R. VERİMLİ 1999.

- On the karyology and morphology of *Sciurus anomalus* (Mammalia: Rodentia) in Turkey. – *Zoology in the Middle East*, 18: 9-15.
- SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. – *Experimental Cell Research*, 75: 304-306.
- TEZ C., R. TEZ, E. ÖZŞERBETÇİ 2006. New data on the karyology of Persian squirrel *Sciurus anomalus* (Mammalia: Rodentia, Sciuridae) in Turkey. – *Acta zoologica bulgarica*, 58: 229-234.
- YİĞİT N., E. ÇOLAK, M. SÖZEN, A. KARATAŞ 2006. Rodents of Türkiye. Meteksan, Ankara. 154 p.
- YİĞİT N., E. ÇOLAK, M. SÖZEN, Ş. ÖZKURT 2003. A study on the geographic distribution along with habitat aspects of rodent species in Turkey. – *Bonner zoologische Beiträge*, 50: 355-368.
- ZIMA J. 2000. Chromosomal evolution in small mammals (Insectivora, Chiroptera, Rodentia). – *Hystrix*, (n.s.), 11: 5-15.
- ZIMA J. 1987. Karyotypes of certain rodents from Czechoslovakia (Sciuridae, Gliridae, Cricetidae). – *Folia Zoologica*, 36: 337-343.

Received: 05.03.2012  
Accepted: 18.04.2012