Taylor & Francis
Taylor & Francis Group

Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics

ISSN: 0008-7114 (Print) 2165-5391 (Online) Journal homepage: https://www.tandfonline.com/loi/tcar20

C-heterochromatin variation and NOR distribution in the karyotype of water vole, *Arvicola terrestris* (Mammalia, Rodentia)

Arslan Atilla, Tarkan Yorulmaz, Kubilay Toyran, Serdar Gözütok & Jan Zima

To cite this article: Arslan Atilla, Tarkan Yorulmaz, Kubilay Toyran, Serdar Gözütok & Jan Zima (2011) C-heterochromatin variation and NOR distribution in the karyotype of water vole, *Arvicola terrestris* (Mammalia, Rodentia), Caryologia, 64:2, 215-222, DOI: 10.1080/00087114.2002.10589786

To link to this article: https://doi.org/10.1080/00087114.2002.10589786

CARYOLOGIA Vol. 64, no. 2: 215-222, 2011

C-heterochromatin variation and NOR distribution in the karyotype of water vole, *Arvicola terrestris* (Mammalia, Rodentia)

Arslan[1] Atilla, Tarkan Yorulmaz[2], Kubilay Toyran[3], Serdar Gözütok[4] and Jan Zima[5,*]

[1]Department of Biology, Faculty of Science, Selçuk University, TR-42031 Konya, Turkey
[2]Department of Biology, Faculty of Science, Çankırı Karatekin University, Çankırı, Turkey
[3]Department of Biology, Faculty of Science and Arts, Bitlis Eren University, Bitlis 13000, Turkey
[4]Department of Biology, Faculty of Science and Arts, Kırıkkale University, Kırıkkale 71450, Turkey
[5]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, CZ-603 65 Brno, Czech Republic

Abstract — A chromosomal study of populations of *Arvicola terrestris* from Anatolia in Turkey and from Central Europe was performed. The diploid number of 36 chromosomes was found in all the specimens examined. The autosomal complement consisted of 12 meta- and submetacentric pairs, two large or medium-sized subtelocentric pairs, and three small acrocentric (Turkey) or subtelocentric (Central Europe) pairs (FNa = 62-68). The X chromosome was medium-sized submetacentric, the Y chromosome was small acrocentric or subtelocentric. All the chromosomes could be reliably identified by their unique G-banding patterns. The C-banding analysis revealed variation in the amount of constitutive heterochromatin in centromeric regions and in short arms of certain autosomes. A unique feature of the C-banded karyotype of individuals from Anatolia was the absence of dark positive regions in most chromosomes. Populations of water vole from Anatolia resemble in their C-band pattern those studied previously in Azerbaijan, and possibly also in the Balkan peninsula, and they are different in this respect from populations in Central Europe and the other parts of the species range. The X chromosome was stained uniformly and C-negatively in populations from Anatolia, whereas a faint dark centromeric C-band was observed in individuals from Central Europe. The Y chromosome was stained C-positively. The active nucleolar organizer regions (NORs) were localized in one pair of small metacentric and two acrocentric autosome pairs in the karyotype of individuals from Anatolia.

Key words: Central Europe, chromosome banding, karyotype differentiation, Turkey.

INTRODUCTION

The genus *Arvicola* of the family Cricetidae occurs in an extensive range in Asia and Europe (Musser and Carleton 2005). The current taxonomic division into two species, *A. sapidus* (Miller, 1908) restricted to south-western Europe, and *A. terrestris* (Linnaeus, 1758) of wide Palaearctic distribution, is often considered to be oversimplified and not congruent with known variation extent (Kryštufek and Vohralík 2005). Panteleyev (2001) and Musser and Carleton (2005) recognised the third species, *A. scherman* (Shaw, 1801), and the internal taxonomic structure of the genus is still under discussion particularly within the highly polymorphic *A. terrestris* (we use this species name in respect of long-established usage, see Corbet 1978). In Turkey, the water vole is relatively rare species with the widely scattered distribution pattern and three subspecies were recognised within the country (Mursaloğlu 1975, Kryštufek and Vohralík 2005).

Chromosomal studies have often significantly contributed to understanding of the phylogeny and systematics of small mammals owing to extensive karyotypic variation recorded in their populations (Zima 2000). Changes in C-heterochromatin amount and distribution are among important mechanisms producing this variation. The conventionally stained karyotype of *A. ter-*

*Corresponding author: phone: ++420 543 422 553, fax: ++420 543 211 346; e-mail: jzima@brno.cas.cz

restris was examined in numerous studies, and the older papers describing chromosomes of this species were summarized by ZIMA and KRÁL (1984). In Turkey, the karyotype of water vole was studied by ÖZKURT *et al.* (1999) from Central Anatolia (Kırşehir) and by GÖZCELIOĞLU *et al.* (2006) from Thrace (Kırklareli). DIAZ DE LA GUARDIA and PRETEL (1978, 1979), PESHEV and BELCHEVA (1978), KULIJEV *et al.* (1978), and GAMPERL *et al.* (1982) investigated the G- and C-banding patterns in the karyotype of water voles from various parts of the species range. BURGOS *et al.* (1989) identified the chromosome rearrangements involved in the karyotypic evolution of three species of the genus *Microtus* and two species of the genus *Arvicola*, and they proposed a phylogenetic tree derived from the pattern of chromosomal divergence. SÁNCHEZ *et al.* (1990) examined the location of the nucleolus organizer regions (NORs) in *Arvicola sapidus*.

The aim of this study is to perform a chromosomal banding analysis of the karyotype of *A. terrestris* from two geographic areas with the use of G- and C-banding and Ag-NORs staining, and to compare the results with previous studies regarding the species. The results obtained may find implications in the systematic treatment of the species studied.

MATERIAL AND METHODS

The animals studied in Turkey (one male and four females) were collected from Bolu (Lake Abant –40° 36′ N; 31° 16′ E), Ankara (Ayaş –38° 15′ N; 34° 17′ E) and Aksaray (Güzelyurt –40° 01′ N; 32° 20′ E) provinces. All these specimens belonged to aquatic type sensu PANTELEYEV (2001). The specimens studied in Central Europe (one male, two females) originated from Žofín, the Novohradské hory Mts., Czech Republic (48° 49′ N; 14° 41′ E), Moravian Karst, Czech Republic (49° 21′ N; 16° 42′ E), and the Belianské Tatry Mts., Slovakia (49° 15′ N; 20° 10′ E). These specimens can be classified within the fossorial type of water voles (PANTELEYEV 2001). Karyotype preparations were obtained from the bone marrow of animals treated with colchicine (FORD and HAMERTON 1956). After preparation of chromosome slides, conventional Giemsa-staining was carried out. G-banding was performed following the technique by SEABRIGHT (1971). Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected in individual autosomal and sex chromosome pairs via C-banding (SUMNER 1972) and Ag-NORs staining (HOWELL and BLACK 1980), respectively. The Ag-NOR staining was performed in specimens from Turkey only. From each specimen, 10 to 20 slides were prepared, and at least 20 well-spread metaphase plates were analysed. Chromosome morphologies were determined after calculating centromeric indices. Standard voucher specimens (skins and skulls) are deposited in the Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey and the Institute of Vertebrate Biology, Academy of Sciences, Brno, Czech Republic.

RESULTS

The karyotype of all the studied specimens consisted of 36 chromosomes. The autosomal complement included 12 metacentric pairs and submetacentric pairs (nos. 1-12), two subtelocentric pairs (nos. 13 and 14) and three smaller pairs which occur either as acrocentric or subtelocentric (nos. 15-17). The first ten pairs of meta- and submetacentric autosomes were large or medium-sized, whereas two autosomal pairs (nos. 11, 12) were distinctly smaller within the metacentric group. Two subtelocentric pairs of autosomes were large or medium-sized, respectively, and the group of acrocentric or subtelocentric autosomes included small-sized elements. In specimens originating from Turkey these autosomes were acrocentric with no apparent short arms (NFa = 62), whereas distinct short arms were present on these autosomes in specimens from Central Europe (NFa = 68). The X chromosome was medium-sized submetacentric (NF = 66), and the Y chromosome was a small acrocentric or subtelocentric, comparable in size with the smallest autosomal pair (Fig. 1).

A distinct difference was observed in the C-banding chromosome pattern between karyotypes of water voles from Turkey and Central Europe (Fig. 2). The amount of C-positive heterochromatin was generally rather low in the karyotype of the specimens from Turkey. Conspicuous C-positive dark bands appeared only in one submetacentric (no. 6) and three acrocentric pairs of autosomes (nos. 15, 16, 17). These C-positive regions were distinct and included relatively large pericentromeric areas. All the other autosomes and the X chromosome were uniformly C-negatively stained.

The C-band karyotype of specimens from Central Europe revealed distinct dark pericen-

tromeric bands in most of autosomes. The only exception was the submetacentric pair no. 2 with overall negative staining. The centromeric dark band was rather faint in the metacentric autosome pair no. 4. The subtelocentric autosome pairs no. 13 and 14 revealed distinct dark centromeric bands but the staining of their short arms was C-negative. The short arms of the small autosomes no. 15, 16 and 17 were stained C-positively. However, the telomeric dark C-band in the short arm no. 16 was apparently separated from the distal dark centromeric C-band by a narrow light euchromatic zone. The X chromosome possessed a dark centromeric band but its size was smaller and staining intensity lower compared to centromeric bands ob-

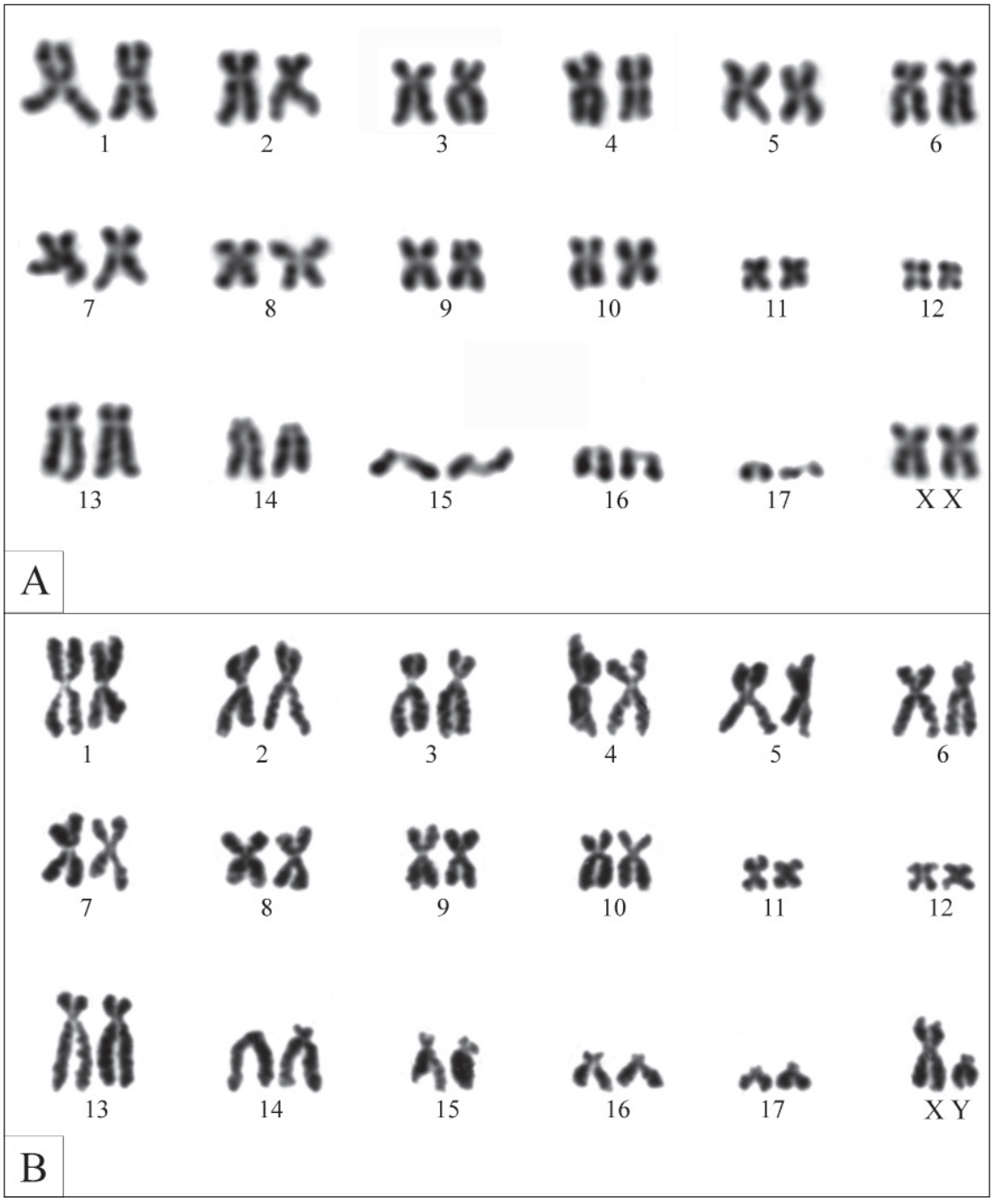

Fig. 1 — Conventionally stained karyotype of a female from Ayaş in Turkey (A), and a male from the Novohradské hory Mts. in the Czech republic (B).

served in most autosomes. The Y chromosome was stained C-positively but the intensity of dark staining was lower than in pericentromeric positive bands in autosomes.

All the autosomes and both the sex chromosomes could be reliably identified on the basis of their unique G-banding patterns. Short arms of the subtelocentric chromosome no. 14 were

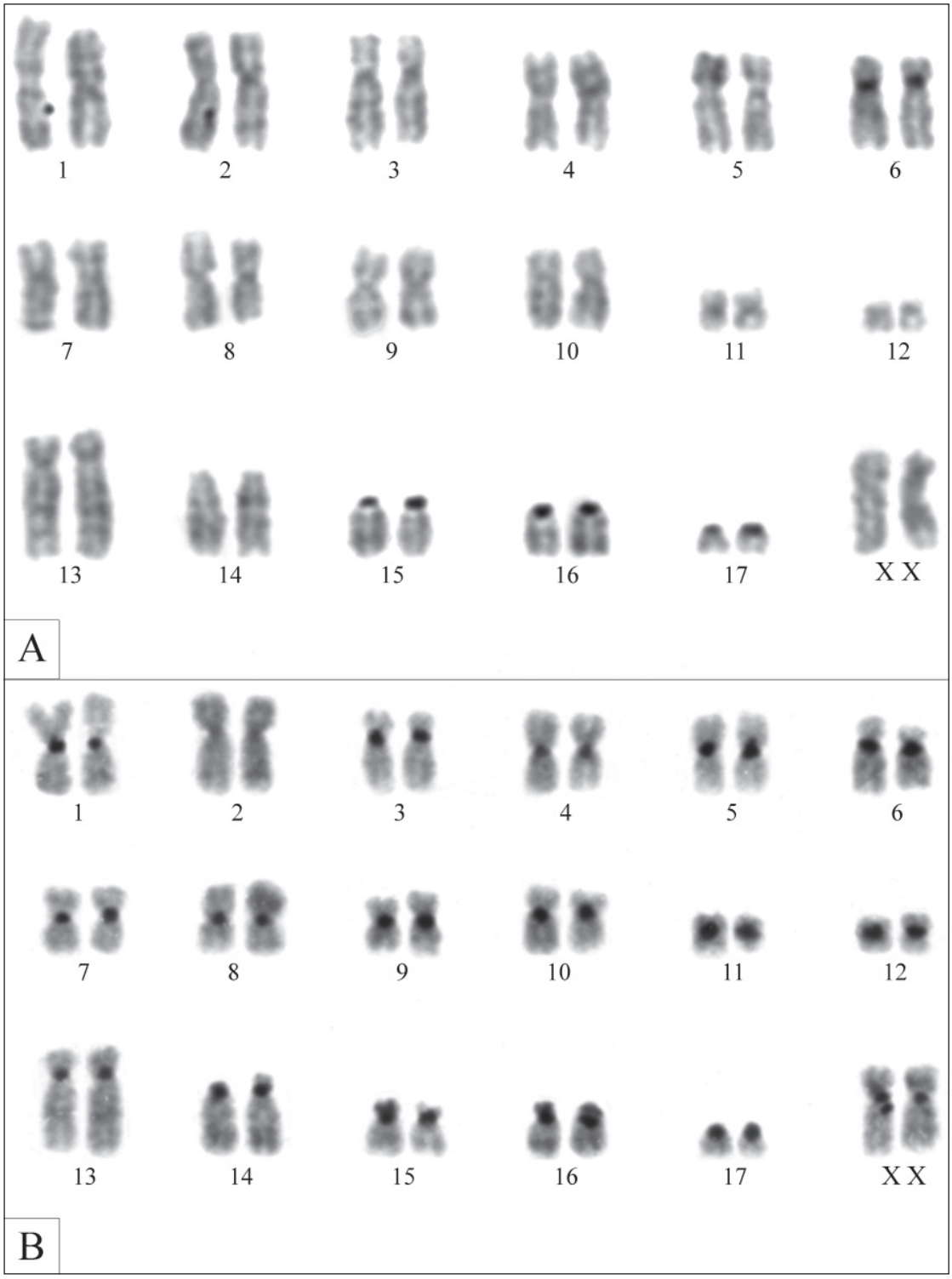

Fig. 2 — C-banded karyotypes of a female from Ayaş in Turkey (A) and a female from the Belianske Tatry Mts. in Slovakia (B).

distinctly visible in the G-banded karyotype of Turkish specimens (Fig. 3A). No apparent differences were observed in the G-banding of euchromatic chromosomal regions between Turkish and European specimens, except of the presence of the darkly stained short arms in pairs no. 15, 16, and 17 in European specimens.

By using silver-nitrate staining in the karyotype of specimens from Turkey, the NORs were localized in the secondary constrictions on the metacentric pair no. 12, and in the pericentromeric C-positive areas of the acrocentric pairs no. 15 and 16. The observed NORs were homomorphic in all the specimens studied (Fig. 3B).

DISCUSSION

Our results show a distinct divergence of the C-heterochromatin amount and distribution between populations of water voles from Turkey and Central Europe. Comparison with previously published data indicates that this divergence can be followed also in other populations and its geographical nature seems apparent. A karyotype similar to that reported here from Anatolia in Turkey was described in a population from Azerbaijan (Pushkin District) by KULIJEV *et al.* (1978). The autosomes nos. 15-17 were characterized as acrocentric with no apparent short arms, and only small centromeric dark bands were revealed on them by C-staining. Unfortunately, the complete C-band karyotype of this population was not presented in figures. The Y chromosome was acrocentric, C-positive, and relatively large. The karyotype constitution of water voles from Bulgaria (PESHEV and BELCHEVA 1978) is difficult to be assessed with certainty because of a low quality of C-banded karyotype but it seems to be similar to the Turkish type. A similar karyotype was described also in other studies reporting only conventionally stained chromosomes, e.g. RAICU *et al.* (1971) from Rumania, SOLDATOVIĆ *et al.* (1967) from Serbia, ÖZKURT *et al.* (1999) from Anatolia, and GÖZCELIOĞLU *et al.* (2006) from Turkish Thrace in Europe.

On the other hand, the karyotype described here from the Czech and Slovak republic resembles closely complements reported from various other parts of the range of water vole, e.g. by FREDGA (1968) from Sweden, SCHMID and LEPPERT (1968) from Switzerland, KRÁL (1972) from former Czechoslovakia, PANTELEYEV and MALYGINA (1974) from Yakutia, ŽIVKOVIĆ and PETROV (1974) from former Yugoslavia, and DIAZ DE LA GUARDIA and PRETEL (1979) from northern Spain. The same C-banding pattern was ascertained also in populations from other studied sites in Azerbaijan and western Siberia (KULIEV *et al.* 1978) and from Austria (GAMPERL *et al.* 1982).

The overall geographical pattern of the distribution of two types of C-heterochromatin distribution in the karyotype of water vole suggests that the complements with low amount of C-positive regions and acrocentric small autosomes are found in populations in Transcaucasia, Anatolia, and the Balkan peninsula. This karyotypic race is, however, not present in the Iberian peninsula, and the status of Apennine populations is not sure because the available data are rather old (MATTHEY 1957). It is important to note that the C-heterochromatin rich karyotype occurs also in high-altitude fossorial populations designated as *A. scherman exitus* or *A. t. monticola* (SCHMID and LEPPERT 1968; DIAZ DE LA GUARDIA and PRETEL 1979, respectively). Both *exitus* and *monticola* were included among synonyms of *A. scherman* by MUSSER and CARLETON (2005).

Distinct variation was found also in the size and centromere position in the Y chromosome. A small subtelocentric Y chromosome was recorded in various European populations (e.g. FREDGA 1968; KRÁL 1972; GAMPERL *et al.* 1982), a small acrocentric Y chromosome was found in populations from western Siberia and in some populations from Azerbaijan (KULIEV *et al.* 1978). The Y chromosome in the Pushkino population with low amount of C-heterochromatin was also acrocentric but considerably larger than in other populations from Azerbaijan. It seems that the increase in the heterochromatic content of the Y chromosomes is not associated with low amount of C-heterochromatin in autosomes, because the largest Y chromosome was described also from a population studied in northern Spain which karyotype possessed three pairs of distinctly subtelocentric small autosomes (DIAZ DE LA GUARDIA and PRETEL 1979).

Nucleolus organizer regions have been detected for the first time in this study for *A. terrestris*. The NOR sites in the autosomes nos. 15 and 16 were associated with heterochromatin regions which are variable in different geographic populations. SÁNCHEZ *et al.* (1990) localized the NORs in the karyotype of a related species, *Arvicola sapidus* (2n = 40). The NOR-carrier pairs were a small metacentric autosomal pair and seven acro- or subtelocentric autosomal pairs.

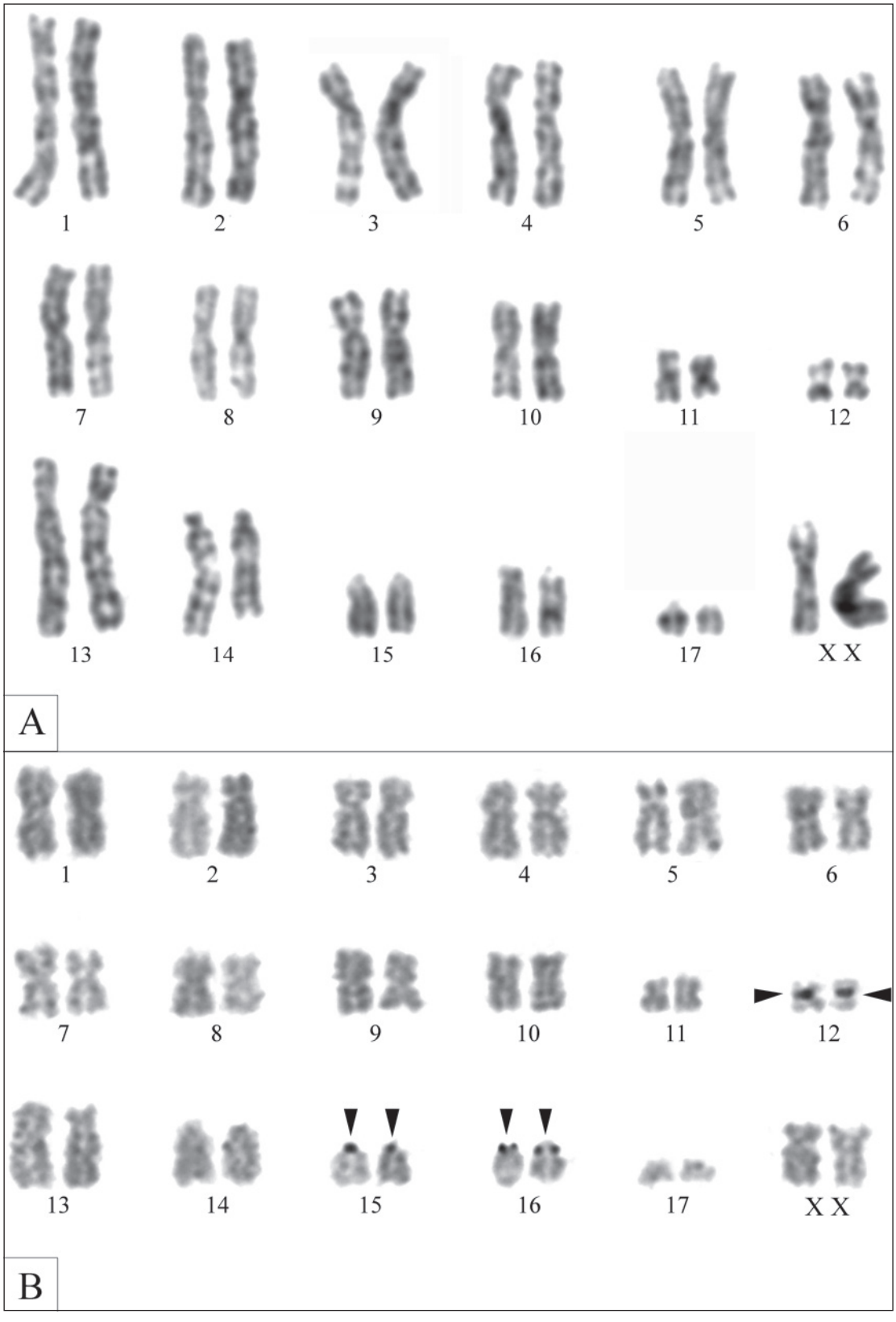

Fig. 3 — G-banded karyotype of a female from Ayaş (A) and silver-stained karyotype of female from Güzelyurt in Turkey (B). Arrows indicate the position of active Ag-NORs.

The comparison of the G-banding patterns suggests that the small metacentric NOR-bearing autosome of *A. sapidus* may be identical with the pair no. 12 of *A. terrestris,* and two acrocentric NOR-bearing autosomes of *A. sapidus* may be identical with the pairs no. 15 and 16 of *A. terrestris* as described in the present study. The other NOR sites reported in *A. sapidus* are apparently not present in the karyotype of the studied populations of *A. terrestris*. Some of these originally NOR-bearing acrocentric autosomes from the complement of *A. sapidus* were probably involved in the chromosomal fusions differentiating the karyotypes of *A. sapidus* and *A. terrestris*.

We can conclude that geographic divergence in the amount and distribution of C-heterochromatin can be demonstrated in the water vole karyotype that is associated rather with latitudinal than altitudinal distribution pattern. It is still not sure if the divergence between the two C-heterochromatin types is precisely correlated with the fossorial or amphibious way of life of individual populations but this cytogenetic marker can be employed in taxonomic considerations of the species internal structure. Additionally, the characteristics of the Y chromosome and the NORs distribution pattern may also be taken into consideration.

Acknowledgment — The authors thank for financial support of this study to the Coordination Committee of Scientific Research Projects (BAP) of Selçuk University and the Ministry of Education of the Czech Republic (grant LC 06073).

REFERENCES

BURGOS M., JIMENEZ R. and DIAZ DE LA GUARDIA R., 1989 — *Comparative study of G- and C-banded chromosomes of five species of Microtidae.* Genetica, 78: 3-12.

CORBET G.B. — *The mammals of the Palaearctic region: a taxonomic review.* British Museum (Natural History), London.

DIAZ DE LA GUARDIA R. and PRETEL A., 1978 — *Karyotype and centric dissociation in water vole (Arvicola sapidus sapidus Miller 1908) (Rodentia, Muridae).* Experientia, 34: 706-708.

DIAZ DE LA GUARDIA R. and PRETEL A., 1979 — *Comparative study of the karyotypes of two species of water vole: Arvicola sapidus and Arvicola terrestris (Rodentia, Microtinae).* Caryologia, 32: 183-189.

FORD C.E. and HAMERTON J.L., 1956 — *A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes.* Stain Technology, 31: 247-251.

FREDGA K., 1968 — *Idiogram and trisomy of the water vole (Arvicola terrestris L.), a favourable animal for cytogenetic research.* Chromosoma, 25: 75-89.

GAMPERL R., EHMANN C.H. and BACHMANN K., 1982 — *Genome size and heterochromatin variation in rodents.* Genetica, 58: 199-212.

GÖZCELIOĞLU B., ÇOLAK E. and ÇOLAK R., 2006 — *Karyotype of Arvicola terrestris (Mammalia: Rodentia) in Turkish Thrace.* Pakistan Journal of Biological Sciences, 9(12): 2387-2388.

HOWELL W.M. and BLACK D.A., 1980 — *Controlled silver staining of nucleolar organizer regions with a protective colloidal developer: a 1-step method.* Experientia, 36: 1014-1015.

KRÁL B., 1972 — *Chromosome characteristics of Muridae and Microtidae from Czechoslovakia.* Acta Scientiarum Naturalium Brno, 6(12): 1-78.

KRYŠTUFEK B. and VOHRALÍK V., 2005 — *Mammals of Turkey and Cyprus. Rodentia I.* Založba Annales, Koper.

KULIJEV G.N., KULIJEV G.K. and RADJABLI S.I., 1978 — *Karyotypical differences between different populations of Arvicola terrestris (Rodentia, Cricetidae).* Zoologicheskii Zhurnal, 57: 1409-1411.

MATTHEY R., 1957 — *Cytologie comparée, systématique et phylogénie des Microtinae (Rodentia-Muridae).* Revue suisse de Zoologie, 64: 39-71.

MURSALOĞLU B., 1975 — *Türkiye su sıçanlarının (Arvicola) cografik varyasyonları.* TÜBİTAK V. Bilim Kongresi Tebligleri: 353-368.

MUSSER G.G. and CARLETON M.D., 2005 — *Superfamily Muroidea.* In D.E. WILSON and D.A.M. REEDER (eds.) Mammal species of the world. A taxonomic and geographic reference. Vol. 2, 3rd ed., p. 894-1531. John Hopkins University Press, Baltimore.

ÖZKURT Ş., ÇOLAK E., YIGIT N., SÖZEN M. and VERIMLI R., 1999 — *Contributions to the karyology and morphology of Arvicola terrestris (Lin., 1758) (Mammalia: Rodentia) in Central Anatolia.* Turkish Journal of Zoology, 23: 253-257.

PANTELEYEV P.A. (ed.), 2001 — *The water vole*. Nauka, Moscow.

PANTELEYEV P.A. and MALYGINA N.A., 1974 — *Chromosomes of Arvicola terrestris L. (Microtinae, Cricetidae) at the eastern border of the species range.* Symposium Theriologicum, II: 355-357.

PESHEV T. and BELCHEVA R., 1978 — *Banding patterns of the water vole (Arvicola terrestris L., Rodentia, Mammalia) chromosomes.* Zoologischer Anzeiger, 200: 132-140.

RAICU P., DUMA D., KIRILLOVA M., and TUTA A., 1971 — *Chromosomal polymorphism in the water vole (Arvicola terrestris L.).* Revue Roumaine de Biologie-Zoologie, 16: 269-296.

SÁNCHEZ A., BURGOS M., JIMÉNÉZ R. and DIAZ DE LA GUARDIA R., 1990 — *Variable conservation of nucleolus organizer regions during karyotypic evolution in Microtidae.* Genome, 33: 119-122.

SCHMID W. and LEPPERT M.F., 1968 — *Mammalian X-chromosomes: a new kind of composite-type X in the vole Arvicola terrestris.* Experientia, 24: 227-279.

SEABRIGHT M., 1971 — *A rapid banding technique for human chromosomes.* Lancet, 1: 971-972.

SOLDATOVIĆ B., SAVIĆ I. and DULIĆ B., 1967 — *Chromosomen bei Arvicola terrestris Linnaeus, 1758 aus Jugoslawien.* Archive of Biological Sciences, Belgrade, 19:17P-18P.

SUMNER A.T., 1972 — *A simple technique for demonstrating centromeric heterochromatin.* Experimental Cell Research, 75: 304-306.

ZIMA J., 2000 — *Chromosomal evolution in small mammals (Insectivora, Chiroptera, Rodentia).* Hystrix (n.s.) 11(2): 5-15.

ZIMA J. and KRÁL B., 1984 — *Karyotypes of European mammals II.* Acta Scientiarum Naturalium Brno, 18(8): 1-62.

ŽIVKOVIĆ S. and PETROV B., 1974 — *Das Chromosomenkomplementen der Wasserwühlmaus Arvicola terrestris stankovici und die taxonomische Stellung dieser Form.* Symposium Theriologicum, II: 343-346.

Received February 7th 2010; accepted June 13th 2011