

Variation in the Conventional and Banded Karyotypes among Populations of *Arvicola amphibius* (L., 1758) (Mammalia: Rodentia) from Turkey

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Abstract: Chromosomal characteristics of water voles (*Arvicola amphibius*) were studied in ten populations including 33 samples from Anatolia, Turkey. The C-banding pattern and NORs distribution were analysed in four samples from Eastern Anatolia. The conventionally stained karyotypes showed the standard complement of the species ($2n = 36$, $NFa = 60-62$, $NF = 64-66$). Variation in the number of autosomal arms originated from the alternative presence of a subtelocentric or an acrocentric autosomal pair. C-banding provided further support for differentiation of the amount and distribution of C-heterochromatin between populations from Central Europe and Asia Minor. Chromosomal variation among Turkish populations was manifested by the number of autosomal arms, positive C-bands and NOR-carrying autosomes.

Key words: *Arvicola amphibius*, chromosomal banding, karyotype, variation, Turkey

Introduction

Water voles of the genus *Arvicola* are distributed in the Palaearctic Region and are represented by three aquatic and (or) fossorial species (WILSON & REEDER 2005). The fossorial species, *Arvicola scherman* Shaw, 1801, has a limited distribution range in mountainous areas of Southern and Central Europe and its taxonomic status is questionable (KRYŠTUFEK et al. 2015). *Arvicola sapidus* Miller, 1908 is an aquatic form with restricted distribution in the Iberian Peninsula and south of France (WILSON & REEDER 2005). The other aquatic species, *A. amphibius*, has the broadest distribution range among all water voles, including both Thrace and Anatolia in Turkey. The alternative usage of two synonymous names, *A. terrestris* and *A. amphibius*, is still questionable (Üstünbaş et al. 2011, TEZ et al. 2011, ARSLAN et al.

2011, KRYŠTUFEK et al. 2015). *Arvicola amphibius* is preferred in this article in conformity with the priority rule for refraining from any conflict between usages of these two names (CORBET 1978, WILSON & REEDER 2005). This species, known as the Eurasian water vole, prefers margins of wetland habitats, enclosed by intense reed mace and bulrush vegetation, e.g. sluggish streams, lakes, dam lakes, marsh areas and irrigation channels in Turkey (MURSALOĞLU 1975, KRYŠTUFEK & VOHRALÍK 2005). The only available study evaluating morphometric and morphological variation between populations of the three subspecies have suggested the following distribution in Turkey: *A. amphibius cernjavskii* in Thrace, *A. a. persicus* in most parts of Anatolia and *A. a. hintoni* in and around of the Hatay Province in the southern

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part of Anatolia (MURSAOĞLU 1975).

Chromosome analyses are assumed to reflect a phenotypic view of the genotype and comparative chromosomal studies enable to clarify problems with regard to phylogeny, taxonomy and identification of new taxa through detection of distinct variability in the number, morphology and banding patterns of chromosomes among populations (ZIMA 2000, DOBIGNY et al. 2004, GÜNDÜZ et al. 2007, KANKILIÇ et al. 2010). To date, chromosomal studies including conventional and staining methods (C, G and AgNOR staining) have revealed that Turkish water voles have the standard karyotype with 36 chromosomes. However, geographical populations showed certain variability in autosomal morphology in their karyotypes (Özkurt et al. 1999, GÖZCELIOĞLU et al. 2006, TEZ et al. 2011, ARSLAN et al. 2011). These studies included just limited geographical sampling and some of them analysed only a single sample. *Arvicola amphibius* is considered a poor disperser because of its dependence on water, thus migration and gene exchange between its populations are apparently limited. We can assume that genetic differentiation between populations increases as the gene flow decreases. In order to study the chromosomal differentiation, we have conducted rather dense geographical sampling of Turkish populations of *A. amphibius* with the aim to reach a more precise understanding of the chromosomal variation within this species.

Materials and Methods

A total of 33 specimens of *A. amphibius* from ten different localities in Turkey were evaluated karyologically (Fig. 1, Table 1). Conventional karyological analyses included samples from localities 1-6 (13 males and nine females in total). Specimens with banded karyotypes were examined from near geographic localities in a previous study performed by ARSLAN et al. (2011). The specimens from localities 7-10 (nine males and two females in total) were used for C-, G- and AgNOR banding analyses. Karyotype preparations were prepared from bone marrow of colchicine treated animals following a modified method of FORD & HAMERTON (1956). G-banding was performed according to the technique proposed by SEABRIGHT (1971). C-banding (SUMNER 1972) and silver staining of nucleolar organizer regions (HOWELL & BLACK 1980) were used to detect constitutive heterochromatin and nucleolus organizer regions (NORs). The number of slides varied between 10 and 20 for each specimen. Usually, 10-20 well spread and non-overlapping metaphase plates were evaluated for each specimen to determine the diploid chromosome number ($2n$) and the morphology of chromosomes. The classification system of chromosomes according to the centromere position proposed by HSU & BENIRSCHKE (1967-1977) was followed and the biarmed (metacentric = M, submetacentric = SM and subtelocentric = ST) and uniarmed (acrocentric = A) chromosomes were determined.

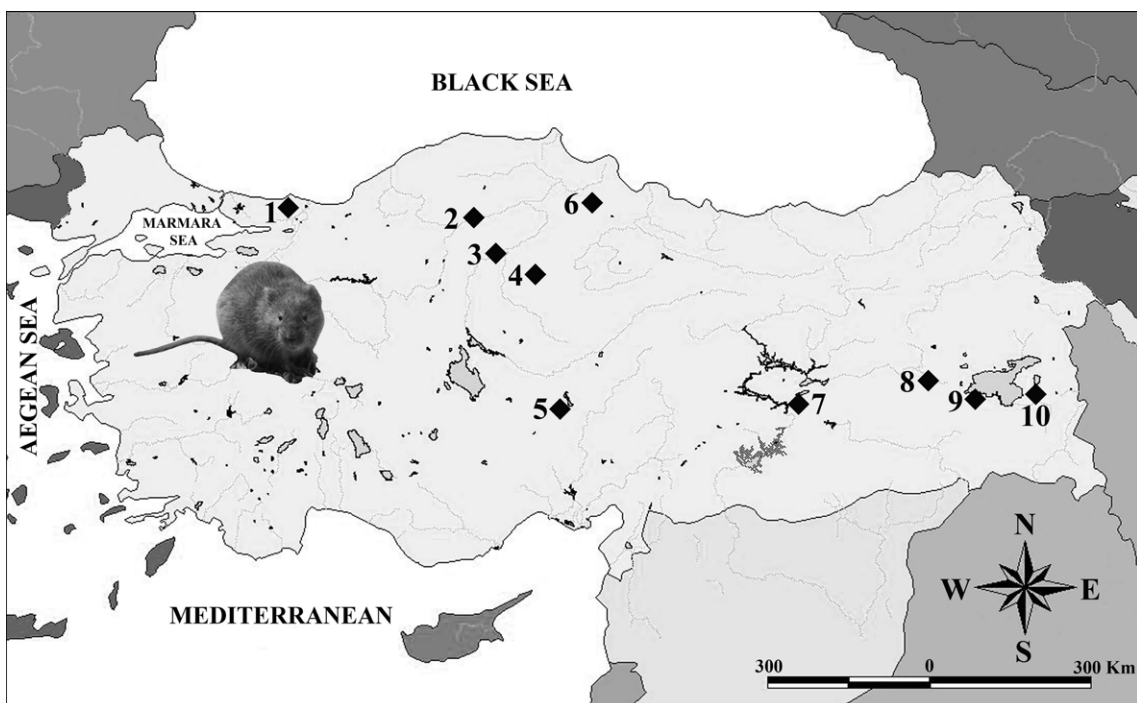


Fig. 1. Map of localities of the collected specimens. The numbers represent the localities given in Table 1.

Table 1. Sampling localities of specimens of *Arvicola amphibius* used in the study. All sites are arranged in order to match with the map numbers in Figure 1. (N= sample size).

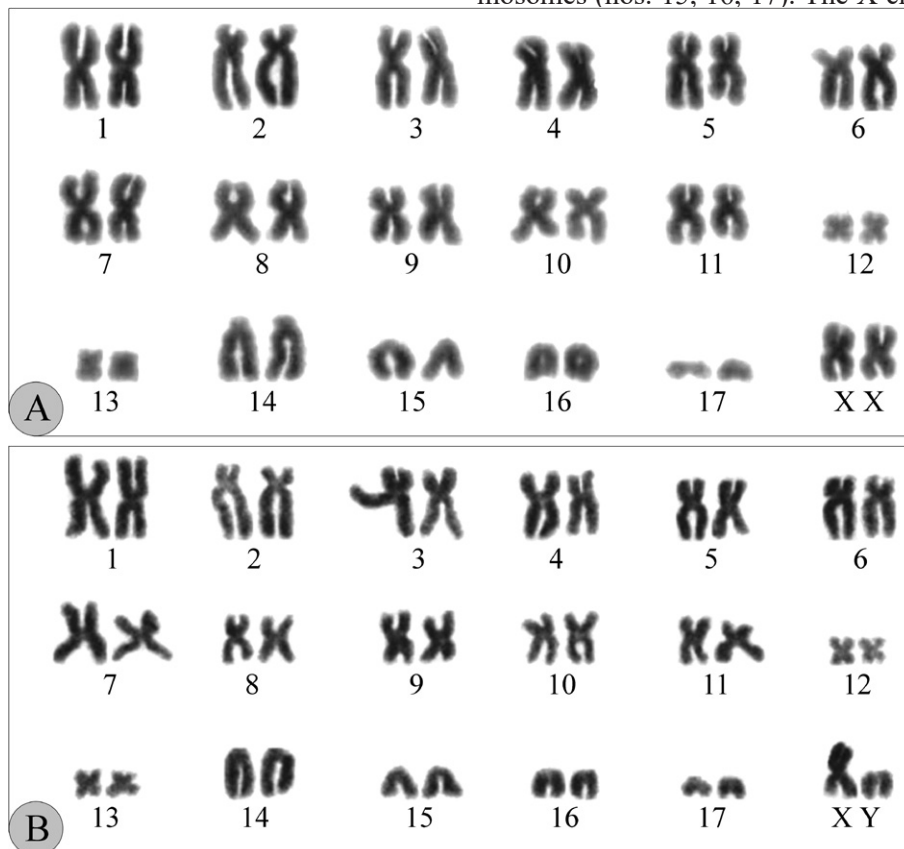
Locality	Locality name, province	N		Coordinates		2n	NFa	NF	X	Y
		♂	♀	North	East					
1	Kocaali, Sakarya	4	2	41° 4'	30°50'	36	60	64	M	A
2	Tosya, Kastamonu	1	-	40°56'	33°51'	36	60	64	M	A
3	Kızılırmak, Çankırı	-	2	40°20'	33°58'	36	62	66	M	-
4	Sungurlu-Alaca Road, Çorum	3	2	40° 8'	34°34'	36	60	64	M	A
5	Ovaçiftliği-Kuşçu, Kayseri	5	1	38°14'	35° 9'	36	60	64	M	A
6	Ladik, Samsun	-	2	40°54'	35°52'	36	60	64	M	A
7	Sivrice, Elazığ	1	1	38°27'	39°16'	36	60	64	M	A
8	Altınova Crossroad, Muş	2	-	38°37'	41°55'	36	60	64	M	A
9	Tatvan, Bitlis	3	-	38°28'	42°28'	36	60	64	M	A
10	Erçek Lake, Van	3	1	38°36'	43°34'	36	60	64	M	A

The fundamental number of autosomal arms (NFa) and the number of all chromosomal arms (NF) in the female complement were determined. Standard voucher specimens (skin, skulls and tissues) were deposited in the Department of Forestry, Artvin Vocational School, Artvin Çoruh University, Artvin, Turkey.

Results

Conventional staining

The diploid number of chromosomes ($2n = 36$) was found in all examined specimens. Two differing karyotypes were found. The autosomal complement ($2n = 36$, NFa = 62 and NF = 66) of the specimens from the Çankırı Province consisted of eight pairs of meta-centric (nos. 1, 7, 8, 9, 10, 11, 12, 13), five pairs of submetacentric (nos. 2, 3, 4, 5, 6), one pair of subtelocentric (no. 14), and three pairs of acrocentric chromosomes (nos. 15, 16, 17). The X chromosome was


Fig. 2. Standard karyotypes of specimens from Kızılırmak (A) and Kocaali (B).

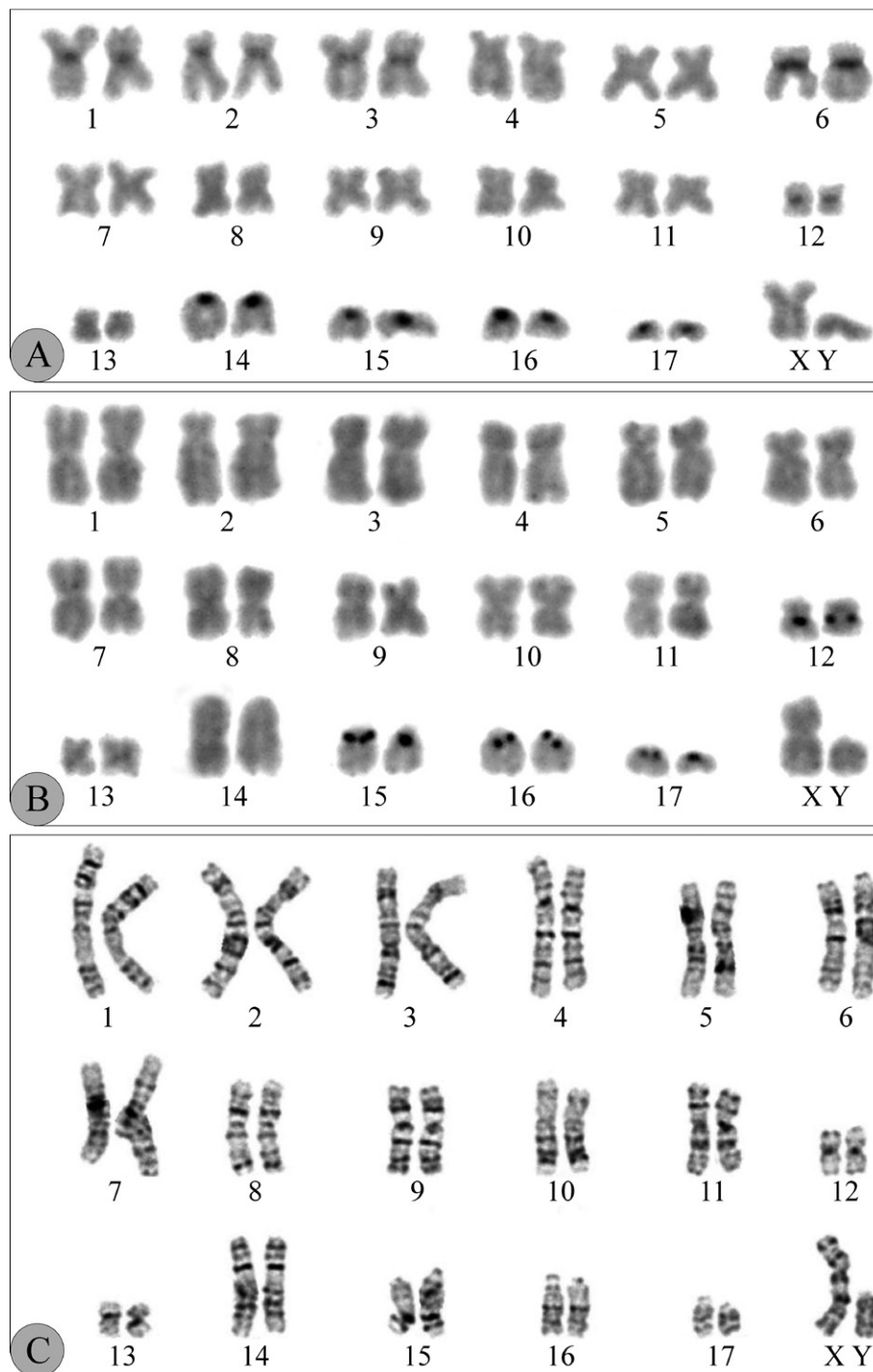


Fig. 3. C-banded (A) and silver stained (B) karyotypes of specimens from Erçek Lake, and G-banded karyotype of a specimen from Altınova crossroad (C).

identified as medium-sized metacentric (Figure 2A).

A slightly different karyotype was determined in the remaining specimens from Central and Eastern Anatolia. Their autosomal set comprised again eight pairs of metacentric and five pairs of submetacentric autosomes. The subtelo-centric pair was missing and four pairs of acrocentric chromosomes were distinguished (nos. 14-17). The X chromosome was

medium-sized metacentric, while the Y chromosome was small acrocentric ($2n = 36$, $NFa = 60$ and $NF = 64$; Fig. 2B).

Chromosome banding patterns

Distinct dark centromeric C-bands were observed in one biarmed (no. 6) and all acrocentric autosomes (nos. 14-17). Additionally, tiny centromeric bands

were recorded in four banded autosomes (nos. 1, 2, 3, 12), whereas the other autosomes and both sex chromosomes were C-negatively stained (Fig. 3A). No variation was found between the studied individuals. In the silver stained karyotypes, active AgNORs were observed near the centromere on metacentric pair no. 12 and within the pericentromeric C-positive areas of three smaller acrocentric pairs (nos. 15, 16, 17). The observed AgNORs were homomorphic in all examined specimens (Fig. 3B). The G-banded karyotype enabled unequivocal identification of autosomal pairs, as well as the X and Y chromosomes (Fig. 3C).

Discussion

The diploid number of 36 chromosomes is found in all previously studied populations of *A. amphibius* (ARSLAN et al. 2011, ARSLAN & ZIMA 2014 for review). The karyotypes with two different numbers of autosomal arms (NFa = 60 or 62) have been already reported in Turkish populations of this species in previous studies for both conventional and banded karyotype. The difference between the two karyotype forms with different NFa values found in Turkish populations results from varying evaluation of a pair of autosomes, which was alternatively designated as subtelocentric (NFa = 62; ARSLAN et al. 2011) or acrocentric (NFa = 60, Özkurt et al. 1999, GÖZCELIOĞLU et al. 2006, TEZ et al. 2011). The karyotype with NFa = 60 was detected in the samples from Kırşehir and two locations in Kırklareli, the karyotype with NFa = 62 in Bolu, Ankara and Aksaray Provinces. The present study confirms that this variation really exists between populations in Anatolia, but it is difficult to derive any distinct geographic pattern of the distribution of both karyotype forms.

An exceptionally large Y chromosome was described from water vole populations studied in the Alps (SCHMID & LEPPERT 1968), the Caucasus (KULIJEV et al. 1978) and the Pyrenees (DIAZ DE LA GUARDIA & PRETEL 1979). However, the size of the Y chromosome seems to be stable within the populations examined in Turkey.

The C-heterochromatin amount and its distribution found in the present study in the samples from Eastern Anatolia were fairly similar to those reported in the specimens from Central Anatolia by ARSLAN et al. (2011). These results are congruent with the conclusion of ARSLAN et al. (2011) that distinct differences in the amount and distribution of C-heterochromatin exist between water vole populations from Central Europe and Anatolia. The Anatolian populations are, in this respect, simi-

lar to those studied in Azerbaijan (KULIJEV et al. 1978). We also assume that the G-banding patterns revealed in karyotypes of specimens from Central (ARSLAN et al. 2011) and Eastern Anatolia (this study) are very similar.

On the other hand, slight but distinct differences can be found in the chromosome banding patterns between water voles from Central and Eastern Anatolia. The karyotype of Eastern Anatolian populations included additional acrocentric autosomal pair possessing a distinct C-positive band. The supposedly homologous subtelocentric autosome recorded in complements from Central Anatolia (ARSLAN et al. 2011) showed no C-positive staining. Tiny C-positive bands on four meta- and subtelocentric autosomes were found in the complements from Eastern Anatolia but similar bands were apparently absent in the karyotypes from Central Anatolia. Finally, the number of NOR-carrying acrocentric autosomal pairs was higher in the complements from Eastern Anatolia (four pairs) as compared to those from Central Anatolia (two pairs) studied by ARSLAN et al. (2011).

Our study shows that karyological variation exists also among *A. amphibius* populations in Turkey. The obtained results of the current and previous studies (Özkurt et al. 1999, GÖZCELIOĞLU et al. 2006, TEZ et al. 2011, ARSLAN et al. 2011) seem to support the assumption that differences in the morphology and banding patterns of chromosomes between *A. amphibius* populations may indicate origin of new chromosomal forms of this species in relation to geographical distributions, as suggested by TEZ et al. (2011).

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References

- ARSLAN A., YORULMAZ T., TOYRAN K., GÖZÜTOK S. & ZIMA J. 2011. C - heterochromatin variation and NOR distribution in the karyotype of water vole *Arvicola terrestris* (Mammalia, Rodentia). *Caryologia* 64(2): 215-222.
- ARSLAN A. & ZIMA J. 2014. Karyotypes of the mammals of Turkey and neighbouring regions: a review. *Folia Zoologica* 63(1): 1-62.
- CORBET G. B. 1978. The mammals of the Palearctic region: a taxonomic review. British Museum (Natural History). Cornell University Press, London and Ithaca. 314 p.
- DIAZ DE LA GUARDIA R. & PRETEL A. 1979. Comparative study of karyotypes of two species of water vole: *Arvicola sapidus* and *Arvicola terrestris* (Rodentia, Microtinae). *Caryologia* 32: 181-189.

- DOBIGNY G., DUCROZ J. F., ROBINSON T. J. & VOLOBOUEV V. 2004. Cytogenetics and cladistics. *Systematic Biology* 53(3): 473-48.
- FORD C. E. & HAMERTON J. L. 1956. 'A colchicine-hypotonic-citrate' squash sequence for mammalian chromosomes. *Stain Technology* 31(6): 247-251.
- GÖZCELİOĞLU B., ÇOLAK E. & ÇOLAK R. 2006. Karyotype of *Arvicola terrestris* (Mammalia: Rodentia) in Turkish Thrace. *Pakistan Journal of Biological Sciences* 9(12): 2387-2388.
- GÜNDÜZ İ., JAAROLA M., TEZ C., YENİYURT C., POLLY P. D. & SEARLE J. B. 2007. Multigenic and morphometric differentiation of ground squirrels (*Spermophilus*, Scuridae, Rodentia) in Turkey, with a description of a new species. *Molecular Phylogenetics and Evolution* 43: 916-935.
- HOWELL W. M. & BLACK D. A. 1980. Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014-1015.
- HSU T. C. & BENIRSCHKE K. 1967-1977. An atlas of mammalian chromosomes. Volumes 1-10. Springer-Verlag, Berlin.
- KANKILIÇ T., KANKILIÇ T., ŞEKER P. S., ÇOLAK R., SELVI E. & ÇOLAK E. 2010. Contributions to the karyology and distribution areas of cytotypes of *Nannospalax leucodon* (Rodentia: Spalacidae) in western Anatolia. *Acta Zoologica Bulgarica* 62(2): 161-167.
- KRYŠTUFEK B. & VOHRALÍK V. 2005. Mammals of Turkey and Cyprus. Rodentia I: Sciuridae, Dipodidae, Gliridae, Arvicolinae. Zložba Annales, Koper. 292 p.
- KRYŠTUFEK B., KOREN T., ENGELBERGER S., HORVÁTH G. F., PURGER J. J., ARSLAN A., CHIŞAMERA G. & MURARIU D. 2015. Fossorial morphotype does not make a species in water voles. *Mammalia* 79(3): 293-303.
- KULIJEV G. N., KULIJEV G. K. & RADJABLI S. I. 1978. Karyotypic differences between different populations of *Arvicola terrestris* (Rodentia, Cricetidae). *Zoologicheskyy Zhurnal* 57: 1409-1411.
- MURSALOĞLU B. 1975. Türkiye su sıçanlarının *Arvicola* coğrafik varyasyonları. TÜBİTAK V. Bilim Kongresi Tebliğleri, 353-368. (In Turkish).
- Özkurt Ş., Çolak E., Yiğit N., Sözen M. & 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.
- SCHMID W. & LEPPERT M. F. 1968. Mammalian X chromosomes: a new kind of composite-type X in the vole *Arvicola terrestris scherman exitus* Miller. *Experientia* 24: 227-279.
- SEABRIGHT M. 1971. A rapid banding technique for human chromosomes. *Lancet* 1: 971-972.
- SUMNER A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research* 75: 304-306.
- TEZ C., İBIŞ O., TEZ R., KILIÇ M. & TELCİOĞLU M. 2011. Distributional, morphological and karyotypic contributions for the Eurasian water vole, *Arvicola amphibius* (Linnaeus, 1758) (Rodentia: Mammalia), from Turkey. *Archives of Biological Sciences* 63: 407-412.
- Üstünbaş S., Çolak R., Karacan G. O & Çolak E. 2011. RAPD-PCR analysis of water vole *Arvicola amphibius* (Linnaeus, 1758) (Mammalia: Rodentia) distributed in Turkey. *Journal of Animal and Veterinary Advances* 10(13): 1673-1677.
- WILSON D.E. & REEDER D. M. 2005. Mammal Species of the World: A Taxonomic and Geographic Reference. 3rd ed. The Johns Hopkins University Press, Baltimore, MD, USA. 2142 p.
- ZIMA J. 2000. Chromosomal evolution in small mammals (Insectivora, Chiroptera, Rodentia). *Hystrix* 11(2): 5-15.

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