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C-heterochromatin and NOR distribution in the karyotype of Persian water vole, *Arvicola persicus* (Mammalia; Rodentia) from Iran

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Abstract

Although recent molecular data has advocated the distinct position of *Arvicola persicus* De Filippi from Iran, karyotypic and differential chromosome staining data, informative tools to describe biological diversity, are lacking. Here we present the first description of the chromosome complement of *A. persicus* from its type locality in Sultaniyeh, southern Alborz Mountains, Iran. Though the diploid chromosome number (2n= 36) and the fundamental number of autosomal arms (FNa= 60) did not deviate from that reported for *Arvicola amphibius* sensu lato in Eurasia (2n= 36, FNa= 60–68), there appear to be significant differences between *A. persicus* and *A. amphibius* s.l. in terms of C-bands and NOR-bearing autosomes. Banded karyology, therefore, provides further evidence for delimiting *A. persicus* as a species, which is distinct from *A. amphibius*.

Key words: Alborz Mountains, *Arvicola*, chromosome number, karyology, rodent species

Water voles of the genus *Arvicola* Lacépède, 1799 (Family Cricetidae), occupy the Palearctic region (Pardiñas et al., 2017), and currently comprise five species: *Arvicola amphibius* sensu stricto (s.str.) (Linnaeus, 1758), *A. italicus* Savi, 1838, *A. monticola* de Sélys-Longchamps, 1838, *A. persicus* De Filippi, 1865, and *A. sapidus* Miller, 1908 (Kryštufek et al., 2015; Castiglia et al., 2016; Mahmoudi et al., 2020). *Arvicola persicus*, which occurs in western and northern Iran, was considered a junior synonym (*Arvicola amphibius persicus*) of *A. amphibius* sensu lato (s.l.) (Thomas, 1907; Hinton, 1926; Ellerman and Morrison-Scott, 1951; Kryštufek et al., 2015). Recent molecular data, however, demonstrated a deep divergence separating Persian water vole from all the remaining *Arvicola* species. *Arvicola persicus* was therefore recognized as a valid species with a restricted range in Iran (Mahmoudi et al., 2020).

Although chromosome characteristics of all the known species were extensively studied throughout Eurasia (e.g. Guardia and Pretel, 1979; Arslan et al., 2011; Arslan and Zima, 2014; Castiglia et al., 2016; Şeker et al., 2018), the Persian water vole, *A. persicus*, has not being karyotyped until now.

In the present study, three male specimens from the type locality of *A. persicus* in Sultaniyeh, southern Alborz Mountains, were analyzed by employing conventional and differential staining techniques (Fig. 1). Taxonomic affiliation of karyotyped voles was assessed from mitochondrial cytochrome *b* sequences (Mahmoudi et al., 2020). Karyotype preparations were obtained from the bone marrow of animals treated with colchicine (Ford and Hamerton, 1956). Approximately 10–20 well-spread Giemsa-staining metaphase plates were analyzed. Constitutive heterochromatin and nucleolus

organizer regions (NORs) were retrieved from C-banded (Sumner, 1972) and Ag-NOR stained chromosomes (Howell and Black, 1980). The classification of chromosomes follows Hsu and Benirschke (1977). The fundamental number of autosomal arms (FNa) and the number of all chromosomal arms (FN) were calculated.

The standard karyotype (2n= 36, FNa= 60) of the analyzed water voles from Sultaniyeh consisted of 36 chromosomes which is in agreement with previous reports for *A. amphibius* s.l. (e.g. Miller, 1912; Arslan et al., 2011; Arslan and Zima, 2014; Şeker et al., 2018) (Fig. 2b) (Table 1).

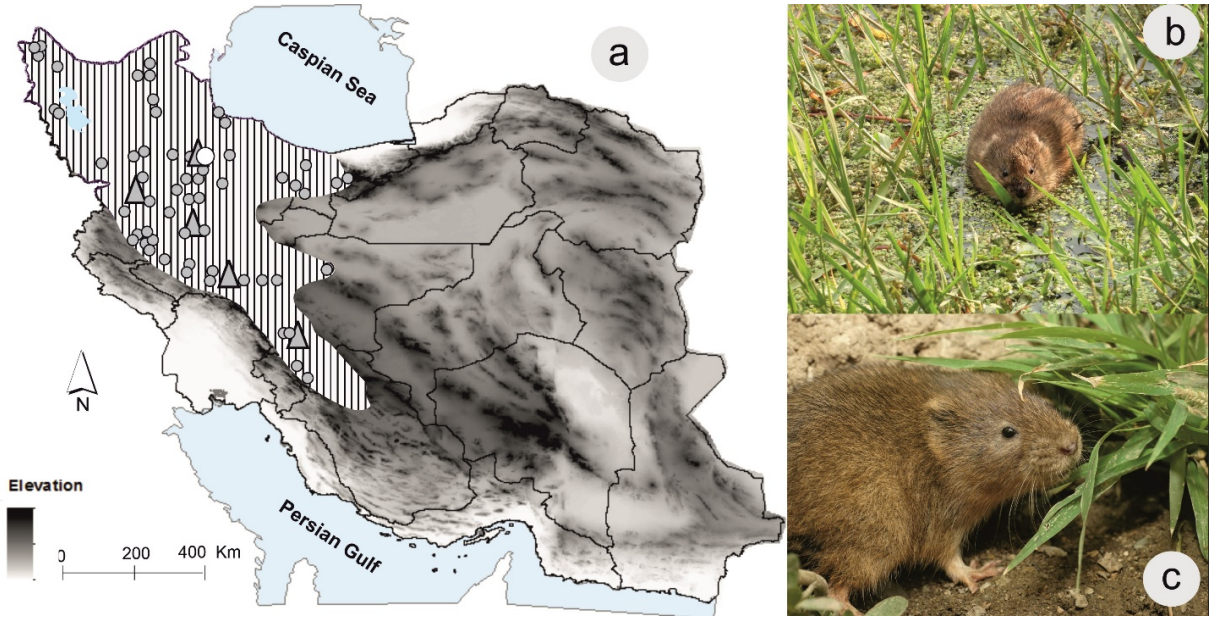

Figure 1: (a) The putative geographic range of *Arvicola persicus* in Iran (modified after Yusefi et al., 2019). The white circle shows the type locality of the species (Sultaniyeh, Alborz Mountains). Grey circles – distribution records; grey triangle – genetically analyzed samples (Mahmoudi et al., 2020). Right insets depict the Persian water vole in its natural habitat. Photo courtesy (b) Seyed Javad Hadi Asl, and (c) Fariborz Heydari.

Table 1: Karyotype characteristics of water voles of the genus *Arvicola*. 2n – Diploid number of chromosomes, NF – The fundamental number, NFa – The number of autosomal arms, M – Metacentric, Sm – Submetacentric, A – Acrocentric, St – Subtelocentric, and sex chromosomes (X and Y).

Species	Locality	2n	NFa	NF	X	Y	Reference
Arvicola amphibius s.l.	Turkey	36	60	64–66	M	A	Şeker et al. (2018)
Arvicola amphibius s.l.	Anatolia, Turkey	36	62	66	M	-	Şeker et al. (2018)
Arvicola amphibius s.l.	Turkey	36	62	66	Sm	A/St	Arslan et al. (2011)
Arvicola amphibius s.l.	Central Europe	36	68	72	Sm	St	Arslan et al. (2011)
Arvicola amphibius s.l.	Europe	36	-	60–68	Sm	A	Zima and Kral (1984)
Arvicola amphibius s.l.	Turkey	36	62	66	Sm	A	Arslan and Zima (2014)
Arvicola amphibius s.l.	Turkey	36	60	64	Sm	-	Tez et al. (2011)
Arvicola amphibius s.l.	Azerbaijan	36	62	66	Sm	A	Kuliev et al. (1978)
Arvicola amphibius s.l.	Novosibirsk, Russia	36	-	72	-	-	Kuliev et al. (1978)
Arvicola amphibius s.l.	Romania	36	60	64	Sm	A	Raicu et al. (1971)
Arvicola amphibius s.l.	Turkish Trace	36	60	64	Sm	A	Gözcelioğlu et al. (2006)
Arvicola amphibius s.l.	Central Anatolia	36	60	64	Sm	A	Özkurt et al. (1999)
Arvicola monticola	Switzerland	36	68	72	Sm	A	Schmid and Leppert (1968)
Arvicola monticola	Spain	36	64	68	M	A	Guardia and Pretel (1979)
Arvicola italicus	Italy	36	68	72	Sm	A	Castiglia et al. (2016)
Arvicola persicus	Iran	36	60	64	Sm	A	**This study**
Arvicola sapidus	Spain	40	-	-	Sm	A	Sánchez et al. (1990)
Arvicola sapidus	Iberian Peninsula	40	64	68	Sm	A	Guardia and Pretel (1979)

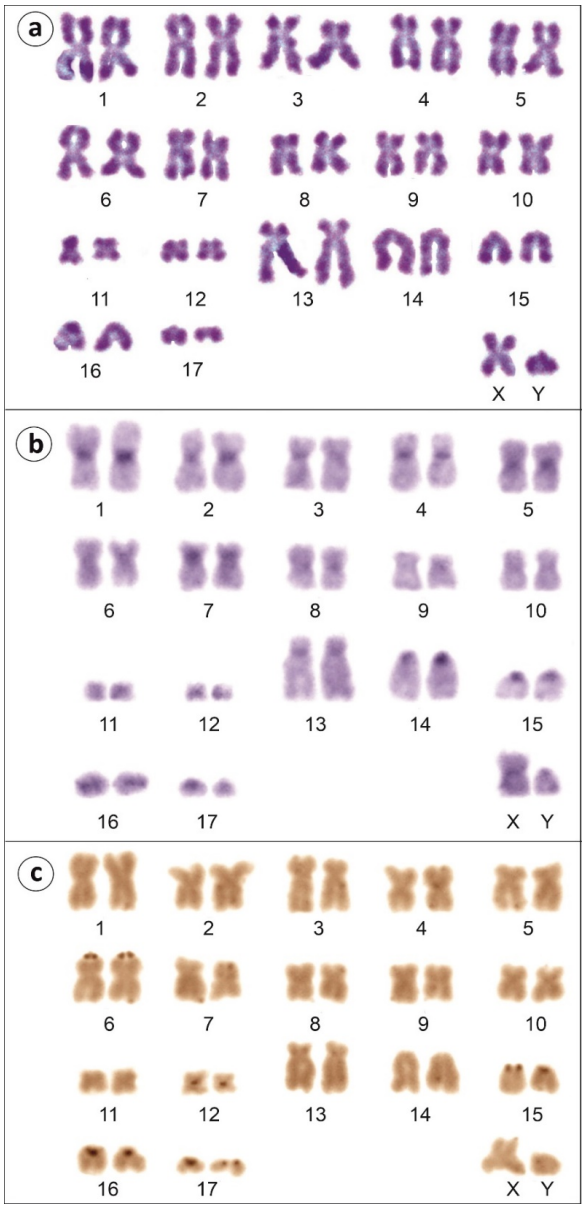

Figure 2: Karyogram of *Arvicola persicus* from Sultaniyeh (the type locality), Zanjan Province, southern Alborz Mountains, Iran. Conventional (a), C-banded (b), and silver-stained karyotypes (c).

The autosomal complement consisted of 12 meta- and submetacentric pairs (nos. 1–12), one pair of large subtelocentric (no. 13), one pair of medium-sized as well as three pairs of small-sized acrocentrics (nos. 14–17). The X chromosome was medium-sized submetacentric, while the Y chromosome was small acrocentric. Five biarmed (nos. 1, 2, 4, 5, 7), and two acrocentric autosomes (nos. 14, 15) showed centromeric C-bands, while the remaining autosomes (nos. 3, 6, 8, 9, 10, 11, 12, 13, 16, 17) and both sex chromosomes stained C-negatively (Fig. 2b). In Turkish populations of *A. amphibius* s.l., centromeric C-bands were observed in only one biarmed (no. 6) and in all acrocentric autosomes (Arslan et al., 2011; Arslan and Zima, 2014; Şeker et al., 2018).

The homomorphic active AgNORs were observed near the centromere on the metacentric pair no. 12, on the telomeric region of submetacentric pair no. 6, and within the pericentromeric C-positive areas of three smaller acrocentric pairs (nos. 15, 16, 17) (Fig. 2c). In contrast to *A. amphibius* s.l. (cf. Fig. 2: Arslan et al., 2011; cf. Fig. 3: Şeker et al., 2018), the Persian water vole is characterized by a small amount of C-positive heterochromatin and a higher number of NORs. In particular, an active NOR on the submetacentric pair no. 6 has not yet been reported for *A. amphibius* s.l. and is unique to *A. persicus*.

This is the first karyotypic description of *A. persicus* from its type locality. The genus *Arvicola* displays two distinct diploid chromosome numbers, namely 2n= 36, and 2n= 40. Populations with 2n= 40 are endemic to the Iberian Peninsula and France and are classified as *A. sapidus*. All the remaining water voles with 2n= 36 and occupying vast expanses between western Europe and Siberia, and from the Arctic circles to the Zagros Mountains belong to 3–4 distinct species (*A. amphibius*, *A. monticola*, *A. italicus*, *A. persicus*) (Kryštufek et al., 2015; Castiglia et al., 2016; Mahmoudi et al., 2020). Given that *A. persicus* holds a basal position in mitochondrial phylogenetic trees (Fig. 3) (Mahmoudi et al., 2020) we suggest that the primitive chromosome number in the genus is 36. *Arvicola persicus* currently has a small restricted range in western and northern Iran, but according to the available records of this species (Fig. 1a), one can expect its wider range including in eastern Turkey, probably Iraq, and parts of Transcaucasia.

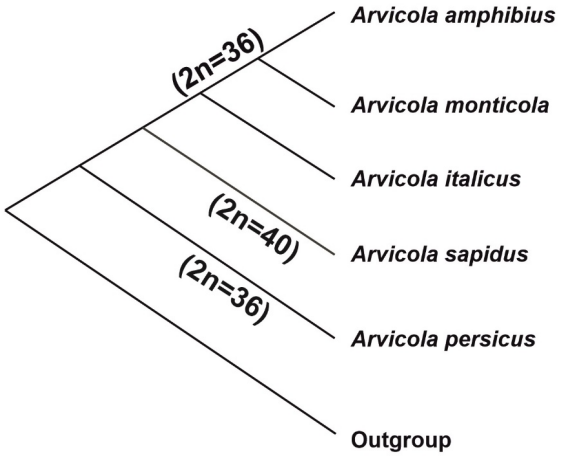

Figure 3: A simplified phylogenetic tree of the genus *Arvicola* (modified after Mahmoudi et al., 2020). Diploid chromosomal numbers of the species are indicated on the relative branches.

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Conflict of interest

The authors declare that there are no conflicting issues related to this short communication.

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