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

Ahmad Mahmoudi¹*, Atilla Arslan², Masoumeh Khoshyar³ and Boris Kryštufek⁴

¹Department of Biology, Faculty of Science, Urmia University, Iran
²Department of Biology, Faculty of Science, Selçuk University, Konya, Türkiye
³Department of Geological Science, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
⁴Slovenian Museum of Natural History, Prešernova 20, SI-1000 Ljubljana, Slovenia

*Corresponding author: a.mahmoudi.bio@gmail.com


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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 Sultaníyeh 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 (Sultaníyeh, 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).

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>2n</th>
<th>NFa</th>
<th>NF</th>
<th>X</th>
<th>Y</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arvicola amphibius</em> s.l.</td>
<td>Turkey</td>
<td>36</td>
<td>60</td>
<td>64–66</td>
<td>M</td>
<td>A</td>
<td>Şeker et al. (2018)</td>
</tr>
<tr>
<td><em>Arvicola amphibius</em> s.l.</td>
<td>Anatolia, Turkey</td>
<td>36</td>
<td>62</td>
<td>66</td>
<td>M</td>
<td>-</td>
<td>Şeker et al. (2018)</td>
</tr>
<tr>
<td><em>Arvicola amphibius</em> s.l.</td>
<td>Turkey</td>
<td>36</td>
<td>62</td>
<td>66</td>
<td>Sm</td>
<td>A/ST</td>
<td>Arslan et al. (2011)</td>
</tr>
<tr>
<td><em>Arvicola amphibius</em> s.l.</td>
<td>Central Europe</td>
<td>36</td>
<td>68</td>
<td>72</td>
<td>Sm</td>
<td>St</td>
<td>Arslan et al. (2011)</td>
</tr>
<tr>
<td><em>Arvicola amphibius</em> s.l.</td>
<td>Europe</td>
<td>36</td>
<td>-</td>
<td>60–68</td>
<td>Sm</td>
<td>A</td>
<td>Zima and Kral (1984)</td>
</tr>
<tr>
<td><em>Arvicola amphibius</em> s.l.</td>
<td>Turkmenistan</td>
<td>36</td>
<td>62</td>
<td>66</td>
<td>Sm</td>
<td>A</td>
<td>Arslan and Zima (2014)</td>
</tr>
<tr>
<td><em>Arvicola amphibia</em> s.l.</td>
<td>Turkey</td>
<td>36</td>
<td>60</td>
<td>64</td>
<td>Sm</td>
<td>-</td>
<td>Tez et al. (2011)</td>
</tr>
<tr>
<td><em>Arvicola amphibia</em> s.l.</td>
<td>Azerbaijan</td>
<td>36</td>
<td>62</td>
<td>66</td>
<td>Sm</td>
<td>A</td>
<td>Kuliev et al. (1978)</td>
</tr>
<tr>
<td><em>Arvicola amphibia</em> s.l.</td>
<td>Novosibirsk, Russia</td>
<td>36</td>
<td>-</td>
<td>72</td>
<td>-</td>
<td>-</td>
<td>Kuliev et al. (1978)</td>
</tr>
<tr>
<td><em>Arvicola amphibia</em> s.l.</td>
<td>Romania</td>
<td>36</td>
<td>60</td>
<td>64</td>
<td>Sm</td>
<td>A</td>
<td>Raicu et al. (1971)</td>
</tr>
<tr>
<td><em>Arvicola amphibia</em> s.l.</td>
<td>Turkish Trace</td>
<td>36</td>
<td>60</td>
<td>64</td>
<td>Sm</td>
<td>A</td>
<td>Gözcelioğlu et al. (2006)</td>
</tr>
<tr>
<td><em>Arvicola amphibia</em> s.l.</td>
<td>Central Anatolia</td>
<td>36</td>
<td>60</td>
<td>64</td>
<td>Sm</td>
<td>A</td>
<td>Özkurt et al. (1999)</td>
</tr>
<tr>
<td><em>Arvicola monticola</em></td>
<td>Switzerland</td>
<td>36</td>
<td>68</td>
<td>72</td>
<td>Sm</td>
<td>A</td>
<td>Schmid and Leppert (1968)</td>
</tr>
<tr>
<td><em>Arvicola monticola</em></td>
<td>Spain</td>
<td>36</td>
<td>64</td>
<td>68</td>
<td>M</td>
<td>A</td>
<td>Guardia and Pretel (1979)</td>
</tr>
<tr>
<td><em>Arvicola italicus</em></td>
<td>Italy</td>
<td>36</td>
<td>68</td>
<td>72</td>
<td>Sm</td>
<td>A</td>
<td>Castiglia et al. (2016)</td>
</tr>
<tr>
<td><em>Arvicola persicus</em></td>
<td>Iran</td>
<td>36</td>
<td>60</td>
<td>64</td>
<td>Sm</td>
<td>A</td>
<td>This study</td>
</tr>
<tr>
<td><em>Arvicola sapidus</em></td>
<td>Spain</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>Sm</td>
<td>A</td>
<td>Sánchez et al. (1990)</td>
</tr>
<tr>
<td><em>Arvicola sapidus</em></td>
<td>Iberian Peninsula</td>
<td>40</td>
<td>64</td>
<td>68</td>
<td>Sm</td>
<td>A</td>
<td>Guardia and Pretel (1979)</td>
</tr>
</tbody>
</table>
The autosomal complement consisted of 12 meta- and submetacentric pairs (nos. 1–12), one pair of large submetacentric (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 AgNORs 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*) (Kryšťufek 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 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).

**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.

References


