Cytogenetic characterization of 23 species of rodents from Iran

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Abstract
Cytogenetic approaches are used in systematic studies implying differences between species and variation between populations. In this study, morphology of chromosomes and chromosome number of 210 specimens of 23 rodent species from different localities of Iran were investigated. Specimens belong to five families comprising Dipodidae (Allactaga elater (2n=48, FNa=92); A. williamsi (2n=48, FNa=92); A. tuossi (2n=48, FNa=92); A. euphratica (2n=48, FNa=92); A. hotsoni (2n=48, FNa=92); Jaculus jaculus (2n=48, FNa=88 and 92); Pygentenus pumilio (2n=48, FNa=82 to 92)), family Cricetidae (Microtus socialis (2n=62, FNa=60); M. qazvinensis (2n=54, FNa=52); M. transcaspicus (2n=52, FNa=50); M. levis (2n=54, FNa=52); M. paradoxus (2n=62, FNa=60); Ellobius talpinus (2n=54, FNa=52); E. fuscocapillus (2n=36, FNa=54); Cricetulus migratorius (2n=22, FNa=38 and 40); Mesocricetus brandti (2n=42, FNa=78)), family Gliridae (Dryomys nitedula (2n=48, FNa=84 to 90)), family Scuridae (Funambulus pennantii (2n=54, FNa=72); Spermophilus fulves (2n=36, FNa=66)) and family Calomyscidae (Calomyscus grandis (2n=44, FNa=64); C. hotsoni (2n=50, FNa=48); C. elbursensis (2n=44, FNa=68, 70, 72 and 76); C. urartensis (2n=28, FNa=44)). In spite of intraspecific variation within some species like Calomyscus elbursensis, Cricetulus migratory, Pygrentenus pumilio, Jaculus jaculus and Dryomys nitedula the results indicated constant chromosome number and fundamental number of chromosomes in the genus Allactaga from Iran.

Key words: Karyology, Chromosome, Dipodidae, Cricetidae, Calomyscidae, Gliridae, Scuridae

INTRODUCTION
Rodentia is the largest order of mammals encompassing at least 43% of recognized mammalian species (Musser and Carleton, 2005). More than 79 rodent species have been recorded from Iran up to present (Karami et al., 2008, Darvish et al., 2010). Rodents are the most diverse order of mammals in Iran (38.2% of species) (Karami et al., 2008). One of the most important features of rodents is a great variation in the chromosome number and morphology of the chromosomes which is very important in systematic studies. This order is one of the most interesting groups for the study of karyology at the level of inter and intraspecies. For example, specimens of Calomyscus elbursensis show intraspecies variation of karyotypes with 2n=44, FN=58 and 2n=30, FN=44 (Graphodatsky et al., 2000). Besides, applying karyotype studies can provide empirical data for systematic research (Robbins and Baker, 1978). In terms of morphological characteristics, rodents show great amount of homogeneity and are hard to be recognized at the level of species based on this characteristic (Matthey,
Karyotypes of some rodents of Iran are known (e.g. Moradi-Gharkheloo and Kyvan, 2003; Darvish and Hosseinie, 2005; Darvish et al., 2006; Moradi-Gharkheloo, 2006, 2008; Mirshamsi et al., 2007; Darvish et al., 2008; Esmaeili et al., 2008), but karyotype of some species and karyological differences between populations are remained to be studied. Since there are few papers concentrated on karyological features of rodents of Iran, the purpose of the present study focused on comparing karyological features of rodent species of different localities of Iran to those previously reported for basic karyotypic characteristics.

**MATERIAL AND METHODS**

A total of 210 specimens of rodents belonging to 23 species were karyologically studied. The list of species is as follows: *Allactaga elater, A. williamsi, A. tuossi, A. euphratica, A. botsoni, Pygeretmus pumilio, Jaculus jaculus, Microtus socialis, M. gaziensis, M. transcaucasicus, M. levis, M. paradoxus, Ellobius talpinus, E. fuscocapillus, Cricetulus migratorius, Mesocricetus brandti, Dryomys nitedula, Funambulus pennantii, Spermophilus fulvus, Calomyscus grandis, C. botsoni, C. elburzensis and C. urartensis*. Specimens of rodents were captured from various localities of Iran using live traps between 2000 to 2012, and were shown in Figure 1 and Table 1. Vouchers were deposited in the Zoology Museum of Rodentology Research Department of Ferdowsi University of Mashhad, Iran (ZMFUM).

Chromosome spreads were achieved according to the conventional bone marrow method. The Vinblastin solution (1 mL/100 g of body weight) (Yosida, 1973) was injected i.p one hour before killing the animal with chloroform. Bone marrow was extracted and incubated for 15 min at 37°C in 8ml KCl 0.075 M. Fixation was performed by methanol: acetic acid (3:1 v/v). Metaphases suspensions were deposited on the slides by Cell Shutting tools made by Rodentology Research Department of Ferdowsi University of Mashhad (RDFUM), and stained with 6% standard Giemsa-solution (PH=7). Well-spread metaphases were photographed using a CCD camera attached to the microscope. At least ten to fifteen well-spread metaphase cells were analysed for each species. Idiograms were prepared from the best metaphase spreads. The chromosomes of all specimens were paired by the Chromosome Image Processing software (CIP), developed by the (RDFUM). For all of rodents the diploid number (2n), the fundamental number (FN) and autosomal fundamental number (FNa) were counted in at least three to four metaphases per specimen. 2n and FNa were obtained to allow comparison between different karyotypes.

**RESULTS**

The karyology of 23 species of rodents from Iran were studied. The information concerning these species is summarized in a synopsis of karyotypic characteristics, in Table 1. This data shows that the levels of chromosomal variations inter and intra species are considerable.

**Karyotype Study**

**Dipodidae Fischer, 1817**

**Genus Allactaga Cuvier, 1837**

The karyological study of all samples from the species of the genus *Allactaga* (*A. elater, A. williamsi, A. tuossi, A. euphratica and A. botsoni*) from different regions revealed that they had the same karyotype with 2n=48, but FNa=92. The X chromosomes in *A. elater* except for samples from Incheborun (Figs. 2a, 5) and *A. tuossi* are large and submetacentric, whereas the Y chromosomes are small and subtelocentric except for *A. elater* from Gonbad that the Y chromosomes was telocentric (Table 1 and Figs. 2a, a1, a2, a3, a4, a6, e). The X chromosome in samples from Incheborun was small metacentric. *A. euphratica* has two medium size submetacentric X chromosomes (Fig. 2b). The X and Y chromosomes in specimens of *A. williamsi* and *A. botsoni* are submetacentric and acrocentric.
respectively (Table 1 and Figs. 2c, d). In two species A. williamsi and A. botoni each autosomal chromosome has two arms.

**Genus Pygeretmus Gloger, 1841**

The karyotypes of male and female *P. pumilio* from Gonbad and Aq Qala of Golestan province in the north of Iran were 2n=48 and FNa from 82 to 92. Males represent 18 pairs of meta-submetacentric and 5 pairs of acrocentric chromosomes (Table 1 and Figs. 4a, 4b, 4c). On the other hand, in females, there were 18 pairs of meta-submetacentric, 3 pairs of acrocentric and 2 pairs of telocentric chromosomes (Fig. 3b). The X chromosome was a medium-sized submetacentric, while the Y was acrocentric.

**Dipodinae Fischer, 1817**

**Genus Jaculus Erxleben, 1777**

The karyotype of male and female *J. jaculus* species showed a diploid number of 2n=48 and the fundamental autosomal arm number (FNa) from 88 to 92. The sexual chromosomes were pair of submetacentric X chromosomes or a X and a small subtelocentric Y (Table 1 and Figs. 4a, 4b, 4c).

### Table 1. Karyotype Characteristics of rodents from different localities of Iran.

<table>
<thead>
<tr>
<th>Species</th>
<th>Samples locality</th>
<th>Latitude</th>
<th>Longitude</th>
<th>2n</th>
<th>FNa</th>
<th>Samples codes</th>
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<td>92</td>
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<td>44</td>
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<td>46° 16'</td>
<td>28</td>
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Genus Microtus Schrank, 1798

Specimens of *Microtus socialis* and *M. qazvinensis* from Zanjan province were studied. The specimens of *Microtus socialis* had 2n=62 and FNa=60. The karyotype of *M. qazvinensis* specimens from Zanjan was 2n=54, FNa=52, FN=54. The X and Y chromosomes were large subtelocentric and telocentric, respectively (Table 1 and Figs. 5a, b). Several specimens of *M. transcaspicus* from Khorasan Razavi province were karyologically studied. Karyological analyses of samples showed the same karyotype 2n=52 and FNa=50, which is in agreement with previously published data for this region (Golenishchev et al., 1999; Mazurok et al., 2001). All autosomes were found to be telocentric. The X chromosome was a large telocentric (Fig. 5c). The karyotype of female and male specimens of *M. levis* which were studied was 2n=54 of acrocentric autosomes with the FNa of 52. The X and Y chromosomes were both telocentric (Figs. 5d, e). Specimens of *M. paradoxus* had a karyotype consisting of 2n=62 and a fundamental number of autosomes (FNa) of 60. All autosomes were found to be telocentric. The X chromosome was a large telocentric (Fig. 5f).
FIGURE 2. Metaphase spreads and idiograms of the genus *Allactaga*. (a) *A. elater* from Aq Qala (a1) Sarakhs (a2) Kavir, Yazd (a3) Tabas (a4) Gonbad (a5) Incheborun (a6) Kashmar (b) *A. euphratica* (c) *A. williamsi* (d) *A. botoni* (e) *A. toussi*. 
Genus *Ellobius* Fischer, 1814

The karyotypes of *E. talpinus* and *E. fuscocapillus* from Chekoudar, Khorasan Razavi province were studied. The specimens of *E. talpinus* demonstrated $2n=54$ and $FNa=52$. All autosomes were found to be telocentric with a size range of medium to small. The X chromosome was an submetacentric, whereas the Y chromosome was approximately subtelocentric (Table 1 and Fig. 6a). *E. fuscocapillus* showed to have 6 pairs of metacentric, 4 pairs of submetacentric and 7 pairs of acrocentric chromosomes. The X chromosome was a large submetacentric while the Y chromosome was a small subtelocentric. In fact, this species characterized by $2n=36$, $FNa=54$ and $FN=58$ which was in concordance with Moradi-Gharkheloo (2003) (Fig. 6b).
Cricetinae Fischer, 1817
Genus Cricetulus Milne-Edward, 1867
In this genus, specimens of C. migratorius from populations of Yazd and two different regions of Khorasan Razavi province (Nayshabur and Gouchan) and the population from Golestan province (Maravehtapeh) were investigated. All specimens of C. migratorius from five areas had the same karyotype with 2n=22, FN=42 to 44 and FNa=38 to 40. Our results from Yazd, Nayshabour and Gouchan consisted of 5 pairs of metacentric chromosomes, 2 pairs of submetacentric and one pairs of acrocentric chromosomes. The X chromosome was large and metacentric, whereas the Y chromosome was medium and submetacentric (Table 1 and Figs. 7a, b, c, d).

Genus Mesocricetus Nehring, 1898
The karyotype of Mesocricetus brandti specimens from Zanjan indicated 2n=42, FNa=78, FN=82. The chromosome combination was made up of 4 pairs of metacentric and 15 pairs of submetacentric and one pairs of acrocentric chromosomes. The X was a submetacentric and the Y was smaller and metacentric chromosome (Table 1 and Fig. 8).

Gliridae Muirhead, 1819
Leithiinae Lydekker, 1896
Genus Dryomys Thomas, 1906
Specimens of Dryomys nitedula had a karyotype consisting of 2n=48 and a fundamental number of autosomes (FNa) of 84 to 90. In this species there were 19 pairs of metacentric and submetacentric, 4 pairs of subacrocentric and acrocentric chromosomes. The X chromosome was metacentric, while the Y was a small telocentric (Table 1 and Fig. 9).

Seuridae Fischer, 1817
Xerinae Osborn, 1910
Genus Spermophilus F. Cuvier, 1825
Spermophilus fulvus from Dargaz was karyologically studied and its 2n, FNa and FN were determined 36, 66 and 70 respectively. This species possessed 16 pairs of meta-submetacentric and one pairs of subtelocentric chromosomes. The X chromosome was a large metacentric and the Y was a large submetacentric (Table 1 and Fig. 10).

Callosciurinae Pocock, 1923
Genus Funambulus Lesson, 1835
Metaphase spread and idiogram of the only specimen of F. pennantii from Sarbaz, Sistan-Baluchistan province in the south-east of Iran was described. The species examined had a karyotype consisting of 2n=54 and a fundamental number of autosomes (FNa) of 72. This species had 10 pairs of meta or submetacentric, 16 pairs of acrocentric chromosome. The X chromosome was submetacentric, while the Y was a large acrocentric (Table 1 and Fig. 11).

Calomyscidae Vorontsov and Potapova, 1979
Genus Calomyscus Thomas, 1905
Specimens of four species of Calomyscus including C. grandis, C. hotsoni, C. elburzensis and C. urartensis from different areas of Iran were karyologically studied. The diploid chromosome number of the species of C. hotsoni from Saravan, Sistan-Baluchistan province in the south-east of Iran was 2n=50 and FNa of 48, including 24 pairs ofacrocentric and 2 medium to large sized submetacentric X chromosomes (Table 1 and Fig. 12a).

C. grandis and C. elburzensis were characterized by 2n of 44 and FNa from 64 to 74 (Figs. 12b, c, c1, c2, c3). Two X chromosomes in C. grandis were subtelocentric (Fig. 12b). The X chromosomes in specimens of C. elburzensis from Aghdarband, Khorasan Razavi province and C. elburzensis from Bojnord, Khorasan Shomali province, were medium to large subtelocentric and the Y chromosome was acrocentric (Figs. 12c, c1). The X chromosome of C. elburzensis from Gelyan, Khorasan Razavi province was medium-sized and submetacentric, whereas Y was a large acrocentric chromosome.
C. elburzensis from Yazd showed 70 autosomal arms. The X chromosome was submetacentric and Y was small chromosome (Figs. 12c3). Also, we report a new karyotype of C. urartensis from Western Azarbayjan Province (Kordasht village, 38°34' N, 46° 16' E). The diploid chromosome number (2n) and the fundamental autosomal arm number (FNa) were 28 and 44 respectively. The autosomal set consisted of 4 pairs of telocentrics, 2 pairs of acrocentrics and 7 pairs of meta and submetacentrics. X chromosome was a large telocentric and the Y was a small submetacentric (Table 1 and Fig. 12d).

**DISCUSSION**

Karological differences between closely related taxa indicated that chromosomal rearrangement is one of the factors which lead to evolution of taxa (Rao et al., 1971). In addition, chromosomal studies can increase our understanding of phylogeny (Graphodatsky et al., 2000,) and help us to identify species, dispersal routes, probable contact zones and hybridizations (Graphodatsky et al., 2000). At the level of species, these data are extremely useful for identification of extensive chromosomal variations within populations, between species and sibling species with a similar morphology and discovery of a new cryptic species (Zima, 2000). The karyological studies for 23 species of rodents from Iran were indicated that karyotypes of some species were similar to those published earlier and some were different (Table 1).

The diploid number (2n) of *Calomyscus* species have been reported between 30 to 52 with FNa between 42 to 60 (Malikov et al., 1999; Graphodatsky et al., 2000; Malikov et al., 2001; Esmaeili et al., 2008). Based on the present study, species of the genus *Calomyscus* from Iran have 2n=44-50 with FNa=48-70. *Calomyscus urartensis* ranges through Transcaucasian region. Graphodatsky et al. (2000) reported 32 chromosome specimens of *Calomyscus* from Naxçivan region in Azerbaijan as *C. urartensis*. In this study 28 chromosome specimens of *C. urartensis* were captured from 100 km of type locality in Dzulfa, Naxçivan. Although, captured specimens have shown different 2n and FNa from the type specimens they were identified as *C. urartensis* because variation in chromosome number and fundamental number of chromosomes in this genus had been reported before (Graphodatsky et al., 2000; Shahabi et al., 2010).

Chromosomal studies along with morphometric and morphological methods could be used as a tool for identification of various species of the genus *Microtus* (Mazurok et al., 2001). In fact, it seems that identification and description of some karyotypic sibling species in this genus refer to higher rate of karyotype evolution in this genus comparing to other mammals. Martinkova et al. (2007) explained that genus *Microtus* represents 2n=17-62. Our investigation indicated that *Microtus* specimens from Iran had 2n of 52-62 with the FNa of 50-60. These interspecific variations might be due to the various kinds of rearrangements such as frequent Robertsonian translocations (chromosomal fusions and fissions), pericentric inversions, heterochromatin changes and supernumerary chromosomes (Yüksel, 1984; Yüksel et al., 2001; Zima, 2000, 2004). The karyotype of *M. levis* has already been described from Turkey (Gözütok and Albayrak, 2009). In this study the karyotypes of male and female specimens of *M. levis* from northeast of Iran has been reported for the first time. These were the same as karyotypes reported from Turkey.

Our result represents low intra and interspecific variations in karyotype of some genus such as *Allactaga* and *Jaculus* with 2n of 48, FNa of 92 and 2n of 48, FNa of 88 to 92, respectively. These results are in agreement with the karyotype of diploid species which has been previously described (Darvish et al., 2006, 2008; Moradi-Gharkheloo, 2008; Darvish and Hosseineie, 2005; Shahin and Ata, 2001, 2004). Comparing our results for the family Dipodidae with those reported for *Allactaga williamsi*, *A. euphratica*, *A. tetradactyla*, *A. elater*, *A. hottoni*, *A. major*, *A. sibirica* and *A. jaculus* from other regions (Vorontsov and Malygina, 1973; Zima and Kral, 1984; Çolak et al., 1997a, 1997b; Çolak and Yigit, 1998; Shahin and Ata, 2001, 2004; Abi-Said, 2004; Darvish and Hosseineie, 2005; Darvish et
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al., 2006; Ata and Shahin, 2006; Sözen et al., 2008; Moradi-Gharkheloo, 2009; Arslan and Zima, 2010) shows that the genus was chromosomally rather conserved as no karyotypic differences could be detected among the species so far. This karyotypic uniformity at the generic level has several reasons, lower phylogenetic age of the taxon, population dynamics and population structure (Darvish et al., 2006).

Species of *C. migratorius* from different geographic regions have 2n of 22 and FNa of 38 to 40, which are similar to data reported from Iran (FNa=38) (Moradi-Gharkheloo, 2006) and Turkey (FNa=40) (Arslan and Akan, 2008) before. These results encourage the idea that there are karyological variations in the chromosomal arms of this species.

The karyotypes of *Dryomys nitedula* were invariant and in agreement with those previously published by Moradi-Gharkheloo (2009). Comparing our results on karyology of *Dryomys nitedula* and those of previous works represents conserved karyotype of 2n=48 and little variation between populations (Doğramaci and Kefelioglu, 1990; Zima et al., 1995). Remarkably the first large pair of chromosomes which had a unique karyotypic feature of *D. nitedula* was in accordance with other studies. The X chromosome of our specimens was a large sized metacentric in agreement with other studies (Zima and Král, 1984; Peshev and Delov, 1995; Zima et al., 1995). However, Graphodatsky and Fokin (1993) and Mitsainas et al. (2008) reported it as submetacentric. On the other hand, Mitsainas et al. (2008) reported dot-like Y chromosome for this species but our result showed telocentric Y chromosome.

Some of different species of the genus *Spermophilus* were introduced based on karyological features (Zima and Král, 1984). The karyological result of *Spermophilus fulvus* from Iran was in agreement with those reported previously by Özкрут et al. (2007) from Turkey.

*Mesocricetus brandti* from Zanjan and central Anatolia, in accordance with Yiğit et al. (2006), have 2n of 42, FNa of 78 and FN of 82 and the Y chromosome, smaller than the X chromosome, and metacentric but the X chromosomes are submetacentric instead of metacentric reported by Yiğit et al. (2006). However, our results were different from those reported from Ardahan and Van, with the FNa of 80, FN of 84 and FNa of 78, and the FN of 80 for Central Anatolia (Yiğit et al., 2000).

Chromosome characteristics of *F. pennantii* have been already described by Sharma et al., (1970) and Roa et al., (1971). The X chromosome has been reported to have large acrocentrics (Chopra and Pai, 1965), a medium metacentric (Srivastava and Bhatnager, 1971), a large submetacentric (Sharma et al., 1970) and larger metacentric (Roa et al., 1971). Y chromosome has been suggested to have small acrocentrics (Roa et al., 1971). The karyotype of this species described by Sharma et al., (1970) was with the 2n of 54 and FN of 74, comprising 10 pairs of meta-submetacentrics and 17 pairs of acrocentrics in females, while Roa et al., (1971) reported 14 pairs of meta-submetacentrics and 9 pairs of acrocentrics in females. They suggested 13 pairs of meta-submetacentrics, 9 pairs of acrocentrics and one pairs including one large metacentric and one smallest acrocentric chromosome for males (Roa et al., 1971). For specimens of this species 2n was in agreement with the data reported previously by Sharma et al. (1970) and Roa et al. (1971), but morphology of chromosomes was different.

The diploid chromosome number of *E. fuscocapillus* recorded by Borisov et al. (1991) was 36. Also, Moradi-Gharkheloo (2003) described 2n of 36, FNa of 54 and NF of 58 for specimens of this species from Iran, with 6 pairs of metacentrics, 4 pairs of submetacentric, 7 pairs of subtelocentric and the medium-sized submetacentric X chromosome, and the subtelocentric Y chromosome. In fact, this species characterized by the 2n of 36, FNa of 54 and FN of 58 was in concordance with Moradi-Gharkheloo (2003) (Fig. 6b). Morphology of chromosomes was also in agreement with those previously reported from Iran by Moradi-Gharkheloo (2003). Specimens of *E.talpinus* had the 2n of 54 and FNa of 52 acrocentric autosomes. For this species 2n and FNa were not in agreement with the data which were reported previously by Moradi Gharkheloo (2003), but 2n was in concordance with Romanenko et al. (2007).
In spite of intraspecific variation within some species like *Calomyscus elburzensis*, *Cricetulus migratorius*, *Pygeretmus pumilio*, *Jaculus jaculus* and *Dryomys nitedula* the results indicated constant chromosome number and fundamental number of chromosomes in the genus *Allactaga* from Iran. As a matter of fact, chromosome studies along with other methods could be a useful method for studying differences between species and populations. However, it is strongly depends on phylogenetic age of the species and populations, evolutionary history of the taxon, ecological and behavioral characteristics of the taxa (Zima et al, 1997).

**FIGURE 5.** Metaphase spreads and idiograms of genus the *Microtus*. (a) *Microtus socialis* (b) *M. gazvinensis* (c) *M. transcaucasicus* (d, e) *M. levis* (f) *M. paradoxus*.

**FIGURE 6.** Metaphase spreads and idiograms of the genus *Ellobius*. (a) *E. talpinus* (b) *E. fuscocapillus*. 
**Figure 7.** Metaphase spreads and idiograms of *Cricetulus migratorius* (a) Yazd (b) Nayshabour (c) Gonbad (d) Maravtapeh.

**Figure 8.** Metaphase spread and idiogram of *Mesocricetus brandti*.

**Figure 9.** Metaphase spread and idiogram of *Dryomys nitedula*.

**Figure 10.** Metaphase spread and idiogram of *Spermophilus fulvus*.

**Figure 11.** Metaphase spread and idiogram of *Funambulus pennantii*. 
FIGURE 12. Metaphase spreads and idiograms of the genus *Calomyscus*. (a) *C. hotsoni* (b) *C. grandis* (c) *C. elborzenis* from Aghdarband (c1) Bojnord (c2) Gelyan (c3) Yazd and (d) *C. urartensis*.

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LITERATURE CITED


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