

Karyologic study of three species of *Calomyscus* (Rodentia: Calomyscidae) from Iran

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The genus *Calomyscus* has long been considered monotypic and represented by the species *C. bailwardi*. Finally in the most recent revision, eight geographic species were recognized: *C. bailwardi*, *C. baluchi*, *C. elburzensis*, *C. grandis*, *C. hotsoni*, *C. mystax*, *C. tsolovi*, and *C. urartensis*. Since the taxonomic status of this genus remains somewhat controversial, karyological analysis may provide important information for evaluating the systematic position of *Calomyscus*. So far, within the range of the genus *Calomyscus* several distinct karyotypes have been identified. The karyotype of *C. hotsoni* has been undocumented until now but its karyotype recorded in this study from vicinity of type locality, Sistan va Baluchistan Province in the south eastern of Iran that was different with the karyotype of another species in this genus. Furthermore, three new karyotypes of *Calomyscus* were studied by conventional staining technique from Khorasan and Yazd Provinces of Iran that represent chromosomal variations in this genus. Chromosome studies show that *Calomyscus* specimens from Yazd Province should be belong to *C. elburzensis*. But more karyological, molecular and morphological studies would be necessary for confirming this decision.

Key words: *Calomyscus*, karyology, Iran

INTRODUCTION

The genus *Calomyscus* Thomas, 1905 has long been considered monotypic and represented by the species *C. bailwardi* (Ellerman and Morrison-Scott, 1966; Corbet and Hill, 1980). Vorontsov et al. (1979) treated most of the subspecies of *C. bailwardi* as distinct species, and in the most recent revision, Carleton and Musser (2005) recognized eight geographic species: *C. bailwardi*, *C. baluchi*, *C. elburzensis*, *C. grandis*, *C. hotsoni*, *C. mystax*, *C. tsolovi*, and *C. urartensis* (Fig. 1). This genus contains a number of geographically isolated populations that inhabit arid, rocky hillsides and mountains ranges in Syria, Azerbaijan, Iran, Turkmenistan, Afghanistan and Pakistan (Lay, 1967; Hassinger, 1973; Vorontsov et al., 1979; Schlitter and Setzer, 1973; Musser and Carleton, 1993; Graphodatsky et al., 2000; Carleton and Musser, 2005). The morphological and geographic integrity of some has been subsequently tested with additional chromosomal data (Graphodatsky et al., 2000; Malikov et al., 1999; Meyer and Malikov, 1996) and multivariate analyses of cranial and dental measurements (Lebedev et al., 1998).

While much research has focused on the geographical distribution of different chromosomal morphologies and their taxonomic significance (Graphodatsky et al., 2000), the multivariate analysis by Lebedev et al. (1998) demonstrated distinct morphological clusters that correspond to the karyotypic differences (Carleton and Musser, 2005). So far, within the range of the genus *Calomyscus*

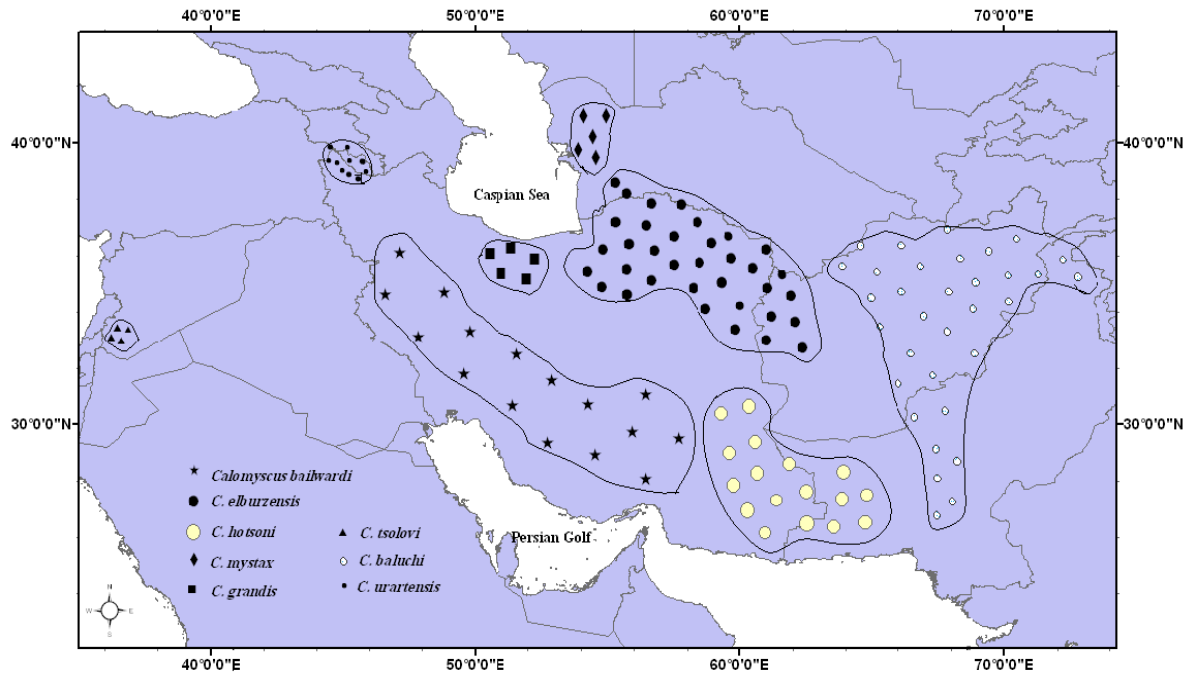


FIGURE 1. Distribution of the eight allopatric species of *Calomyscus*.(modified from Vorontsov et al. (1979) with addition of data in Graphodatsky et al. (2000), Meyer and Malikov (2000), Carleton and Musser (2005), Norris et al. (2008).

several distinct karyotypes have been identified: *Calomyscus baiwardi* has three distinct karyotypes from Zagros Mountains, Iran with $2n=37$, $FNa=44$; $2n=50$, $FNa=50$; $2n=52$, $FNa=56$, *C. grandis* from Tehran, Iran with $2n=44$, $FNa=46$, *C. elburzensis* from Kopet Dag Mountain, Iran and Akal Region, central and eastern Kopet Dag, Mary Region, western Badkhyz, Turkmenistan with $2n=44$, $FNa=58$; from Khorasan Province, Iran with $2n=44$, $FNa=60$ and from Akal Region, central Kopet Dag Turkmenistan with $2n=30$, $FNa=44$, *C. urartensis* from Naxcivan, Azerbaijan with $2n=32$, $FNa=42$ and *C. mystax* from Balkhan Region, Turkmenistan with $2n=44$, $FNa=46$ (Graphodatsky et al., 2000; Malikov et al., 2001; Esmacili et al., 2008). Since the taxonomic status of this genus remains somewhat controversial, karyological analysis may provide important information for evaluating the systematic position of *Calomyscus*. We report several different karyotypes in this genus from various localities of Iran as well as the karyotype of *C. hotsoni* from south eastern of Iran that has been undocumented until now.

MATERIAL AND METHODS

Specimens of genus *Calomyscus* were collected between March 2004 and August 2009 from various locations of Iran (Fig. 1) by various live and snap traps. Live trapped specimens were transferred to the Ferdowsi University where chromosome metaphases were obtained from bone marrow. Chromosome spreads from the femoral and tibial bone marrow cells of the one hour vinblastin-treated specimens (1 mL/100 g of body weight) were prepared by air drying method, according to the method of Dutrillaux et al (1982). Metaphases were stained by the Giemsa standard method ($pH=7$). About 20 metaphase spreads from each specimen were examined at $\times 100$ magnification and photographed using a CCD camera connected to a computer. The karyotype was determined on the basis of five to ten well-prepared metaphase spreads by the chromosome processing software made

in Rodentology research Department of Ferdowsi University of Mashhad, Iran. Terminology for the description of the centromeric position on each chromosome follows Shahin and Bandata (2001), considering a chromosome as metacentric if the ratio of the length of longer arm (P) is equal to 1.00. However, it was considered subtelocentric if the ratio was higher than 1.79 and submetacentric if the ratio was higher than 1.00 and equal to or lower than 1.79. If the ratio is 0.00, a chromosome is considered telocentric (acrocentric). The results were compared according to variations in $2n$ and FNa values. All specimens were deposited and housed in the Zoological Museum, Ferdowsi University of Mashhad and Rodentology research Department (ZMFUM.)

RESULTS

We record new karyotypes for three species of the present study from Iran as well as the karyotype of *C. botsoni* from vicinity of type locality (Saravan, Sistan va Baluchistan Province in the south eastern of Iran) that has been undocumented until now.

KARYOTYPES

Karyotype of *Calomyscus elburzensis*; A single male specimen from Bojnord, Khorasan Province, ($37^{\circ} 29' N$, $57^{\circ} 17' E$). The karyotype contained 44 chromosomes (Fig. 2A), including 10 pairs of subtelocentric autosomes, 11 pairs of acrocentric autosomes ($FNa = 62$). The X chromosome was subtelocentric and the Y chromosome was acrocentric.

Karyotype of *C. elburzensis*; A single male specimen from Gelyan, Khorasan Province ($37^{\circ} 20' N$, $57^{\circ} 56' E$). The karyotype possessed 44 chromosomes (Fig. 2B), including three pairs of subtelocentric autosomes, six pairs of acrocentric autosomes, six pairs of metacentric autosomes and six pairs of submetacentric autosomes ($FNa = 72$). The X chromosome was submetacentric and the Y chromosome was acrocentric.

Karyotype of *C. elburzensis*; The karyotype of two female specimens from Aghdarband, Khorasan Province ($36^{\circ} 30' N$, $61^{\circ} 7' E$) contained 44 chromosomes (Fig. 2C), including 10 pairs of subtelocentric autosomes, 11 pairs of acrocentric autosomes ($FNa = 62$). The two X chromosomes were subtelocentric. This karyotype was similar to karyotype 1.

Karyotype of *C. grandis*; The karyotype of two female and male specimens from type locality, Fasham, Tehran Province ($35^{\circ} 56' N$, $51^{\circ} 31' E$) as Graphodatsky et al. (2000) recorded from lower station of the Telecaban road, Tehran Province, contained 44 chromosomes (Fig. 2D) and including two pairs of subtelocentric autosomes, 19 pairs of acrocentric autosomes ($FNa = 46$). Two X chromosomes in the female and male specimens were subtelocentric and the Y chromosome in the male was acrocentric.

Karyotype of *C. botsoni*; Two female specimens from vicinity of type locality, Saravan, Sistan va Baluchistan Province in the south eastern of Iran ($27^{\circ} 18' N$, $61^{\circ} 46' E$) presented a complement of 50 chromosome and 48 autosome arms ($2n=50$, $FNa = 48$), including 24 pairs of acrocentric autosomes and the two X chromosomes were subtelocentric (Fig. 2E).

Karyotype of *C. botsoni*; A diploid number of 48 chromosomes and 48 autosome arms from vicinity of type locality, Saravan, Sistan va Baluchistan Province in the south eastern of Iran ($27^{\circ} 18' N$, $61^{\circ} 46' E$) recorded ($2n=48$, $FNa = 48$), including one pair subtelocentric autosome, 22 pairs of acrocentric autosomes (Fig. 2F). The two X chromosomes were subtelocentric.

Karyotype of *C. elburzensis*; One male specimen from Yazd Province ($31^{\circ} 40' N$, $54^{\circ} 19' E$) that its karyotype contained 44 chromosomes and 70 autosome arms. The X chromosome was submetacentric and the Y chromosome was acrocentric (Fig. 2G).

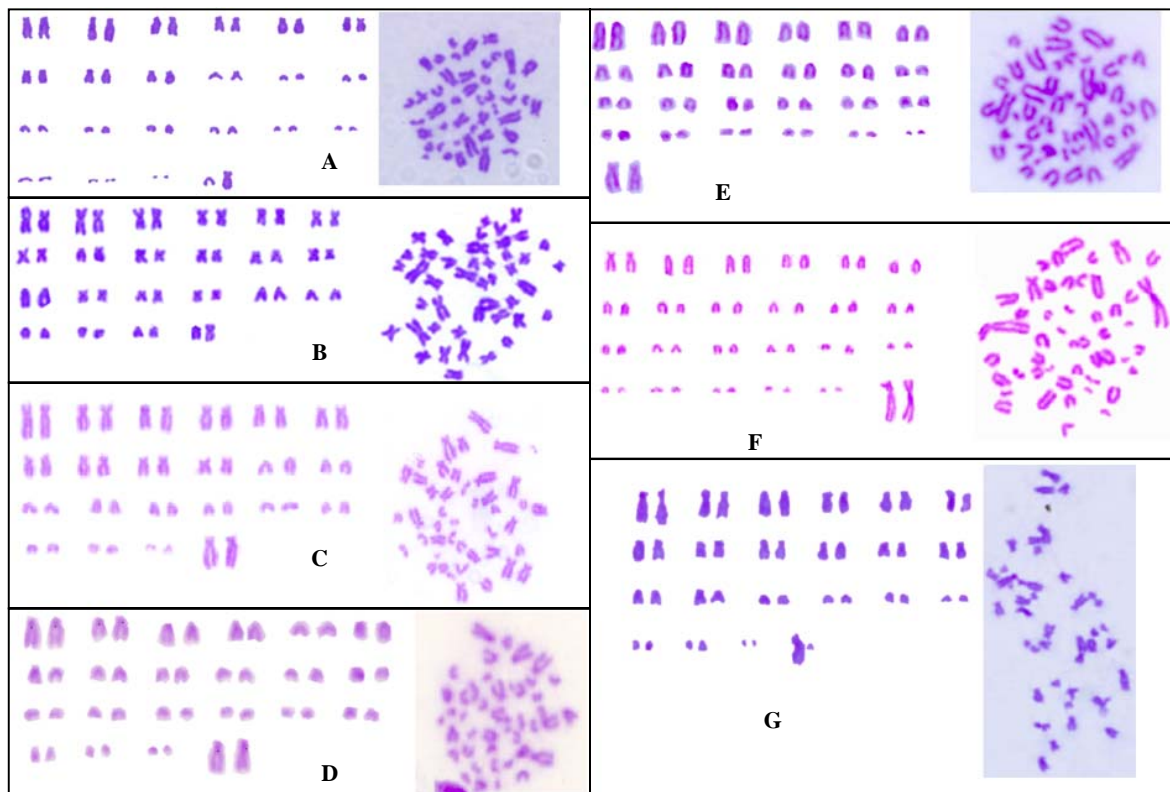


FIGURE 2. The karyotypes of *Calomyscus* species from different localities of Iran. *C. elburzensis* (A, B, C and G); *C. grandis* (D); *C. hotsoni* (E and F).

DISCUSSION

The chromosome number of the known karyotypes of *Calomyscus* varies from $2n = 30$ to $2n = 52$, and the number of arms (FNa) from 42 to 60 (Graphodatsky et al., 2000; Malikov et al., 2001; Esmaeili et al., 2008). In this study, the karyotype of *C. grandis* from type locality compared with the karyotype which was described by Graphodatsky et al. (2000) showing both of them contained 44 chromosomes and 46 autosome arms, including two pairs of subtelocentric autosomes and 19 pairs of acrocentric autosomes that represent an unique karyotype for *C. grandis*. About *C. elburzensis*, we reported three new karyotypes that diploid number of chromosomes was 44 and similar to which recorded by Graphodatsky et al. (2000) but this study found the autosomal numbers of 62 and 72 in Khorasan Province that were different from another specimens of *C. elburzensis*. We also recorded the karyotype with chromosome number of 44 and autosome number of 70 from Yazd Province and considered it belonging to *C. elburzensis*. Our subsequent morphometric and molecular studies confirmed the karyology results. It represents a noteworthy chromosomal change that it may be a reason to speciation in this genus but nothing shows evidence for speciation and may be just a chromosomal polymorphism. Graphodatsky et al. (2000) identified centric and tandem fusions and heterochromatin changes as the major modes of karyotype evolution in this group. Furthermore they recorded natural hybrids between individuals with different karyotypes. They also indicated that chromosomal repatterning occurred recently within the genus by fluorescence in situ hybridization

with a 353-bp *Bsp*RI complex tandem repeat (Graphodatsky *et al.*, 2000). *Calomyscus hotsoni* is endemic to southwestern Pakistan and southeastern Iran. This species is smaller than the other species in Pakistan (*C. baluchi*), has a smaller hind foot and black-tipped, pale yellow-brown pelage and its karyotype has been undocumented until now. Based on size, *C. hotsoni* is allied with *C. mystax* and *C. elburzensis* and is known from the vicinity of the type locality (Baluchistan, Gwambuk Kaul, about 30 miles southwest of Panjgur) and from Sistan va Baluchistan Province of southeastern Iran (Carleton and Musser, 2005; Norris *et al.*, 2008). Two different chromosome numbers of 48 and 50 with the equal autosome number of 50 for *C. hotsoni* were found in the same place from Baluchistan Province that represent a deletion in the only pair biarm autosome for karyotype 6 has transformed to karyotype 5 that does not have any biarm autosome or vice versa. The chromosome number of 50 and autosome number of 50 recorded in this species is the same as recorded by Graphodatsky *et al.* (2000) in *C. bailwardi* from Zagros Mountains in Fars Province, shows it may be semispecies or a subspecies of *C. bailwardi*. But regarding to high variation in the chromosome and autosome number of *Calomyscus* species, decision about systematic status of this species is difficult and need more karyological and molecular studies. However Robertsonian translocations have played an important role in karyotype evolution of this genus. Without an extensive karyological study, we could not determine if karyotypic variation recorded in *Calomyscus* indicate recent speciation events or intraspecific polymorphism caused by chromosomal repatterning. So far, *C. elburzensis* has been recorded from South and Southwest of Turkmenistan and in Iran from north and northwest mountains (Carleton and Musser, 2005). With considering Yazd specimens as *C. elburzensis*, there will be plenty of arguments about distribution, origin and how *C. elburzensis* exists in Yazd Province. We recommend collecting more specimens for karyological studies especially by G-banding and fluorescence in situ hybridization techniques from Yazd and other localities of Iranian Plateau.

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