Research Article

Karyological study of the Caspian bent-toed Gecko *Cyrtopodion caspium* (Sauria: Gekkonidae) from North and North-Eastern of Iran

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Abstract

The diploid number among Gekkonid lizards ranges from 2n=16 to 2n=46. The majority of geckos' chromosomes are small which makes the study of their chromosomes very difficult. For this reason, karyotype of *Cyrtopodion caspium* and some similar gecko are still not known. The close relatives of *C. caspium* have variation in the chromosomal number in their populations. So to find out possible variability among these populations in Iran. We described the karyotype of testis and bone marrow of 14 specimens of this species from seven provinces and 10 localities in north and north-eastern parts of Iran in 2011. They showed 2n=38 for all populations. There is not intraspecific chromosome polymorphism in *Cyrtopodion caspium*. The karyotype consists of one pair of metacentric element and 17 pairs of telocentric or subtelocentric elements and one pair of three arm chromosomes. The number of haploid chromosomes was 21 for three male specimens. The chromatid separation did not show heteromorphic sex chromosomes. Our results highlight the need for continued research into the basic biology and taxonomy of *Cyrtopodion caspium* in Iran.

Keywords: Chromosome structure, Karyology, Sex chromosome, Lizard, Iran, and Caspian bent toed gecko

Introduction

The family Gekkonidae is the most diverse and the oldest group of reptiles which have a worldwide distribution (Anderson, 1999). It is the largest family of lizards, comprising 100 genera and 943 species. Although there are several studies on this species, a few of them are related to the karyotype of this family (Ahmadzadeh *et al.*, 2004, 2005, 2008; Hojati *et al.*, 2009).

Cyrtopodion caspium is a small sized oviparous nocturnal lizard, as a house gecko, it is distributed in many cities and villages in Iran. It consists of two subspecies, *Cyrtopodion caspium caspium* (Eichwald, 1831) and *Cyrtopodion caspium insularis* (Akhmedov and Szczerbak, 1978). The main habitats of *C. c. caspium* in Iran are located in the Gorgan region of Mazandaran, to northern and eastern Khorasan, extending south to Sistan and west to Azerbaijan. *C. c. insularis* has only been reported from a limited region in the Caspian Sea. (Rastegar-Pouyani *et al.*, 2008; Rhodin *et al.*, 2010).

The diploid number among Gekkonid lizards ranges from 2n=16 to 2n=46 (Schmidt *et al.*, 1994).The typical karyotype consists of a gradual series of acrocentric chromosomes which there is no difference between macro and micro chromosomes.

The sex determination mechanisms in Saurian have not been completely understood. Sex chromosome evolution in recent data from some lizard's families suggests that they have multiple origins (Beak, 1983). There are species with chromosomal sex determination mechanisms ascribed to male heterogamete in the family's Iguanidae (Frost & Etheridge, 1989), Lacertidae, Teiidae, Scincidae, and Pygopodidae. Female heterogamety is known in the family's Gekkonidae, Varanidae, and Lacertidae (Peccinini-Seale *et al.*, 1981). In the genus *Cnemidophorus*, Cole *et al.* (1969) and Bull (1978) reported a chromosomal sex determination mechanism of the type XX: XY for *Cnemidophorus tigris*. We performed a comparative karyological analysis among different populations of *Cyrtopodion caspium*, from North and North-Eastern Iran, in order to find out possible variability among these populations.

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Material and Methods

During the field work from May to June 2011, we have collected 14 adult specimens (9 males and 5 females) of *Cyrtopodion caspium* from seven provinces and 10 localities of north and north-eastern of Iran (Table 1). All individuals of these species were transferred to the laboratory at the IAU branch of Mashhad, and their karyotypes were determined from all specimens.

Chromosomal preparations were obtained from suspension of bone marrow cells from femur and vertebral column and of spleen cells according to the procedures of Porter and Sites (1986). Meiotic chromosomal spreads were prepared from testis suspension adapted for lizards by Peccinini-Seale *et al*. (1971). Metaphase plates were obtained by the squash method, with treatment of vinblastine specimens. For 9 male individuals at least 11-21 complete metaphases were analyzed. Slides were stained by 4% gimsa with pH 7.2. The photographs were taken on a Zeiss microscope with 100X magnification. Chromosome number and properties were determined by MIP Software.

Table 1.Sampling localities of *Cyrtopodion caspium*

	Locality- province	Longitude	Latitude	Altitude (m)
1	Anzali port- Gilan	49 º 25/	37 º 28/	15
2	Siyahdarreh- Gilan	49 º 08/	37 º 07/	10
3	Fereydounkenar- Mazandaran	53 º 23/	36 º 50/	-10
4	Zaghmarz- Mazandaran	53 º 33/	36 º 43/	-10
5	Ziyarat village-Golestan	54 º 24/	36 º 54/	250
6	Alagol wetland- Golestan	54 º 36/	37 º 17/	00
7	Kalat- Northern Khorasan	59 º 40/	37 º 00/	1000
8	Ahmad Abad village – Semnan	56º 42/	35º 48/	1000
9	Shandiz – Razavi Khorosan	59 º 25/	33 º 56/	1360
10	Byrjand - Southern Khorasan	59 º 13/	32 º 53/	1491

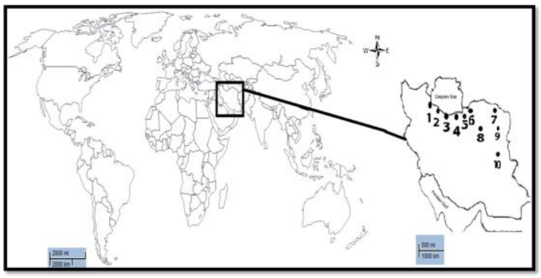

Figure 1.The map of sampling localities.

Results

The karyotype of *Cyrtopodion caspium* is shown in figure 2. Metaphase analyses showed a diploid number of 38 chromosomes (2n=38). The fifth pair was metacentric and other pairs were sub-telocentric or telocentric, and there is a pair with three chromatids. Macro and microchromosomes were indistinguishable and no sex chromosome polymorphism could be observed (Figure 1and Table2 and 3).

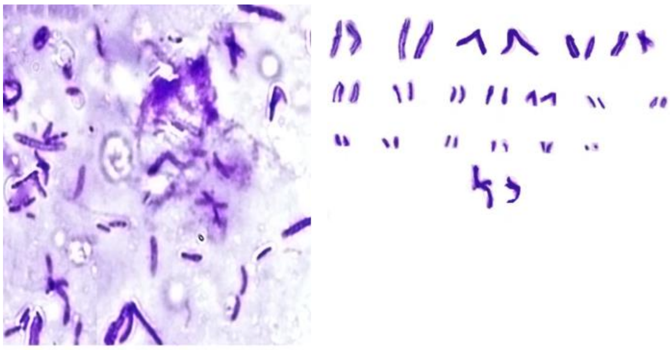

Figure 2. Karyotype of Cyrtopodion caspium.

Table 3. Calculations on karyotype of Cyrtopodion caspium.

Order	Variable name	Value
1	2n	38
2	Number of Arms	46
3	Fn	44
4	Fna	2
5	All Chromosome Lenght	1490.08 µm
6	Total Metacentric	0
7	Total Submetacentric	8
8	Total Acrocentric	24
9	Total Telocentric	6

No heteromorphic sex chromosomes were observed in the karyotype of Cyrtopodion caspium. Meiotic analysis showed a haploid number of 21 chromosomes (n=21) for male specimens (Figure 3).

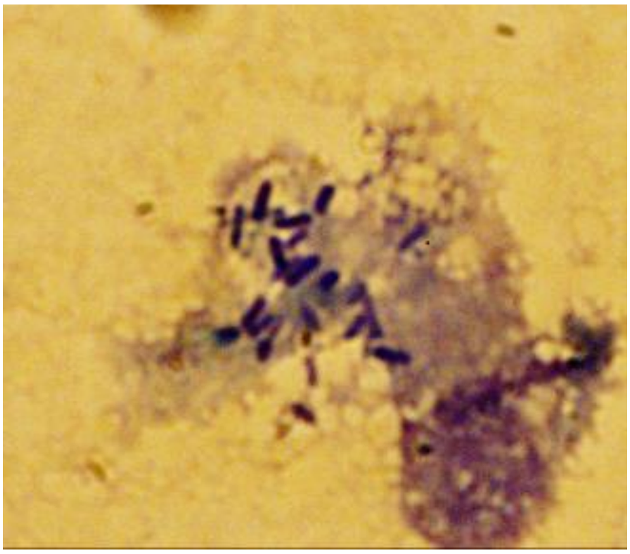

Figure 3.Karyotype of spermatid cell in *C. caspium.*

There was variability in number (1-2 nucleoli) and size (small, medium and large) of nucleoli in the 50 analyzed interphase cells (Figure 4).

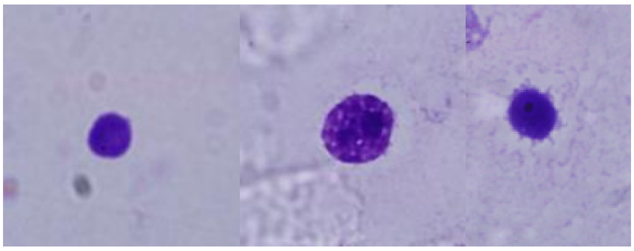

Figure 4.Interphasic nuclei of bone marrow cells.

However, T.F-factor that stands for symmetry is lower than 50% and shows that chromosomes are not in symmetrical position and are located very far from chromosomal symmetry.

$$T.F\% = \frac{\text{Short arms lengths}}{\text{Total arms lengths}} \times 100$$

T.F. = 1.594

Moreover, to show the symmetrical position the DRL index is employed whichit's amaximum relative length of chromosomes minus from minimum relative length of chromosomes. DRL index equals 0.1069 that confirms lack of symmetrical condition in the species.

Table 2. Description of Cyrtopodion caspium chromosomes.

No.	Length (μm)	Up Arm (μm)	Down Arm (μm)	Lcn/L total	Centromer Index (μm)	Chromosome Type
1	93.66	24.6	69.07	0.063	0.26	Acrocentric
2	76.48	29.07	47.41	0.051	0.38	Acrocentric
3	72.47	33.06	39.41	0.049	0.46	Sub-metacentric
4	74.09	25.08	49.01	0.05	0.34	Sub-metacentric
5	83.41	5.39	78.03	0.056	0.06	Telocentric
6	80.35	6.32	74.03	0.054	0.08	Telocentric
7	50.18	5	45.18	0.034	0.1	Acrocentric
8	50.43	5.83	44.6	0.034	0.12	Acrocentric
9	43.05	5.1	37.95	0.029	0.12	Telocentric
10	47.01	5	42.01	0.032	0.11	Telocentric
11	35.45	12.04	23.41	0.024	0.34	Submetacentric
12	18.3	8.25	10.05	0.012	0.45	Submetacentric
13	40.54	5.1	35.44	0.027	0.13	Telocentric
14	41	5.1	35.9	0.028	0.12	Telocentric
15	34.96	10.44	24.52	0.024	0.3	Submetacentric
16	37.25	12.17	25.08	0.025	0.33	Submetacentric
17	33.14	9.06	24.08	0.022	0.27	Acrocentric
18	35.06	12.04	23.02	0.024	0.34	Acrocentric
19	31	5	26	0.021	0.16	Acrocentric
20	39.11	5.1	34.01	0.026	0.13	Acrocentric
21	26.49	9.49	17	0.018	0.36	Submetacentric
22	32.7	7.07	25.63	0.022	0.22	Submetacentric
23	26	5	21	0.017	0.19	Acrocentric
24	24.41	5.1	19.31	0.016	0.21	Acrocentric
25	22.36	6.08	16.28	0.015	0.27	Acrocentric

26	22.19	5.1	17.09	0.015	0.23	Acrocentric
27	21	5	16	0.014	0.24	Acrocentric
28	20.96	5.83	15.13	0.014	0.28	Acrocentric
29	18.57	6.4	12.17	0.012	0.34	Acrocentric
30	19.66	8	11.66	0.013	0.41	Acrocentric
31	18.73	5.39	13.34	0.013	0.29	Acrocentric
32	19	5	14	0.013	0.26	Acrocentric
33	18.34	5	13.34	0.012	0.27	Acrocentric
34	16.67	7.62	9.06	0.011	0.46	Acrocentric
35	12.22	3	9.22	0.008	0.25	Acrocentric
36	9.06	0	9.06	0.006	0	Acrocentric
37	59.74	5	54.74	0.04	0.08	Acrocentric
38	85.04	7.81	77.23	0.057	0.09	Acrocentric

Discussion

Chromosomal evolution has been occurred in all vertebrates. The results of other survey reveal that many minor chromosome rearrangements have been occurred. However, minor deletions throughout the genome of the ancestors of reptiles and birds have led to reduce birds' genome to 50% of reptiles 'genome (Swanson *et al.*1981; Edwards 2009; Iturra*et al.*, 1994).

Single or multiple centric fissions are the main chromosome rearrangement found in the evolution of lizard karyotype, including *Anolis*, *Scelopurus grammicus* and *Liolaemus* (Webster *et al.*, 1972; Lamborot andAlvares-Sarret,1989; Sites, 1983; Lamborot, 1991)

There is not intraspecific chromosome polymorphism in *Cyrtopodion caspium*, which have not shown considerable chromosome variations in several examined populations. Although Intra-specific chromosomal polymorphism is generally between 10-50 % in lizards (Iturra*et al.*, 19940).

Some authors have argued that the 2n=38 is ancestral for the family Gekkonidae (and perhaps the entire suborder), although others have expressed different opinions (Shibaike *et al.*, 2009; Oliver *et al.*, 2007).

However all specimens from seven provinces were monomorphism. The diploid number of 2n=38 found in *Cyrtopodion caspium* is situated within the range of the family.

Different specimens of *Diplodactylus tessellatus* from Australia exhibit 2n=28, 30, and 38 that 2n= 38 is the ancestral case. 38 chromosomes are reduced to 30 and 28 through minor fissions of some acrocentric chromosomes. Moreover, 2n=38, 36, 34, 30 are reported from *Vittatus diplodactylus* (Oliver et *al*., 2007).

In *Tarento lamauritanica* (Gekkonidae) only one chromosome number has been reported (2n=42). Many herpetologists believe that more studies need to investigate variation in chromosome number (Shibaike *et al.*, 2009 and Hidestonhi*et al.*2001). For example, many studies on *Diplodactylus vittatus* show high variation in chromosome number in members of Gekkonidae. The above species exhibits 3 different chromosome numbers from different regions. (King 1977) Geckoes' karyotypes are 2n=38 in many cases. However, diversity in chromosome numbers has been observed in geckos. It should be emphasize that different chromosome numbers are available in populations which are similar morphologically. (Shibaike *et al.*, 2009).

Gekko gecko (one of the near relatives of *C. caspium*) exhibits only 2n=38 and there is no variation in chromosome number. However, it should be considered that only one specimen of G. gecko has been investigated to study the karyotype of this species. (Cohen *etal.*1967)

Evolution of karyotypes occurs in neighboring geographical regions; for example, chromosome evolution of 9 Gekkonidae species are reported from Eastern Asian islands (*G. shibatai*, *G. tawaensis*, *G. vertebral* is, *G. yakuensis*, and 3 non-described species). Moreover the number of chromosomes are 2n=38 in all of specimens. However different karyotypes have been investigated from different populations (Shitake and Takahashi, 2009).

There are many acrocentric chromosomes in all of reptiles (Hidetoshi *et al.*, 2001). The majority of geckoes' chromosomes are small which makes the study of their chromosomes very difficult (Katia andMachado, 1995).

Karyotypes with no distinction between macro and micro chromosomes, as the one we found in *C. caspium* are also typical of the Gekkonidae.

Heteromorphic sex chromosomes were not observed in *C. caspium.* It was corroborated by the absence of a distinctive heteromorphic bivalent in male cells. The presence of heteromorphic sex chromosome in Gekkonidae is known only from 10 species belonging to five genera (Shitake and Takahashi, 2009).However as far as we know; there is no reference between sexual chromosomes in *Cyrtopodion*. (Katia andMachado, 1995)

Most Gekkonids have a chromosomal mechanism

of sex determination of the ZZ:ZW type (Kawai *et al*., 2009) , in which the heterogametic sex is the female, such as in *Heteronotia binoei* (Mortiz,1990), *Gehyra australis* (King 1983), *Gehyra purpuracens* (Moritz,1984) and *Cyrtodactylus pudisulcus* (Shitake and Takahashi 2009). But, in a few species, the mechanism is of the XX: XY type, such as in *Gekko gecko* (Solleder and Schmid 1984), *Gekko japonica* (Mortis 1990) and *Gonato desceciliae* (McBee*et al.,* 1987). *G. hokouensis* demonstrates high diversity of sex chromosomes and their rapid evolution; however, mechanisms of sex determination are evolved rapidly (Ezaz *et al.,* 2009).
Temperature is the most important factor in determination of sex in lizards ,for example ancestral Lephodosaur (Janes*et al.,* 2009) but in some cases sex chromosomes determine gender (Janes *et al.,* 2009). For example, there are sex chromosomes in *Gekko hokouennsis* and sex determination is not related to environmental factors in these species (Kawai *et al.,* 2009).

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