Scientific Reports in Life Sciences 4 (3): 1-12
DOI: http://doi.org/10.5281/zenodo.10154943

Scientific reports in life sciences
http://www.scientific-reports.com

Karyological studies of three Iranian lizards of the family Gekkonidae from Iran

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Received: 12 September 2023 / Revised: 29 October 2023 / Accepted: 13 November 2023/ Published online: 18 November 2023.

How to cite: Mahmoudi, M., Ghaffari, S.M., Salehi, H., Sari, A. (2023). Karyological studies of three Iranian lizards of the family Gekkonidae from Iran , Scientific Reports in Life Sciences 4(3), 1-12. **DOI:** http://doi.org/10.5281/zenodo.10154943

Abstract

The Gekkonidae is the most diverse and ancient group of reptiles and has a worldwide distribution, it constitutes the largest family of lizards, comprising 110 genera and about 1553 species. The Gekkonidae family is characterized by a rich variety of species, different modes of sex determination and diverse karyotypes. In the present study, three lizard species from Iran (Semnan province) belonging to the family Gekkonidae were Karyotyped using bone marrow technique. Two male specimens of *Tenuidactylus caspius caspius* from Semnan province in this study showed (2n = 43). The karyotype consists of 43 acrocentric chromosomes. Chromosomes range in size from 3.88 to 1.77µm. It seems that one macroacrocentric may be a sex chromosome in the male species. This count is a new chromosome number report for this subspecies *Hemidactylus flavivirdis* was diploid (2n =40) with one pair of macro metacentric, one pair of macro submetacentric, one pair of median metacentric and others were acrocentric chromosomes. This is the first chromosome number report for the fauna of Iran. *Hemidactylus robustus* showed *2n* =46 including 23 pairs of acrocentric chromosomes which is reported here for the first time.

Keywords: Chromosome, Gekkonid, Karyotype, Reptile

Introduction

The Gekkonidae is the most diverse and ancient group of reptiles and has a worldwide distribution, it constitutes the largest family of lizards, comprising 110 genera and about 1553 species (Uetz et al., 2023). In this study, the karyotype analysis of three species of the genus *Tenuidactylus* and *Hemidactylus* are presented. The genus *Tenuidactylus* consists of eight recognized species distributed in southwest Asia, central Asian republics, and east Asia (Uetz et al., 2023), of which *T. caspius* (Eichwald, 1831), *T. longipes* (Nikolsky, 1896), and *T. turcmenicus* (Szczerbak, 1978) occur in Iran (Safaei-Mahroo et al., 2015).

The house geckos (*Hemidactylus*) are a member of family Gekkonidae. It was about 188 species. They distributed from India to southern China and Southeast Asia. Some species of house geckes are introduced into central and South America , southern Africa, Australia and another area of the world (Das, 2010; Carranza andArnold, 2012; Šmíd et al., 2014; Uetz et al., 2023). Information on chromosomes and cytogenetic behavior can be used to identify the phylogenetic relationship between species and the population of animals (Lauhajinda and Taksintum, 2006). Therefore, it is necessary to study the karyology of this group. Many recent studies on the chromosomes of lizards provide useful taxonomic data. Significant karyotypic variation has been used to interpret relationships within genera and among genera within a family (Deakin and Ezaz, 2019).

Despite the great number of species in the family, only a few karyotypes have been reported. Based on analyses of published chromosomal data for 74 gekkonine species. King (1987) recognized eight putative ancestral karyomorphs (2n = 32, 34, 38, 40, 42, 44, and 46 all acrocentric or telocentric chromosomes) within the subfamily, and postulated that each of the known karyotypes has derived from one of these prototypes through chromosomal rearrangements by fusion, inversion, and/or heterochromatin addition. The diploid number among gekkonid lizards ranges from 2n =16 to 2n =46(Gorman, 1973; Schmid, et al., 1994). Most gekkonid chromosome complements consist of acrocentric elements (sometimes with a few metacentrics) that gradually decrease in size. The karyotypic evolution within the group is accompanied by Robertsonian fissions, fusions and pericentric inversions (Trifonov et. al., 2011).

Materials and methods

Karyological analyses were carried out on three species of lizards, which are collected from various parts of Iran (Table 1). They were intraperitoneally injected with 0.1 ml of phytohemagglutinin (PHA) per gram body weight for 24 h and with 0.1 ml colchicine solution

(2mg/ml) per gram body weight for 5 h before sacrifice. The bone marrow cells were treated with 0.075M KCl for 20 min and fixed in an acetic acid-methanol (1:3) solution. Mitotic chromosome preparations were made by an air-dry method and stained by the Geimsa solution. The karyotype was determined for each specimen based on comparing photographs of 10 metaphase cells. The photographs of chromosomes enlarged were measured and numbered. Calculation for centromeric index and arm ratio on each chromosome is used according to Levan et al. (1964).

Results and discussion

Tenuidactylus caspius caspius (Eichwald, 1831)

Tenuidactylus caspius is the most common gecko in northern Iran and comprises two subspecies in the Caspian Sea region (Anderson, 1999). *Tenuidactylus caspius caspius* is widely distributed in the Transcaucasia, S Russia, Kazakhstan, Uzbekistan, Tajikistan, Turkmenistan, N Afghanistan and N and E Iran (Kami, 2005; Uetz et al., 2023). In Iran it is known to occur W of the Caspian Sea, the eastern Alborz an Kopet Dagh ranges, along the Afghan border to the Zabol region in Sistan and Baluchistan province, two isolated and dubious records from Kermanshah and Fars provinces.(Smid et al., 2014; Besharati et al., 2021). The subspecies *Tenuidactylus caspius insularis* (Akhmedov and Szczerbak, 1978) has only been reported from a limited region in the Caspian Sea. (Rastegar-Pouyani et al., 2008; Rhodin et al., 2010). Only one Previous chromosome number for *Tenuidactylus caspius* (syn. *Cyrtopodion caspium*) has been reported as 2n=38 in somatic cells and n =21 in gametic cells of male species by Molavi et al.(2014) from Iran which is very questionable. The shape of chromosomes was not appropriate (see Molavi et al., 2014). The range in size of chromosomes for this species is reported 9.6 to 93.66 µm µm. While in our sample the range in size of chromosomes was 1.77 to 3.88µm. This size of chromosome length which was reported by Molavt et al. (2014) has not been seen in all lizard, plant and animal species (see all the books and papers in this regard). Also, the authors did not mention any explanation for the difference between 2n =38 and n =21. Geckos' karyotype are 2n=38 and 42 in some species but, diversity in chromosome numbers has been reported in geckos by Shibaike et al. (2009). Our two male specimens of *Tenuidactylus caspius caspius* from Semnan province in this study showed (2n = 42 + one sex chromosome). The karyotype consists of 43 acrocentric chromosomes (Fig. 1A). Chromosomes range in size from 3.88 to 1.77µm. It seems that one macroacrocentric may be a sex chromosome in the male species (Fig. 1A). Some species of the Gekkonidae family show variation in somatic chromosome number (King, 1977). This count is a new chromosome number report for this subspecies.

***Hemidactylus flaviviridis* (Rüppell 1840)**

The four *Hemidactylus* species reported so far from Iran are: *H. faviviridis, H. persicus, H. robustus*, and *H. romeshkanicus* (Anderson, 1999; Bauer et al., 2006; Rastegar-Pouyani et al., 2006 Šmíd, et al., 2014). Only one of them (*H. romeshkanicus*) is endemic to Iran (Šmíd, et al., 2014). The yellow-bellied house gecko, *Hemidactylus flaviviridis* (Rüppell 1840) has been reported to occur from the northeastern African and Arabian shores of the Red Sea and around the coast of Arabia and Iran, across Pakistan, eastern Afghanistan and northern India to West Bengal and south to the vicinity of Bombay (Anderson, 1999). In Iran, *H. flaviviridis* has already been reported from the coastal towns and villages of southern Baluchistan, Kerman, Fars, and Khuzestan Provinces (Anderson, 1999). Very few *Hemidactylus* species have been studied by molecular cytogenetic techniques. It was revealed that chromosome numbers vary from 2n=16 to 2n=46, with most of the karyotypes composed of 38 - 42 chromosomes (Trifonov et. al., 2011). However, diploid chromosome number as high as 2n =70 has also been reported in a parthenogenetic species, *H. garnotti* (Gorman, 1973). Previous chromosome reports for *H. flaviviridis* are 2n =40 (20 bi-armed and 20 telocentric chromosomes) by Asana and Mahabale (1941), 2n =46 (46 telocentric chromosomes) by Makino and Momma (1949) and 2n =40 (12 bi-armed and 28 telocentric chromosomes) by Branch (1980). Our male specimen was diploid with 2n=40 including one pair of macro metacentric, one pair of macro submetacentric, one pair of median metacentric and others were telocentric chromosomes, which one pair was micro chromosome (Fig. 1B).). This karyotype differs from previous reports in two metacentric chromosomes and one submetacentric chromosome. Chromosomes have a range in size from 5.27 to 0.32µm (Table 3). The cause of chromosomal changes in this species has not yet been explained. This is the first chromosome number report for the fauna of Iran.

Table 1. Sampling localities

Species	Geographic Coordinates	Altitude	Locality
Tenuidactylus caspius caspius	35°40′N, 53°37′E	1555m.	Semnan province: Neck of Ahovan, Kalateh Souhan
Tenuidactylus caspius caspius	35°35′N, 53°26′E	1182m	Semnan province: East of Semnan sity
Hemidactylus flavivirdis	25°17′N,60°37′E	7m	Sistan & Baluchestan province: Chahbahar, Shilat square

Hemidactylus robustus	25°27′N, 59°30′E	10m	Sistan & Baluchestan province: 250 km west of Chahbahar

Table 2. Measurements and classification of chromosomes in *Tenuidactylus caspius caspius*

No. of chromosome	long arm (μ)	short arm (μ)	Arm ratio L/S= r	Chromosome type
1	3.88	0.0	∞	Acrocentric
2	3.69	0.0	∞	Acrocentric
3	3.42	0.0	∞	Acrocentric
4	3.38	0.0	∞	Acrocentric
5	3.34	0.0	∞	Acrocentric
6	3.15	0.0	∞	Acrocentric
7	2.89	0.0	∞	Acrocentric
8	2.85	0.0	∞	Acrocentric
9	2.84	0.0	∞	Acrocentric
10	2.52	0.0	∞	Acrocentric
11	2.34	0.0	∞	Acrocentric
12	2.17	0.0	∞	Acrocentric
13	2.08	0.0	∞	Acrocentric
14	2.02	0.0	∞	Acrocentric
15	1.75	0.0	∞	Acrocentric
16	1.62	0.0	∞	Acrocentric
17	1.49	0.0	∞	Acrocentric
18	1.40	0.0	∞	Acrocentric
19	1.35	0.0	∞	Acrocentric
20	1.29	0.0	∞	Acrocentric
21	1.17	0.0	∞	Acrocentric
23	2.74	0.0	∞	Sex acrocentric

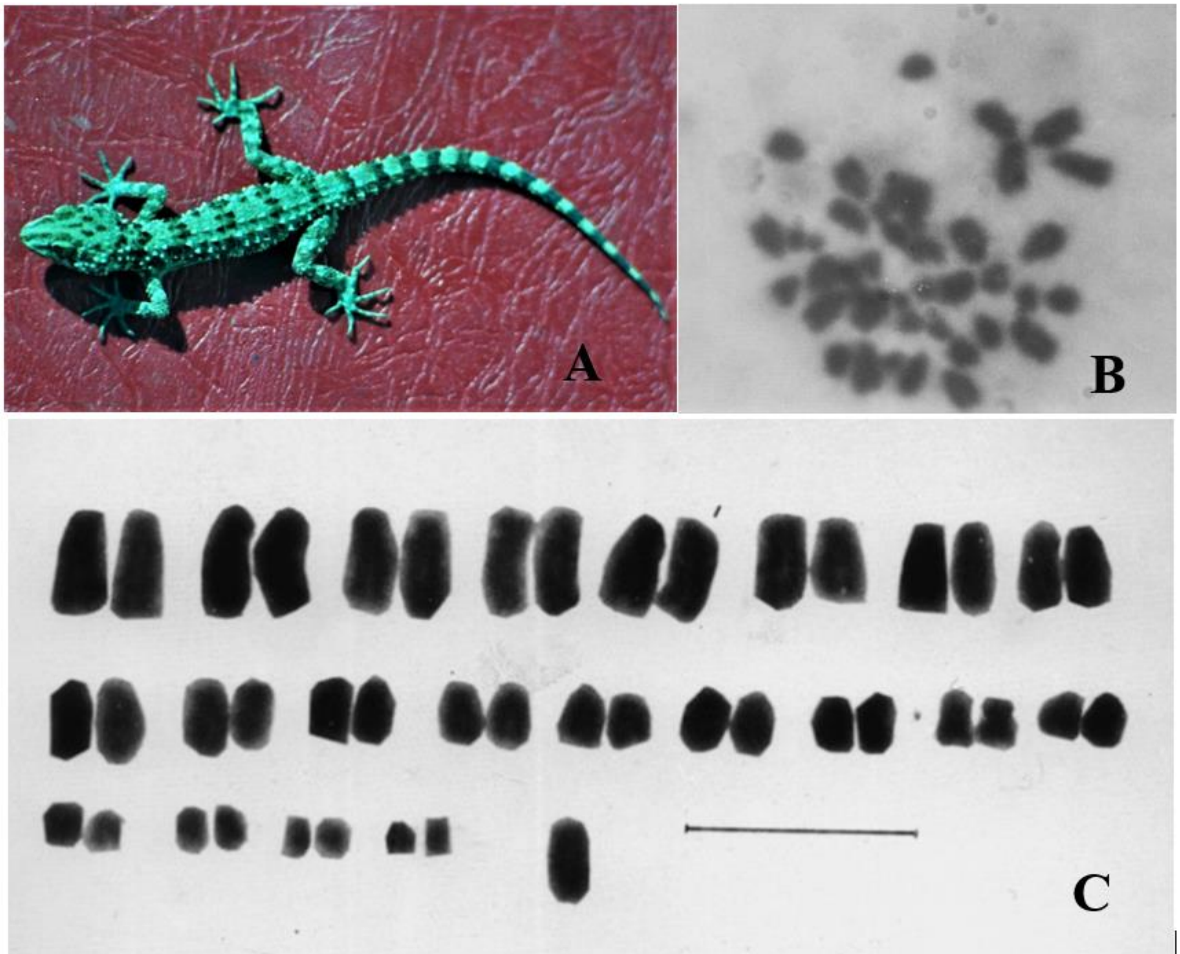

Figure 1. *Tenuidactylus caspius caspius* (Eichwald,1831), A: Caspian bent toad gecko (male). B: metaphase plate (2n=43). C: karyotype(43 acrocentric chromosomes). Bar =10μm.

***Hemidactylus robustus* (Heyden, 1827)**

Hemidactylus robustus (Heyden, 1827) is a widespread species inhabiting coastal areas along the Red Sea, Arabian Sea and Persian Gulf and occurring also in the hinterland of the Horn of Africa and Arabia (Sindaco and Jeremcenko, 2008).

Hemidactylus robustus was long considered a junior synonym of *H. turcicus* (Linnaeus), a species widespread in the Mediterranean (Kluge, 1993; Lanza, 1978; Sch€atti and Gasperetti, 1994). This was the result of their overall morphological similarity and the lack of a thorough taxonomic revision of both species. Although some authors have treated *H. robustus* as a separate species (Baha El Din, 2005; Lanza, 1990; Moravec and B€ohme, 1997), its recognition as a separate taxon was fully confirmed only by analysis of genetic data (Carranza and Arnold, 2006) and further elaborated by other studies (Carranza andArnold, 2012; Smid et al., 2013). Therefore, all records of *H. turcicus* in Iran are referred to *H. robustus* (Sindaco and Jeremčenko, 2008; Šmíd et al., 2014). Chromosome number for seven species of the genus *Hemidactylus* are 2n =40, 42, 44 and 46, which more of them have 2n =40 (Asana and Mahahale, 1941; Makino and Memma, 1949; Bharnagar, 1962; Branch, 1980; Trifonov, 2011; Patawng and Tanomtong, 2015).). The difference in previous chromosome numbers is related to the number of microchromosomes. Our male specimen was diploid (2n =46) and showed 23 pairs of acrocentric chromosomes of gradually decreasing size (Fig. 3A, B. Table 4). This count is reported here for the first time.

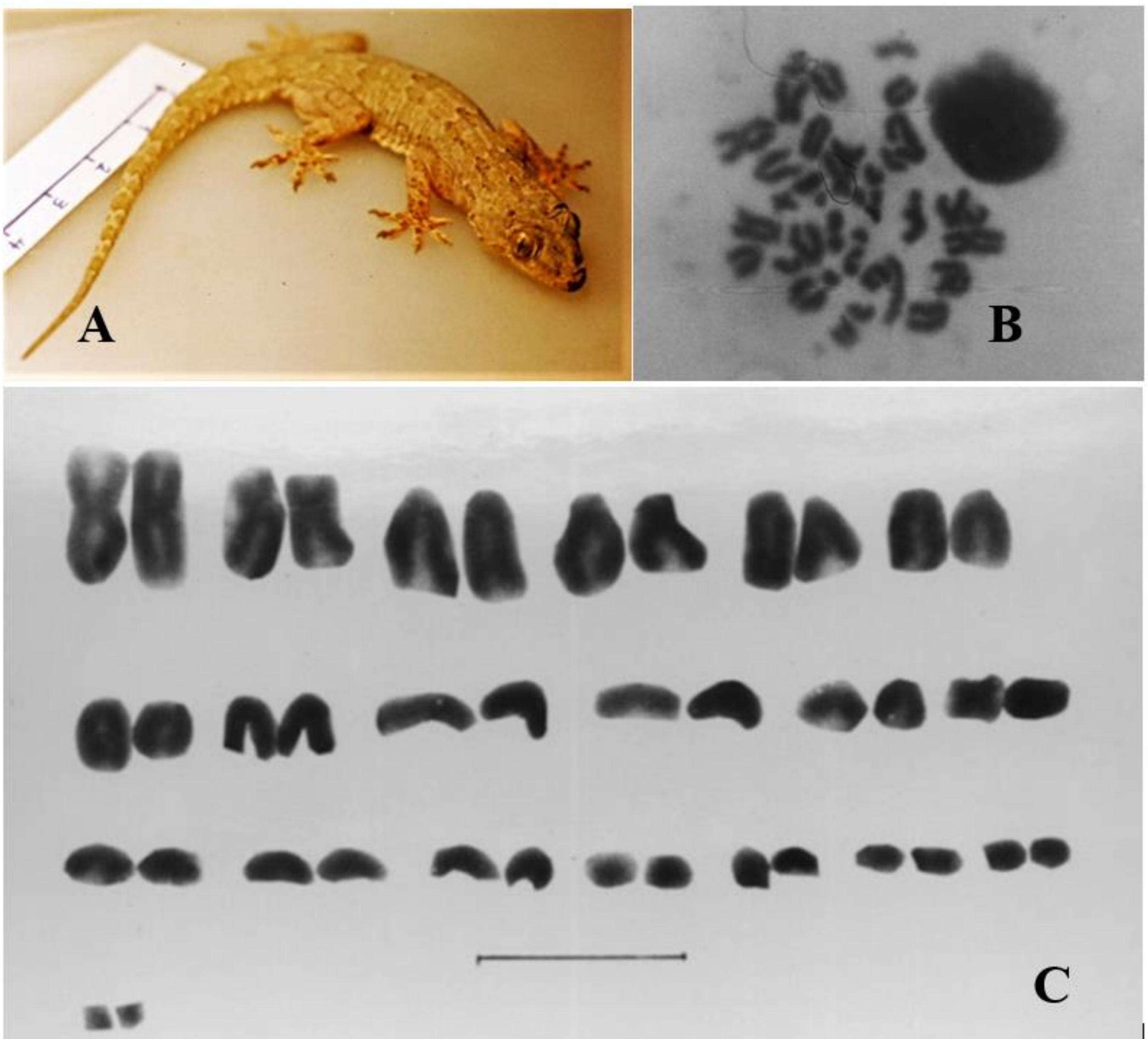

Figure 2. *Hemidactylus flaviviridis* Rüppell,1835, A: Yellow bellied Gecko(male). B: metaphase plate (2n=40). C: karyotype. Bar =10µm.

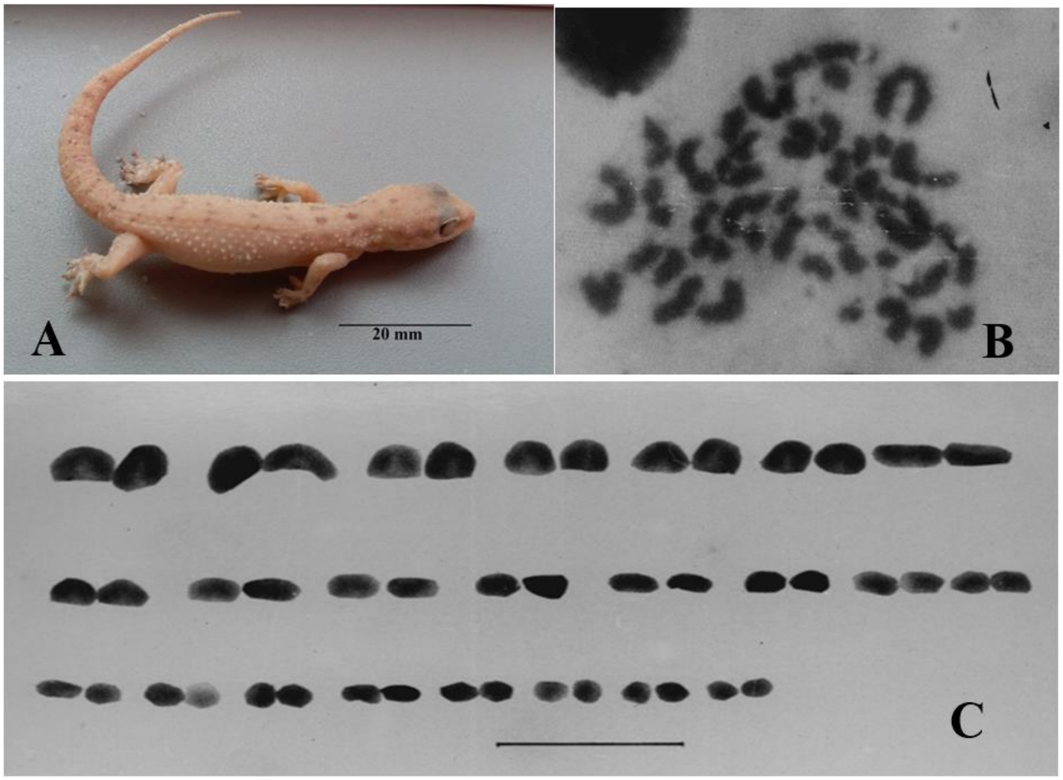

Figure 3. *Hemidactylus robustus*. A: adult male. B: metaphase plate (2n=46). C: karyotype (23 pairs of acrocentric chromosomes). Bar =10µm.

Table 3. Measurements and classification of chromosomes in *Hemidactylus flavivirdis*

No. of chromosome	long arm (µ)	short arm (µ)	Arm ratio L/S= r	Chromosome type
1	2.74	2.53	1.08	Metacentric
2	2.74	1.46	1.87	Submetacentric
3	4.3	0.0	∞	Acrocentric
4	4.27	0.0	∞	Acrocentric
5	3.98	0.0	∞	Acrocentric
6	3.31	0.0	∞	Acrocentric
7	2.76	0.0	∞	Acrocentric
8	2.43	0.0	∞	Acrocentric
9	2.34	0.0	∞	Acrocentric
10	2.04	0.0	∞	Acrocentric
11	2.02	0.0	∞	Acrocentric
12	1.01	1.01	1	Metacentric
13	1.74	0.0	∞	Acrocentric
14	1.65	0.0	∞	Acrocentric
15	1.56	0.0	∞	Acrocentric
16	0.95	0.0	∞	Acrocentric
17	0.93	0.0	∞	Acrocentric
18	0.89	0.0	∞	Acrocentric
19	0.76	0.0	∞	Acrocentric
20	0.32	0.0	∞	Micro

Table 4. Measurements and classification of chromosomes in *Hemidactylus flavivirdis*

No. of chromosome	long arm (μ)	short arm (μ)	Arm ratio L/S= r	Chromosome type
1	2.96	0.0	∞	Acrocentric
2	2.72	0.0	∞	Acrocentric
3	2.36	0.0	∞	Acrocentric
4	2.10	0.0	∞	Acrocentric
5	2.07	0.0	∞	Acrocentric
6	1.58	0.0	∞	Acrocentric
7	1.54	0.0	∞	Acrocentric
8	1.48	0.0	∞	Acrocentric
9	1.35	0.0	∞	Acrocentric
10	1.27	0.0	∞	Acrocentric
11	1.26	0.0	∞	Acrocentric
12	1.12	0.0	∞	Acrocentric
13	1.10	0.0	∞	Acrocentric
14	1.06	0.0	∞	Acrocentric
15	0.94	0.0	∞	Acrocentric
16	0.93	0.0	∞	Acrocentric
17	0.92	0.0	∞	Acrocentric
18	0.90	0.0	∞	Acrocentric
19	0.81	0.0	∞	Acrocentric
20	0.80	0.0	∞	Acrocentric
21	0.71	0.0	∞	Acrocentric
22	0.70	0.0	∞	Acrocentric
23	0.62	0.0	∞	Acrocentric

Acknowledgements

This work was supported by the Research Council, University of Tehran.

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