Journal of Animal Diversity

Online ISSN 2676-685X

Volume 5, Issue 2 (2023)

Short Communication

http://dx.doi.org/10.61186/JAD.5.2.7

Karyological analyses of four species of the families Lacertidae and Scincidae (Sauria) from Iran

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Citation: Ghaffari, S. M., Mahmoudi, M., Salehi, H. and Sari, A. R. (2023). Karyological analyses of four species of the families Lacertidae and Scincidae (Sauria) from Iran. *Journal of Animal Diversity*, 5 (2): 57–62. http://dx.doi.org/10.61186/JAD.5.2.7

Received: 19 December 2022
Accepted: 10 June 2023
Published online: 30 June 2023

Abstract

Karyological studies were conducted using bone marrow cell preparations from four species of Lacertidae and Scincidae from Iran: *Eremias persica* Blanford, *Eremias kopetdaghica* Szczerbak, *Ophisops elegans* Ménétries (Lacertidae), and *Eumeces schneiderii princeps* (Eichwald) (Scincidae). *Eremias persica* was diploid with 2n = 38 chromosomes. The karyotype consisted of eighteen pairs of acrocentric macrochromosomes and two microchromosomes. *Eremias kopetdaghica* was also diploid with 2n = 38 comprising one pair of metacentric macrochromosomes, seventeen pairs of acrocentric macrochromosomes, and one pair of microchromosomes. The chromosome count for these two species is reported for the first time herein. The chromosome count for *Ophisops elegans* (2n = 38) was in agreement with a previous report and was the first for the herpetofauna of Iran. *Eumeces schneiderii princeps* showed 32 macro- and microchromosomes, representing the first report for this subspecies.

Key words: Chromosome, *Eremias*, *Eumeces schneiderii princeps*, karyotype, lacertids, *Ophisops elegans*

Lacertid lizards (Sauria: Lacertidae), which comprise 42 genera with 373 species (Uetz et al., 2023), inhabit Africa and almost all of Eurasia. The family Lacertidae with nine genera and forty-nine species, is the most diverse family in the Iranian Plateau (Nasrabadi et al., 2017). Lacertid chromosomes generally share the same acrocentric morphology and gradually decrease in length; therefore, individual chromosomes are difficult to distinguish (Lisachov et al., 2020). Most of the studied species present a karyotype with thirty-six unarmed macrochromosomes and two microchromosomes with the karyotype consisting of eighteen pairs of acrocentric macrochromosomes and two microchromosomes (Peccinini-Seale, 1981).

The family Scincidae represents the most morphological diversified squamate reptiles with great variation in body size and form. Representatives are found worldwide in a variety of habitats. Scincidae consists of approximately 146 genera and about 1,650 species (Uetz et al., 2023). In Iran, Scincidae with 7 genera, 18 species, and 2 subspecies follows Lacertidae and Gekkonidae in species richness (Nasrabadi et al., 2017). Scincidae, despite its importance due to the large number of species and high degree of differentiation, is rather poorly known regarding karyotypes. Currently, about 8% of scincid species have been karyotyped (Greer and Shea, 2000; Olmo and Signorino, 2005; Kaewsri et al., 2014; Kostmann et al., 2021). The diploid chromosome numbers in this family range from 2n = 22 to 2n = 36 (Giovannotti et al., 2010; Patawang, 2017).

Karyological analyses were conducted on four species of lizards collected from Semnan Province of Iran in 2010 (Table 1). They were intraperitoneally injected with 0.1 ml of phytohemaglutinin (PHA) per gram body weight for 24 h and with 0.1 ml colchicine solution (2 mg/ml) per gram body weight for 5 h before sacrifice. The bone marrow cells were treated with 0.075 M KCl for 20 min and fixed in

acetic acid–methanol (1:3) solution. Mitotic chromosome preparations were made using an air-dry method and stained with Giemsa solution (Adegoke and Nadesan, 1986). The karyotype was determined for each individual based on at least twenty well-spread cells. Calculation for centromeric index and arm ratio on each chromosome was used according to Levan et al. (1964).

Family Lacertidae

***Eremias persica* Blanford, 1875 (Fig. 1A)**

The genus *Eremias* Fitzinger, 1834, generally known as racerunner lizards, belongs to the family Lacertidae (Arnold et al., 2007). Today, the genus *Eremias* consists of 42 recognized species found in Middle and Central Asia, inhabiting steppes, sand dunes, and stony and mountain deserts at various elevations in the vast arid territories from southeastern Europe in the west to Mongolia, China, and the Korean Peninsula in the east (Arnold et al., 2007; Mozaffari and Parham, 2007; Uetz et al., 2023). Twenty-one species of *Eremias* are known from Iran, six of which are endemic to the country (Nasrabadi et al., 2017; Eskandarzadeh et al., 2018; Uetz et al., 2023). Blanford described *Eremias persica* in 1875, but the type locality was not precisely reported, limited to just "near Esfahan". Rastegar-Pouyani et al. (2010) believed that *Eremias persica* represents a species complex as there are many local populations inhabiting a variety of habitats and exhibiting considerable morphological, genetic, and ecological variations. Previous chromosome counts for six species of the genus *Eremias* from China by Dai et al. (2004) showed nineteen pairs of chromosomes with the karyotype formula 2n = 38 (36 macro + 2 micro). Singh and Banerjee (2004), in their review of the chromosomal diversity of Indian mammals, amphibians, and reptiles, indicated 2n = 38 for *E. persica*. It seems that this count is not accurate because, according to the literature (Ananjeva et al., 2006; Bahmani et al., 2014), this species is not distributed in India. The chromosomal study of our two samples that were accurately identified as females showed that the chromosomal set of this species was 2n = 38. (Fig. 1B). The karyotype consisted of eighteen pairs of acrocentric macrochromosomes and two microchromosomes (Fig. 1C). The macrochromosomes ranged in size from 1.21 to 3.66 µm (Table 2). This count is reported for the first time here.

***Eremias kopetdaghica* Szczerbak, 1972 (Fig. 1D)**

Eremias strauchi Kessler, 1878 comprised two subspecies, *Eremias strauchi strauchi* Kessler, 1878 and *Eremias strauchi kopetdaghica* Szczerbak, 1972 (Firouz, 2000). These two subspecies, are distributed allopatrically in both northern corners of eastern and western Iran (Anderson, 1999; Rastegar-Pouyani et al., 2007; Hosseinian Yousefkhani et al., 2013). Šmíd et al. (2014) reported *Eremias kopetdaghica* as an independent species from Iran and the south of Turkmenistan. Molecular phylogenies by Rastegar-Pouyani et al. (2015) confirmed that *E. kopetdaghica* is distinct from *Eremias strauchi*, supporting its independent species status. Hosseinian Yousefkhani et al. (2016) believed that ecological niche differentiation and taxonomic distinction between *Eremias strauchi strauchi* and *Eremias strauchi kopetdaghica* confirm the taxonomic suggestion of Rastegar-Pouyani et al. (2015) that both subspecies can be upgraded to the species level. We found this species from new localities in Semnan Province (Cherzkouh, Cheshmeh–Ali, north of Damghan). Our male specimen presented a karyotype consisting of one pair metacentric macrochromosomes, seventeen pairs of acrocentric macrochromosomes, and one pair of microchromosomes (Fig. 1E, F). The macrochromosomes ranged in size from 0.88 to 3.38 µm (Table 2) and gradually decreased in size. This count is reported for the first time here.

***Ophisops elegans* Ménétries, 1832 (Fig. 1G)**

The genus *Ophisops* Ménétries, 1832 is distributed in southeastern Europe, North Africa, and Asia, with 11 currently recognized species (Uetz et al., 2023). *Ophisops elegans* is widely distributed throughout the eastern Mediterranean region and southwestern Asia and has also been recorded from North Africa (Oraie et al., 2012). In Iran, *O. elegans* is considered as one of the most common lacertid lizards, being distributed eastward through the western parts of the central Iranian Plateau and in the south as far as Kerman (Anderson, 1999). *Ophisops elegans* is also known from Taftan Mountain (Oraie et al., 2012). The first chromosome count for *O. elegans* was reported by Arronet (1968) from Armenia and showed 38 chromosomes in this species in both sexes with 36 acrocentric macrochromosomes and 2 microchromosomes. A similar number of chromosomes was also described by Gorman (1969) in the *O. elegans* male; however, he did not study the karyotype of the female. Bhatnagr and Yoniss (1976) recognized a difference in the karyotypes of *O. elegans* males and females; the male karyotype was 36 Macro + 2 micro = 38 and the female karyotype was 35 Macro + 3 micro. Odierna et al. (1993) reported the female sex microchromosome. Our specimen was male and showed eighteen pairs of acrocentric macrochromosomes and one pair of microchromosomes. We did not find heteromorphic sex chromosomes in this species (Fig. 1H, I), which is in agreement with the previous report by Arronet (1968). The macrochromosomes in *O. elegans* ranged in size from 0.84 to 3.27 µm (Table 2). This is the first chromosome number report for this species as part of the herpetofauna of Iran.

Table 1: Summary of the sampling information for lizards in this study.

Species	2n	NF	Geographic coordinates	Numbers of specimens	Date of collection	Altitude (meters a. s. l.)	Locality
Eremias persica Blanford	38	38	53°37′ E, 35°42′ N	5	2 June 2010	1,558	Semnan Province: Neck of Ahovan
Eremias kopetdaghica Szczerbak	38	40	53°40′ E, 35°43′ N	2	2 August 2010	1,549	Semnan Province: Attaran, towards neck of Ahovan
Ophisops elegans Ménétries	38	38	53°37′ E, 35°42′ N	3	1 April 2010	1,558	Semnan Province: Neck of Ahovan
Eumeces schneiderii princeps Eichwald	32	40	53°39′ E, 35°43′ N	1	3 August 2010	1,561	Semnan Province: Around the neck of Ahovan

Table 2: Measurements and classification of chromosomes in four species of lizards.

No. of chromosome	*Eremias kopetdaghica*				*Eremias persica*				*Ophisops elegans*				*Eumeces schneiderii princeps*			
	long arm (µm)	short arm (µm)	Arm ratio L/S= r	Chromosome type	long arm (µm)	short arm (µm)	Arm ratio L/S= r	Chromosome type	long arm (µm)	short arm (µm)	Arm ratio L/S= r	Chromosome type	long arm (µm)	short arm (µm)	Arm ratio L/S= r	Chromosome type
1	1.82	1.56	1.16	Metacentric	3.66	0.0	∞	Acrocentric	3.27	0.0	∞	Acrocentric	2.82	2.82	1	Metacentric
2	2.77	0.0	∞	Acrocentric	3.29	0.0	∞	Acrocentric	3.12	0.0	∞	Acrocentric	2.62	2.62	1	Metacentric
3	2.73	0.0	∞	Acrocentric	2.89	0.0	∞	Acrocentric	2.46	0.0	∞	Acrocentric	1.39	1.08	1.28	Metacentric
4	2.49	0.0	∞	Acrocentric	2.75	0.0	∞	Acrocentric	2.34	0.0	∞	Acrocentric	1.32	1.10	1.20	Metacentric
5	2.32	0.0	∞	Acrocentric	2.62	0.0	∞	Acrocentric	2.17	0.0	∞	Acrocentric	1.34	0.91	1.47	Metacentric
6	2.20	0.0	∞	Acrocentric	2.61	0.0	∞	Acrocentric	2.14	0.0	∞	Acrocentric	1.33	0.88	1.51	Metacentric
7	2.12	0.0	∞	Acrocentric	2.38	0.0	∞	Acrocentric	1.90	0.0	∞	Acrocentric	1.53	0.61	2.50	Submetacentric
8	1.97	0.0	∞	Acrocentric	2.14	0.0	∞	Acrocentric	1.70	0.0	∞	Acrocentric	1.07	0.97	1.10	Metacentric
9	1.90	0.0	∞	Acrocentric	2.03	0.0	∞	Acrocentric	1.55	0.0	∞	Acrocentric	1.75	0.0	∞	Acrocentric
10	1.87	0.0	∞	Acrocentric	1.98	0.0	∞	Acrocentric	1.48	0.0	∞	Acrocentric	1.55	0.0	∞	Acrocentric
11	1.84	0.0	∞	Acrocentric	1.85	0.0	∞	Acrocentric	1.28	0.0	∞	Acrocentric	1.51	0.0	∞	Acrocentric
12	1.70	0.0	∞	Acrocentric	1.55	0.0	∞	Acrocentric	1.26	0.0	∞	Acrocentric	1.48	0.0	∞	Acrocentric
13	1.58	0.0	∞	Acrocentric	1.32	0.0	∞	Acrocentric	1.20	0.0	∞	Acrocentric	1.41	0.0	∞	Acrocentric
14	1.54	0.0	∞	Acrocentric	1.23	0.0	∞	Acrocentric	1.19	0.0	∞	Acrocentric	1.37	0.0	∞	Acrocentric
15	1.43	0.0	∞	Acrocentric	1.22	0.0	∞	Acrocentric	1.11	0.0	∞	Acrocentric	1.36	0.0	∞	Acrocentric
16	1.21	0.0	∞	Acrocentric	1.22	0.0	∞	Acrocentric	0.96	0.0	∞	Acrocentric	1.27	0.0	∞	Acrocentric
17	1.19	0.0	∞	Acrocentric	1.21	0.0	∞	Acrocentric	0.88	0.0	∞	Acrocentric				
18	0.88	0.0	∞	Acrocentric	1.21	0.0	∞	Acrocentric	0.84	0.0	∞	Acrocentric				

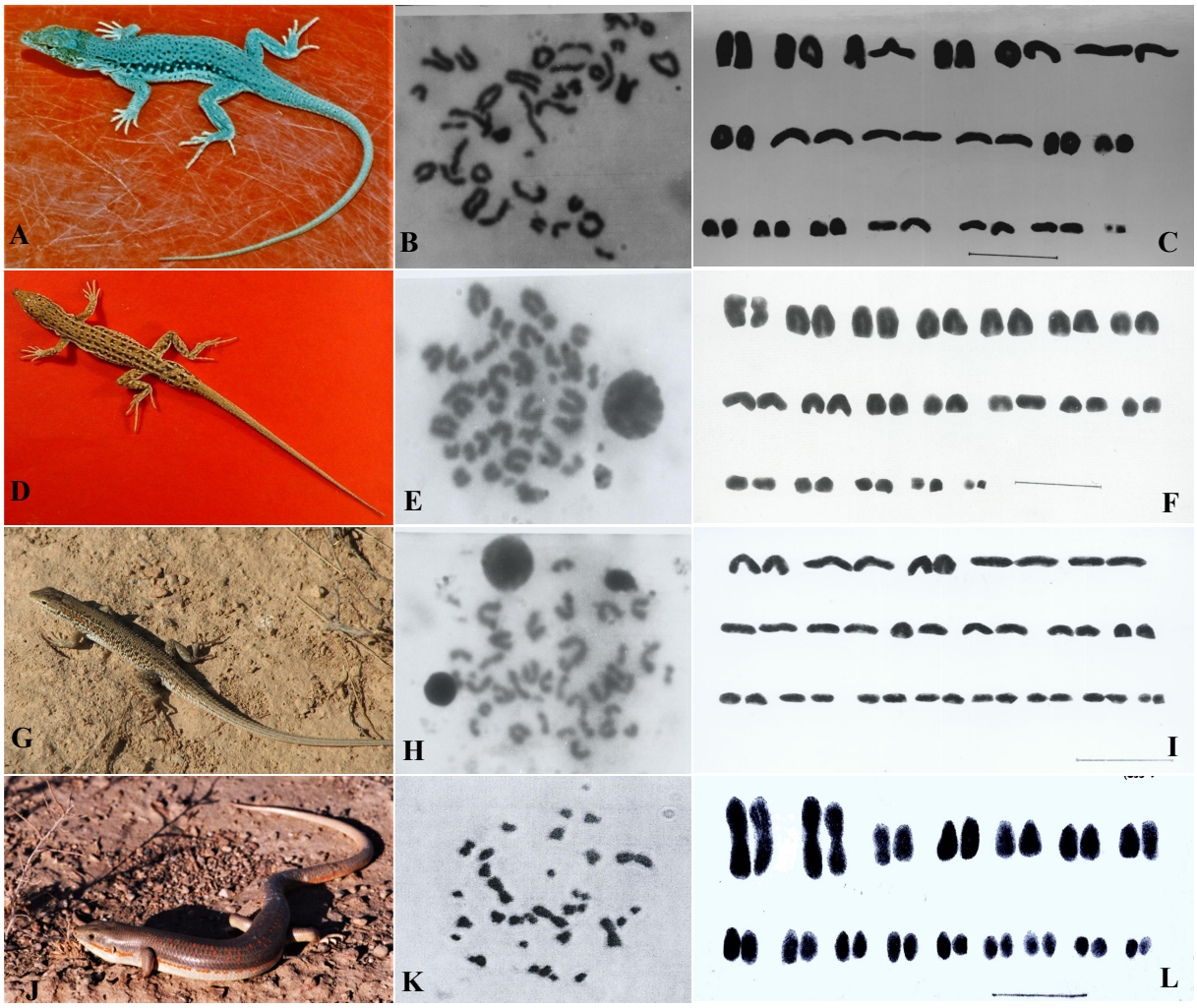

Figure 1: Images, metaphase plates and karyograms of four species of lizards. Scale bar = 10 µm.

Family Scincidae

***Eumeces schneiderii princeps* (Eichwald, 1839) (Fig. 1J)**

Eumeces Wiegmann, 1834 is considered to be the most archaic of the extant scincid genera, and it is widely distributed in subtropical and temperate Eurasia, northern Africa, and North America (Greer, 1970). This genus is divided into two sections, of which the *Eumeces* section is distributed in northern Africa, west Asia, and Central America (Taylor, 1936). The *Eumeces schneiderii* group consists of five subspecies: *E. s. barani*, *E. s. pavimentatus*, *E. s. princeps*, *E. s. schneiderii*, and *E. s. zarudnyi* (Uetz et al., 2023). The recognized Iranian members of the genus are *E. schneiderii princeps* and *E. s. zarudnyi* (Nasrabadi et al., 2017). The distribution of the skink *Eumeces schneiderii* extends from North Africa over Sinai, Jordan, Lebanon, Syria, Anatolia, and Cyprus to west and Central Asia with a vertical distribution to 1,800 m (Faizi et al., 2016). In recent years, a new species *Eumeces persicus* Faizi et al. (2017) was described from Iran. Caputo et al. (1994) reported the chromosome number for *E. schneiderii* as 2n = 32 in somatic cells. Our specimen of this subspecies was male and showed 32 macro- and microchromosomes. However, the metacentric chromosomes of the first two pairs were considerably larger than the remaining fourteen (Fig. 1K, L), which is in agreement with the previous report by Caputo et al. (1994). The chromosomes of the third, fourth, fifth, sixth, and eight pairs were metacentric, the seventh pair was submetacentric, and the others were telocentric (Fig. 1L). This is the first chromosome number report for this subspecies (Table 2).

Acknowledgments

This work was supported by the Research Council of the University of Tehran. We sincerely thank three anonymous reviewers for constructive comments that improved the manuscript.

Authors contribution

Writing of original manuscript, S.M.G.; Methodology, M.M. and H.S.; Supervision, S.M.G. and A.S.

Conflict of interest

The authors declare that there are no conflicting issues related to this short communication.

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