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THE FIRST CASE OF TRIPLOIDY IN SKINKS OF THE GENUS *Ophiomorus* (REPTILIA: SCINIDAE)

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Polyploidy is a well-known phenomenon among various groups of animals, plants and fungi. However, the occurrence and study of this phenomenon among reptiles is still underestimated. Currently, polyploid (3 – 4*n*) and mosaic (2/3*n*) individuals are known in eleven families of reptiles. The paper presents a unique case of polyploidy in a skink of the genus *Ophiomorus*. Using DNA flow cytometry, we detected diploid and triploid specimens of *O. tridactylus* in a population near Jask in Southern Iran. This may be the result of hybridization or natural spontaneous autotriploidy. Further studies of these lizards in the region will make it possible to determine the causes of this phenomenon.

Keywords: polyploidy; DNA flow cytometry; genome size; lizards; Iran.

INTRODUCTION

Polyploidy is interesting, but poorly studied phenomena occurring in some animal, plant, and fungal lineages (Mason and Pires, 2015; Dar and Rehman, 2017). Two categories of polyploids are able to be recognized: allopolyploids are hybrids and autopolyploids have chromosome sets derived from a single species (Borkin et al., 1996). Polyploids have been documented across a wide range of vertebrate species (Gregory and Mable, 2005). In reptiles, polyploidy is relatively common. Triploid species and lineages have been identified in about twenty species complexes of seven families: Agamidae Spix, 1825, Gekkonidae Gray, 1825, Gymnopthalmidae Fitzinger, 1826, Liolaemidae Frost et Etheridge, 1989, Scincidae Gray, 1825, Teiidae Gray, 1827, and Typhlopidae Merrem, 1820 (Bogart, 1980; Kearney et al., 2009; Trifonov et al., 2015; Abdala et al., 2016; Stöck et al., 2021). Their origin is usually associated with interspecific hybridization and parthenogenetic reproduction (Kearney et al., 2009). Sometimes, triploid parthenogenetic females interbreed with males of diploid bisexual species, which can result in polyploid individuals. Such cases were noted in the genus *Aspidoscelis* Fitzinger, 1843 (Teiidae), where hybridization of triploid parthenogenetic females with diploid males can lead to the formation of tetraploid offspring (Hardy and Cole, 1998; Lutes et al., 2011; Cole et al., 2014, 2017). Sometimes, in the genus *Darevskia* Arribas, 1999 (Lacertidae Bonaparte, 1831) diploid parthenogenetic females interbreed with males of bisexual species, which can lead to the formation of triploid and tetraploid hybrids (Darevsky et al., 1973; Danielyan et al., 2008; Freitas et al., 2019).

Occasionally, species with sharp differences in the number of chromosomes can be observed within a reptilian family. However, such differences are not always associated with polyploidy. Apparently, a similar case was observed in the family Chamaeleonidae Rafinesque,

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1815, where *Rieppeleon brevicaudatus* (Matschie, 1892) has 62 chromosomes, while rest species of the family have 28 – 38 chromosomes (Mezzasalma et al., 2023). Some authors (Hall, 1970) believed that this case could be associated with polyploidy, but recently such an increase in the number of chromosomes is associated with massive chromosomal rearrangements (Mezzasalma et al., 2024).

Non-hybrid mixoploidy (2/3*n*) and spontaneous autopolyploidy (3*n*) were also observed among reptilian species. A sex-related diploid-triploid mosaicism was observed in populations of two bisexual species: *Platemys platycephala* (Schneider, 1792) from the family Chelidae Gray, 1831 and *Liolaemus chiliensis* (Lesson, 1830) from the family Liolaemidae. In both species, triploidy was positively or negatively (respectively) associated with the number of males and could therefore play a role in sex determination (Bickham et al., 1985; Lamborot et al., 2006; Bickham and Hanks, 2009). Also, a mixture of diploid and triploid somatic cells was observed in a parthenogenetic female of *Lepidophyma flavimaculatum* Duméril, 1851 from the family Xantusiidae Baird, 1858 (Bezy, 1972). A case of spontaneous autotriploidy has recently been found in *Saltuarius cornutus* (Ogilby, 1892) from the family Carphodactylidae Kluge, 1967 (Pensabene et al., 2024).

Our study focuses on members of the genus *Ophiomorus* A. M. C. Duméril et Bibron, 1839, which includes 12 species of relatively small skink lizards with short limbs and often reduced fingers and toes (Nabizadeh et al., 2023; Uetz, 2024). The distribution of the genus is disjunct and divided into isolated areas in the Balkans, Turkey, the Levant, Iran, Pakistan and India. 7 – 8 species inhabit Iran, where they live mostly in sand dunes (Šmíd et al., 2014). The karyotype, phylogeny, range, and ecology of these skinks are extremely insufficiently studied. The purpose of this paper is to describe a unique case of triploidy in these rare and poorly studied skinks.

MATERIAL AND METHODS

During a travel to Iran in 2018, we collected twelve adults of *O. tridactylus* (Blyth, 1853), *O. maranjabensis* Azemi, Farhadi Qomi, Kami et Anderson, 2011, and *O. streeti* Anderson et Leviton, 1966 from four localities (Table 1; Fig. 1). The amount of DNA per nucleus (genome size) was measured by DNA flow cytometry. Flow cytometry was performed by using of microscope-based flow fluorimeter with mercury arc lamp as a light source constructed at the Institute of Cytology, Russian Academy of Sciences, St. Petersburg (https: //patents.google.com/patent/SU1056008A1/ru). DNA-histograms were acquired with a multichannel analyzer connected with a microcomputer. The analysis rate was 100 – 200 cells per second; four runs were done on each sample, with the total number of cells measured per sample being above 10,000. The peaks of DNA histograms were plotted to the Gaussian curves by means of the least-square technique.

Red blood cells were taken by a micropipette from the heart. Each individual was anaesthetized by chloroform. Tested cells were mixed with reference cells and assayed simultaneously. Spleenocytes of *Mus musculus* Linnaeus, 1758 (C57B1) males were used as a reference standard. In arbitrary units, genome size of an individual under the study was determined as the ratio of its cell peak mean to that of *M. musculus* (6.8 pg by Bianchi et al., 1983). The cell samples were suspended in Versene solution (pH 7.3 – 7.7; Biolot, St. Petersburg), with a total cell concentration of approximately 10^6 cells/ml and stored before study at 4°C. The cells were lysed by addition of Triton X-100 (Ferak, Berlin) at a final concentration of 0.1%, and stained with a mixture of olivomycin (OM, Moscow Medicine Plant) and ethidium bromide (EB, Calbiochem) at the following final concentration: 40 µg/ml OM, 20 µg/ml EB, and 15 mM $MgCl_2$. The stained samples were measured after 4 – 6 h. Other details of the methodology were published earlier (Vinogradov et al., 1990; Rozanov and Vinogradov, 1998).

TABLE 1. The Nuclear DNA Content (pg) in Erythrocytes of Three Species of the Genus *Ophiomorus*

No.	Species	Ploidy	Locality	Coordinate	Altitude	Nuclear DNA content		
						n	mean ± SD	range
1	*maranjabensis*	2*n*	“Safari sands”	34°18′ N 51°51′ E	827	1	4.564	
2	–”–	2*n*	Kashsan	33°53′ N 51°49′ E	957	1	4.572	
3	*streeti*	2*n*	Kerman	27°56′ N 58°05′ E	451	5	4.494 ± 0.014	4.468 – 4.502
4	*tridactylus*	2*n*	Bahu Kalat	25°46′ N 61°25′ E	38	3	4.509 ± 0.010	4.503 – 4.520
5	–”–	2*n*	Jask	25°48′ N 57°49′ E	75	1	4.532	
	–”–	3*n*	–”–	–”–	–”–	1	6.850	

RESULTS AND DISCUSSION

Among two individuals of *O. tridactylus* from Jask, one was diploid and the other was triploid. The nuclear DNA content of the triploid individual was equal to 6.850 pg, while in diploid *O. tridactylus* was 4.532 pg. Genome size in the other *O. tridactylus* population we studied and in two other *Ophiomorus* species ranged from 4.503 to 4.572 pg (Table 1). The triploid specimen was adult, normally developed and had a normal color pattern.

In the family Scincidae, triploidy is known only in the *Menetia greyii* species complex consisting of 2 – 3 diploid bisexual species (from south-central Australia) and three triploid hybrid parthenogenetic lineages widespread throughout Australia (Adams et al., 2003). Our record of triploidy in the genus *Ophiomorus* is the second for the family. Unfortunately, due to small number of individuals studied, it is difficult to classify the type of polyploidy (allo- or auto-) which we discovered. It could be the result of hybridization with closely related species (perhaps, *O. brevipes*; see Fig. 1) or a case of spontaneous autotriploidy (e.g., as a result of the fusion of the first polar nucleus with the nucleus of the secondary oocyte; Stenberg and Saura, 2013). However, among terrestrial vertebrates the latter type of polyploidy is much more common in amphibians than in reptiles (Litvinchuk et al., 2016; Pensabene et al., 2024). Only new studies of the species in the region will help to resolve this issue.

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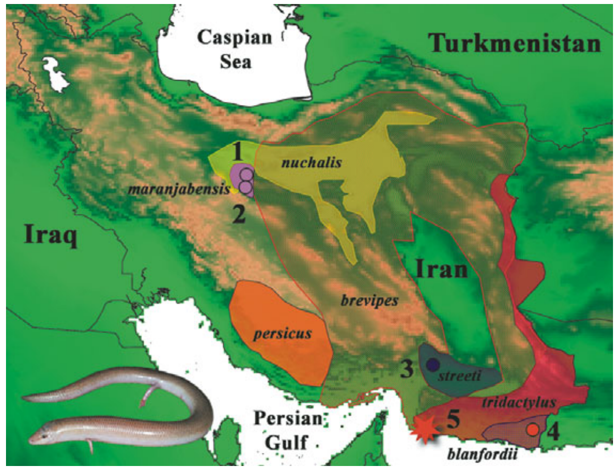

Fig. 1. Map showing ranges of seven species and studied localities of *Ophiomorus* in Iran. The red asterisk marks the location (Jask), where the triploid individual of *O. tridactylus* was found. Violet circles are localities of *O. maranjabensis*, blue circle is the locality of *O. streeti*, and red circle and asterisk are localities of *O. tridactylus*. Numbers for localities are given in Table 1. Photo credit: DAM (*O. tridactylus* from Bahu Kalat).

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