

Original paper

Detection of *Serpentoplasma* sp. (Apicomplexa: Haemohormidiidae) in dice snake (*Natrix tesellata*) (Reptilia: Colubridae) in Azerbaijan

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ABSTRACT. The aim of this study was to determine the presence of blood parasites in dice snake (*Natrix tesellata*) from Pirallahi Island, located off the western coast of the Caspian Sea of Azerbaijan. The dice snake is one of the most widespread and common species of the herpetofauna of Azerbaijan. This species of snakes represents an important link in the natural balance, since included in various biocenotic connections. A total of 67 specimens of dice snake individuals were captured during spring and summer 2021–2024. Blood sampling was carried out from the caudal vein by insulin syringe. The blood smears were stained with Giemsa solution and examined by light microscopy for haemoparasites. Microscopical examination of the blood smears revealed that 23 snakes of 67 (15.4%) were infected with *Serpentoplasma* sp. Multiply infected erythrocytes with one to three parasites were unusual, with most host cells containing a single inclusion. Infected thrombocytes were not detected. The parasites contained round-to-oval inclusions from small, anaplasmod-like bodies, vacuoles with a dot or two of apparent chromatin on their margin or without chromatin dots. The structures were intracytoplasmic, measured $3\text{--}3.8 \times 2.5\text{--}3.3 \mu\text{m}$ and occurred as rounded vacuole-like structures, one in each infected erythrocyte. This is the first finding of haemoparasites in dice snakes in Azerbaijan.

Keywords: blood parasite, morphology, *Natrix tesellata*, *Serpentoplasma* sp.

Introduction

Serpentoplasma is a genus of parasitic alveolates belonging the phylum Apicomplexa. The genus was erected in 1962 by Pienaar [1]. It appears as an apparent piroplasmoid parasite of snakes in which infection begins in thrombocytes, where dense chromatin masses produce nuclei that then infect erythroid cells. Division in both types of cells is by budding, binary fission, or perhaps by merogony, sometimes resulting in Nuttallia-like tetrads of nuclei. The parasites invade erythroid cells, where they may appear as vacuoles with a dot of chromatin at the margin, or as irregularly shaped nuclei within the cytoplasm. Biological cycle and vectors of the representatives of the genus *Serpentoplasma* are still unknown, but due to their classification as

piroplasms, they are likely transmitted by ticks and mites [2].

Colubridae Oppel, 1811 is a large and diversified snake family that encompasses seven subfamilies [3], of which only two, Colubrinae Oppel, 1811 and Natricinae Bonaparte, 1838, occur in Azerbaijan [4]. Among colubrid snakes, the dice snake (*Natrix tesellata*, Laurenti, 1768) is one of the most widespread and common species of the herpetofauna of Azerbaijan. This species of snakes represents an important link in the natural balance, since it is included in various biocenotic connections. Its most significant role is manifested by participation in trophic chains. As a consumer of the highest rank and mainly a batracho- and ichthyophag, the dice snake partly plays a positive role of an “orderly” in ecosystems, eating primarily



Figure 1. Dice snake (*Natrix tesellata*) individuals from Pirallahi Island (Caspian Sea, Azerbaijan)

sick and weakened animals. The dice snakes themselves serve as an additional source of food for other vertebrates: predatory fish, birds and mammals.

In the present study, we screened the blood of dice snakes using light microscopy. The investigation resulted in the first report of piroplasmorid parasite in *N. tesellata* in Azerbaijan.

Materials and Methods

A total 67 dice snake individuals were captured during spring and summer 2021–2024 on Pirallahi Island, located off the western coast of the Caspian Sea. The island is 11 kilometres (6.8 m) long and has a maximum width of 4 kilometres (2.5 m). The total square of the island is 14.6 km². Administratively, Pirallahi Island belongs to the Pirallahi district of Baku. The island is a part of the Absheron archipelago Important Bird Area (IBA), designated as such by BirdLife International because it supports significant populations of wintering whooper swans, common pochards, tufted ducks, great crested grebes and common coots, as

well as breeding Caspian gulls [5].

The snakes were collected by hand, transferred to the laboratory for examination, and then released at the place of capture (Fig.1). Blood samples were collected from each snake individual by puncture of the ventral caudal vein using insulin syringes. A drop of each blood sample was used to prepare thin blood smears on microscopical slides, air-dried, fixed in absolute methanol for 5 min, and stained with Giemsa (diluted 1:10 in distilled water, pH = 7) for 20 min as described in Šíroky et al. [6]. All procedures for snakes handling, blood sampling was carried out in accordance with the valid legislation of Azerbaijan, i.e. the Act on “About the Animal World” and the Amendment of Certain Related Acts no. 1/643-IIQD (30 April, 2004) (<https://e-qanun.az/framework/3850>).

The presence of parasites was examined by light microscopy using Leica DM1000 microscope with a 100× magnification objective lens and immersion oil. Photomicrographs were captured by Leica DFC425 digital camera, morphometrical parameters were measured using the ImgeScope M imaging software.

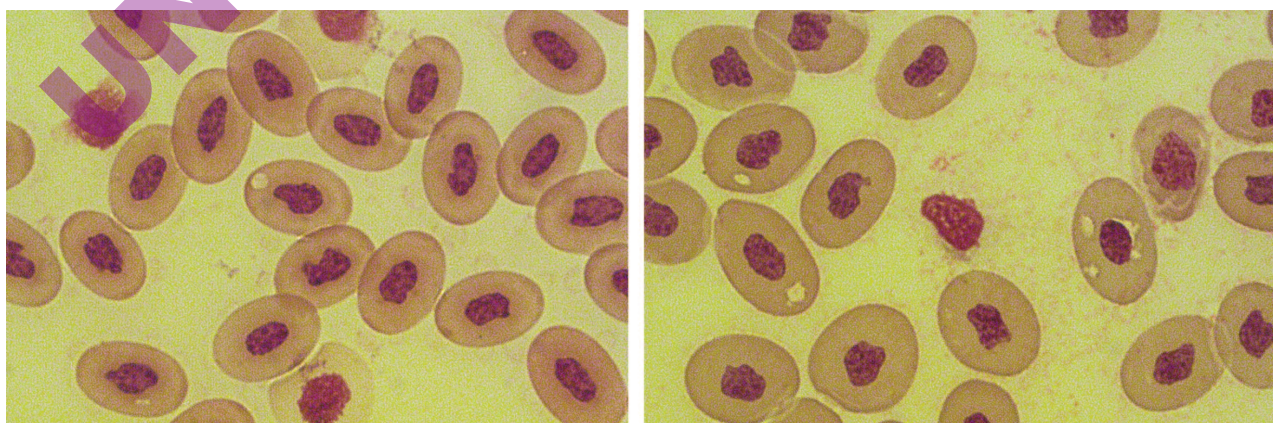


Figure 2. *Serpentoplasma* sp. in blood cells of *Natrix tesellata* (Giemsa stained blood smears)

Table 1. *Serpentoplasma* spp. reported in snake species and in *Natrix tesellata* in Azerbaijan and their measurements; na – data not available

Host species	Type locality	n infected (% infected)	Measurements of parasites (μm)		Reference
			Thrombocytic parasites	Erythrocytic parasites	
<i>Crotaphopeltis hotamboeia</i>	Free State, Africa	6/na	na	1.8 ± 0.93 (1.4–2.3) × 1.94 ± 0.7 (1.2–2.10)	[11]
<i>Lycophidion capense</i>	Free State, Africa	2/na	na	1.12 ± 0.84 (0.4–1.5) × 0.97 ± 0.71 (0.5–1.64)	[11]
<i>Elapsoidea sundevallii</i>	Free State, Africa	3/na	na	0.67 ± 0.57 (0.4–1.2) × 1.16 ± 0.6 (0.6–1.67)	[11]
<i>Hemachatus haemachatus</i>	Lesotho, Africa	2/na	na	0.67 ± 0.57 (0.4–1.2) × 1.16 ± 0.6 (0.6–1.67)	[11]
<i>Causus rhomheatus</i>	Free State, Africa	1/na	na	2.68 ± 1.4 (1.89–2.90) × 3.89 ± 1.1 (2.24–4.2)	[11]
<i>Bitis arietans</i>	Free State, Africa	1/na	na	1.1 ± 0.99 (0.9–1.4) × 1.4 ± 0.81 (0.91–1.35)	[11]
<i>Trimorphodon biscutatus</i>	Colima state, Mexico	1/13 (7.7%)	na	3–5	[2]
<i>Boa constrictor</i>	Colima state, Mexico	1/57 (1.8%)	2.5 and 3.5×3	$1 \times 1-1.5 \times 1.3$	[2]
<i>Python regius</i>	Ghana, Africa	1/18 (5.6%)	$3-4 \times 2-3$	na	[2]
<i>Opheodrys semicarinatum</i>	Ryukyu Island, Japan	4/5 (80.0%)	1 and 4–4.5	6×3.5	[2]
<i>Nerodia clarkii</i>	Levy Country, Florida	2/6 (33.3%)	$1 \times 1-4 \times 2.5$	$3 \times 2.5-3$	[2]
<i>Storeria occipitomaculata</i>	Alachua Country, Florida	1/2 (50.0%)	1 and 2×1.5	$3.5 \times 1-1.5$	[2]
<i>Natrix tesellata</i>	Guilan province, Iran	2/30 (6.67%)	na	na	[12]
<i>Natrix tesellata</i>	Pirallahi Island, Azerbaijan	23/67 (15.4%)	na	$3-3.8 \times 2.5-3.3$	This study

Results

Microscopical examination of the blood smears revealed that 23 snakes of 67 (15.4%) were infected with *Serpentoplasma* sp. They were found in mature erythrocytes of which 2,700/10,000 (27%) cells were infected. The parasites were contained inclusions round-to-oval inclusions from small, anaplasmod-like bodies, vacuoles with a dot or two of apparent chromatin on their margin or without chromatin dots. The structures were intracytoplasmic, measured $3-3.8 \times 2.5-3.3$ μm and occurred as rounded vacuole-like structures, one in each infected erythrocyte (Fig. 2).

Multiply infected erythrocytes with one to three parasites were unusual, with most host cells containing a single inclusion. Infected thrombocytes were not detected.

Discussion

Snakes are known to be parasitized by a diverse community of endo- and ectoparasites such as acanthocephalans, nematodes, trematodes, monogeneans, cestodes, protozoans, microsporidians, myxozoans, pentastomids, annelids, crustaceans, acari, ticks, and dipterans [7]. With regards to the protozoans, *Hepatozoon* spp. are considered the most frequently reported intracellular haemoparasites in snakes, however, their diversity and systematics are poorly understood [2,8]. Piroplasms appear in erythrocytes as small inclusions, round to amoeboid in shape [9], containing granules of chromatin associated with a vacuole [2]. Because of their small size and vacuole-like look, an infection with *Sauroplasma* sp. (occurring in lizards) or *Serpentoplasma* sp.

(occurring in snakes) can easily be overlooked or mistaken as artefacts or infections with bacterial organisms or virus particles and, of course, also vice versa [2]. *Serpentoplasma* sp. has low prevalence and low degrees of parasitaemia, and it seems that their prevalence is generally low [2]. Our study showed 15.4% of specimens in a sample of snakes were infected with *Serpentoplasma* sp. The natural vectors of *Serpentoplasma* sp. are not known, but it seems likely that, similarly to other piroplasmid taxa (*Babesia*, *Theileria*), ticks serve as definitive hosts [10]. The distribution of *Serpentoplasma* sp. is cosmopolitan, as indicated by infections in elapid, boid, and colubrid snakes (Table 1) from different parts of the world. Given the rarity of *Serpentoplasma* sp. infections, it will be difficult to obtain adequate material for ultrastructural and genomic studies, which must be done to determine the systematic position of these parasites.

Although various blood parasites species have been already identified in snakes, our study may further contribute to the information on the *Serpentoplasma* genus, which is believed to have not received enough attention.

In conclusion, the prevalence of blood parasites in snakes in Azerbaijan is still greatly underestimated. The present study provides the first detection of *Serpentoplasma* sp. in *Natrix tessellata*. We believe that it is also important to investigate the transmission routes of this parasite.

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