

Pathogenic *Leishmania* sp. (*Sauroleishmania*) (*Trypanosomatida*: *Trypanosomatidae*) detected in rapid fringe-toed lizard (*Eremias velox*) from Azerbaijan

Turkan Gurbanova

Institute of Living Systems Research, Ministry of Science and Education of the Republic of Azerbaijan, 115 A.Abbaszade Str., AZ1004, Baku, Azerbaijan

For correspondence: *turkan.qurbanova@gmail.com*

Received: April 30, 2026; Reviewed: June 10, 2026; Accepted: June 16, 2026

Leishmaniasis, caused by protozoan parasites of the genus *Leishmania*, remains a significant global public health concern, particularly in tropical and subtropical regions. Although mammals are recognized as the primary reservoir hosts, reptiles, especially lizards, may also harbor members of the subgenus *Sauroleishmania*, with increasing evidence suggesting their potential epidemiological relevance. In this study, six individuals of the rapid fringe-toed lizard (*Eremias velox*) were collected from Pirallahi Island (Azerbaijan) and examined for the presence of blood parasites using light microscopy. Promastigotes of *Leishmania* sp. were detected in one specimen. The observed forms exhibited morphological characteristics typical of promastigotes, including an elongated body, a centrally located nucleus, a distinct kinetoplast, and a free flagellum. This study represents the first report of *Leishmania* infection in *E. velox* in Azerbaijan, constituting both a new host and a new geographic record. These findings contribute to the growing body of evidence indicating that reptiles may play a role in the natural maintenance and circulation of *Leishmania* parasites, particularly in arid and semi-arid ecosystems.

Keywords: *Eremias velox*, parasitology, *Leishmania* sp., promastigote, Azerbaijan

INTRODUCTION

Reptiles are among the most diverse groups of animals that inhabit almost all continents and environments (Roll et al., 2017). Reptiles are an important part of Azerbaijan's wildlife and can be found in various habitats across the country. According to the latest official reports, the reptile fauna of Azerbaijan comprised 63 species, including 28 snakes (Serpentes), 32 lizards (Sauria), and 3 turtles and 1 tortoise (Testudines) (Information System of the Fauna of Azerbaijan: Vertebrates, 2023). Among these species, the rapid fringe-toed lizard (*Eremias velox* (Pallas, 1771)) represents one of the widely distributed lizard taxa, occurring in the eastern Fore-Caucasus and South Caucasus, the Lower Volga region, Central Asia, northern Afghanistan, China, and northern and northeastern Iran. In

Azerbaijan, it occurs in the Mil and Salyan plains, Zuvand (Lerik district), Gobustan, and in the southeastern and central-western parts of the Absheron Peninsula. It inhabits foothill areas with sparse vegetation dominated by wormwood, as well as dry riverbeds. The name given to this lizard reflects its agility; it moves very quickly and, when sensing danger, hides in cavities around plant roots, among shrubs, and in cracks and crevices. It can rapidly burrow into sand and dig its own shelter. It feeds on flies, beetles, and ants. Lepidopterans (especially caterpillars) and grasshoppers constitute the main part of its diet. In Azerbaijan, it does not have a special conservation status (Information System of the Fauna of Azerbaijan, Vertebrates, 2023).

Lizards are known reservoirs for numerous parasites, such as kinetoplastids and apicomplexan parasites, including coccidia and

haematozoa (Telford, 1995; Sloboda et al., 2007), and thus play important roles in ecological processes. Among these parasites, species of *Leishmania* are particularly noteworthy, as one of their most remarkable features is their ability to successfully parasitize the host's macrophages - cells that are ordinarily responsible for eliminating invading pathogens. Protozoans of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) have a digenetic life cycle, circulating between a wide range of reservoir hosts and phlebotomine sand flies (Diptera: Psychodidae). Members of this genus were recently divided into four subgenera: *Leishmania*, *Viannia*, *Sauroleishmania*, and *Mundinia* (Espinosa et al., 2016). *Sauroleishmania* was first described as a separate genus in 1973 (Ranque, 1973), later the same subgeneric name was used to separate all reptile-infecting species from those that infect mammals (Saf'janova, 1982). The phylogenetic position of *Sauroleishmania* was unclear for a long time, based on recent molecular data, it is now generally accepted that *Sauroleishmania* forms a monophyletic group that belongs within the genus *Leishmania* (Akhoundi et al., 2016). Currently, 21 species belonging to the subgenus *Sauroleishmania* are described, including two unnamed species (Akhoundi et al., 2016).

The mechanism of *Sauroleishmania* transmission remains unclear; it is considered that reptiles are infected by sand fly bite and/or by its ingestion (Killick-Kendrick, 1986; Telford, 2009). Proven natural vectors are reptile-biting sand flies of the genus *Sergentomyia* (Killick-Kendrick, 1986; Maroli et al., 1988). *Sauroleishmania* development in sand flies is localized in the hindgut and thus is described as hypopylarian (Lainson & Shaw, 1987). However, there are some older records of the anterior migration of these parasites in the sand fly gut (Adler & Theodor, 1935; Heisch, 1985; Killick-Kendrick, 1979), which indicates that some *Sauroleishmania* parasites might be transmitted by bite via the mechanism known for mammalian species (Bates, 2007). The cycle begins when a female Phlebotomine sandfly takes a blood meal from an infected host. During this process, it ingests macrophages containing the intracellular amastigote form of the parasite. Inside the

sandfly's midgut, these amastigotes transform into promastigotes, which are elongated, flagellated, and motile. The promastigotes multiply by binary fission and migrate toward the proboscises of the insect, becoming infective. When the infected sandfly bites another host, it injects the infective promastigotes into the skin. Once inside the host, promastigotes are rapidly phagocytosed by macrophages. Within these cells, the parasites transform into the amastigote form, which is non-motile and adapted to survive intracellularly. The amastigotes multiply by binary fission, eventually causing the host cell to rupture. The cycle is completed when another sandfly feeds on an infected individual and ingests the amastigote-laden macrophages. These parasites then undergo transformation back into promastigotes in the sandfly, repeating the cycle.

Lizards can act as natural reservoirs of *Leishmania*. This means that the parasite is able to persist in their bodies for long periods without causing pronounced symptoms, thereby maintaining the circulation of the pathogen in nature and potentially contributing to its transmission to humans and other animals.

The study of *Leishmania* in lizards is an important component in understanding natural foci of Leishmaniasis, the evolution of the parasite, and the development of effective control and prevention strategies for this disease.

In the present study, we screened the rapid fringe-toed lizard in Azerbaijan using microscopical examination to identify potential zoonotic pathogens that these animals may harbor. In addition, this is the first record of *Leishmania* sp. in the blood sample of *E. velox* in Azerbaijan.

MATERIALS AND METHODS

A total of six individuals (Fig. A) were captured during spring and summer 2024 from Pirallahi Island, located on the western coast of the Caspian Sea. The lizards were collected by hand, physically examined to assess their health status, anamnestic data (e.g., species, biological stage, sex, physical abnormalities such as tail loss or predator-induced wounds) and sampled before release in the original home range. A small amount of blood (~200µl) obtained via

cardiocentesis 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 (Široký et al., 2007).

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

Morphometric measurements are presented as mean±standard deviation (SD). Statistical differences between groups were evaluated using Student's t-test, with significance accepted at $p < 0.05$.

All procedures for lizard handling, blood sampling were performed with the approval of the Ethics Committee of the Institute of Zoology, Ministry of Science and Education of the Azerbaijan Republic (protocol No. 06, 22.10.2022).

RESULTS AND DISCUSSION

Of the six captured lizards, four were males and two were females. The body length of males ranged from 55 to 74 mm, whereas that of females ranged from 52 to 69 mm. These measurements were used to estimate the age of the individuals, indicating that all examined lizards were sexually mature.

No visible signs of tail regeneration were observed in five individuals. Only one female exhibited evidence of tail autotomy, characterized by an orange tail with a regenerated yellowish tip. This suggests that tail regeneration likely occurred during adulthood.

None of the samples collected during the study period tested positive for *Phlebotomus* spp. Of the six examined specimens, promastigotes of *Leishmania* sp. were identified in one individual (Fig.B). The individual infected with *Leishmania* sp. was a sexually mature male. The observed forms were elongated and exhibited a clearly defined nucleus, kinetoplast, and flagellum. The nucleus was predominantly located centrally within the cell, although in some cases it was positioned closer to the flagellar end, occupying a substantial portion of the cell body. The kinetoplast was typically oriented perpendicular to the longitudinal axis of the parasite and appeared as a small rod-shaped structure. The body length of the promastigotes ranged from 10.10 ± 0.25 to 12.33 ± 0.08 μm , and the width from 1.30 ± 0.07 to 2.02 ± 0.07 μm . The nucleus measured from 1.46 ± 0.11 to 1.94 ± 0.08 μm in length and from 1.14 ± 0.04 to 1.46 ± 0.09 μm in width. The flagellum length ranged from 12.79 ± 0.32 to 21.45 ± 0.36 μm , while the kinetoplast length was 1.10 ± 0.00 μm , indicating that this parameter remained constant.



Fig. A - rapid fringe-toed lizard (*Eremias velox*); **B** - promastigote of *Leishmania* sp. in blood sample of *E. velox* (stained by Giemsa, 1000× magnification).

Approximately 20–21 species of *Leishmania* associated with reptiles have been described to date, primarily belonging to the subgenus *Sauroleishmania*, which includes parasites infecting lizards across different geographical regions (Lozano et al., 2018; Ticha et al., 2021; Bandi et al., 2023). Earlier taxonomic studies reported even greater diversity, with up to 17 species and numerous unidentified forms described in reptilian hosts, although many of these require revision (Ovezmukhammedov & Safianova, 1989).

This study represents the first confirmed report of *Leishmania* infection in the *E. velox* in Azerbaijan, thereby expanding the known host range of the parasite in the region. The detection of infection in this species is particularly important given its wide distribution and ecological adaptability, which may facilitate interactions with sand fly vectors and contribute to parasite transmission.

Prior to this study, Popov reported the detection of promastigotes in blood smears from three Caspian geckos (*Gymnodactylus caspius*) in Azerbaijan (Popov, 1930; Popov, 1940). In addition, in the early 1970s, Belova suggested that at least 21 lizard species, including *E. velox*, from six different families could harbor *Leishmania* promastigotes (Belova, 1971). Although these early observations were primarily based on morphological identification and lacked molecular confirmation, they provided important preliminary evidence for the potential involvement of reptiles in the life cycle of *Leishmania*.

The results of this study are consistent with findings from neighboring regions, including Iran, Turkey, and Central Asia, where reptiles have been implicated in the epidemiology of leishmaniasis. In Iran, a comprehensive review reported the presence of *Leishmania* (including *Sauroleishmania*) in reptilian hosts and highlighted the potential role of lizards and snakes in maintaining zoonotic species such as *Leishmania tropica* (Sazmand et al., 2024). Additionally, “lizard *Leishmania*” strains have been isolated from reptile species in Iran and are increasingly studied for their biological and epidemiological significance (Keshavarzian et al., 2020).

Similarly, studies conducted in Mediterranean regions, including Turkey, have demonstrated the presence of reptile-associated *Leishmania* species such as *Leishmania tarentolae* in geckos, as well as their overlap with pathogenic species like *L. infantum* in endemic areas (Mendoza-Roldan et al., 2022). These findings indicate that reptiles may contribute to the maintenance and circulation of *Leishmania* parasites in environments where sand fly vectors are abundant.

Recent evidence from Central Asia and adjacent regions further supports the possible role of reptiles as reservoir hosts in the epidemiology of leishmaniasis. Molecular investigations conducted in countries such as China, Kazakhstan, and Uzbekistan have confirmed the presence of both non-pathogenic and pathogenic *Leishmania* species in lizard populations. In particular, *Leishmania* DNA, including zoonotic species such as *L. tropica* and members of the *L. donovani* complex, has been detected in multiple lizard species, sometimes with relatively high infection prevalence (Zhang et al., 2019).

These findings are strongly supported by earlier molecular studies from northwestern China, where a high prevalence of *Leishmania* infection (up to ~58%) was reported in diverse lizard species, including detection of pathogenic taxa such as *L. tropica*, *L. turanica*, and the *L. donovani* complex (Gao et al., 2015; Guan et al., 2016). The occurrence of mixed infections and multiple haplotypes within individual hosts further suggests complex transmission dynamics involving reptiles. Additional evidence from reptile studies in the same endemic regions indicates that not only lizards but also other ectothermic vertebrates, such as snakes, may harbor *Leishmania* species, including medically important taxa like *L. donovani* and *L. turanica* (Chen, 2019). This broad host range supports the hypothesis that reptiles could contribute to the maintenance of *Leishmania* in natural foci.

Historically, the potential role of reptiles in leishmaniasis transmission has been proposed for decades, as sand fly vectors readily feed on lizards and experimental infections have demonstrated parasite persistence in reptilian hosts (Belova, 1971). More recent experimental studies have also shown that lizards can mount

immune responses to *Leishmania donovani* infection, with patterns partly comparable to those observed in mammals (Xiao et al., 2024), further supporting their biological compatibility as hosts.

Taken together, these regional findings, combined with the present study, support the hypothesis that reptiles, including *E. velox*, may play a broader and previously underappreciated role in the natural cycle of leishmaniasis, particularly in arid and semi-arid ecosystems. Therefore, the detection of *Leishmania* infection in *E. velox* in Azerbaijan aligns with regional patterns and reinforces the idea that reptiles may play a more significant role in the epidemiology of leishmaniasis than previously recognized. This finding highlights the need to consider reptilian hosts in future epidemiological studies and control strategies in the region.

CONCLUSION

This study provides the first evidence of *Leishmania* infection in *Eremias velox* in Azerbaijan. The results highlight the potential role of reptiles as reservoir hosts and emphasize the need for further molecular and epidemiological studies.

FUNDING

This study did not receive any specific grant from the state, commercial or non-commercial organizations.

CONFLICT OF INTEREST

The authors confirm the absence of a conflict.

AI STATEMENT

The author declares that no artificial intelligence (AI) tools were used to generate, analyze, interpret, or validate the scientific data and findings presented in this study. Any AI-assisted technologies, if used, were limited to language editing, grammar correction, or formatting support. The author takes full responsibility for the originality, accuracy, and integrity of the manuscript.

ETHICAL APPROVAL

All procedures involving animal sampling and examination were conducted in accordance with applicable institutional and national guidelines for the ethical treatment of animals and wildlife research.

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ORCID:

Turkan Gurbanova: <https://orcid.org/0000-0002-5923-5600>

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