Herpetology Notes 19: 843–847, published on 28 November 2026

First record of blood parasites *Hemolivia* sp. (Apicomplexa: Karyolysidae) parasitising the Cururu Toad, *Rhinella diptycha* (Cope, 1862) in the Caatinga, northeastern Brazil

Carla A.C. Carvalho[1,2,3,*], Lucas G.A. Rodrigues[2], João V.G. Brandão[2,3], Fabiano M. Vieira[2,3], Leonardo B. Ribeiro[2,3], and Gabriela Felix-Nascimento[2,3]

The life history and habitat of different vertebrate hosts influence their exposure to potential haemoparasite vectors. Frogs are closely associated with aquatic environments and are exposed to a variety of haematophagous vectors, such as mosquitoes, ticks, or leeches, which may themselves harbour a wide diversity of haemoparasites (Barta and Desser, 1984; Cabagna Zenklusen et al., 2014).

The Caatinga Morphoclimatic Domain, commonly referred to as the Caatinga, is an ecological region in northeastern Brazil characterised predominantly by Seasonally Dry Tropical Forest, encompassing at least 13 distinct physiognomies as adaptations to xeric environmental conditions of the semiarid climate (Silva et al., 2017). Knowledge of parasitism in Caatinga frogs is largely restricted to publications that provide sporadic records of infections and lists of helminth fauna for a few host species (Soares et al., 2022). Despite the limited data available, new species have been described (Felix-Nascimento et al., 2020; Vieira et al., 2023). When it comes to haemoparasites, the available information is even more restricted.

The Order Anura currently comprises 8053 known species (Frost, 2026). Brazil harbours the greatest anuran diversity worldwide, with 1226 recorded species (Drummond et al., 2026; Frost, 2026), of which 115 occur in the Caatinga Morphoclimatic Domain (Soares et al., 2026). Among these, only 3.15% of species are known to be infected with haemoparasites, including *Trypanosoma herthameyeri* in *Leptodactylus fuscus* (Attias et al., 2016) and members of the genus *Hepatozoon* in *Rhinella diptycha* and *R. granulosa* (Carvalho et al., 2024). We here provide the first documentation of parasitism by a member of the genus *Hemolivia* in *R. diptycha* (Fig. 1) in the Caatinga Morphoclimatic Domain.

Toads were collected from January 2024–March 2025 at two sites in Petrolina Municipality, Pernambuco State, Brazil. The first was a conventional agricultural area located within the Projeto de Irrigação Senador Nilo Coelho – Núcleo 01 (9.3346°S, 40.5865°W,



Figure 1. Adult Cururu Toad (*Rhinella diptycha*) from the municipality of Petrolina, state of Pernambuco, Brazil. Photo by Leonardo B. Ribeiro.

[1] Laboratório de Estudos Morfofisiológicos e Parasitários, Departamento de Ciências Biológicas e da Saúde, Universidade Federal do Amapá, Rodovia Josmar Chaves Pinto KM 02, Jardim Marco Zero, Macapá, Amapá 68903-419, Brazil.

[2] TETRAPODA – Grupo de Estudo em Morfofisiologia Animal, Universidade Federal do Vale do São Francisco, Rodovia BR-407, KM 12, Lote 543 S/N, Projeto de Irrigação Senador Nilo Coelho, Petrolina, Pernambuco 56300-000, Brazil.

[3] Laboratório de Fisiologia Animal, Universidade Federal do Vale do São Francisco, Rodovia BR-407, KM 12, Lote 543 S/N, Projeto de Irrigação Senador Nilo Coelho, Petrolina, Pernambuco 56300-000, Brazil.

[*] Corresponding author. E-mail: carlaadriellecc@hotmail.com

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elevation 380 m). The second was situated on the Campus de Ciências Agrárias of the Universidade Federal do Vale do São Francisco (UNIVASF), an area of anthropogenically modified Caatinga (9.3281°S, 40.5497°W, elevation 376 m). Sampling was conducted through active searches near intermittent and perennial waterbodies. A total of 65 *R. diptycha* was collected.

Following collection, animals were transported to the Laboratório de Fisiologia Animal at UNIVASF, their snout–vent length (SVL) was measured using callipers, and they were weighed using a digital precision scale. Blood samples were obtained via intracardiac puncture or from the ventral abdominal vein for the preparation of blood smears. Four slides were prepared for each specimen. Two smears were fixed in absolute methanol for 10 min and stained with a 10% Giemsa solution for 30 min. In addition, some smears were stained using Fast Panoptic. Smears were examined under a light microscope equipped with a digital camera. Morphometric analyses were performed using ToupView software, based on measurements of parasite and nuclear length (µm), width (µm), and area (µm^2). Data are presented as mean ± standard deviation with ranges in parentheses. Parasitism prevalence was calculated according to Bush et al. (1997), and the parasites were counted per 2000 erythrocytes (Garrido and Pérez-Mellado, 2013). Hosts were euthanised for subsequent helminthological analyses. Specimens were deposited in the Coleção Herpetológica of the Museu de Fauna da Caatinga (MFCH) at the Centro de Conservação e Manejo de Fauna da Caatinga of UNIVASF (accession numbers 6132–6135, 6145, 6152, 6158, 6162, 6165, 6167, 6249–6303).

Of the 65 *R. diptycha* collected (SVL 135.1 ± 24.8 mm; weight 284.2 ± 168.3 g), two adult males were infected with haemoparasites (prevalence: 3.1%). These gametocytes were identified as members of the genus *Hemolivia* based on the characteristics described for *Hemolivia stellata* gametocytes by Petit et al. (1990). Young gametocytes, which lack a capsule, are elongated and relatively robust, with one rounded extremity and the other tapering to a point (Fig. 2A, B). Their nuclear chromatin is condensed into transverse bands (Fig. 2A), as if migrating towards

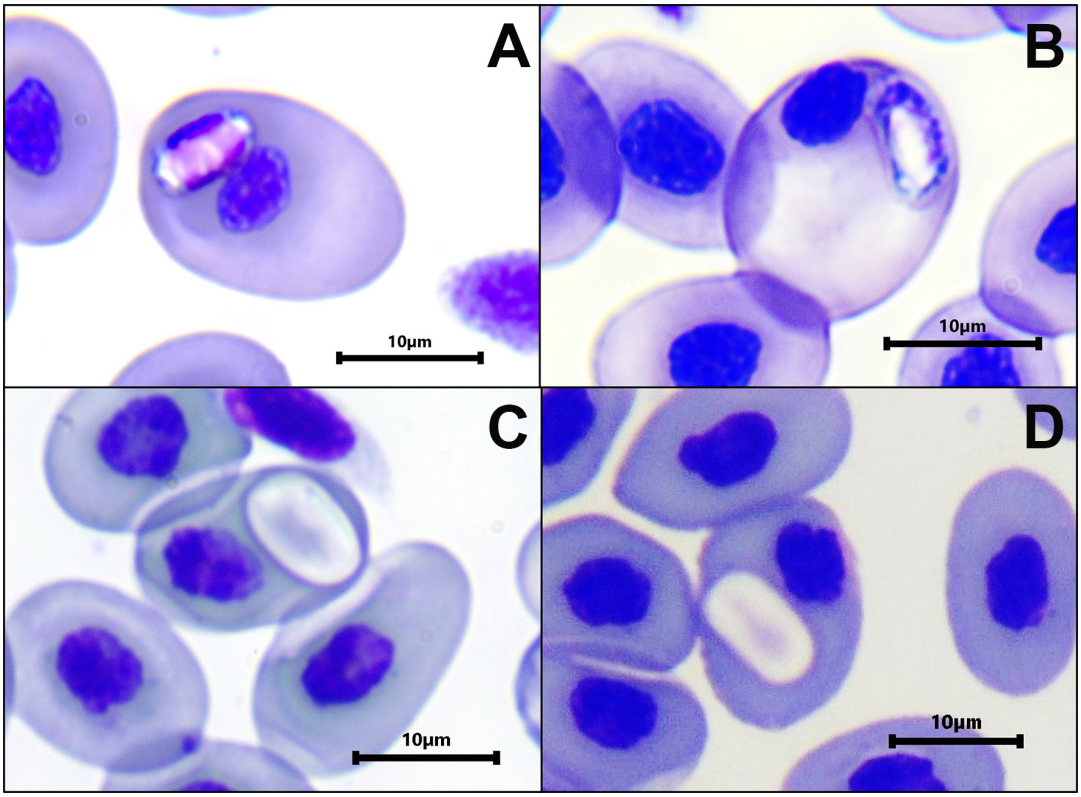


Figure 2. Gametocytes of *Hemolivia* sp. parasitising *Rhinella diptycha* (Cope, 1862) from Caatinga, Brazil. (A, B) Immature gametocytes showing the chromatin around the periphery. (C, D) Mature gametocytes. Scale bars = 10 µm.

the periphery (Fig. 2B). As maturation progresses, the capsule thickens and becomes impermeable to stain (Fig. 2C, D). It was possible to observe different stages of gametocyte maturation in the parasites. Initially, chromatin organisation was evident, with chromatin migrating from the central region towards the periphery of the gametocyte (Fig. 2A, B). After maturity, these parasites exhibited an oval, non-staining capsule containing a single gametocyte. The capsule remained impermeable to staining, preventing visualisation of the nucleus or any internal structures (Fig. 2C, D). The gametocytes appeared to displace the nucleus of the host erythrocyte. Morphological data of the parasites and host erythrocytes are provided in Table 1.

In Brazil, several records of haemoparasites infecting *R. diptycha* exist. Kattar (1986) reported gametocytes resembling *Hemolivia*, identified as *Haemogregarina sensu lato*, from *R. diptycha* (reported as *Bufo paracnemis*) in João Pessoa, Paraíba, in the Atlantic Forest. The genus *Hepatozoon* was reported from the Atlantic Forest of São Paulo based on a molecular identification (Ferreira et al., 2020) and a record with morphological identification of gametocytes came from the Caatinga of Pernambuco (Carvalho et al., 2024). Further records include the description of *Dactylosoma amphibia* from a population in the Cerrado biome of Cocalinho, Mato Grosso (Úngari et al., 2022) and the description of *Lankesterella oliviactarinae* and a record of *Hemolivia* from the Amazon biome in Marabá, Pará (Alves-Coêlho et al., 2025).

Apart from the descriptions of *Hemolivia pulcherrima* and *Hemolivia* sp. in Nicaragua (Živčicová et al., 2024), neotropical records of the genus are concentrated in Brazil (Petit et al., 1990; Oliveira et al., 2020; Alves-Coêlho et al., 2025). All studies on *Hemolivia* in Brazilian frogs have been conducted in the Amazon, specifically in Pará State. These studies include *Hemolivia stellata*, the type species of the genus, first described in *R. marina* (Petit et al., 1990), as well as two records of unidentified *Hemolivia*, one from *R. major* (Oliveira et al., 2020) and one from *R. diptycha* (Alves-Coêlho et al., 2025).

Table 1. Morphometric values of gametocytes of *Hemolivia* sp. (n = 15) and non-parasitised erythrocytes of *Rhinella diptycha* (n = 15) from Caatinga, Brazil. Length measurements are in µm, areas in µm^2, values are provided as means ± standard deviation with ranges below them.

Measurement	Immature Gametocyte (n = 10)	Mature Gametocyte (n = 5)	Non-Parasitised Erythrocytes (n = 15)
Length	9.9 ± 1.5 (7.8–12.1)	10.5 ± 0.5 (9.8–11.1)	–
Width	4.3 ± 0.9 (2.2–5.1)	6.0 ± 0.8 (4.6–6.8)	–
Area	38.4 ± 10.5 (13.8–54.4)	53.9 ± 6.9 (42.2–62.4)	–
Length of erythrocyte	17.8 ± 2.2 (14.4–21.0)	18.4 ± 1.5 (16.6–20.2)	17.0 ± 1.2 (14.8–19.0)
Width of erythrocyte	14.1 ± 1.0 (12.1–15.5)	12.2 ± 1.2 (11.1–14.0)	12.1 ± 0.9 (10.8–13.4)
Area of erythrocyte	205.0 ± 36.7 (152.9–258.2)	188.0 ± 32.2 (154.8–219.3)	160.1 ± 20.2 (129.2–193.9)
Length of erythrocyte nucleus	6.5 ± 1.1 (4.8–7.7)	7.3 ± 0.4 (7.0–8.0)	7.1 ± 0.9 (5.8–8.5)
Width of erythrocyte nucleus	4.6 ± 0.4 (3.9–5.9)	5.3 ± 0.4 (4.9–5.9)	4.8 ± 0.4 (4.3–5.4)
Area of erythrocyte nucleus	25.9 ± 5.3 (16.9–31.1)	31.2 ± 2.2 (28.7–34.5)	27.9 ± 3.9 (18.8–31.5)

The maturation of *Hemolivia* gametocytes from *R. diptycha* showed similarities to the maturation of other parasites of this genus that occur in turtles. In studies by Cook et al. (2015), *H. parvula* gametocytes were observed with intensely stained chromatin around the periphery, as in the present study (Fig. 2A, B); however, the authors noticed that when mature, the gametocyte presented a terminal nucleus, as also reported by Živčicová et al. (2024) in *Hemolivia pulcherrima*, *Hemolivia* sp., and *Hemolivia mauritanica*, which was not observed in the present study.

Infection by species of *Hemolivia* in frogs occurs through ingestion of infected ticks (Petit et al., 1990). In the description of the life cycle of *H. stellata*, the tick *Amblyomma rotundatum* was identified as the vector (Petit et al., 1990). Although no ticks were observed on the individuals sampled in this study, a previous study documented *A. rotundatum* parasitising *R. diptycha* (syn. *R. jimi*) in rural Petrolina, Pernambuco, Brazil (Horta et al., 2015), near the collection sites of the present study. Tick parasitism by *A. rotundatum* in *R. diptycha* from the Atlantic Forest has also been reported (Nossa et al., 2019). However, none of these studies examined blood smears to assess infection by *Hemolivia* or other haemoparasites.

The Caatinga Morphoclimatic Domain is unique to Brazil and has undergone continuous modification since colonisation, with many areas degraded for agricultural use through aggressive and rudimentary practices, including burning and soil exposure (Andrade et al., 2005). This ongoing degradation increases vulnerability to extreme climatic events and desertification processes, potentially causing local biodiversity loss and impairing Caatinga regeneration (Macedo et al., 2023). Research on haemoparasites in anurans from the Caatinga is still incipient, and given the severe threats facing this biome, haemoparasite species may disappear before they are even documented. Thus, further research on *Hemolivia* parasitism in Brazilian herpetofauna is essential, alongside expanded studies of haemoparasitism more broadly within the Caatinga. Moreover, additional research is needed to clarify the biology of infection, including identification of definitive hosts, as well as molecular and histological investigations of intermediate hosts.

Acknowledgements. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) under Finance Code 001. Carla Adrielle Costa de Carvalho was supported by a CAPES doctoral fellowship (Process: 88887.111960/2025-00). João Vitor Gonçalves Brandão was supported by a Bolsa de Iniciação Científica fellowship from the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE; Process: BIC-0322-2.13/25). Fabiano Matos Vieira was supported by FACEPE (Processes: DCR-0023-2.13/24 and APQ-0625-2.13/24) and by a research grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Process: 303233/2024-9). Our research permit was provided by Instituto Chico Mendes de Conservação da Biodiversidade/ Sistema de Autorização e Informação em Biodiversidade (Processes 91121-1 and 93464-1). The collection of specimens of *Rhinella diptycha* was approved by the ethics committee on the use of animals of the Universidade Federal do Vale do São Francisco (Processes 0005/271023 and 0003/280824).

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Accepted by Hinrich Kaiser