Journal of Animal Diversity

Online ISSN 2676-685X

Volume 5, Issue 4 (2023)

Short Communication

http://dx.doi.org/10.22034/JAD.2023.5.4.6

Blood parasite of the genus *Dactylosoma* Labbé, 1894 in Marsh frog *Pelophylax ridibundus* (Pallas, 1771) from the north of Iran

Zahra Rahimi , Reyhaneh Hajiyan and Hossein Javanbakht*

Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran
**Corresponding author*: *h.javanbakht@guilan.ac.ir*

Citation: Rahimi, Z., Hajiyan, R. and Javanbakht, H. (2023). Blood parasite of the genus *Dactylosoma* Labbé, 1894 in Marsh frog *Pelophylax ridibundus* (Pallas, 1771) from the north of Iran. *Journal of Animal Diversity*, 5 (4): 57–62. http://dx.doi.org/10.22034/JAD.2023.5.4.6

Received: 25 August 2023
Accepted: 26 October 2023
Published online: 31 December 2023

Abstract

Blood parasites of the order Adeleiorina (protozoan phylum Apicomplexa) are a diverse group of haemoparasites reported from almost all vertebrate classes. The most commonly recorded haemoparasites of anurans are species of *Dactylosoma* Labbé, 1894. To date, six *Dactylosoma* species have been described from anurans and fishes. In the present study, we used molecular characterization to identify haemoparasites detected by microscopy in blood smears of *Pelophylax ridibundus*, a frog in the north of Iran. Blood samples were examined from four adult individuals. Smears were prepared and stained with Giemsa. Microscopy investigation revealed that one individual was positive for blood parasites. According to morphological characteristics, it was identified as belonging to genus *Dactylosoma*. In genetic analyses, the blast of obtained partial 18S rRNA gene sequences showed 100% identity with *Dactylosoma* sp. and *D. ranarum* (Kruse, 1890). Phylogenetic analysis showed the *Dactylosoma* sp. of the present study as a monophyletic group in *Dactylosoma* species, clustered with *Dactylosoma* sp. and *D. ranarum* (support > 80%). This study is the first report of a dactylosomatid parasite in *Pelophylax ridibundus*.

Key words: Coccidia, haemogregarine, haemoparasites, Ranidae

Anurans play an important role in the ecosystem, preying on various invertebrates and serving as prey for different animal groups (Faggioni et al., 2017). Studies in several geographical regions have indicated that anurans, in their aquatic and terrestrial habitats, are exposed to a variety of haemoparasites, including protozoans, microfilariae, rickettsia, and viruses (Barta and Desser, 1984). Protozoan haemoparasites in amphibians comprise a large group of apicomplexan blood parasites belonging to the genera *Haemogregarina* Danilewsky, 1885; *Hepatozoon* Miller, 1908; *Lankesterella* Labbé, 1899; *Schellakia* Reichenow, 1919; *Babesioma* Jakowska and Nigrelli, 1956; and *Dactylosoma* Labbé, 1894. Their form may be oval, round, or banana-shaped inclusions in red blood cells or even in leucocytes (Poynton and Whitaker, 2001; Ferreira et al., 2020). Dactylosomatidae is a small family of apicomplexan haemogregarines that currently comprises *Dactylosoma* and *Babesiosoma*. According to Barta (2012), the systematics of Dactylosomatidae are not clear and reclassifications and systematic revisions are needed. To date, six species of *Dactylosoma* have been described; two of them are fish parasites, and four are found in anurans (Úngari et al., 2020). *Dactylosoma ranarum* (Kruse, 1890) was the first described from an anuran, the European frog *Pelophylax esculentus* (Linnaeus, 1758) (Úngari et al., 2020).

Parasites and pathogens have deleterious effects in some frog populations, e.g., decreased growth, reduced development, and behavioral modification (Ponton et al., 2009). The infection of blood cells by hematic protozoa can cause changes in the blood cell morphology (Davies and Johnston, 2000), but no pathological effect has been described in their natural hosts (Bernal and Pinto, 2016). However, it is suggested that haemoparasite infections can cause clinically significant inflammatory disease in the visceral organs of rice field frogs, and the severity of lesions is tentatively related to levels of parasitemia (Sailasuta et al., 2011).

[DOI: 10.22034/JAD.2023.5.4.6]

Over the last century, there has been a greater focus on parasites of medical or veterinary importance (Davies and Johnston, 2000). However, literature regarding the haemoparasites of amphibian wildlife is scare, especially regarding anurans (Davies and Johnston, 2000). Currently, 7,166 anuran species have been described worldwide (Frost, 2021); of which 19 species occur throughout Iran. In Iran, there have been few studies on anuran haemoparasites, e.g., the presence of *Hepatozoon* in the Marsh frog *Pelophylax ridibundus* by Rajabi et al. (2017). The Marsh frog *P. ridibundus* is a member of the family Ranidae that represents a species complex that has been previously known as "*Rana ridibunda*", and it is the largest type of frog in most of its range (Frost, 2021). This species lives in mixed and deciduous forests, forest-steppe, steppe, semi-desert, and desert zones. It is reported from all over Iran except in the southeast and central deserts. The latest report of Iranian water frogs of the genus *Pelophylax* Fitzinger, 1843 suggested the marsh frog recorded from the Caspian Sea border to be *Pelophylax* sp. (Pesarakloo et al., 2016). The aim of this study was to report haemoparasite species in four Marsh frog (*Pelophylax ridibundus*) individuals from the north of Iran using the traditional morphological screen of peripheral blood stages and molecular techniques.

This study was conducted on the border of Rasht, which is located in the northern region of Iran (37.1531 N, and 49.6523 E). Four frogs were collected between May and June 2021. The specimens were captured by hand and net. The frogs were identified according to the descriptions by Yousefi Siahkalroodi et al. (2013). Blood was obtained from the heart, and blood smears were prepared, air-dried, and then fixed in absolute methanol for 2 minutes, then dried in the air and stained with Giemsa (1 part Giemsa's stain: 9 parts phosphate buffer saline pH 7.0) in a staining trough for 15 minutes. The slide was examined by light microscopy at ×10 and ×40. Micrographs were taken using a digital camera (UCMOS10000KPA, China) with a Nikon microscope (Alphaphoto, YS, Japan). The intensity of infection in the blood smears was determined by counting the total number of cells infected per 10^4 erythrocytes.

Genomic DNA was extracted from the blood sample using buffer detergent (Triton X- 100). We amplified fragments of the 18S rRNA gene using universal primers for Protista: HepF300 (5′-GTT TCT GAC CTA TCA GCT TTC GACG-3′) and HepR900 (5′-CAA ATC TAA GAA TTT CAC CTC TGA C-3′). PCR reactions were run targeting a fragment (approximately 600 bp) of the 18S rDNA gene (Ujvari et al., 2004) in a 25 µl reaction mixture using 12.5 µl Thermo Scientific DreamTaq PCR master mix (2×) (2× DreamTaq buffer, 0.4 mM of each dNTP, and 4 mM $MgCl_2$), 1.25 µl of each primer, and at least 25 ng DNA (Cook et al 2015). The PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles, entailing a 95 °C denaturation for 30 s, annealing at 60 °C for 30 s with an end extension at 72 °C for 1 min, and following the cycles a final extension of 72 °C for 10 min as detailed according to previous methods (Netherlands et al., 2014).

After confirmation under ultraviolet light on a 1% agarose gel, the amplified product was sent to a commercial sequencing company (Macrogen, South Korea) for sequencing in both directions. The obtained sequence was compared with the GenBank database, and similar sequences were downloaded and aligned to the sequence generated in this study. Sequences were aligned using Clustal W in BioEdit v 7.0.5.3 (Hall, 2005). The final alignment consisted of 26 sequences with 538 bps (Table 1). The model of evolution was estimated using the AIC criteria using the jModelTest v 0.1.1 program. Two different analyses (Maximum likelihood and Bayesian inference) formed the phylogenetic analyses. The best evolutionary model for ML analysis was identified as MTV+I+G. Maximum likelihood (ML) analysis with random sequence addition (100 replicate heuristic searches) was used to assess evolutionary relationships using the software PhyMl 3.0 (Guindon et al., 2010). Bayesian analysis was implemented using MrBayes v.3.2 with parameters estimated as part of the analysis. The best-fit model for BI identified by AIC was GTR+I+G. The analysis was run for 10,000,000 generations, saving one tree every 1,000 generations. *Adelina grylli*, *Klossia helicina* and *Adelina dimidiata* were used as outgroups following Ferreira et al. (2020). The FigTree v1.4.0 Program was used to visualize the phylogenetic tree. The new sequences were uploaded to GenBank with accessing number PP355366. All stages of this research complied with Iranian laws and with authorization from the University of Guilan.

One individual was determined as positive for blood parasites of the genus *Dactylosoma* using microscopy screening of blood smears. The diagnosis was initially based on the hyaline appearance of the variable oval and amoeboid forms occurring in red blood cells (Fig. 1). The intensity of parasites was about 0.02% per 10^4 red blood cells. Red blood cells were infected with trophozoites. Trophozoites are oval, round, or lobate; their cytoplasm does not stain (Fig. 1). These intra-erythrocyte parasites do not change the shape of the erythrocyte's nucleus nor the shape of the red blood cells.

The blast of obtained sequences from partial 18S rRNA gene sequences showed 100% identity with *D. ranarum* that were previously detected in the frog species *Rana esculenta* (Linnaeus, 1758) and *Dactylosoma* sp. from *Pelophylax lessonae* in Europe (Reis Ferreira et al., 2020). The genetic distance between the species of *Dactylosoma* ranged between 0 and 0.01%. Bayesian inference (*p*-value= 0.99) and Maximum likelihood (*p*-value= 82) analyses had similar topologies and showed that obtained sequence clustered in the group *D. ranarum* (Fig. 2).

[DOI: 10.22034/JAD.2023.5.4.6]

Table 1: The data set of *Dactylosoma*, *Karyolysus*, *Hepatozoon*, and *Hemogregarina* species that are reported in this study. The information includes the accession numbers, locality, and origin of the 18S rRNA gene.

No.	Species	Accession numbers	Locality	Origin
1	*Dactylosoma* sp.	PP355366	Rasht, Iran	This study
2	*Dactylosoma piperis*	MW264134.1	Brazil	Ungari (2018)
3	*Dactylosoma kermiti*	MN879395.1	South Africa	Netherlands et al. (2020)
4	*Dactylosoma kermiti*	MN879398.1	South Africa	Netherlands et al. (2020)
5	*Dactylosoma ranarum*	HQ224958.1	France	Barta et al. (2012)
6	*Dactylosoma ranarum*	HQ224957.1	France	Barta et al. (2012)
7	*Dactylosoma* sp	MN879399.1	Belgium	Netherlands (2020)
8	*Karyolysus* sp.	KJ461940.1	Poland, Odolanow	Haklova (2014)
9	*Karyolysus* sp.	KJ461941.1	Hungary, Godollo	Haklová-Kočíková et al. (2014)
10	*Karyolysus* sp.	KJ461939.1	Slovakia, Cabrad	Haklová-Kočíková et al. (2014)
11	*Hepatozoon* sp.	JX531941.1	Spain, Rambla los Vaquerizos	Maia et al. (2012)
12	*Hepatozoon* sp.	JX531928.1	Portugal, Viana do Castelo	Maia et al. (2012)
13	*Hepatozoon canis*	AY471615.2	Brazil	Criado-Fornelio et al. (2006)
14	*Hepatozoon* sp.	EF222257.1	Spain, Burgos	Criado-Fornelio et al. (2009)
15	*Babesiosoma stableri*	HQ224961.1	France	Barta et al. (2012)
16	*Haemogregarina* sp.	KX507248.1	USA, Tyler, Texas	Alhaboubi (2017)
17	*Haemogregarina* sp.	KX507250.1	USA, Tyler, Texas	Alhaboubi (2017)
18	*Haemogregarina* sp.	MT754268.1	Colombia	Gutiérrez-Liberato et al. (2021)
19	*Haemogregarina* sp.	MT345290.1	Tunisia	Attia El Hili et al. (2020)
20	*Haemogregarina pellegrini*	KM887508.1	VietNam	Dvorakova et al. (2015)
21	*Haemogregarina sacaliae*	KM887507.1	VietNam	Dvorakova et al. (2015)
22	*Haemogregarina balli*	HQ224959.1	France	Barta et al. (2012)
23	*Haemogregarina stepanowi*	KF992697.1	Turkey, Diyarbakir	Kvicerova et al. (2014)
24	*Klossia helicina*	HQ224955.1	France	Barta et al. (2012)
25	*Adelina grylli*	DQ096836.2	Bulgaria	Kopecna (2006)
26	*Adelina dimidiata*	DQ096835.1	Bulgaria	Kopecna (2006)

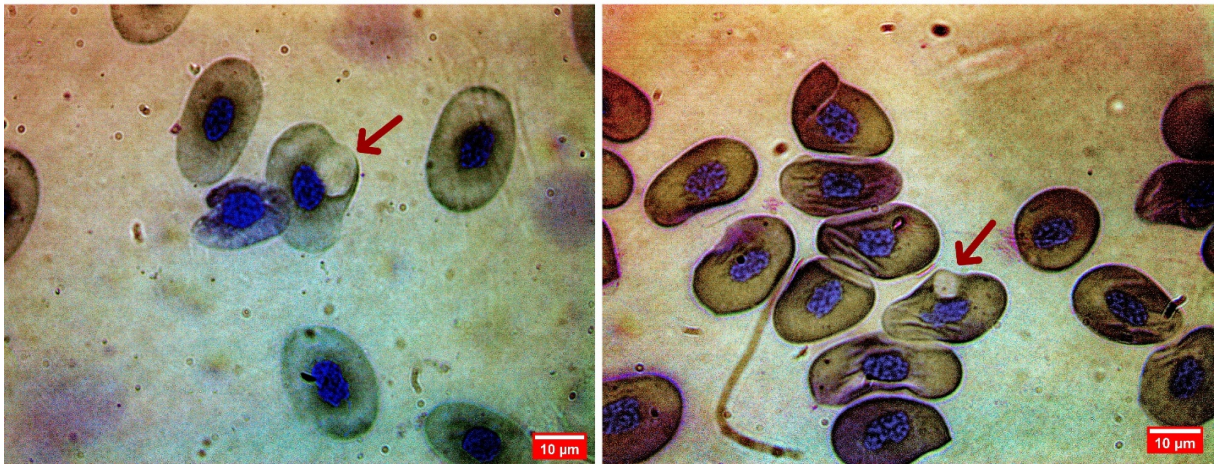

Figure 1: Photographs of the erythrocytes of Marsh frog (*Pelophylax ridibundus*) infected with a *Dactylosoma* blood parasite (arrows) from Iran.

They formed a sister clade with *Dactylosoma* sp. and *Babesiosoma stableri*. All dactylosomids species including *Dactylosoma kermiti* parasitizing *Ptychadena anchietae* and *Sclerophrys gutturalis* from South Africa and *Dactylosoma piperis* parasitizing *Leptodactylus labyrinthicus* from Brazil clustered together in one clade, as a sister group to the other haemoparasite clades. The interspecific distance between the *Dactylosoma* sp. presented in this study and *D. kermiti* was 0.01%, while the distance with *D. ranarum* and *Dactylosoma* sp. from *Pelophylax lessonae* was 0% (Table 2). This is the first report of parasitism by *Dactylosoma* species in *Pelophylax ridibundus.*

Amphibians are among the vertebrate groups with the highest rate of decline in species diversity. According to the IUCN Global Amphibian Assessment, the main causes are linked to human activities, environmental changes, fragmentation, and loss of habitat including climate change, habitat destruction, pollution, and emerging diseases that greatly affect these animals, (Frost, 2019). In addition, this group of vertebrates is disposed to host a wide variety of haemoparasites, such as bacteria, fungi, helminths, and haemoparasites, such as haemogregarines and trypanosomatids (Netherlands et al., 2018). *Dactylosoma ranarum* is a cosmopolitan species reported from several anuran species in Europe, Central and South America, Africa, and Asia (Netherlands et al., 2020). However, this study is the first report of *Dactylosoma* in *Pelophylax ridibundus*.

In the present study, the prevalence of *Dactylosoma* was considerably lower (25%) as compared to the results of a frog blood parasite survey from South Africa by Netherlands et al. (2020), in which 38% (61/160) of the *Ptychadena anchietae* screened were reported infected. However, the present rate was higher than in *Pelophylax lessonae* from Belgium where 7% of individuals were infected. These findings could be due to the droughts in

[DOI: 10.22034/JAD.2023.5.4.6]

South Africa that potentially affect on water-borne invertebrate vector numbers (Archer et al., 2017). Moreover, the present sample size is small (25% infection rate may be higher than in others studies as only four individuals were examined, which can cause a difference in the percent positive).

Parasitaemia of some parasites such as *Dactylosoma* may remain or even potentially increase over time within the host without reinfection. This is due to their ability to multiply asexually in the red blood cells, allowing them to outcompete parasites of the genus *Hepatozoon*, which require reinfection. This may be a possible explanation for species of *Dactylosoma* that were found to be the most prevalent blood parasite in anurans (Netherlands et al., 2020). Certain species of *Hepatozoon*, such as *Hepatozoon ixoxo* Netherlands, Cook, and Smit, 2014 may maintain a constant and high infection over long periods without re-infection (Netherlands et al., 2014). As we expected, *Dactylosoma* sp. from the present study formed a clade with *D. ranarum* [GenBank: HQ224957, Hq224958], completely distinct from the other species of *Dactylosoma* for which sequences were available. It can be separated based on the conservative 18S rRNA marker. *P*-uncorrected distance ranges between 0 and 0.01% in dactylosomids species (Table 2).

Table 2: Uncorrected *p*-distances between 31 haplotypes of *Dactylosoma*, *Haemogregarina*, *Karyolysus*, and *Hepatozoon* species using the 18S rRNA gene. Table S1 includes the names of species with GenBank accession numbers.

Sequences	*p*-distances
Hepatozoon sp. this study	-
Hepatozoon canis	0.084
Adelina dimidiata	0.119
Adelina grylli	0.104
Hepatozoon sp.	0.065
Klossia helicina	0.125
Dactylosoma ranarum	0.000
Dactylosoma ranarum	0.000
Haemogregarina balli	0.062
Babesiosoma stableri	0.002
Hepatozoon sp.	0.086
Hepatozoon sp.	0.081
Haemogregarinastepanowi	0.062
Karyolysus sp.	0.077
Karyolysus sp.	0.075
Karyolysus sp.	0.073
Haemogregarina sacaliae	0.067
Haemogregarina pellegrini	0.060
Haemogregarina sp.	0.042
Haemogregarina sp.	0.046
Dactylosoma kermiti	0.006
Dactylosoma kermiti	0.006
Dactylosoma sp.	0.000
Haemogregarina sp.	0.048
Haemogregarina sp.	0.051
Dactylosoma piperis	0.009

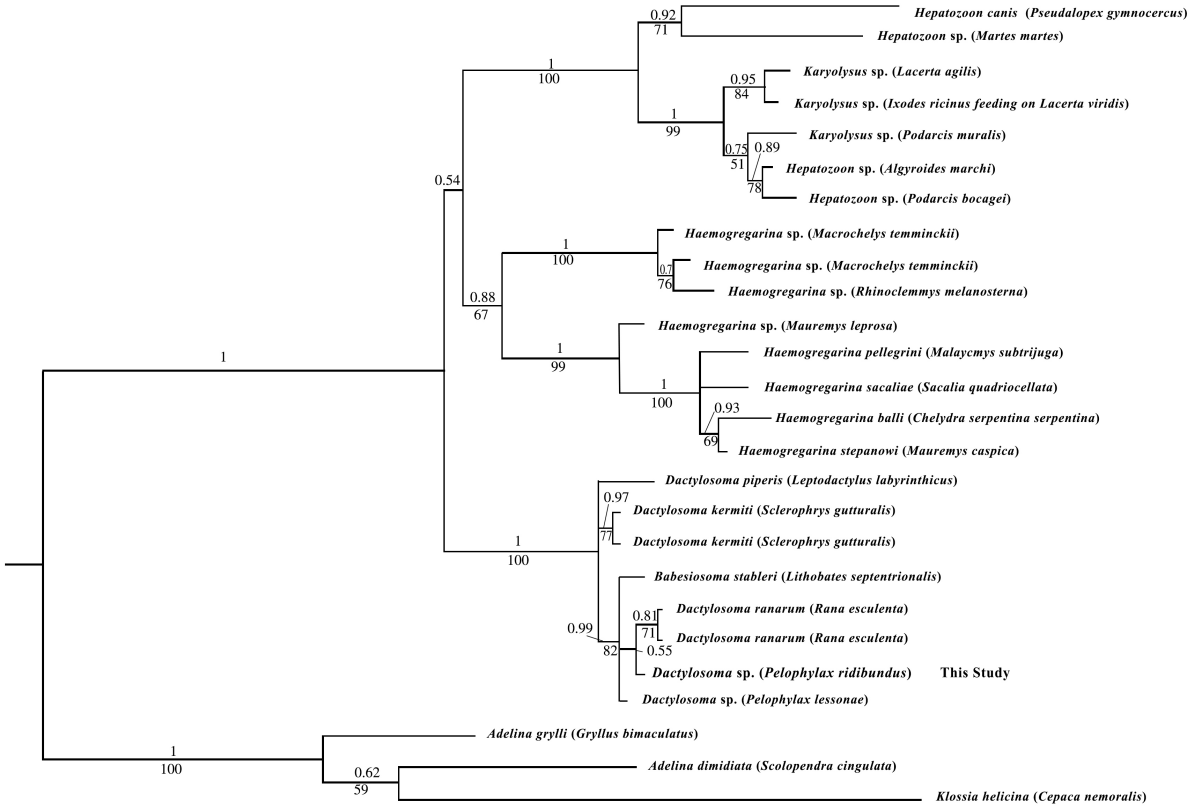

Figure 2: The phylogenetic tree of *Dactylosoma*, *Hepatozoon*, *Karyolysus*, and *Haemogregarina* species implemented in PhyML and MrBayes based on the 18S rRNA gene. Posterior probabilities are shown above branches and bootstrap values are shown below.

[DOI: 10.22034/JAD.2023.5.4.6]

Regarding the low support and lack of genetic distance between *Dactylosoma* sp. of this study and *Dactylosoma* sp. of *Pelophylax lessonae*, it can be concluded that they are the same species. To date, a low interspecific divergence value (0.01% and below) based on the conservative 18S rRNA gene sequence comparisons is known between Anurans dactylosomids. Similarly, a low interspecific divergence value (less than 1%) was detected between other haemogregarines such as *Haemogregarina*, *Karyolysus*, *Hemolivia*, and *Hepatozoon* (Netherlands et al., 2020).

Therefore, it is imperative to increase the sampling of *Dactylosoma* species, particularly from diverse potential hosts, especially fish hosts. This expanded sampling will help ascertain whether the observed pattern of low interspecific divergence values is common among all dactylosomatids, or if it is specific to those associated with anuran hosts.

This study provides new haemoparasite geographical records and a new anuran host record. Future studies should be performed to obtain more sequences from regions to better the phylogenetic identification and clarify the true diversity in these taxa which are fundamental for the conservation programs of anurans that are particularly sensitive to habitat fragmentation and habitat loss, climate change, and pathological effects.

Acknowledgments

We are grateful to anonymous reviewers for their valuable inputs and Professor Theodore Papenfuss and Dr. Erica DeMilio for their valuable scientific comments and language editing.

Authors contribution

Hossein Javanbakht performed project planning, management, and manuscript writing, Zahra Rahimi conducted sample analysis, and Reyhaneh Hajian carried out data analysis.

Conflict of interest

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

Ethical approval

All stages of this research complied with Iranian laws and with authorization from the University of Guilan.

References

Archer, E. R. M., Landman, W. A., Tadross, M. A., Malherbe, J., Weepener, H., Maluleke, P. and Marumbwa, F. M. (2017). Understanding the evolution of the 2014–2016 summer rainfall seasons in southern Africa: Key lessons. *Climate Risk Management*, 16 (C): 22–28. https://doi.org/10.1016/j.crm.2017.03.006

Barta, J. R. and Desser, S. S. (1984). Blood parasites of amphibians from algonquin park, ontario. *Journal of Wildlife Diseases*, 20 (3): 180–189. https://doi.org/10.7589/0090-3558-20.3.180

Bernal, X. E. and Pinto, C. M. (2016). Sexual differences in the prevalence of a new species of trypanosome infecting túngara frogs. *Parasites and Wildlife*, 5 (1): 40–47. https://doi.org/10.1016/j.ijppaw.2016.01.005

Davies, A. and Johnston, M. R. (2000). The biology of some intraerythrocytic parasites of fishes, amphibia, and reptiles. *Advances in Parasitology*, 45: 1–107. https://doi.org/10.1016/S0065-308X(00)45003-7

Faggioni, G. P., de Souza, F. L., Uetanabaro, M., Landgref-Filho, P. and Prado, C. P. A. (2017). Diet of *Leptodactylus bufonius* BOULENGER, 1894, in the Brazilian Chaco. *Herpetozoa*, 30 (1/2): 72–76 .

Frost, D. R. (2019). *Amphibian Species of the World*. American Museum of Natural History: New York, USA. Accessible at: http://research.amnh.org/herpetology/amphibia/index.html

Frost, D. R. (2021). Amphibian Species of the World: an Online Reference. Available from: Https://Amphibians of the world. Amnh. Org/Index.Php. American Museum of Natural History, New York, USA. https://doi.org/doi.org/10.5531/db.vz.0001

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, 59 (3): 307–321. https://doi.org/10.1093/sysbio/syq010

Hall, T. (2005). Bioedit sequence alignment editor, v. 7.0. 5.3. Carlsbad, California: Ibis Biosciences. http://www.mbio.ncsu.edu/BioEdit/bioedit.html

Netherlands, E. C., Cook, C. A. and Smit, N. J. (2014). *Hepatozoon* species (Adeleorina: Hepatozoidae) of African bufonids, with morphological description and molecular diagnosis of *Hepatozoon ixoxo* sp. nov. Parasitising three *Amietophrynus* species (Anura: Bufonidae). *Parasites and Vectors*, 7 (1): 1–12. https://doi.org/10.1186/s13071-014-0552-0

Netherlands, E. C., Cook, C. A., Du Preez, L. H., Vanhove, M. P. M., Brendonck, L. and Smit, N. J. (2018). Monophyly of the species of *Hepatozoon* (Adeleorina: Hepatozoidae) parasitizing (African) anurans, with the description of three new species from hyperoliid frogs in South Africa. *Parasitology*, 145 (8): 1039–1050. https://doi.org/10.1017/S003118201700213X

Netherlands, E. C., Cook, C. A., Du Preez, L. H., Vanhove, M. P. M., Brendonck, L. and Smit, N. J. (2020). An overview of the Dactylosomatidae (Apicomplexa: Adeleorina: Dactylosomatidae), with the description of *Dactylosoma kermiti* n. sp. parasitising *Ptychadena anchietae* and *Sclerophrys gutturalis* from South Africa. *International Journal for Parasitology: Parasites and Wildlife*, 11: 246–260. https://doi.org/10.1016/j.ijppaw.2019.12.006

[DOI: 10.22034/JAD.2023.5.4.6]

Pesarakloo, A., Rastegar-Pouyani, E., Rastegar-Pouyani, N., Kami, H., Najibzadeh, M., Khosravani, A. and Oraie, H. (2016). The first taxonomic revaluation of the Iranian water frogs of the genus *Pelophylax* (Anura: Ranidae) using sequences of the mitochondrial genome. *Mitochondrial DNA Part A*, 28 (3): 392–398. https://doi.org/10.3109/19401736.2015.1127362

Ponton, F., Duneau, D., Sánchez, M. I., Courtiol, A., Terekhin, A. T., Budilova, E. V., Renaud, F. and Thomas, F. (2009). Effect of parasite-induced behavioral alterations on juvenile development. *Behavioral Ecology*, 20 (5): 1020–1025. https://doi.org/10.1093/beheco/arp092

Poynton, S. L. and Whitaker, B. R. (2001). Protozoa and metazoan infecting amphibians, *In*: Wright, K. M. and Whitaker, B. R. (Eds.), *Amphibian Medicine and Captive Husbandry*. Malabar FL: Krieger Publishing Company. pp 193–221. https://www.cabdirect.org/cabdirect/abstract/20013162987

Rajabi, F., Javanbakht, H. and Sajjadi, S. S. (2017). A preliminary study of haemoparasites in marsh frogs, *Pelophylax ridibundus* (Ranidae) from Iran. *Journal of Entomology and Zoology Studies*, 5 (4): 1314–1317.

Reis Ferreira, D., Perles, L., Machado, R. Z., Prado, C. P. A. and André M. R. (2020). Molecular detection of Apicomplexan hemoparasites in anurans from Brazil. *Parasitology Research*, 119 (10): 3469–3479. https://doi.org/10.1007/s00436-020-06835-9

Sailasuta, A., Satetasit, J. and Chutmongkonkul, M. (2011). Pathological study of blood parasites in Rice Field Frogs, *Hoplobatrachus rugulosus* (Wiegmann, 1834). *Veterinary Medicine International*, 2011 (2): 850568. https://doi.org/10.4061/2011/850568

Ujvari, B., Madsen, T. and Olsson, M. (2004). High prevalence of *Hepatozoon* spp. (Apicomplexa, Hepatozoidae) infection in Water Pythons (*Liasis fuscus*) from Tropical Australia. *Journal of Parasitology*, 90 (3): 670—672. https://doi.org/10.1645/GE-204R

Úngari, L. P., Netherlands, E. C., Quagliatto Santos, A. L., Alcantara, E. P. D., Emmerich, E., Da Silva, R. J. and O'Dwyer, L. H. (2020). A new species, *Dactylosoma piperis* n. sp. (Apicomplexa, Dactylosomatidae), from the pepper frog *Leptodactylus labyrinthicus* (Anura, Leptodactylidae) from Mato Grosso State, Brazil. *Parasite*, 27, 73: 1–13. https://doi.org/10.1051/PARASITE/2020070

Yousefi Siahkalroodi, S., Saeedi, H., Sadat-Behfar, M., Fallahi, R and Izadian, M. (2013). *Atlas of amphibians of Iran*. Department of Environment of Iran, Tehran, Iran. 104 pp.

[DOI: 10.22034/JAD.2023.5.4.6]