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Hemoparasites of the genus *Trypanosoma* (Kinetoplastida: Trypanosomatidae and hemogregarines in Anurans of the São Paulo and Mato Grosso do Sul States – Brazil

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ABSTRACT

Wild animals are exposed to numerous pathogens, including hemoparasites. The *Trypanosoma* and hemogregari group are frequently reported as parasites in anurans (frogs, tree frogs and toads). The identification of these hemopa asites is usually made through stage observation of their morphology in the peripheral blood of the host. There a no studies, however, based on the biological cycle of these hemoparasites. The objective of the present study was evaluate the presence of hemogregarines and *Trypanosoma* spp. in anurans captured in the States of São Paulo a Mato Grosso do Sul – Brazil and to perform the morphological and morphometric characterization of these hemopa asites. The species of anurans examined were: *Dendropsophus nanus*, *D. minutus*, *Leptodactylus chaquens L. podicipinus*, *L. labyrinthicus*, *L. fuscus*, *Bufo granulosus*, *B. schneideri*, *Phyllomedusa hypocondrialis*, *Trac cephalus venulosus*, *Scinax fuscovarius* and *Hypsiboas albopunctatus*. Of the total of 40 animals studied, four (10% were positive for hemogregarines and eight (20%) were positive for *Trypanosoma* spp. Hemogregarine gamon showed variable morphology and, in addition to intra-erythrocytic forms, extra-erythrocytic forms were also observe Extremely different forms of *Trypanosoma* were observed, as described in the literature, with the broad and oval forn being the most common.

Key words: Anuran, hemogregarine, *Trypanosoma*, hemoparasites.

INTRODUCTION

The *Trypanosoma* genus and the hemogregarines have been reported among the hemoparasites infecting anurans. Biological and molecular characterization data are almost inexistent for these parasites in these animals and species nomination is difficult. The term, hemogregarine, is used to describe blood parasites collectively

plexa (Jakes et al. 2003). The main genera of this are the *Hepatozoon* (Family Hepatozoidae) and *H gregarina* (Family Haemogregarinidae). Differen between these genera is generally made through servation of the sporogonic stage of the parasite vector. However, most descriptions have been only on the blood stage in the host, while the b

in addition to its forms in the vertebrate host, a sporogonic development in the Malpighian tubule of the insect vector (Desser et al. 1995) has also been described. In Brazil, there is only one report on hemogregarine in the toad (*Bufo crucifer*) (Souza and Filho 1974).

The genus *Trypanosoma* belongs to the Kinetoplastida order and the family Trypanosomatidae. Species classification of anurans *Trypanosoma* is confusing, due to the great polymorphism present in this genus and the absence of studies on the biological cycle. Since numerous species have been described, it is plausible that the same parasite has received different names in different hosts and different geographic localizations. Moreover, exogenous influences, such as temperature, maturity and density of the population of hosts and environment pollution can influence the different shapes of the hemoparasite (Bardsley and Harmsen 1973, Mackenzie 1999, Silva et al. 2005).

The possible vectors of *Trypanosoma* spp. have not yet been identified, but according to previous descriptions, they may include hematophagous dipteran and leeches (Barrow 1953, Woo 1969, Desser et al. 1975). Species of *Phlebotomus* have been shown to be vectors of this genus in frogs from China (Feng and Chao 1943) and in California (Ayala 1971). In Brazil, a recent report has described the infection of this genus in anurans. Ferreira et al. (2007) observed the morphology of the trypanosomes of different biomes in Brazil, and also genetically studied the genera of 25 isolated species of anurans. The authors demonstrated that the restriction of the parasite to the host is not absolute and showed that some genotypes of *Trypanosoma* are shared by more than one species of different genera and, sporadically, by species from distinct families of anurans. Some genotypes were also seen to be restricted to certain localities and were different from those of other trypanosomes previously studied in the rest of the world.

The knowledge of the hemoparasites of anurans is of importance, since these animals are in a process of extinction and also are extremely sensitive to environmental variations (Silvano and Segalla 2005). The objective of this work was to observe the occurrence of hemogre-

MATERIALS AND METHODS

Forty amphibian specimens, captured in São Paulo State (Botucatu, Fartura and Chavantes) and Mato Grosso do Sul State (Pantanal-Corumbá) were studied. The animals were captured from October to November, 2005 and October to November, 2006. The species belong to the family Hylidae (tree frog), family Leptodactylidae (frog) and family Bufonidae (toad). Animals were collected by hand, blood was taken from their hearts and blood smears were prepared. Smears were fixed in absolute methanol for 3 minutes and stained with 10% Giemsa for 30 minutes. The blood smears were examined with an optical microscope, 1,000X magnification, for the diagnosis of hemoparasites. The morphometrical analysis of the parasites was performed using a computerized system of image analysis (Lite Qwin 3,01 – Leica).

The variables recorded for hemogregarines were: Body Area (BA), Body Length (BL) and Body Width (BW); Area (NA), length (NL) and width of the nucleus of the parasite (NW). For the *Trypanosoma* genus, the following traits were analyzed: Body Area (BA), Body Length excluding the free flagellum (BL) and width of the parasite (BW); Width at the widest part of body including the undulating membrane (W); Area of the nucleus (NA); Distance from the end anterior to the middle of the nucleus (A-N); Distance from the posterior end to middle of the nucleus (P-N); Distance from the anterior end to the kinetoplast (K-A); Distance from the posterior end to the kinetoplast (K-P); Distance from the kinetoplast to the middle of the nucleus (K-N); Distance of the kinetoplast to the posterior end of the nucleus (K-Np); Total length including free flagellum (TF); Length of the undulating membrane (LMO) and Free flagellum (FF).

The experiments related to this paper have been approved by the Unesp Bioscience Institute's Ethics Committee approval. The animals were captured according to the IBAMA (Brazilian Institute of Environment and Renewable Natural Resources) license no. 099/99-DIFAS. And they were sacrificed with overdose of anaesthetic.

RESULTS

Of the 40 animals examined, 10% were positive for

Number of species by family	Positive individuals for hemogregarines and *Trypanosoma*	Positive individuals for hemogregarines	Positive individuals for *Trypanosoma*
Leptodactylidae* (n = 5)	2	—	1
Bufonidae* (n = 11)	—	1	—
Hylidae* (n = 24)	1	—	4

(*) Leptodactylidae: *Leptodactylus chaquensis*, *L. podicipinus*, *L. labyrinthicus*, *L. fuscus*; Bufonidae: *Chaunus granulosus*, *C. schneideri*; Hylidae: *Phyllomedusa hypocondrialis*, *Trachicephalus venulosus* (synonym: *Phrynohyas venulosa*), *Scinax fuscovarius*, *Dendropsophus nanus* (synonym: *Hyla nana*), *Dendropsophus minutus* (synonym: *Hyla minuta*), *Hypsiboas albopunctatus* (synonym: *Hyla albopunctata*).

Fig. 1 – Hemogregarines and Trypanosoma spp. present in anurans captured in São Paulo and Mato Grosso do Sul States – Brazil.

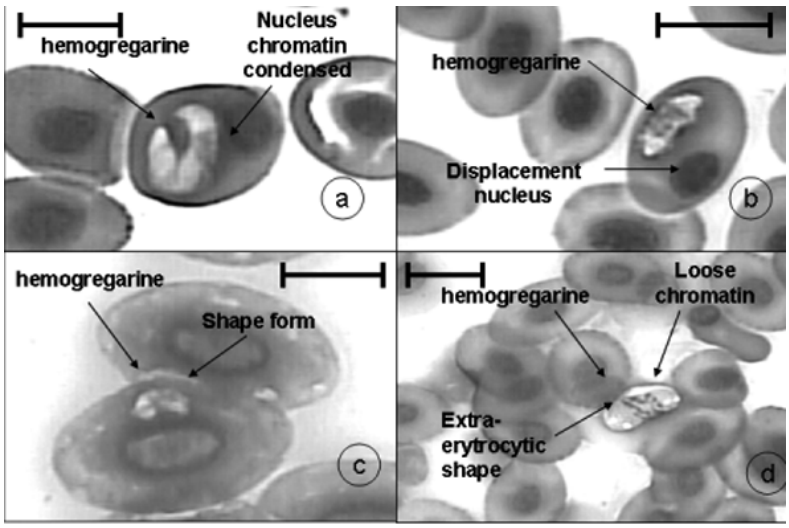


Fig. 2 – Gamont of hemogregarine in a blood smear: a) hemogregarine from *Leptodactylus podicipinus*; b) hemogregarine from *Leptodactylus chaquensis*; c) hemogregarine from *Phyllomedusa hypocondrialis*; d) hemogregarine extraerythrocytic from *L. podicipinus*. Scale bar = 10μm. Giemsa stained thin blood films.

The hemogregarines were generally observed inside the red blood cells. Gamonts were observed with variable morphology; some presented a broad shape, others had a narrow and elongated shape (Fig. 2a) or a pear drop aspect. The position of the host cell nucleus was central or slightly displaced towards one of the extremities.

ment of the nucleus of the erythrocyte was obse
the presence of some gamonts (Fig. 2b). Smalle
of the parasite were also seen, in which the nucl
cupied a central position and the nucleus chroma
condensed. In these parasites, the displacement
nucleus of the erythrocyte did not occur (Fig. 2c)

each hemogregarine and its respective hosts are listed in Figure 3.

The *Trypanosoma* forms observed were extremely polymorphic, with oval, elongated or slender shapes. Some parasites presented rounded extremities in the region previous to the kinetoplast (Fig. 4a), or were slender in the region after the kinetoplast. The presence of a surface costate was sometimes observed in the body of the parasite (Fig. 4b). The nucleus, when present, had a varying format that was round to elongate. The kinetoplast, when visible, was located before, after or in the region of the nucleus. The undulating membrane, when presented, was seen extended along the whole body of the parasite, or along half the body or only at the extremity of the *Trypanosoma* sp. The flagellum, when presented, had dimensions that varied from short to long (Fig. 4c and 4d). The measurements of each described form and its respective hosts are detailed in Figure 5.

Due to a lack of more complete data in the literature, although some forms were similar to those already described, small morphological differences did not allow the identification of the *Trypanosoma* species.

DISCUSSION

In the present study, the frequency of hemoparasites obtained in anurans was relatively high, especially for *Trypanosoma* spp. (20%). For hemogregarines, the frequency was lower than 10%, but prominent nevertheless. Mohammed and Mansour (1963, 1966a, b, c) observed a higher frequency of hemogregarines in toads than was seen in the present study (30.5%). With regard to the frequency of *Trypanosoma* in anurans, the results differed according to the group studied. Lehmann (1959) diagnosed 44.4% of *R. boyli* as infected with *Trypanosoma*, while Ayala (1970) found 15.6% of *B. boreas* parasitized; Werner et al. (1988) observed prevalences that varied from 1.5% to 26.9% in toads, according to the species of *Trypanosoma*, and Woo and Bogart (1984) found 16.7% of tree frogs to be infected with *Trypanosoma*.

The results obtained in our study, however, are sim-

Trypanosoma spp. than by hemogregarines (Barta and Desser 1984). In a study on anurans of Costa Rica, results were similar (Desser 2001). Although it was not possible to compare the results of the animals collected in São Paulo with those collected in the Mato Grosso do Sul, due to the differences among the species collected in the two states, the number of positive animals was greater in the Mato Grosso do Sul State than in the São Paulo State. One of the explanations for the lower number of parasites could be the strong association of these parasites with the environment. In environments that are more ecologically modified, animals tend to have a reduced number of hemoparasites (Mackenzie 1999, Silva et al. 2005), possibly explaining the decreased number seen in the State of São Paulo, although other factors must be taken in consideration, such as climate and density of vectors.

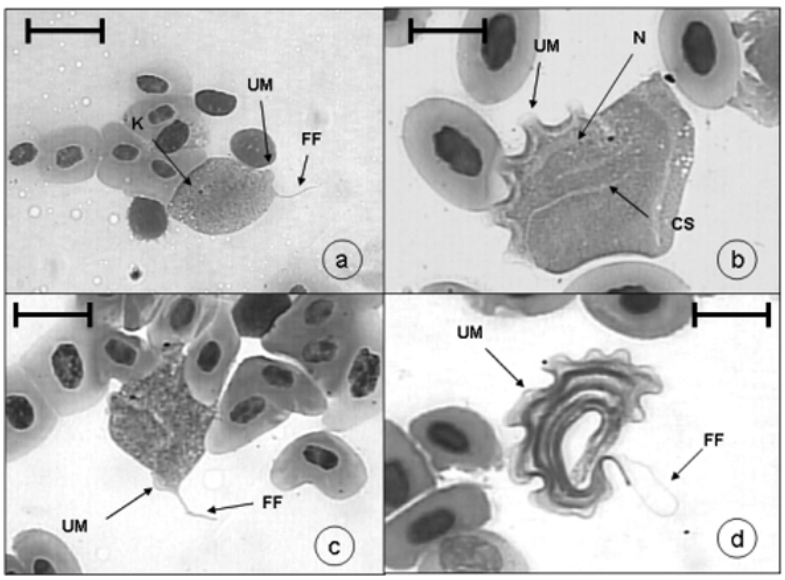
Hemogregarines and *Trypanosoma* observed showed variability of morphological forms. Sixteen different forms of hemogregarines were detected in four species of anurans and 28 different forms of *Trypanosoma* spp. in seven species of anurans. Generally, these parasites could not be compared to those already described in the literature, since the previous descriptions of these protozoa are outdated and insufficient, due to the lack of recent studies on these parasites from anurans. According to the literature, hemogregarines are commonly found in frogs (Ranidae) and toads (Bufonidae) in the whole world, whereas in tree frogs (Hylidae) there are no records of these hemoparasites (Smith 1996). In the present study, animals of the family Leptodactylidae and of the family Hylidae were observed infected with hemogregarines; according to Smith (1996), arborous anurans may be less subjected to these hemoparasites. For the Bufonidae, where accounts of hosting hemogregarines are common (Smith 1996), we found only one positive species (*Chaunus schneideri*) (Fig. 3).

It was not possible to classify the species based only on the forms and dimensions of the hemogregarines found; therefore, these forms may represent the same species of parasite or several different species. Since the biological cycles of these hemogregarines have not

Parasitised animals	Hemogregarine nucleus					
	BA (μm^2)	BL (μm)	BW (μm)	NA (μm^2)	NL (μm)	NW (μm)
*Chaunus schneideri***	17.61	10.81	2.11	—	—	—
	21.34	7.84	3.07	—	—	—
Leptodactylus chaquensis	21.32	7.66	3.62	10.18	3.62	2.33
	34.28	11.04	3.76	17.56	4.89	3.85
	35.15	10.87	4.10	12.28	2.71	3.15
Leptodactylus podicipinus	17.32	9.68	2.51	6.56	3.34	2.30
	33.60	12.15	5.02	11.73	3.43	3.62
	40.32	13.14	2.97	11.24	3.68	2.97
	40.67	12.29	3.87	18.73	3.97	3.51
	41.27	19.27	4.19	7.08	4.19	3.37
	43.86	11.37	3.88	9.77	2.48	3.50
	44.38	12.63	4.08	18.65	5.48	4.13
	44.98	12.40	4.20	10.70	3.97	2.56
	46.72	13.77	4.33	13.67	4.33	2.81
	65.59	12.45	6.23	21.51	4.82	3.62
Phyllomedusa hypocondrialis	20.61	7.40	2.82	6.64	2.32	2.17

(**) The nucleus was not clear.

Fig. 3 – Hemogregarine morphometrical parameters observed in four species of anurans captured in São Paulo and Mato Grosso do Sul States – Brazil.



Hosts	Parasite dimensions													
	BA (μm^2)	BL (μm)	BW (μm)	L (μm)	W (μm)	FF (μm)	TF (μm)	NA (μm^2)	A-N (μm)	P-N (μm)	K-A (μm)	K-P (μm)	K-N (μm)	K-Np (μm)
Dendropsophus minutus	93.11	25.26	6.57	42.42	8.06	23.02	48.69	—	—	—	22.32	2.81	—	—
	246.09	23.16	14	56.12	15.57	—	—	22.47	12.45	10.62	10.81	9.33	4.99	10.20
	246.39	22.49	13.93	24.39	13.93	—	—	15.68	10.30	11.20	15.80	7.76	4.33	7.39
	257.58	21.82	12.45	59.43	12.97	12.95	41.34	8	12.35	18.05	18.15	4.82	6.17	8.36
	267.43	24.92	14.20	68.50	19.77	11.16	37.64	19.86	11.81	14.801	17.36	2.52	5.80	8.05
	329.94	25.13	17.58	63.31	17.58	11.26	40.10	17.02	16.49	6.46	18	8.70	7.67	11.20
*Hypsiboas**** *albopunctatus*	313.39	42.61	8.09	32.64	9.90	—	—	—	—	—	12.71	27.63	—	—
	371.92	37.31	17.58	90.31	15.81	—	—	—	—	—	28.14	9.45	—	—
	393.46	41.70	17.44	79.44	17.79	—	—	—	—	—	30.24	9.58	—	—
	413.14	25.75	17.17	77	20.37	—	—	—	—	—	18.63	7	—	—
	672.51	57.83	24.42	103.13	25.91	—	—	—	—	—	47.45	4.13	—	—
	683.35	36.25	28.38	77.97	30.36	—	—	22.84	20.79	19.24	18.32	13.18	8.62	16.25
	690.18	26.64	27.95	—	—	—	—	7.57	9.60	16.93	8.58	17.58	1.42	2.15
	1078.49	52.33	29.96	24.57	29.96	—	—	33.98	29.27	16.83	25.22	18.35	3.11	6.73
Trachicephalus venulosus	157.44	27.44	8.43	51.80	9.96	—	—	—	—	—	—	—	—	—
	172.52	29.20	9.24	56.56	9.08	20.83	44.24	—	—	—	—	—	—	—
	213.88	25.45	10.08	70.53	13.04	24.36	43.57	23.25	12.71	12.72	20.16	2.71	8.61	5.58
	511.20	38.15	14.82	80.55	16.47	7.5	54.66	—	—	—	—	—	—	—
	642.37	46.72	21.05	77.31	20.65	—	—	—	—	—	—	—	—	—
	916.28	38.80	29.95	98.91	35.61	—	—	39.69	11.51	25.49	13.01	22.58	8.61	5.58
Phyllomedusa hypocondrialis	272.03	37.49	11.29	61.03	10.39	—	—	—	—	—	25.27	9.46	—	—
Leptodactylus chaquensis	80.75	14.37	8.10	34.38	11.07	21.85	38.41	—	—	—	—	—	—	—
	165.85	12.13	8.17	33.90	15.32	—	—	—	—	—	—	—	—	—
	333.37	31.33	14.20	78.19	16.75	—	—	—	—	—	—	—	—	—
	406.17	29.37	21.46	88.43	18.15	15.45	47.39	—	—	—	19.64	6.44	—	—
	463.56	26.60	18.54	47.48	18.54	11.43	55.76	—	—	—	—	—	—	—
Leptodactylus labyrinthicus	267.87	66.04	6.93	99.62	9.30	27.17	94.43	—	—	—	—	—	—	—
Leptodactylus podicipinus	238.57	21.06	13.73	51.96	18.06	19.59	44.02	—	—	—	—	—	—	—

(***) Two specimens were positive.

Fig. 5 – *Trypanosoma* spp. morphometrical parameters observed in seven species of anurans captured in São Paulo and Mato Grosso do Sul States – Brazil.

With regard to the forms of *Trypanosoma* spp. found, a great polymorphic variability prevailed, similarly to descriptions made in the literature. We experienced difficulty in classification, even utilizing the morphology and morphometrical data. Miyata (1978) described many specific details of each species; species found sometimes had a similar morphology to those species described in the literature, but differed in some corporal dimensions. These authors raised the following question: "Are the great majority of *Trypanosoma* observed new forms or a phase of development of the parasite?", since few studies have been carried out with sition of the kinetoplast, the position of the nucleus and the stretch of the free flagellum, amongst others. In spite of all these parameters, it is very difficult to characterize these species, since these structures were sometimes not visible.

Of the animals positive for hemogregarines and *Trypanosoma*, some were infected by the two hemoparasites: *L. chaquensis*, *L. podicipinus* e *P. hypocondrialis*. The great pleomorphism observed could indicate distinct species, but we could not discard the possibility of the existence of different stages in the same species. Moreover, different species of anurans can be

Although *T. rotatorium* is possibly the most described species in the literature, none of the forms of *Trypanosoma* observed in the present study corresponded faithfully to this species. According to our results, we may conclude that for the characterization of these species of *Trypanosoma* and hemogregarines in anurans, molecular studies of the blood stages associated with ecological and biological aspects are necessary. In addition, the possible impacts of these infections in these animals and the importance of the presence of hemoparasites as an indicator of the environmental conditions need to be considered.

RESUMO

Os animais silvestres estão expostos a inúmeros patógenos, dentre eles estão os hemoparasitas. Podem-se destacar espécies do gênero *Trypanosoma* e do grupo das hemogregarinas, que ocorrem com freqüência parasitando anuros (rãs, pererecas e sapos). Normalmente, a descrição destes hemoparasitas é feita através da morfologia dos estágios observados no sangue periférico do hospedeiro e as pesquisas sobre o ciclo biológico desses hemoparasitas são escassas. Os objetivos do presente estudo foram avaliar a presença de hemogregarinas e *Trypanosoma* spp. em anuros capturados nos Estados de São Paulo e Mato Grosso do Sul e fazer a caracterização morfológica e morfométrica dos seus hemoparasitas. As espécies de anuros examinadas foram: *Dendropsophus nanus*, *D. minutus*, *Leptodactylus chaquensis*, *L. podicipinus*, *L. labyrinthicus*, *L. fuscus*, *Bufo granulosus*, *B. schneideri*, *Phyllomedusa hypocondrialis*, *Trachicephalus venulosus*, *Scinax fuscovarius* e *Hypsiboas albopunctatus*. Dos 40 animais estudados, foram encontrados quatro (10%) positivos para hemogregarinas e oito (20%) positivos para *Trypanosoma* spp. Foram observados gamontes de hemogregarinas com morfologia variável e, além das formas intra-eritrocíticas, também foram observados gamontes fora das hemácias. As formas de *Trypanosoma* encontradas eram muito polimórficas, conforme é descrito na literatura, sendo na sua maioria, larga e oval.

Palavras-chave: Anuros, hemogregarinas, *Trypanosoma*, hemoparasitas.

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