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NATURAL AND EXPERIMENTAL INFECTION OF THE LIZARD *AMEIVA AMEIVA* WITH *HEMOLIVIA STELLATA* (ADELEINA: HAEMOGREGARINIDAE) OF THE TOAD *BUFO MARINUS*

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Summary:

Developmental stages of a haemogregarine in erythrocytes of the lizard *Ameiva ameiva* (Teiidae), from Pará State, north Brazil, were shown to be those of *Hemolivia* by the nature of the parasite's sporogonic cycle in the tick *Amblyomma rotondatum*. The type species, *Hemolivia stellata* Petit *et al.*, 1990 was described in the giant toad *Bufo marinus* and the tick *Amblyomma rotondatum*, also from Pará State, and in view of the fact that *A. ameiva* and *Bufo marinus* share the same habitat and are both commonly infested by *A. rotondatum*, the possibility that the parasite of *A. ameiva* is *H. stellata* had to be considered. Uninfected lizards fed with material from infected ticks taken from *B. marinus*, and others fed with liver of toads containing tissue-cysts of *H. stellata*, were shown to subsequently develop typical *Hemolivia* infections, with all stages of the development similar to those seen in the naturally infected lizards. Conversely, a juvenile, uninfected toad became infected when fed with sporocysts of *Hemolivia* in a macerated tick that had fed on an infected *A. ameiva* and pieces of liver containing tissue-cysts from the same lizard. The remarkable lack of host specificity shown by *H. stellata*, in hosts so widely separated as an amphibian and a reptile, is discussed.

KEY WORDS : *Hemolivia stellata*, Apicomplexa, haemogregarine, *Bufo marinus*, *Ameiva ameiva*, amphibian, lizard, *Amblyomma rotondatum*, ticks, transmission, Brazil.

Résumé : INFECTION NATURELLE ET EXPÉRIMENTALE DU LÉZARD *AMEIVA AMEIVA* PAR *HEMOLIVIA STELLATA* (ADELEINA: HAEMOGREGARINIDAE) PARASITE DU CRAPAUD *BUFO MARINUS*

Les stades de développement d'une hémogrégarine trouvés dans les érythrocytes du lézard Ameiva ameiva *(Teiidae), de l'état de Pará au nord du Brésil, se sont avérés être ceux d'une* Hemolivia, *d'après le cycle sporogonique du parasite chez la tique* Amblyomma rotondatum. *L'espèce type* Hemolivia stellata *Petit* et al., *1990, a été décrite chez le crapaud géant* Bufo marinus *et la tique* Amblyomma rotondatum, *également de l'état de Para. Étant donné que les hôtes* A. ameiva *et* B. marinus *partagent le même habitat et que tous deux sont infestés par* A. rotondatum, *la possibilité que l'hémogrégarine trouvée chez* A. ameiva *soit* H. stellata *était à considérer. Des lézards non infectés, nourris les uns avec du matériel provenant de tiques infectées, prélevées chez* B. marinus, *les autres avec du foie de crapauds contenant des kystes d'*H. stellata *ont tous développé des infections à* Hemolivia *typiques, avec des stades de développement semblables à ceux observés chez les lézards infectés dans les conditions naturelles. Réciproquement, un jeune crapaud initialement non infecté, l'est devenu après avoir ingéré les sporocystes d'*Hemolivia *présents dans des macérats de tiques nourries à partir d'un* A. ameiva *infecté et avec des fragments de foie de ce même lézard, contenant des kystes tissulaires. L'absence de spécificité d'hôte remarquable d'*H. stellata, *espèce retrouvée chez des hôtes aussi éloignés qu'un amphibien et un reptile, est discutée.*

MOTS CLÉS : Hemolivia stellata, *Apicomplexa, haemogregarine,* Bufo marinus, Ameiva ameiva, *amphibien, lézard,* Amblyomma rotondatum, *tiques, transmission, Brésil.*

INTRODUCTION

To date, three species of the haemogregarine *Hemolivia* have been described: the type species, *H. stellata*, in the giant toad *Bufo marinus* from Pará, north Brazil (Petit *et al.*, 1990); *H. mauritanica* (Michel, 1973) Landau & Paperna, 1997, Paperna (2006) from the Old World tortoises *Testudo graeca, T. mauritanica* and *T. marginata*; and *H. mariae* Smallridge & Paperna, 1997 from the Australian lizard *Tiliqua rugosa*. Diagnostic characters of the genus include the development of schizonts, cysts containing one or two cystozoites, and encapsulated gametocytes all within erythrocytes of the peripheral blood. Tissue-cysts, similar to those in the erythrocytes, are also found in reticulo-endothelial cells of the viscera, together with exoerythrocytic schizonts. In the tick vectors, sporogony takes place in the cells of the gut, with the ultimate production of curious star-shaped oocysts. These produce a large number of sporokinetes which, on liberation, enter other intestinal cells and give rise to sporocysts containing sporozoites. Principal modes of transmission are by ingestion of the tick vector containing mature sporocysts, or sporocysts excreted by the tick and contaminating the environment. Experimental transmission has also been obtained by feeding clean toads with the tissue-cysts of *H. stellata* (Petit *et.al.*, 1990)

In a recent paper on haematozoa of the teiid lizard *Ameiva ameiva* (Teiidae) from Amazonian Brazil (Lainson, De Souza & Franco, 2003) we described a haemogre-

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garine producing schizonts and encapsulated stain-resistant forms, considered to be gametocytes, in the erythrocytes: exoerythrocytic schizonts were abundant in the liver macrophages. These findings led us to suggest that the parasite was probably a species of *Hemolivia*. In this communication we describe more fully the developmental stages of the parasite in *A. ameiva* and its production of typical *Hemolivia* oocysts in ticks that had engorged both naturally and experimentally on infected lizards. Evidence is provided indicating that the parasite of *A. ameiva* is, in fact, *H. stellata*.

MATERIALS AND METHODS

Further specimens of *A. ameiva* were captured in the vicinity of Capanema, Pará State, north Brazil (1° 12´ S-47° 11´ W) in the same area that had previously provided infected lizards. They were maintained in our animal house on a diet of adult and larval *Tenebrio* and given water *ad. lib.*

Blood from the orbital sinus was used to prepare thin blood films, and one lizard showing abundant intraerythrocytic parasites was sacrificed in order to make impression smears of the internal organs. Smears were prepared from the contents of a tick (*Amblyomma rotondatum*) that had fed on one of the infected lizards. Lizard and tick smears were air-dried, fixed in absolute methyl alcohol for three minutes and stained by Giemsa's method for one hour. Photomicrographs were prepared using a Zeiss "Photomicroscope III" and Kodak TMX 100 film.

Nymphs from our laboratory-bred colony of *A. rotondatum* were fed on one of the infected lizards that was showing moderate numbers of parasites suspected to be gametocytes of *Hemolivia* in the red blood cells. One engorged tick was examined for possible developmental stages of the haemogregarine on the day of detachment, after 10 days of feeding. Others were examined from 3-40 days following spontaneous detachment.

The type locality of *H. stellata* in *B. marinus* is in the same region of Brazil as that in which our *A. ameiva* were captured, and where both animals share the same habitat and are commonly fed on by the tick *A. rotondatum*. Although we suggested (Lainson *et al.*, 2003) that it was unlikely that the parasite in *A. ameiva* was also *H. stellata* due to the wide difference of hosts, the possibility nevertheless needed to be considered. Attempts were therefore made to transmit the parasite from *B. marinus* to uninfected *A. ameiva* and, conversely, to infect an uninfected toad with the parasite from a lizard.

Five lizards that had shown no evidence of infection following the periodic examination of blood films during a period of two months, were force-fed with pieces of liver containing abundant tissue-cysts of *H. stellata* from an infected toad. Three other negative lizards, one very juvenile and two adults, were fed with macerated ticks that had fed on infected toads and which contained mature sporocysts of this parasite. To avoid the risk of losing lizards due to repeated bleeding, we based our timing for the first examination of Giemsa-stained blood films on the observations of Petit *et al.* (1990), who showed gametocytes of *H. stellata* to appear in the peripheral blood of toads fed with sporocysts from ticks, or cysts from the liver of infected toads, from 40-45 days later. The experimental lizards were, therefore, first examined on day 50 post-feeding (p.f.).

A very young *B. marinus* (Fig. 26), showing no parasites in blood films prepared during a period of one month, was force-fed with material from a macerated tick that had fed on an infected *A. ameiva* and which contained mature sporocysts of *Hemolivia*. 14 days later, the toad was then fed with pieces of liver from the same lizard, and which contained tissue-cysts of the parasite. Blood films of the toad were prepared at 30 days p.f. and again at 44 days p.f., stained by Giemsa's method, and examined for parasites.

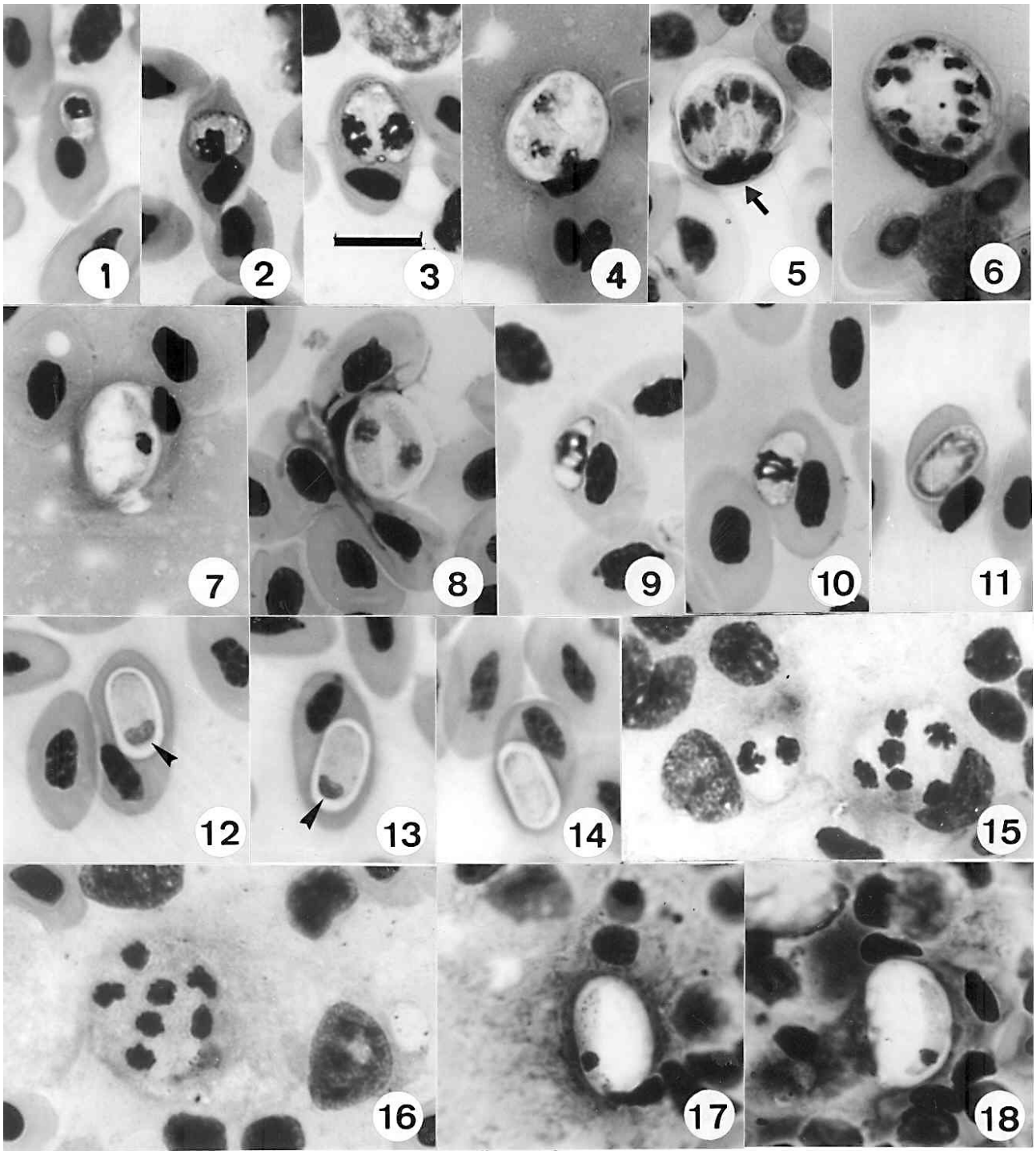
RESULTS

Development of the intraerythrocytic haemogregarine in naturally infected *A. ameiva*

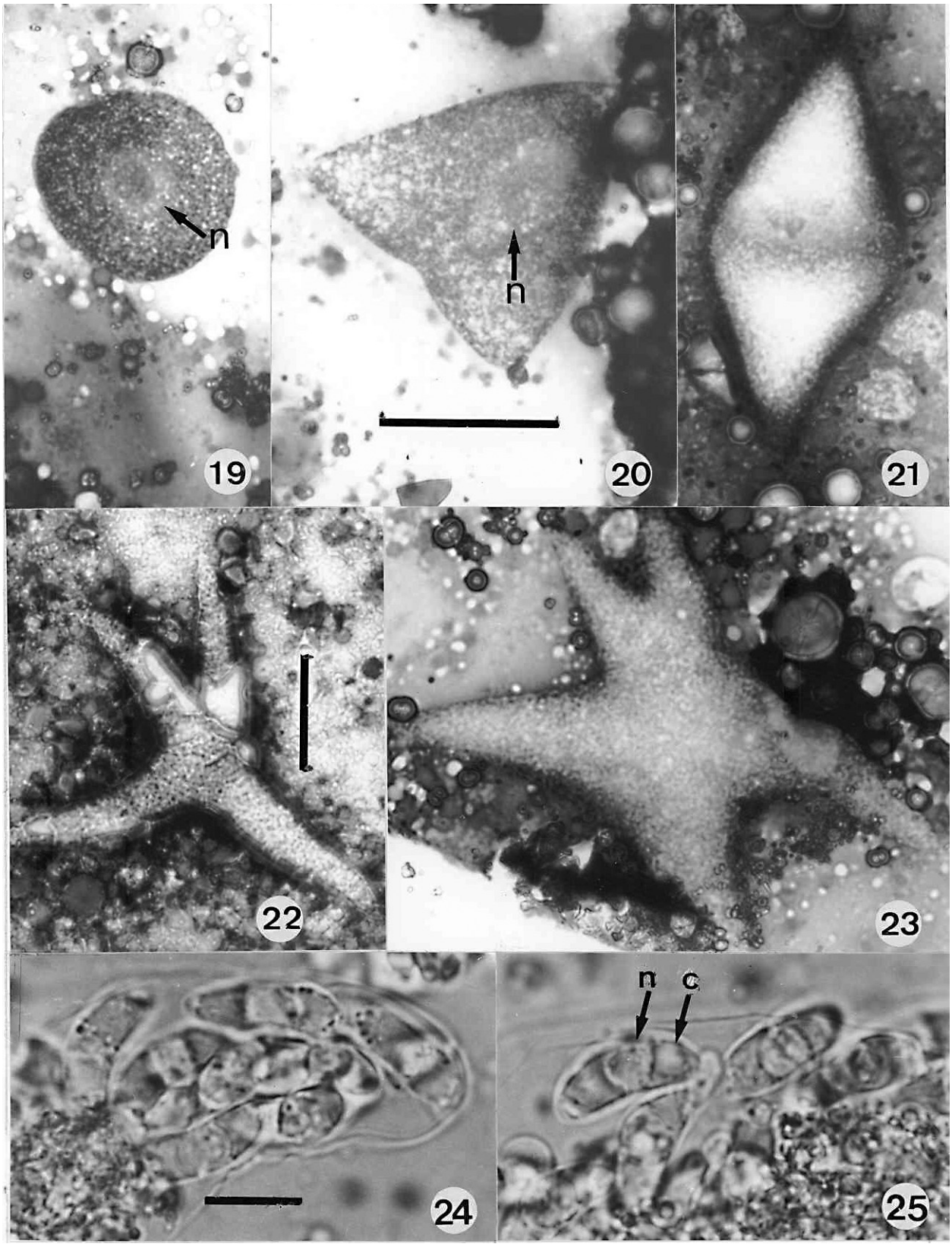
Infections were detected in only three of the 20 newly captured lizards (15 %), with variable numbers of developing and mature schizonts (Figs 1-6), cysts containing a single cystozoite (rarely two) (Figs 7, 8) and the stages suspected to be gametocytes (Figs 9-14), all in erythrocytes of the peripheral blood. Exoerythrocytic schizonts were present in the liver smears of the sacrificed lizard (Figs 15, 16) and occasional tissue-cysts were present in the spleen and liver (Figs 17, 18). All stages of development were morphologically indistinguishable from those described for *H. stellata* of the toad *B. marinus* by Petit *et al.* (1990).

Development in the tick

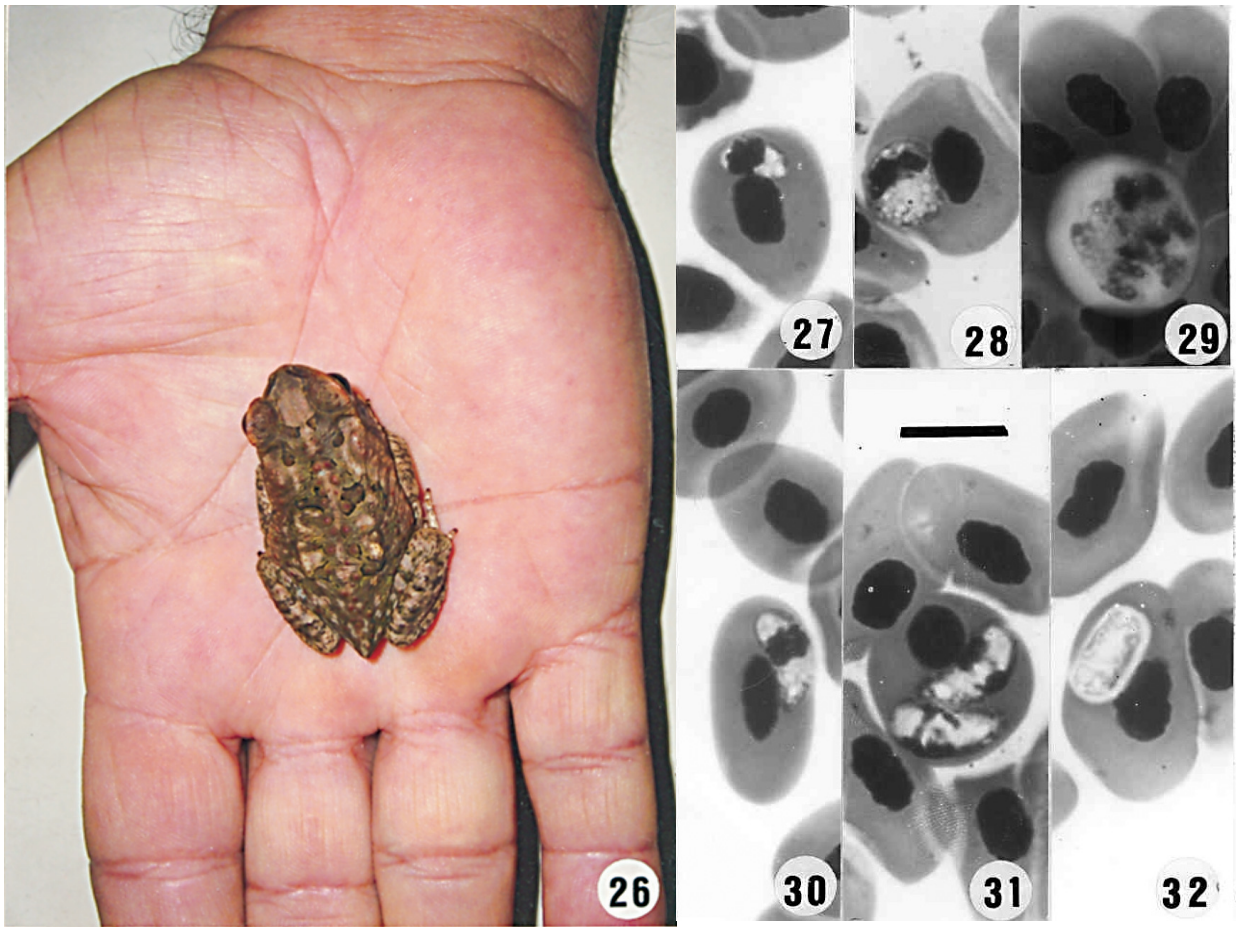
Ticks were allowed to detach naturally: in the case of the experimentally fed ticks, this was usually after 8-14 days of feeding on the infected lizard. Developmental stages of oocysts characteristic of a *Hemolivia* species were abundant in smears of ticks that had detached from a naturally infected lizard, and their development was followed in the experimentally infected ticks (Figs 19-23) up to the formation of sporokinetes (Figs 24,25) and mature sporocysts.



Figs 1-18. – Natural infection of *Hemolivia stellata* in the teiid lizard *Ameiva ameiva*. Figs 1-4. Early stages of schizogony in erythrocytes of the peripheral blood. Figs 5, 6. Mature, segmented schizonts (erythrocyte nucleus arrowed). Figs 7, 8. Intra-erythrocytic cysts with one and two cystozoites. Figs 9, 10. Young, intra-erythrocytic gametocytes. Figs 11-14. Encapsulation of mature gametocytes: morphology of the nucleus (arrow heads) suggests the parasite to be doubled-up on itself within the capsule. Figs 15, 16. Exoerythrocytic schizonts in cells of the R-E system in the liver. Figs 17, 18. Monozoic cysts in the spleen. Giemsa-stained smears. Bar in Figure 3 = 10 µm and applies to all other Figures.



Figs 19-25. – Development of *Hemolivia stellata* in the tick *Amblyomma rotondatum* fed on an infected *Ameiva ameiva*. Fig. 19. Young, spherical oocyst. Figs 20, 21. Development of future 3- and 4-pointed oocysts. Figs 22, 23. Later formation of undifferentiated 4- and 5-pointed oocysts. Figs 24, 25. Formation of sporokinetes in the oocyst's arms. Bar in Figure 20 = 20 µ and also applies to Figures 19, 21 and 23. Bar in Figure 22 = 20 µm. That in Figure 24 = 10 µm and also applies to Figure 25: n = nucleus, c = crystalloid body.



Figs 26-28. – Transmission of *Hemolivia* from the lizard *Ameiva ameiva* to the toad *Bufo marinus*. Fig. 26. A young toad, experimentally infected following ingestion of sporocysts from a macerated tick removed from an infected lizard and with liver tissue, containing tissue-cysts, from the same lizard. Figs 27-29. Developmental stages in erythrocytes of the toad 30 days after the infective meal. Fig. 27. Trophozoite. Fig. 28. Young, binucleate schizont. Fig. 29. Maturing schizont. Fig. 30-32. Developing gametocytes 44 days post-infection. Figs 30, 31. Young gametocytes. Fig. 32. Mature encapsulated and stain-resistant gametocyte. Bar = 10 µm. Giemsa staining.

Transmission of *H. stellata* from the toad *Bufo marinus* to the lizard *Ameiva ameiva*

All five uninfected lizards that were fed with toad liver containing tissue-cysts of *H. stellata* showed intraerythrocytic stages of the parasite, including gametocytes, on day 50 p.i.

Of the three uninfected lizards that were fed with the suspension of macerated ticks containing sporocysts of this parasite, the juvenile specimen and one adult showed intraerythrocytic stages, including gametocytes, at 50 days p.f. Parasites were particularly abundant in the immature lizard. The third, adult lizard of this group showed no evidence of infection, probably due to the small number of sporocysts in the suspension of macerated ticks.

Transmission of the parasite in the lizard *A. ameiva* to the toad *Bufo marinus*

Developmental stages of *Hemolivia* (Figs 27-29) were present in the blood film of the toad prepared 30 days after feeding the animal.with the macerated tick containing sporocysts of the parasite in *A. ameiva*. Developing and mature gametocytes were abundant in the blood film made at 44 days p.f. (Figs 30-32).

DISCUSSSION

From the results of this study we are led to the conclusion that the *Hemolivia* species infecting the lizard *A. ameiva* in north Brazil is *H. stellata*, the type species of the genus described in the giant toad *Bufo marinus* by Petit *et al.* (1990). The morphology and pattern of development of the parasite is very similar in the two vertebrate hosts and the tick vector *A. rotundatum*, which infests both animals in the same habitat. Finally, any doubt regarding this conclusion was removed by the successful transmission of the parasite from a toad to lizards and from a lizard to a toad.

In the absence of laboratory-bred *A. ameiva* or *B. marinus* we were obliged to use wild-caught animals for experimental transmission. Repeated examination of their blood had shown no evidence of infection, however, and we discount the remote possibility that the experimental lizards were all harbouring inapparent infections which all became patent at the same time. Our past examination of more than 200 *A. ameiva* from Capanema (Lainson *et. al.*, 2003) has shown the natural infection-rate to be consistently low and comparable with the 15 % noted in the batch of lizards used in the present study. For similar reasons we dismiss the possibility of a prior, natural infection in the juvenile experimental toad. The much higher infection rate of *H. stellata* in the toad *B. marinus*, given as 30 % of 120 specimens examined by Petit *et al.* (1990), compared with that in the lizard *A. ameiva*, clearly indicates the toad as the principal host.

That a protozoan species can naturally infect hosts as widely separated as an amphibian and a reptile is remarkable, and indicates care in giving different specific names to *Hemolivia* based solely on host difference. This is especially the case in habitats frequented by *B. marinus* which, in attempts to use this toad in biological control of various insect pests, has been imported from its native habitat in the American tropics to many other geographical localities, including the West Indies, Bermuda, the Hawaiian Islands, tropical Australia, New Guinea, the Solomons and the Pacific Islands.

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REFERENCES

LAINSON R., DE SOUZA M.C. & FRANCO C.M. Haematozoan parasites of the lizard *Ameiva ameiva* (Teiidae) from Amazonian Brazil: a preliminary note. *Memórias do Instituto Oswaldo Cruz*, 2003, *98*, 1067-1070.

LANDAU I. & PAPERNA I. The assignment of *Hepatozoon mauritanicum*, a tick-transmitted parasite of tortoises, to the genus *Hemolivia*. *Parasite*, 1997, *4*, 365-367.

PAPERNA I. *Hemolivia mauritanica* (Haemogregarinidae: Apicomplexa) infection in the tortoise *Testudo graeca* in the Near East with data on sporogonous development in the tick vector *Hyalomna aegyptium*. *Parasite*, 2006, *13*, 267-273.

PETIT G, LANDAU I, BACCAM D. & LAINSON R. Description et cycle biologique d'*Hemolivia stellata* n. g., n. sp., hémogrégarine de crapauds brésiliens. *Annales de Parasitologie Humaine et Comparée*, 1990, *65*, 3-15.

SMALLRIDGE C. & PAPERNA I. The tick-transmitted haemogregarinid of the Australian sleepy lizard *Tiliqua rugosa* belongs to the genus *Hemolivia*. *Parasite*, 1997, *4*, 359-363.

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