PARASITES OF THE SQUIRREL SCIURUS SPADICEUS (RODENTIA: SCIURIDAE) FROM AMAZONIAN BRASIL, WITH PARTICULAR REFERENCE TO EIMERIA DAMNOSA N. SP. (APICOMPLEXA: EIMERIIDAE)

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Summary:

A description is given of the mature oocysts and endogenous stages of Eimeria damnosa n. sp. from the small intestine of the red squirrel, Sciurus spadiceus, from the State of Acre, north Brazil. Ten of 12 animals examined were infected. Oocysts ovoid to ellipsoidal, occasionally cylindrical but not with parallel sides, 30.2 × 20.0 µm (18.0 × 15.0-40.2 × 30.0), shapeindex (ratio length/width) 1.5 (1.3-1.8), n = 40. Oocyst wall smooth, colourless, with no micropyle, apparently of a single layer measuring approximately 1.0-1.5 µm thick. No oocyst residuum, but approximately 50 % of the oocysts with a single spherical, ovoid or dumbbell-shaped polar body. Sporocysts pear-shaped, 15.0 × 8.0 µm (11.0 × 6.0-16.0 × 8.0), shape index 1.9 (1.8-2.0), n = 33. Stieda body, if it merits this name, appears only as a slight thickening of the sporocyst wall at the more pointed extremity. Endogenous stages intracytoplasmic in the epithelial cells of the duodenum and throughout the ileum, above the host cell nucleus. Sporulation frequently completed in the lumen of the intestine, but most oocysts mature outside the host at some time within 24 hours. Massive infections may result in extensive desquamation of the gut epithelium, and sometimes in the death of the animal. In addition to this coccidian, one squirrel showed abundant trophozoites of a Giardia sp., in the ileum. The liver of two others contained developing and mature meronts, producing large numbers of slender merozoites, and other cyst-like bodies containing a small number of large zoites (sporozoites?). No parasites were detected in the blood of any of the squirrels that could be associated with this unidentified protozoan. Histological sections of the ileum of one squirrel revealed a globidium-like parasite in the lamina propria: it contained a very large number of slender, curved zoites. Three animals were with a sheathed microfilaria in the peripheral blood and liver smears. Finally, a *Trypanosoma cruzi*-like trypanosome was isolated from the blood of one squirrel and a *T. lewisi*-like trypanosome from two others.

KEY WORDS : *Eimeria damnosa* n. sp., coccidia, *Sciurus spadiceus*, squirrel, *Giardia*, unidentified protozoan parasite, globidium, microfilaria, trypanosome, Brazil. **Résumé :** Les parasites de l'écureuil *Sciurus spadiceus* (Rodentia: Sciuridae) de l'Amazonie au Brésil, avec une référence particulière à *Eimeria damnosa* n. sp. (Apicomplexa: Eimeridae)

Description des oocytes matures et des stades endogènes d'Eimeria damnosa n. sp. de l'intestin grêle de l'écureuil rouge, Sciurus spadiceus, de l'État d'Acre au nord du Brésil. Dix des 12 écureuils examinés sont infectés. Les oocytes sont ovoïdes ou ellipsoïdes, occasionnellement cylindriques, mais non symétriques : 30,2 × 20,0 µm (18,0 × 15,0-40,2 × 30,0), index longueur/largeur de 1,5 (1,3-1,8), n = 40. La paroi de l'oocyte, lisse, incolore, sans micropyle, apparemment constituée d'une seule couche, est de 1,0 à 1,5 µm d'épaisseur. Il n'y a pas de résidu, mais environ 50 % des oocytes présentent un corpuscule polaire unique, sphérique, ovoïde ou en forme d'haltères. Les sporocytes sont en forme de poire : 15,0 × 8,0 µm (11,0 × 6,0-16,0 × 8,0), index de 1,9 (1,8-2,0), n = 33. Le corps de Stieda, si on peut l'appeler ainsi, n'apparaît que comme un discret épaissement de la paroi du sporocyte à son extrémité. Les stades intracytoplasmiques, supranucléaires, sont observés dans les cellules épithéliales du duodénum et de l'iléon de l'hôte. La sporulation est souvent achevée dans la lumière intestinale, mais nombre d'oocytes matures sont évacués à tout moment par l'hôte dans les 24 heures. Des infestations importantes peuvent engendrer une desquamation massive de l'endothélium intestinal, et parfois la mort de l'animal. En plus de cette coccidie, l'un des écureuils était porteur de nombreux trophozoïtes de Giardia sp. dans son iléon. Le foie de deux autres contenait des mérontes en développement produisant de nombreux et fins mérozoïtes et d'autres "zoïtes" en faible nombre (sporozoïtes ?). Aucun parasite, qui puisse être en rapport avec ce protozoaire non identifié, n'a été détecté dans le sang d'aucun des écureuils. Les coupes histologiques de l'iléum de l'un des écureuils ont révélé la présence d'un parasite "globidium-like" au niveau de la lamina propria : présence d'un grand nombre de "zoïtes" fins et recourbés. Trois animaux présentaient une "microfilaire" au niveau du sang périphérique et de coupes de foie. Enfin, un trypanosome – "Trypanosoma cruzi-like" – a été isolé du sang d'un écureuil, et un autre – "T. lewisi-like" – du sang de deux autres.

MOTS CLÉS : Eimeria damnosa n. sp., coccidie, Sciurus spadiceus, écureuil, Giardia, protozoaire parasite non identifié, globidium, microfilaire, trypanosome, Brésil.

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INTRODUCTION

The first description of an *Eimeria* species in squirrels (Rodentia: Sciuromorpha: Sciuridae) would appear to be that of Galli-Valerio (1922), who gave the name of *Eimeria sciurorum* to a parasite of

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the alpine red squirrel, *Sciurus vulgaris* var. *alpinus*, in Switzerland. Subsequently this coccidian has been recorded in different species of *Sciurus* in various countries of Europe (Pellérdy, 1974; Levine & Ivens, 1990) and in Belize, Central America. Lainson (1968) described the endogenous development of the parasite in the small intestine of *S. aureogaster hypopyrrhus* from the latter locality. In a recent paper (Lainson, Brígido & Silveira, 2004) we recorded the presence of an *Eimeria* sp. in another neotropical squirrel, *S. spadiceus*, from north Brazil. It is the object of the present paper to describe the mature oocysts and endogenous stages of this new species of *Eimeria* and record the presence of some other parasites encountered in this animal.

MATERIALS AND METHODS

welve specimens of S. spadiceus were captured in fruit-baited traps placed in the lower branches of trees in road-side forest along the AC 40 Highway (km 70) in the locality of Birroque, municipality of Plácido de Castro, State of Acre, North Brazil. On arrival in Belém, they were maintained, in separate cages, on a diet of fruit and nuts. Blood taken from the femoral vein was used for the preparation of thin blood films, which were air-dried, fixed in absolute methyl alcohol and stained for one hour by Giemsa's method. Blood was also cultured in Difco B45 medium. Faecal samples were lightly triturated in 2 % aqueous potassium dichromate solution (K₂Cr₂O₇) and spread as a thin layer in covered Petri dishes kept at approximately 24-26°C. The suspensions were examined at once for the presence of coccidial oocysts, using normal light microscopy at magnifications of \times 160 and \times 400, and then periodically to determine the sporulation time of the oocysts detected. Bile, removed from the gall bladder of two heavily infected animals that had died in captivity, was also examined for oocysts. The site of development of the coccidian in the intestine was determined by the examination of fresh cover-slip preparations of scrapings from the gut epithelium at different positions along the small intestine. Smears of this material and impression smears of the liver, spleen, lung and kidney were rapidly air-dried and either fixed in absolute methyl alcohol and stained directly by Giemsa's method, or fixed in aqueous Bouin's fluid for 20 minutes and stained by a modified Giemsa method (Lainson, 1958). Tissues for histology were fixed in 10 % buffered formol-saline, embedded in paraffin wax and examined in sections cut at 4 µm and stained with haematoxylin and eosin. Oocysts and sporocysts were measured using a \times 100 neofluar objective, × 10 eyepieces and an ocular micrometer. Photomicrographs were prepared using a Zeiss "Photomicroscope III" and Kodak TMX 100 film. All measurements are given in µm: for the oocysts and sporocysts, these are given as means, with the range in parentheses, followed by the shape-index (ratio of length/width) and the number measured (n).

RESULTS, DESCRIPTIONS AND DISCUSSION

Parasites encountered in the 12 squirrels are given in Table I. Ten of the 12 animals (83.3 %) were passing abundant oocysts of a single type (Figs 17-21, 41), considered to be those of a previously undescribed species of *Eimeria*.

Development of the endogenous stages of the parasite was followed in histological sections of the small intestine (Figs 3-16), in which massive infection resulted in extensive desquamation of the gut epithelium (Fig. 2). In addition to this parasite, one squirrel also showed a large number of trophozoites of a *Giardia* sp. in the lumen of the small intestine (Fig. 22) and, in two animals, abundant developmental stages

Squirrel No.	Eimeria damnosa n. sp.	<i>Giardia</i> sp.	Unidentified protozoan in liver	"Globidium" in intestinal propria lamina	Microfilaria	<i>T. cruzi</i> -like trypanosome	<i>T. lewiwi</i> -like trypanosome
1861	+	_	_	_	_	_	_
1865	+	_	_	_	_	_	_
1866	+	_	+	-	-	_	_
1871	+	+	_	+	+	_	_
1883	+	_	_	-	+	_	_
1884	+	_	_	_	_	_	_
1886	+	-	+	-	-	-	-
1914	_	_	_	-	-	_	_
1915	_	_	_	-	-	_	_
1925	+	-	_	_	+	+	_
1939	+	-	_	_	-	_	+
2138	+	_	_	_	-	_	+

Table I. - Parasites encountered in 12 specimens of the Amazonian red squirrel Sciurus spadiceus.

of an unidentified protozoan were detected in the liver smears (Figs 23-35). Histological sections of the ileum of one squirrel revealed a single large globidiumlike body in the *lamina propria* (Fig. 36): it contained many hundreds of slender, curved zoites (Fig. 37). The same squirrel, and two others, showed a large sheathed microfilaria measuring approximately $200 \times 7 \mu m$ in the peripheral blood and smears of the liver (Fig. 38). Finally, a *Trypanosoma cruzi*-like parasite was isolated in blood cultured from one squirrel (Fig. 39), while culture of blood from two others gave rise to epimastigotes which, when inoculated into laboratory mice and hamsters, produced transient infections with a *T. lewisi*-like trypanosome (Fig. 40) (Lainson *et al.*, 2004).

EIMERIA DAMNOSA N. SP. (Figs 1-21, 41)

Description of the oocyst (Figs 17-21, 41). With the characters of the genus. Mature oocysts ovoid to broadly cylindrical, but without parallel sides, 30.2×20.0 (18.0×15.0 - 40.2×30.0), shape-index 1.5 (1.3-1.8), n = 40. Oocyst wall apparently of a single layer from 1.0-1.5 thick, smooth, colourless and with no micropyle. No oocyst residuum, but approximately 50 % with a single spherical to dumbbell-shaped polar body. Sporocysts pear-shaped, 15.0×8.0 (11.0×6.0 - 16.0×8.0), shape-index 1.9 (1.8-2.0), n = 33. Stieda body very inconspicuous, appearing merely as a slight thickening of the sporocyst wall at the more pointed extremity. Sporocyst residuum a spherical mass of fine granules and globules lying between the two strongly recurved sporozoites.

Endogenous stages (Figs 1-16): these are intracytoplasmic in the epithelial cells of the small intestine, with development above the nucleus of the host cell. In two animals that had died in captivity, massive infection occurred in the duodenum and throughout most of the ileum (Figs 1-4). Asexual stages were particularly abundant, resulting in vast numbers of intracellular merozoites in all stages of rounding-up and developing into further meronts or gamonts. Three distinct types of meronts were discernable in both histological sections and Giemsa-stained smears of the ileum (Figs 5-10 : I, II, III): one producing very small merozoites of about $4.0-5.0 \times 1.0-1.5 \ \mu m$ (Figs 5, 8), another giving rise to merozoites of about 5.0-8.0 µm (Figs 6, 9), and the third forming much larger ones of about 10.0-12.0 \times 2.0 µm (Figs 7, 10). The number of merozoites produced by each type of meront varied from as few as four or six to as many as 30. Macrogamonts are spherical to subspherical throughout their growth. In stained smears the larger and apparently mature forms were from 15.0×14.0 to 20.0×18.0 , while in sections they appear considerably smaller $(10.0-12.0 \times 8.0-10.0 \text{ (Fig. 12)})$. Developing microgamonts were less abundant. In sections (Fig. 11) they appear as spherical to ovoid bodies

averaging $12.0 \times 12.0 \,\mu$ m, with the dividing nuclei forming an intensely staining ring around the periphery of an homogeneous, finely vacuolated cytoplasm: towards maturity they are less regular in shape (Fig. 12). In stained smears some intact or ruptured immature forms (Fig. 13) were estimated to contain up to 150 nuclei. Fully mature forms shed very small microgametes about 2.0-3.0 µm long (Fig. 13), and leave a bulky residuum. Zygotes contain the usual small and large wall-forming bodies (Fig. 14) and eventually assume an ovoid shape (Figs 7, 15). The oocyst wall is formed while the young oocyst is still intracellular (Fig. 16).

Sporulation: some oocysts mature within the gut lumen. The majority, however, are expelled unsporulated (Fig. 17) but are fully mature in less than 24 hours. Host: the Amazonian red squirrel *Sciurus spadiceus* Olfers, 1818 (Rodentia: Sciuridae).

Type locality: Km 70, the AC 40 Highway, Birroque, municipality of Plácido de Castro, State of Acre, north Brazil.

Type material: phototypes of oocysts, histological sections and stained smears of the endogenous stages held in the Department of Parasitology, Instituto Evandro Chagas under the depository Nos 1871 and 1888.

Prevalence: 10 of 12 squirrels examined (83.3 %) were infected.

Pathology: in captive squirrels, massive infection may result in death of the animals following extensive damage to the epithelium of the small intestine.

Etymology: the specific name is from the Latin adj. *damnosus*: causing damage or injury.

Discussion

Levine & Ivens (1990) gave a list of 73 species of *Eimeria* recorded among 17 genera of the family Sciuridae, and, as far as we are aware, only four more have since been added (Wilber *et al.*, 1994, 1998; Fuller & Duszynski, 1997). Wilber *et al.*, (1998), however, concluded that among the species described from hosts in the tribe Marmotini (marmots, ground squirrels, prairie dogs, chipmunks) the oocysts of many differently named parasites were morphologically indistinguishable, and that some other descriptions were inadequate under the rulings of the International Code of Zoological Nomenclature.

As a result, they considered that the number of named eimerian species from members of the Marmotini should be reduced from 40 to only 26 valid species. This leads one to speculate as to whether or not a similar reduction of valid species is necessary among the extensive number of *Eimeria* spp. described from other rodents.

There is a remarkable variation in the size and shape of the oocysts of *E. damnosa* n. sp. (Figs 17-21). The



Figs 1-4. – Endogenous stages of *Eimeria damnosa* n. sp. in the epithelial cells of the small intestine of the squirrel *Sciurus spadiceus*. Fig. 1. Low power view showing enormous numbers of developing parasites. Bar = 50.0μ m. Fig. 2. Low power view of epithelial cells and parasites sloughed into the gut lumen. Bar = 50.0μ m. Figs 3, 4. High power view showing abundance of mature and developing meronts and macrogamonts. Bar = 10.0μ m. L = gut lumen.



Figs 5-13. – Endogenous stages of *Eimeria damnosa* n. sp. in smears and sections of the ileum of the squirrel *Sciurus spadiceus*. Figs 5-7. In sections, showing the type I, II and III meronts producing merozoites of very different size. Figs 8-10. Type I, II, and III meronts as seen in Giemsa-stained smears. Note budding off of merozoites from the residual mass of cytoplasm. Fig. 11. Section showing a young microgamont with peripherally disposed nuclei. Fig. 12. Two nearly mature macrogamonts (Ma) and a mature microgamont (Mi) shedding microgametes. Fig. 13. Above, a nearly mature microgamont and, below, a mature one shedding microgametes (arrowed). R = bulky residuum. Bar in Figure 9 = 10.0 μ m and applies to all other figures.



Figs 14-21. – *Eimeria damnosa* n. sp. of the squirrel *Sciurus spadiceus*. Fig. 14. Young zygote with outer, small wall-forming bodies and inner large wall-forming bodies. Fig. 15. Two zygotes (Z) assuming the ovoid shape of the future oocyst. Fig. 16. Young intracellular oocyst (O), already with a well developed wall. Sections: haematoxylin and eosin staining. Fig. 17. Unsporulated oocyst in newly passed faeces. Figs 18-21. Mature oocysts, less than 24 hours later: note variable size and shape. P = polar body, Sp = sporozoite, Sr = sporocyst residuum, St = Stieda body. Fig. 22. Trophozoite of a *Giardia* sp. in a smear of the small intestine. Bar in Figure 21 = 10.0 μ m and applies to all figures.



Figs 23-35. – An unidentified parasite in the liver of the squirrel, *Sciurus spadiceus*. Figs 23-28, as seen in Giemsa-stained smears. Figs 23, 24. Mature meronts. Figs 25-28. Developing stages apparently producing eight zoites (sporozoites?). Figs 29-35. The same stages as seen in histological sections. Fig. 29. Meront budding off merozoites. Figs 30, 31. Mature meronts. Figs 32-35. Development of the cyst-like bodies containing eight zoites. Bar in Figure 24 = $10.0 \mu m$ and applies to all other figures.



Figs 36-40. – Parasites of the squirrel *Sciurus spadiceus*. Figs 36, 37. Low and high power views of a globidium-like parasite in the *lamina propria* of the ileum. Bar in Figure 36 = 50.0 μ m. Bar in Figure 37 = 10.0 μ m. Fig. 38. A large, sheathed microfilaria in the peripheral blood. Bar = 10.0 μ m and also applies to Figures 39 and 40. Fig. 39. A *Trypanosoma cruzi*-like parasite in the blood of mice inoculated with culture forms of the parasite isolated from a squirrel. Fig. 40. A *T. lewisi*-like parasite isolated in the same way.



Fig. 41. – Line-drawing of a mature oocyst of *Eimeria damnosa* n. sp., from the squirrel *Sciurus spadiceus*. Bar = 10.0 µm.

possibility of mixed infections was discounted, however, due to gradation in the dimensions of both oocysts and sporocysts.

Among those Eimeria spp., of sciurid rodents with elongate-ovoid to cylindrical oocysts, E. damnosa n. sp., can be differentiated from E. sciurorum of the European squirrel S. vulgaris and the Central American S. aureogaster hypopyrrhus by its larger oocysts (means $30.2 \times 20.0 vs 25.0 \times 17.0$). The cylindrical oocysts of E. sciurorum are described as predominantly with parallel sides (Levine & Ivens, 1990), whereas among the few virtually cylindrical oocysts of E. damnosa n. sp. that were seen, none could be said to have parallel sides (Fig. 19). The sporulation time of *E. sciurorum* has been given as three-four days by several authors (Pellerdy, 1974; Levine & Ivens, 1990) whereas that of E. damnosa n. sp. is remarkable in being less than 24 hours, with some oocysts even reaching maturity in the gut lumen. E. kniplingi develops principally in the large intestine of the fox squirrel, S. niger: the oocysts measure only $24.2 \times 14.2 \,\mu\text{m}$, lack polar bodies but have an oocyst residuum, and undergo sporulation in two-three days. Oocysts of E. moelleri, from S. carolinensis, have a micropyle and lack polar bodies: sporulation time is three days. Those of E. arctomysi, in the marmot Marmota marmota, also possess a micropyle and measure only 24.0×20.0 . In addition,

the description of *E. arctomysi* was based on oocysts with incomplete sporulation (Galli-Valerio, 1931) and, for this reason, has been relegated to *species inquirendae* by Wilber *et al.*, (1998). *E. yukonensis*, of the ground squirrel *Spermophilus undulatus*, differs in its much smaller oocysts, their possession of a micropyle and a brownish coloured oocyst wall. Finally, although oocysts of *E. tamiasciuri* of the spruce squirrel *Tamiasciurus hudsonicus* approximate most closely to the morphology of *E. damnosa* n. sp., the sporocysts have very prominent cone-shaped Stieda bodies and the sporulation time is recorded as three days.

There is no doubt regarding the considerable pathogenicity of both E. sciurorum (Pellérdy, 1954, 1974; Lainson, 1968) and E. damnosa n. sp. (present observations), particularly in caged squirrels in which infection is probably exacerbated under the stress of captivity. Stress is also a common feature of wildlife, however, particularly among small mammals that face the constant threat of predators in addition to natural or manmade environmental changes. It should come as no surprise, therefore, to find that acute coccidiosis may be a common cause of death in the populations of squirrels, especially when the infection-rate is as high as has been shown in the present study. In Croatia, Rajković-Janje & Auslender (1991) considered E. sciurorum to be the cause of fatal gastroenteritis in a specimen of S. vulgaris found dead in its woodland habitat.

AN UNIDENTIFIED PROTOZOAN IN THE LIVER OF S. SPADICEUS (Figs 23-35)

The host cell of the parasite was difficult to ascertain, but development appears to be within the parenchyma cells of the liver and it was followed in both smears and sections. No stages were seen in the other tissues and the peripheral blood cells.

The youngest stages were broadly sausage-shaped, with rounded ends, a highly vacuolated cytoplasm and a single bulky nucleus placed at one extremity (Fig. 25): they measured approximately 15.0×8.0 µm. and appeared to be enclosed by an ovoid membrane. Subsequent development follows a rounding-up of the parasite, which at this stage is still uninucleate and about 15.0 µm in diameter (Fig. 26), and two distinctly different stages are produced. Meronts produce 50 or more long, slender merozoites measuring approximately 10.0 × 1.5 µm (Figs 23, 24, 30, 31). These are budded off from a bulky mass of residual cytoplasm (Fig. 29). Other parasites divide to produce a small number (estimated as eight) larger and stouter zoites measuring about $15.0 \times 4.0 \ \mu m$ (Figs 25-28, 32-35). The presence of many developmental stages which stained poorly, or not at all, suggests that they are enclosed by an ovoid, stain-resistant capsule which remains intact and without deformation, even after the considerable pressure exerted in making an impression smear of the liver.

The development of this unidentified protozoan is reminiscent of that seen in some members of the family Lankesterellidae. Schellackia undergoes both merogony and sporogony in the intestine of the vertebrate host, while these stages of Lainsonia are found in viscera such as the liver and lungs. Both parasites finally produce oocysts containing eight sporozoites and the cyst-like bodies seen in the liver of our squirrels are, therefore, highly suggestive of oocysts. Members of the Lankesterellidae are principally parasites of cold-blooded vertebrates (amphibians and reptiles), but as parasites with the characters of the family are also known in birds (Lainson, 1959; Dissanaike, 1967) it seems reasonable to suppose that the parasite of S. spadiceus could be a related organism. The oocysts of Schellackia and Lainsonia are of a transient nature and soon rupture to release the sporozoites, which invade cells of the peripheral blood and also accumulate in the macrophages of the internal organs. If the apparently octozoic stages of the parasite of S. spadiceus are oocysts of a lankesterellid it therefore remains difficult to account for our failure to demonstrate microgamonts, the process of fertilization, and the presence of sporozoites in the peripheral blood cells and the liver macrophages.

OTHER PARASITES

The presence of abundant *Giardia* trophozoites in one of our squirrels calls for no particular comment. The genus occurs in amphibians, reptiles, birds and mammals and opinion is still divided regarding classification and nomenclature. Among mammals the parasite has been recorded in a wide range of animals, including canine and feline hosts, a variety of farm animals, rodents and man. That human giardiasis is a zoonosis has still to be firmly established, but frequent reports of giardiasis among persons drinking water from streams in areas far from human habitation has led to the suspicion that wild animals may be a source of infection, particularly beavers, muskrats and other rodents (Dykes *et al.*, 1990).

Pellérdy (1974), Paperna (1999) and Lainson (2003) have discussed the enigmatic nature of the taxon *Globidium* Flesch, 1883. In mammals, globidia have been described in the *lamina propria* of the digestive tract of armadillos, marsupials, horses, sheep and goats. There are rare reports of their occurrence in reptiles: in a snake (Harant & Cazal, 1934), a gekkonid lizard (Paperna, 1999) and an amphisbaenid lizard (Lainson, 2003). The most popular theory is that they represent giant meronts of known intestinal coccidians of these hosts, but this is still open to doubt. In our infected squirrel, for example, we were able to detect only a sin-

gle globidium, measuring approximately $50.0 \times 30.0 \,\mu$ m, in the many sections of ileum examined, all of which were showing vast numbers of asexual and sexual stages of *E. damnosa* n. sp. If it indeed represents a giant meront of this parasite we would have expected to find other examples and their developmental stages. Unfortunately, the microfilariae were only noted in the blood films of the infected squirrels a considerable time after their autopsy, when a search for the adult nematodes was no longer possible. It is hoped that acquisition of further specimens of *S. spadiceus* will enable detection and identification of the adult worms, and further observations on the other parasites described here.

Characterization and descriptions of the trypanosomes will form the subject of another paper. From its behaviour in blood-agar culture medium and morphology of the trypomastigotes produced in experimentally infected mice, one of these parasites is indistinguishable from T. cruzi. From the morphology of the trypanomastigotes of the other trypanosome, in infections produced in experimentally infected mice, we at first thought that the parasite was T. rangeli. The fleeting nature of the infection in mice and our failure to establish the parasite in Rhodnius robustus fed on these animals, however, now lead us to suspect that we are dealing with another species of trypanosome within the subgenus Herpetosoma. The type host of T. rangeli is the triatomine *Rhodnius prolixus*, which is so closely related to *R. robustus* that doubts have been raised, by some, that they represent two distinct species. We are unaware of any previous record of a T. lewisi-like trypanosome in neotropical squirrels.

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