ON *EIMERIA BRAGANCAENSIS* N. SP. (APICOMPLEXA: EIMERIIDAE) AND TISSUE-CYSTS OF AN UNIDENTIFIED PROTOZOAN IN THE BAT *PEROPTERYX MACROTIS* (CHIROPTERA: EMBALLONURIDAE) FROM AMAZONIAN BRAZIL

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Summary :

A description is given of the mature oocysts and endogenous stages of *Eimeria bragancaensis* n. sp., from the small intestine of the bat *Peropteryx macrotis* from north Brazil. Oocysts are spherical to subspherical, 15.9 × 14.6 µm (range 14-17 × 14-17.7). The wall is of two layers: an outer, thicker one which is of a brownish-yellow colour and striated (pitted), and an inner one which is thin and colourless. The outer layer is frequently lost. No micropyle or oocyst residuum present, but the oocyst usually contains one or two polar bodies. Sporocysts 8.4 × 5.3 µm (range 6.25-9 × 4-6) with inconspicuous Stieda and substiedal bodies. Endogenous stages are intracytoplasmic in the epithelial cells of the small intestine, above the host-cell nucleus. Stages of merogony and gametogony are described. Abundant tissue-cysts of an unidentified protozoan, containing from 1-4 zoites, were found in the parenchyma cells of the liver and, less frequently, in the *lamina propria* of the small intestine. Their possible nature is discussed.

KEY WORDS : *Eimeria bragancaensis* n. sp., oocysts, endogenous stages, *Peropteryx macrotis*, Chiroptera, bat, unidentified protozoan, tissue-cysts, Brazil.

Résumé : *EIMERIA BRAGANCAENSIS* N. SP. (APICOMPLEXA : EIMERIIDAE) ET KYSTES VISCÉRAUX D'UN PROTOZOAIRE NON IDENTIFIÉ CHEZ *PEROPTERYX MACROTIS* (CHIROPTERA : EMBALLONURIDAE) DE L'AMAZONIE BRÉSILIENNE

*Description des oocystes matures et du stade de développement endogène d'*Eimeria bragancaensis *n. sp. au niveau de l'intestin grêle de la chauve-souris* Peropteryx macrotis *du nord du Brésil. Les oocystes sont sphériques ou subsphériques : 15,9 × 14,6 µm (14-17 × 14-17,7). La paroi comporte deux couches : l'externe, la plus épaisse, de couleur jaune-brun, est striée ; l'interne, plus fine, est incolore. La couche externe est souvent absente. Les oocystes comportent habituellement un ou deux corps polaires. Les sporocytes mesurent 8,4 × 5,3 µm (6,25-9 × 4-6). Les stades de développement endogène sont intracytoplasmiques dans les cellules épithéliales de l'intestin grêle, au-dessus du noyau de la cellule hôte. Les stades de mérogonie et de gamétogonie sont décrits. De nombreux kystes viscéraux d'un protozoaire non identifié et contenant de un à quatre zoïtes, ont été observés dans les cellules du parenchyme hépatique et moins souvent dans la* lamina propria *du grêle. Leur nature est discutée.*

MOTS CLÉS : Eimeria bragancaensis *n. sp., oocystes, développement endogène,* Peropteryx macrotis, *chiroptère, protozoaire sans identité, kystes viscéraux, Brésil.*

INTRODUCTION

As far as we are aware, only eleven valid specific names have been given to *Eimeria* species of bats (Lainson & Naiff, 1998), in spite of the wide range of genera and species within the order Chiroptera and the huge biomass they represent. For this reason descriptions of additional chiropteran eimeriids are particularly welcome.

Coccidial oocysts found in the faeces of one of three specimens of the bat *Peropteryx macrotis* from north Brazil are considered to be those of a previously unrecorded *Eimeria* species, and descriptions are given of the mature oocysts in the faeces and the endogenous stages of the parasite as seen in histological sections of the small intestine. Occasional tissue-cysts, containing from 1-4 zoites, were found in the l*amina propria* of the small intestine and, more abundantly, in the parenchyma cells of the liver. We consider these not to be an extra-intestinal stage of the new *Eimeria* sp., but part of the life-cycle of some other unidentified parasite. Their possible nature is discussed.

MATERIALS AND METHODS

Three specimens of *P. macrotis* were captured in primary forest near Bragança, Pará, north Brazil (1° 03´S, 46° 46´W) in August, l998: one was taken from a hollow tree-trunk and two from the branches of a nearby tree. Abundant oocysts were detected in faeces removed from the rectum of the first bat and this material was suspended in a small quantity of 2 % (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) and maintained at room temperature (23-24° C). No oocysts could be detected in faeces from the other two animals. Further examination of the

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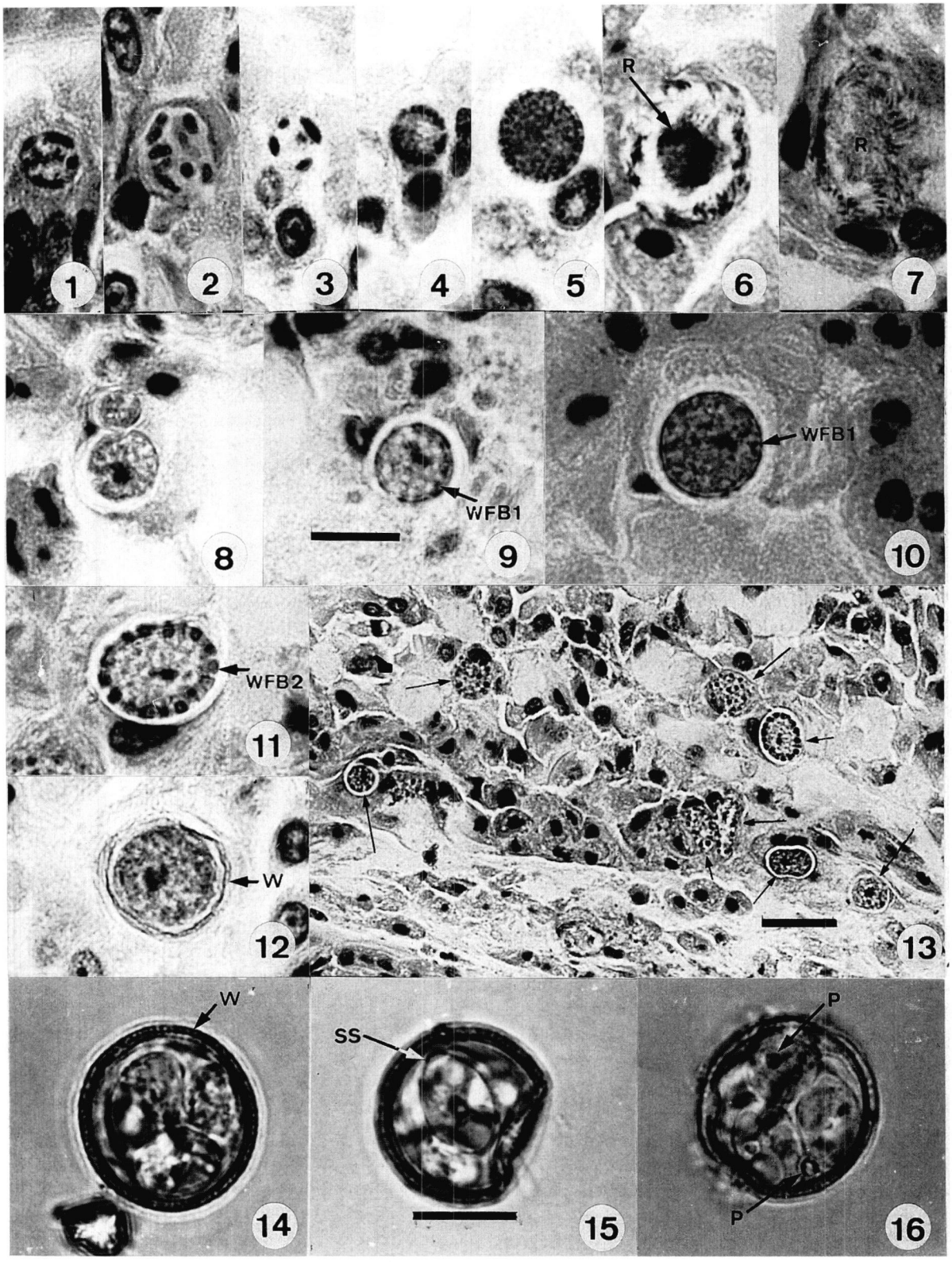
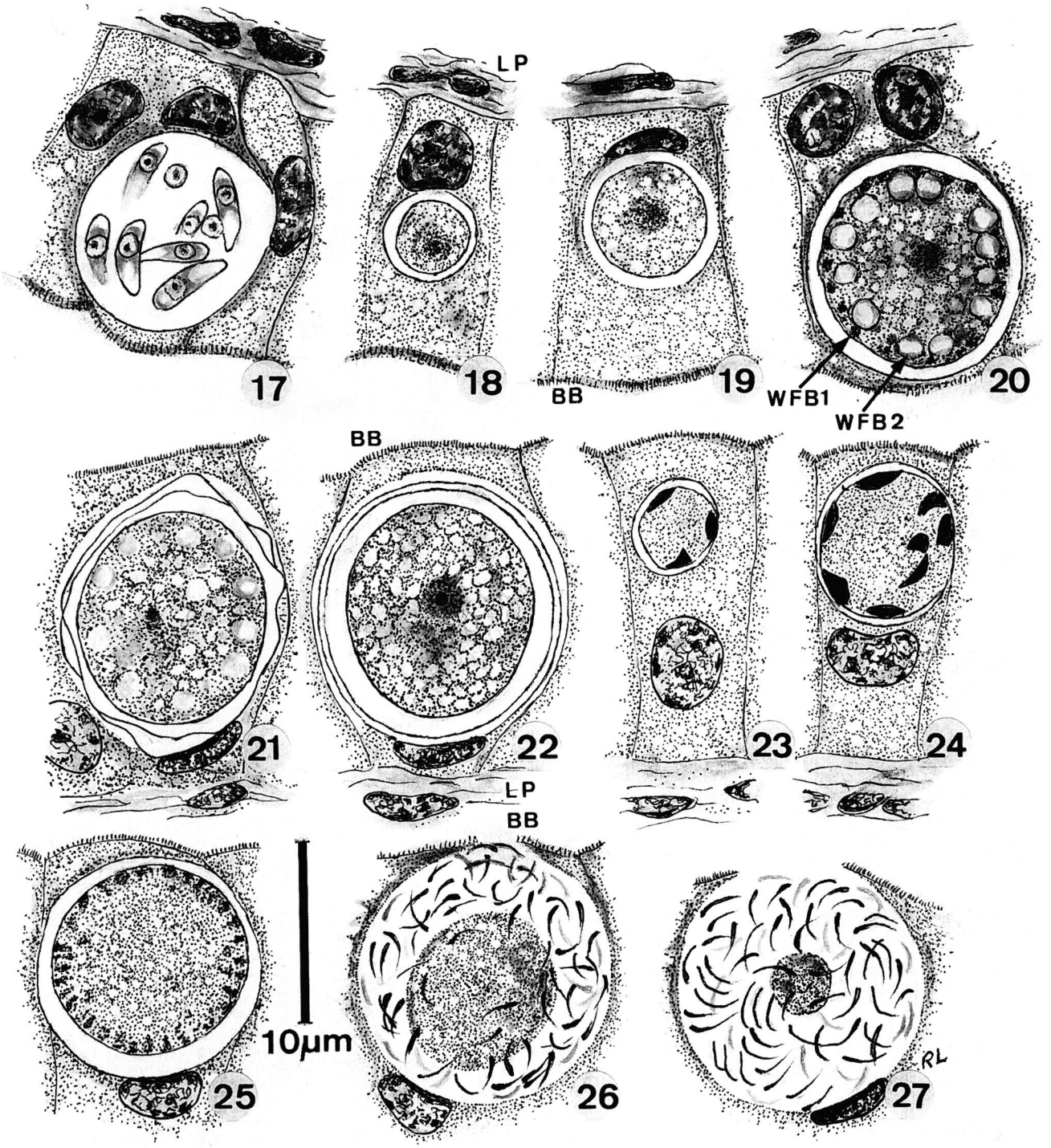


Figs 1-13. – Endogenous stages of *Eimeria bragancaensis* n. sp., in the epithelial cells of the small intestine of the bat, *Peropteryx macrotis*. Figs 1, 2. Developing and segmented meronts. Figs 3-5. Growing microgamonts. Figs 6, 7. Mature microgamonts shedding microgametes. Fig. 8. Young macrogamonts. Figs 9,10. Growing macrogamonts: note large numbers of cytoplasmic inclusions, probably amylopectin granules. Fig. 11. Mature macrogamont. Fig. 12. Young oocyst. Fig. 13. Sloughing of epithelial cells and parasites (arrowed) into the gut lumen. Figs 14-16. – Mature oocysts in the faeces.

P = polar bodies; R = microgamont residuum; SS = Stieda and substiedal bodies; W = inner oocyst wall layer; WFB 1 = small type 1 wall-forming bodies; WFB 2 = large type 2 wall-forming bodies. Bars = 10 µm. That in Fig. 9 also applies to Figs 1-12.



Figs 17-27. – Line-drawings of the endogenous stages of *Eimeria bragancaensis* n. sp. in the small intestine of the bat *Peropteryx macrotis*. Fig. 17. Segmented meront. Figs 18, 19. Developing macrogamonts. Fig. 20. Mature macrogamont. Figs 21, 22. Young oocysts, with thin, inner layer of the oocyst wall. Figs 23-25. Developing microgamonts. Figs 26, 27. Mature microgamonts shedding microgametes and leaving a residuum of variable size.

positive material was only possible several weeks later, by which time most of the oocysts were fully sporulated. We were unable, therefore, to record the sporulation time.

Twenty-five oocysts and 35 sporocysts were measured by normal light-microscopy with a × 100 neofluar objective, × 10 eyepieces and an ocular micrometer. The entire intestine was removed and fixed in 10 % neutral buffered formalin, together with pieces of liver, spleen and lung. Tissues were paraffin-wax embedded and sections, cut at 5 µm, were stained with haematoxylin and eosin. Photomicrographs were prepared using a Zeiss Photomicroscope III and Kodak TMX 100 film. All measurements are given in µm: for the oocysts these are given as means, with the range in parentheses, followed by the shape-index (ratio of length/width).

DESCRIPTIONS

EIMERIA BRAGANCAENSIS N. SP. (Figs 1-29)

Description of the oocyst (Figs 14-16; 28,29). With the characters of the genus. Mature oocysts spherical (50 %) to subspherical, 15.9 × 14.6 (14-17.7 × 14-17.7), shape-index 1.0 (1-1.2). Intact oocyst wall about 1.0 thick and of two layers (Figs 14, 28). The outer layer is prominently striated (pitted), yellow-brown in colour and approximately 0.75 thick. It is frequently lost, leaving only the thin, colourless and non-striated inner layer (Fig. 29). There is no micropyle or oocyst residuum, but one or two polar bodies. These are rather irregular in shape and measure approximately 1-2 × 1. Sporocysts pear-shaped, 8.4 × 5.3 (6.25-9 × 4-6), shape index 1.6 (1.2-1.9), and with inconspicuous Stieda and substiedal bodies. Sporocyst residuum composed of a relatively small number of globules and finer granules. Sporozoites with anterior (smaller) and posterior (larger) refractile bodies.

Endogenous stages (Figs 1-13). Merogony and gametogony stages are intracytoplasmic in the epithelial cells of the small intestine, above the host-cell nucleus. Segmented meronts (Figs 2, 17) averaged 10 × 8: from cross-sections it was estimated that they produce only from 10-20 merozoites measuring approximately 5 × 1.5, and that little or no residuum remains. Young macrogamonts measuring about 4 × 4 are recognizable by their voluminous nucleus containing a prominent karyosome (Figs 8, 9, 18, 19). Mature macrogamonts are about 11 × 10 and, with growth, contain the usual eimeriid small (type I) and large (type II) wall-forming bodies: the latter are particularly conspicuous (Figs 11, 20). The cytoplasm of the growing macrogamonts and zygotes (or young oocysts) becomes packed with small, ovoid, colourless bodies (Figs 10, 20-22) which probably represent the amylopectin granules commonly recorded in the macrogamonts and zygotes of other eimeriids. Early microgamonts (Figs 3, 23, 24) are readily differentiated from young schizonts and macrogamonts by their intensely staining and frequently angular nuclei, located around the periphery of the parasite. Towards maturity the microgamonts measure approximately 10, and produce a large number of microgametes measuring about 3 × 0.5. There is a conspicuous residuum, of variable size (Figs 6, 7,26, 27).

Host: the bat *Peropteryx macrotis* (Wagner, 1843) (Chiroptera: Emballonuridae).

Location in host: in the cytoplasm of the epithelial cells of the small intestine.

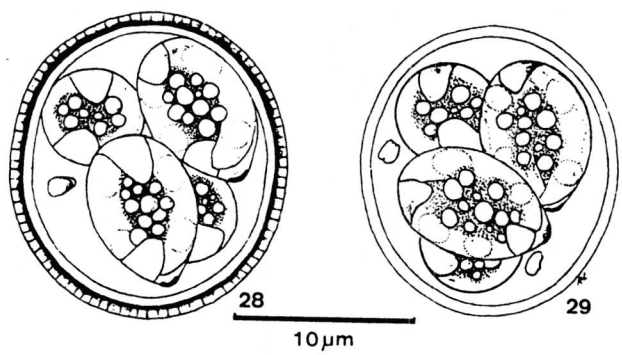
Type locality: primary forest, near Bragança, Pará State, north Brazil (1° 03´S 46° 46´W).

Sporulation time: not ascertained.

Prevalence: uncertain. One of three *P. macrotis* examined was infected.

Pathology: there was considerable sloughing of the intestinal epithelium in the major developmental regions of *E. bragancaensis* in the small intestine, with dissociated cells and parasites present in the gut lumen (Fig. 13). The infected bat, however, appeared to be in good health.

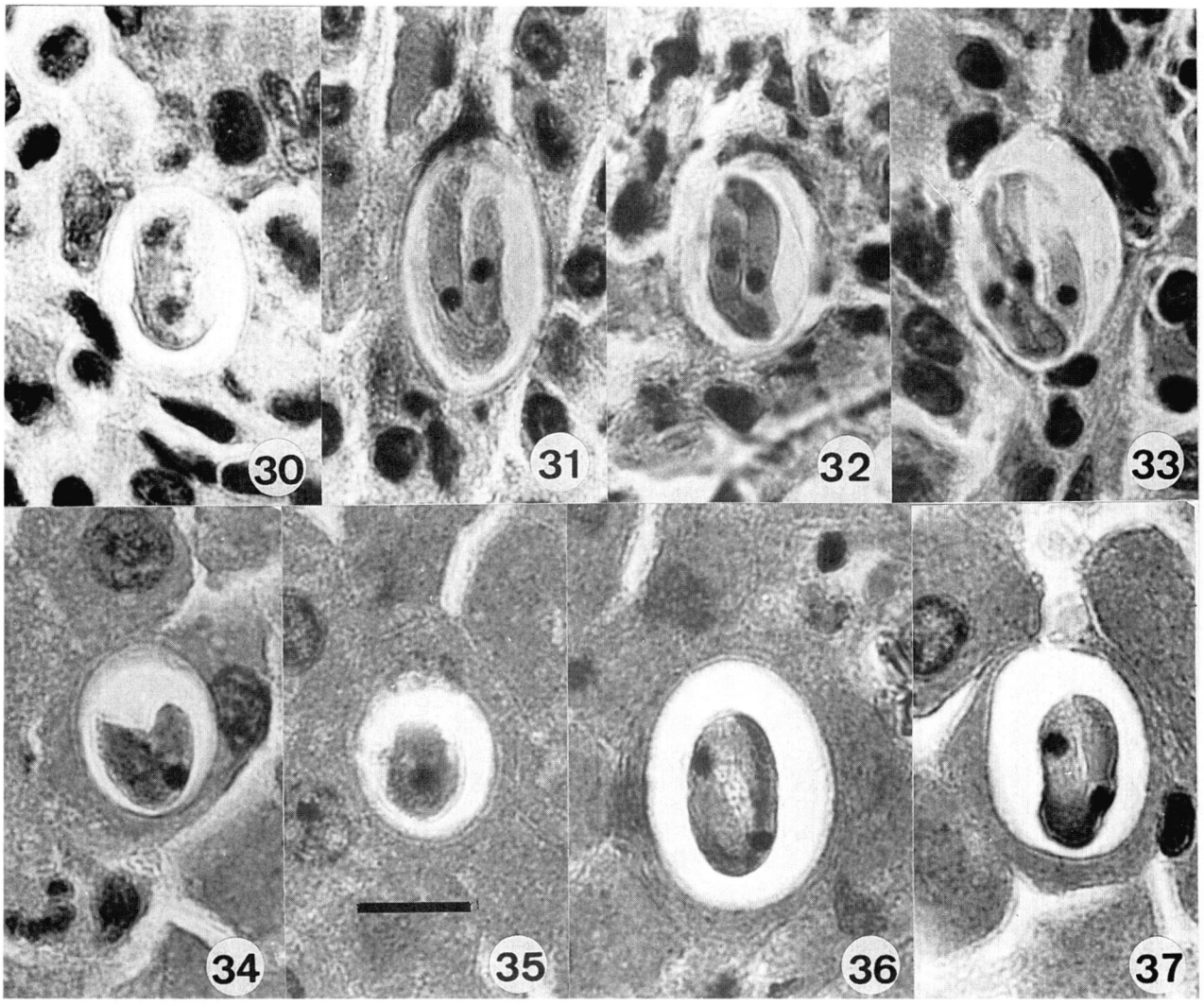
Etymology: The specific name is derived from the type locality of the parasite in Bragança, Pará, north Brazil, where the infected bat was captured.



Figs 28, 29. – Line-drawings of mature oocysts of *Eimeria bragancaensis* n. sp., in faeces of the bat *Peropteryx macrotis*. Fig. 28. Oocyst with intact, two-layered wall. Fig. 29. Oocyst, having lost the thicker, outer striated wall layer.

TISSUE-CYSTS OF AN UNIDENTIFIED PARASITE IN THE LIVER AND *LAMINA PROPRIA* OF THE BAT *PEROPTERYX MACROTIS*

During our examination of histological sections of tissues from the bat infected with *E. bragancaensis* n.sp., we noted the occasional presence of cysts in the *lamina propria* of the small intestine (Figs 30-33), and similar bodies were abundant in parenchyma cells of the liver (Figs 34-37). They measured approximately



Figs 30-33. – Developing tissue-cysts of an unidentified protozoan (probably *Hepatozoon*) in the *lamina propria* of the small intestine of the bat *Peropteryx macrotis*, containing from 2-4 endozoites. Figs 34-37. – Similar cysts in the parenchyma cells of the liver. Sections stained with haematoxylin and eosin. Bar = 10 µm.

16.7 × 13.7 (12.5-22.5 × 8.7-16.2), shape-index 1.2 (1-1.6) for 30 cysts examined, and contained either an undivided parasite with a single nucleus (Figs 34, 35) or stages in the division to form from 2-4 zoites (Figs 30-33, 36, 37). Fully separated zoites measured approximately 12.5 × 2.5.

DISCUSSION

Table I lists the generally accepted species of *Eimeria* previously described from bats. Among a number of features, *Eimeria bragancaensis* n.sp. can be differentiated from these as follows. From *E. andamanensis* Mandal & Nair 1973, *E. dukei* Lavier 1927 and *E. levinei* Bray 1958 by the absence of a micropyle and/or an oocyst residuum: from *E. eumops* Marinkelle 1968, *E. molossi* Lainson & Naiff 1998, *E. rhynchonycteridis* Lainson 1968, *E. vespertilii* Mussaiev & Weissov 1961, *E zakirica* Mussaiev 1967 and *E. mebelyi* Mussaiev & Gauzer 1971 by its much smaller oocysts.

Morphologically, the oocysts of E. *bragancaensis* n. sp., are closest to those of *E. macyi* Wheat 1975 and *E. bessei* Lavier 1924. The oocyst of *E. macyi* is only slightly larger (19 × 17.6, range 16-21 × 15-19), has a striated wall (but which is described as unilayered) and further resembles *E. bragancaensis* n. sp. in having no oocyst residuum, but 1-2 polar bodies. Its sporocysts, however, are considerably larger (11 × 7, range 10-12 × 6-8 versus 8.4 × 5.3, range 6.2-9 × 4-6). In addition, the sporocysts have "prominent" Stieda and substiedal bodies, which contrast markedly with the inconspicuous ones of *E. bragancaensis* n. sp. The

Species	Host	Measurements (µm), morphology[a], oocyst	Measurements (µm), Sporocyst[b]
E. andamanensis Mandal & Nair 1973	*Taphozous melanopogon*	M, OR	?
E. dukei Lavier 1927	*Nyctinomous limbatus, N. pumilus*	20-24, S; 23-25 × 18-22, O; OR, W1	7-9 × 6-7
E. eumopos Marinkelle 1968	*Eumops trumbulli*	34.9 × 28 (34-36 × 27-28), O-S; PP, W2	11 × 7.9 (10.5-11.8 × 6.5-8.1), St
E. hessei Lavier 1924	*Rhinolophus hipposideros*	16-22, S & 15-18 × 13-15, O; W1	11 × 5.5
E. levinei Bray 1958	*Tadarida bemmelini*	21.6 × 18.2 (19-24 × 17-19), O, M, W1	8.5 × 7.4 (8-9 × 7-8) St
E. macyi Wheat 1975	*Pipistrellus subflavus*	19 × 17.6 (16-21 × 15-19), E, P, W1	11 × 7 (10-12 × 6-8) St, Sb
E. mehelyi Mussaiev & Gauzer 1971	*Rhinolophus mehelyi*	Larger than 19 × 17.6 (Wheat, 1975)	?
E. molossi Lainson & Naiff 1998	*Molossus ater*	23.4 × 17.5 (18-30 × 15-22.5), E, PP, W3	10.2 × 7.5 (10-12.5 × 7.5), St
E. rhynchonycteridis Lainson 1968	*Rhynchonycteris naso*	25.5, S; W1	15.2 × 8.1; St, Sb
E. vespertilii Mussaiev & Weissov 1961	*Vespertilio kublii*	25.1 × 22 (20-27 × 18-25), S-SS; O, W2, P	6-10 × 4-6, St
E. zakirika Mussaiev 1967	*Vespertilio kublii*	25 × 22.5 (20-30 × 16-26), O; P, W1	11.2 × 7.6 (8-14 × 6-10), St
E. bragancaensis n. sp. (this paper)	*Peropteryx macrotis*	15.9 × 15.2 (14-17.7 × 12.5-17.7), S-SS; W2	8.4 × 5.3 (6.2-9 × 4-6), St, Sb

[a] E = ellipsoidal; M = with micropyle; O = ovoid; OR = with oocyst residuum; P/PP = with polar body (ies); S = spherical; SS = subspherical; W = number of oocyst wall layers.
[b] St = with Stieda body; Sb = with substiedal body.

Table I. – *Eimeria* species recorded in bats.

endogenous stages of *E. macyi* were not described. The oocyst of *E. hessei* has the large size range of 16-22 for spherical forms and 15-18 × 13-15 for those described as "ovoid". According to Lavier's description and illustrations, there is a single-layered, non-striated wall, and the oocyst contains no polar bodies. Measurements of sporocysts are not given in text, but from the µm scale accompanying the illustrations they are approximately 11 × 5.5 and larger, therefore, than those of *E. bragancaensis* n. sp: no Stieda or substiedal bodies are figured. Additional differential features could not be found when comparing the endogenous stages of the two parasites.

The mature oocysts of *E. bragancaensis* n. sp. are fragile and soon become considerably deformed (Fig. 15). In many cases the thicker, striated wall becomes detached, with the resulting impression that there are two different oocysts: one with a two-layered, brown and striated wall, and another with a single, thin and colourless wall which has no striations (Figs 28, 29). The frequent loss of one or more layers of the oocyst wall of a given coccidian may create difficulties or even errors in diagnosis, as the nature of the wall is a character commonly used to separate species (e.g. "rough" *versus* "smooth"; Wheat, 1976). When such modifications of the oocyst wall are suspected, particular attention needs to be given to all additional morphological characters.

We are of the opinion that the tissue-cysts present in the *lamina propria* of the small intestine and, more frequently, in the parenchyma cells of the liver, are not extra-intestinal stages of *E. bragancaensis* n. sp., but that they belong to another unidentified protozoan parasite. They bear a striking resemblance to the latent cysts ("kystes endogéniques") of *Hepatozoon*, found in various organs of snake and lizard hosts, particularly the liver (Landau *et al.*, 1972). In the lizard, these cysts arise when the animal ingests an infected arthropod vector (e.g. a mosquito) and sporozoites invade cells of the liver and other organs. Here they encyst and produce a small number of endozoites (usually from 2-6) by the process of endodyogeny. These latent infective forms then serve as a source of infection for snakes preying on the lizards.

The absence of haemogregarines in the blood of the bat under study suggests that it is not the definitive mammalian host, and that the cysts and contained

endozoites may represent a "dead-end" for the parasite. *Hepatozoon*, probably *H. didelphydis* (d'Utra e Silva & Arantes, 1916), has been recorded in a number of neotropical marsupials including *Didelphis marsupialis, Philander opossum*, and *Metachirus nudicaudatus* (d'Utra e Silva & Arantes, 1916; Regendanz & Kikuth, 1928; Garnham & Lewis, 1958; Deane & Deane, 1961; Ayala *et al.*, 1973): in addition, another unnamed species has been noted in specimens of the cricetid rodents *Holochilus brasiliensis* and *Oryzomys capito* from Amazonian Brazil (Lainson, 1974), and unidentified haemogregarines have also been recorded in Amazonian birds (Lainson *et al.*, 1970) All of these hosts are abundant in north Brazil, and as *P. macrotis* is an insectivorous bat, its infection may well have originated following the ingestion of mosquitoes or another insect vector carrying the sporozoites of any one of these parasites. Oocysts containing mature sporocysts, typical of the genus *Hepatozoon* have been encountered in a freshly dissected phlebotomine sandfly, *Lutzomyia* sp., from Pará (unpublished observation). Finally, if the parasite can tolerate the change from a cold-blooded to a warm-blooded host there could be a snake-bat-snake life-cycle for the parasite. The infected bat was resting in a hollow tree-trunk – a favourite retreat for some snakes.

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