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Eimeria spp. (Apicomplexa: Eimeriidae) in Black Caiman *Melanosuchus niger* (Crocodilia: Alligatoridae) from the Amazon Region, Brazil, with a Description of Two New Coccidian Species

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Abstract. During November 2011, faeces from 23 black caimans (*Melanosuchus niger*) were collected from the Brazilian Amazon, and coccidians were found in 83% of the individuals examined. Three *Eimeria* species were found in the black caiman faeces, two of which were recognised as new species and are subsequently described in the present study. *Eimeria paraguayensis* was found in 17 of 23 animals sampled. *Eimeria nigeri* n. sp were found in 16/23 animals examined. Oocysts were spherical to sub-spherical, 29.2 × 28.1 (23–32 × 23–30) µm, and 12.1 × 11.0 µm spherical oocyst residuum was present. Sporocyst was ovoid, 15.5 × 9.7 (8–11 × 12–17) µm, and had a Stieda body at a slightly pointed end. The sporocyst residuum consisted of a compact mass of refractile granules. *Eimeria portovelhensis* n. sp. were found in 16/23 animals examined. Oocysts were spheroidal, 19.5 × 19.1 (16–21) µm, and had 9.0 × 7.8 µm spherical oocyst residuum present. The spindle-shaped sporocysts were 11.0 × 4.0 (8–13 × 2–5) µm and had two apparent Stieda bodies, one at each pole and with one filament curved at each pole. Sporocyst residuum was present, with four or five small refractile granules. The two new species are differentiated from the five species of *Eimeria* recorded within the crocodilian family Alligatoridae.

Key words: Coccidia, Amazon, crocodilian, Rondonia, *Melanosuchus.*

INTRODUCTION

Order Crocodylia contains only nine described species and two species of the genus *Eimeria* that has not been formally described (Duszynski 2013). In South America, *Eimeria crocodyli* Lainson, 1968 and *Isospora wilkiei* Lainson, 1968 have been isolated from *Crocodylus acutus* Cuvier, Belize. In Brazil, *E. caimani* Aquino-Shuster and Duszynski, 1989 and *E. paraguayensis* Aquino-Shuster and Duszynski, 1989 have been isolated from *Caiman yacare* Daudin, and *I. jacarei* Carini and Biocca, 1940 was found to parasitise *C. latirostris* Daudin. *E. pintoi* Carini, 1932 must be considered a *species inquirenda*, needing further investigation

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because neither a line drawing nor a photomicrograph was published, and the host was never classified beyond being a *Caiman* spp.

Eimerians found in *C. yacare* have been described for a population of this crocodilian in Paraguay (Aquino-Shuster and Duszynski 1989), and the same eimerians were recently reported for the same host in Brazil, in the state of Mato Grosso do Sul's Pantanal region (L. Viana, unpublished date). These data show that information about coccidians in crocodilians remains limited in Brazil and throughout the world.

The black caiman, *Melanosuchus niger* Spix, is the largest Neotropical crocodilian, reaching a total length of approximately 4–5 metres (Ross 1998). The black caiman is an opportunistic predator whose diet includes vertebrates such as amphibians, birds and small mammals (Horna *et al*. 2001). The species has a wide distribution in the Amazon area, including regions of Ecuador, Colombia, French Guiana, Peru, Bolivia, and Brazil (Thorbjarnarson 2010). Population studies on the black caiman in the Brazilian Amazon region were conducted together with experimental culling for a controlled exploration of *M. niger*, targeting the meat and skin markets. This exploration provided easy access to crocodilian faecal samples, which would be difficult to obtain under normal conditions in nature. The current study reports the coccidians found in association with the population of the black caiman *M. niger* from the Amazon region, Brazil, and includes a description of two new *Eimeria* species.

MATERIALS AND METHODS

The study was conducted in November 2011 at the Extractive Reserve Lake Cuniã (RESEX Cuniã) (08°19′05.0″S and 63°29′48.9″W), which is located in the municipality of Porto Velho, State of Rondonia, Brazil. Wild caimans were captured using a noose on a pole and taken to a processing house registered by the Sanitary Inspection of Porto Velho (number 0010/2011), which was built specifically to process caimans. The caimans were euthanised at the alligator slaughter house in the Silva Lopes Araujo Community, near the RESEX headquarters. The animals were anesthetised using a ZILKA airgun (Campos 2000) and then euthanised by cutting the sinus venosus. Then, the faeces were removed directly from the rectum.

The collected samples were placed in plastic vials containing 2.5% (w/v) $K_2Cr_2O_7$ solution in a 1:6 ratio (v/v). Samples were analysed at the Laboratório de Ecologia de Vertebrados, Universidade do Estado do Rio de Janeiro (UERJ). The faecal material was placed on a thin layer (~ 5 mm) of 2.5% $K_2Cr_2O_7$ solution in Petri dishes, and incubated at 23–28°C for two weeks. Oocysts were recovered by flotation in Sheather's sugar solution (S.G. 1.20) and microscopically examined using the technique described by Duszynski and Wilber (1997).

Morphological observations and measurements (in µm) were performed using a Carl Zeiss binocular microscope with an apochromatic oil immersion objective lens and (MOV K15X) ocular micrometre. Oocysts were photographed with a Zeiss universal photomicroscope equipped with Nomarski-interference 100 × objective lenses. The size ranges are shown in parentheses, followed by the average and shape index (L/W ratio). Abbreviations: micropyle [M], oocyst residuum [OR], polar granule [PG], Stieda body [SB], Substieda body [SSB], parastieda body [PSB], sporocyst residuum [SR], sporozoite [SZ], refractile body [SRB], nucleus [N], length [L] and width [W].

This study was approved by SISBIO (Authorization System and Information on Biodiversity) and the Brazilian Ministry of Environment under permit number 10633-9.

RESULTS

We took one sample from each of the 23 black caimans, leading to a final sample size of 23. Nineteen of the examined samples (83%) were found to contain coccidians, representing three *Eimeria* species. The prevalences were 74% for *E. paraguayensis*, 70% for *E. nigeri* n. sp., and 17% for *E. portovelhensis* n. sp. Mixed infections were found in 79% of the black caimans examined, with most coinfections caused by *E. paraguayensis* + *E. nigeri* (N = 11; 58%) (Table 1).

Two of the coccidians examined differed consistently from previously described species, suggesting that they might represent two novel species. Below, we describe these two new *Eimeria* species. The morphometric and morphological features from the *Eimeria* spp. oocysts found in the black caimans, as well as those from *E. nigeri* n. sp. and *E. portovelhensis* n. sp. are presented in Table 2.

Eimeria nigeri n. sp. (Figs 1–2)

Description of sporulated oocysts: Oocysts spheroidal to subspheroidal, wall composed of 2 layers (n = 49), ~1.2 (1.0–1.3) thick; outer is slightly sculptured, colorless, ~ 2/3 of total thickness, while inner appears as a dark line; sporulated oocysts (n = 50) 29.2 × 28.1 (23–32 × 23–30), L/W ratio 1.0 (1.0–1.1); M, PG: both absent; OR: mostly spheroidal globule, 12.1 × 11.0 (9.3–14 × 8–13), composed of at least 3 different globules, each inside the next larger body. Distinctive feature of oocyst: the complex OR composed of multiple bodies.

Description of sporocysts and sporozoites: Sporocysts ovoidal, slightly pointed at one end, (n = 48)

Table 1. The prevalence of simple and mixed infections of *Eimeria* spp. sampled from 23 black caimans from the Reserva Extrativista Lago Cuniã (RESEX Cuniã), within the Brazilian state of Rondonia.

Infection	Species	N	Prevalence (%)
Simple	*E. paraguayensis*	3	16
	E. nigeri	1	5
	E. portovelhensis	0	0
	Total	4	21
Mixed	*E. paraguayensis* + *E. nigeri*	11	58
	E. paraguayensis + *E. nigeri* + *Eimeria portovelhensis*	3	16
	E. nigeri + *Eimeria portovelhensis*	1	5
	Total	15	79

15.5 × 9.7 (12–17 × 8–11), L/W ratio 1.6 (1.2–2.0); SB: prominent, 2.2 × 1.7 (1–3 × 1–2); SSB, PSB: both absent; SR: condensed group of large globules that appear to be enclosed by a dense matrix of tiny granules. Distinctive feature of sporocyst: SR of large globules that may be membrane bound. Sporozoite with 2 large refractile bodies spheroid and located in the middle and posterior end.

Type-host: The black caiman *Melanosuchus niger* Spix, 1825 (Crocodylia, Alligatoridae).

Type-locality: Extractive Reserve Lake Cuniã (08°19′05.0″S and 63°29′48.9″W), Porto Velho County, Rondonia State, Brazil.

Site of infection: Not investigated.

Sporulation: Unknown. Oocyst suspensions were kept in vials of 2.5% $K_2Cr_2O_7$ solution for two weeks prior to examination.

Prevalence: 16/23 (70%).

Etymology: The specific name is designated by specific name of the host.

Type-specimens: One-half of the oocysts were kept in 10% aqueous buffered formalin (v/v) and the other half in 70% ethanol, according to Duszynski and Gardner (1991). Both samples were deposited in the Parasite Collection at the Department of Animal Parasitology, which is located at the Universidade Federal Rural do Rio de Janeiro, within the municipality of Seropédica, Rio de Janeiro, Brazil. Phototypes and line drawings were deposited as well, under repository number P-43/2012.

Remarks

Only five *Eimeria* species (*E. alligatori*, *E. hatcheri*, *E. paraguayensis*, *E. pintoi* and *E. caimani*) were previously recorded to parasitise crocodilians of the Alligatoridae family (Table 2). The *Eimeria nigeri* oocysts have a slightly rough wall and can be readily differentiated from the smooth *E. crocodyli* and *E. hatcheri* oocysts. Another characteristic of *E. nigeri* is the subspheric formation of their oocysts, which differs from the ellipsoidal oocysts of *E. paraguayensis, E. pintoi* and *E. alligatori.* The most similar coccidia to *E. nigeri* are those of *E. caimani* from the caiman *C. yacare*. However, in *E. caimani*, the oocyst is spherical and consistently smaller (22.4) than that of *E. nigeri*, which has spheroidal to subspheroidal oocysts with dimensions of 29.2 × 28.1. Additionally, *E caimani* has a markedly rougher oocyst wall than the slightly sculptured *E. nigeri* oocyst wall. The differential characteristics of the oocysts described above are consistent to designate *E. nigeri* as a new species.

Eimeria portovelhensis n. sp. (Figs 3–4)

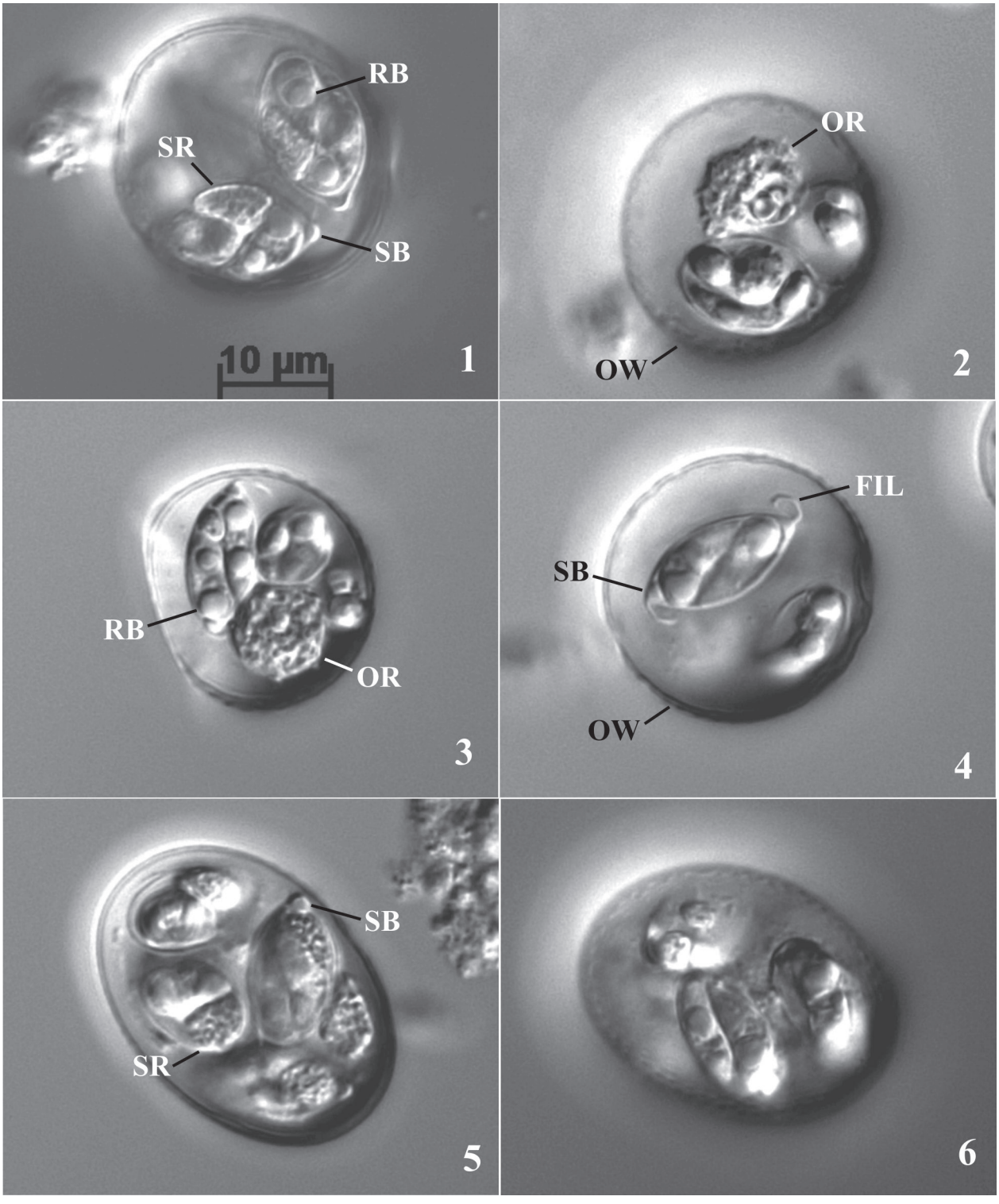
Description of sporulated oocyst: Oocysts spheroidal, wall composed of two layers (n = 50), ~ 0.9 (0.9–1.1) thick; outer is slightly sculptured, colorless, inner appears as a slightly dark line. Sporulated oocysts (n = 50) 19.5 × 19.1 (16–21), L/W ratio 1.0 (1.0); M, PG: both absent; OR: spheroidal globule, 9.0 × 7.8 (3–12 × 2–12), composed of globules of different size.

Description of sporocyst and sporozoites: Sporocysts spindle-shaped, (n = 48) 11.0 × 4.0 (8–13 × 2–5), L/W ratio 2.8 (2.6–4.5); SB: apparently double, one at each pole and with one filament curved at each pole; SSB, PSB: both absent; SR: spheroid compact mass composed of tiny granules. Distinctive feature of sporocyst: spindle-shaped and SB with one filament curved. Sporozoite with 2 large refractile bodies spheroid and located in middle and at posterior end.

Table 2. Comparative morphology of *Eimeria* spp. recorded in different species within the crocodilian family Alligatoridae.

			Oocysts					Sporocysts			
Coccidia	Host	Reference	Shape	Measurements (µm)	Shape index	Wall (µm)	Oocyst residuum (µm)	Shape index	Measurements (µm)	Stieda body	Substieda body
E. alligatori	*Alligator mississippiensis*	McAllister and Upton 1990	Ellipsoidal	25.3 × 20.0 (22–31 × 18–22)	1.3 (1.1–1.6)	One-layered, pitted, 0.6	Spherical, 13.2 (10–15)	1.8 (1.6–2.1)	14.3 8.1 (12–15 × 7–9)	Present	Absent
E. hatcheri	*Alligator mississippiensis*	McAllister and Upton 1990	Spheroid or sub-spheroid	16.1 × 13.7 (12–18 × 12–15)	1.2 (1.0–1.4)	One-layered, smooth, 0.6	Subspheroid, 6.4 × 6.1 (5–7 × 5–7)	1.8 (1.5–2.1)	10.5 × 5.8 (10–13 × 5–6)	Present, as minute point	Absent
E. paraguayensis†	*Caiman yacare*	Aquino-Shuster and Duszynski 1989	Ellipsoidal	34.0 × 23.6 (26–38 × 20–29)	1.4 (1.1–1.7)	Bi-layered, pitted, 1.0	Present, granules that form a diffuse, irregular mass ≤15 in diameter	1.9 (1.5–2.4)	14.0 × 7.1 (10–19 × 6–10)	Present	Absent
E. pintoi†	*Caiman* sp.	Carini 1932	Ellipsoidal	30–33 × 20–22	?	?	?	?	?	?	?
E. caimani	*Caiman yacare*	Aquino-Shuster and Duszynski 1989	Spheroid	22.4 (19–29)	1.0	Bi-layered, pitted, 1.0	Compact spheroidal mass 9–10	1.9 (1.2–2.1)	12.9 × 6.5 (8–17 × 5–8)	Present, as slight point end	Absent
E. nigeri n. sp.	*Melanosuchus niger*	Current work	Spheroid or sub-spheroid	29.2 × 28.1 (23–31 × 23–30)	1.0 (1.0–1.1)	Bi-layered, outer is slightly rough, 1.2	Spherical, 12.3 × 11.0 (9–17 × 8–13)	1.6 (1.2–2.0)	15.5 × 9.7 (12–17 × 8–11)	Present	Absent
E. portovelhensis n. sp.	*Melanosuchus niger*	Current work	Spheroid or sub-spheroid	19.5 × 19.1 (16–21 × 16–21)	1.0 (1.0)	One layer, outer is slightly rough, 0.9 (0.9–1.1)	Spherical, 12.3 × 11.0 (9–17 × 8–13)	2.8 (2.6–4.5)	10.9 × 3.8 (8–13 × 1–5	Absent	Absent

† May be synonyms (McAllister and Upton 1990).



Figs 1–6. Nomarski interference micrographs of sporulated coccidia oocysts found in black caimans faeces × 1,000. **1–2** – *Eimeria nigeri* n. sp.; **3–4** – *Eimeria portovelhensis* n. sp.; **5–6** – *Eimeria paraguayensis*. FIL – filament from the area of the Stieda body, OR – oocyst residuum, OW – oocyst wall, RB – refractile body, SB – Stieda body, SR – sporocyst residuum.

Type-host: *Melanosuchus niger* Spix, 1825 (Crocodylia, Alligatoridae).

Type-locality: Extractive Reserve Lake Cuniã (08°19′05.0″S and 63°29′48.9″W), located within Porto Velho County in the Rondonia state of Brazil.

Site of infection: Not investigated.

Sporulation: Unknown. Oocyst suspensions were kept in vials of 2.5% $K_2Cr_2O_7$ solution for two weeks prior to discovery.

Prevalence: 4/23 (17%).

Etymology: The name combines the name of the county from which the infected animals were taken and "–ensis" (belonging to).

Type-specimens: One-half of the oocysts were kept in 10% aqueous buffered formalin (v/v) and the other half in 70% ethanol, according to Duszynski and Gardner (1991). Both samples were deposited in the Parasite Collection at the Department of Animal Parasitology, Universidade Federal Rural do Rio de Janeiro, which is located in the municipality of Seropédica, Rio de Janeiro, Brazil. Phototypes and line drawings were deposited as well under the repository number P-44/2012.

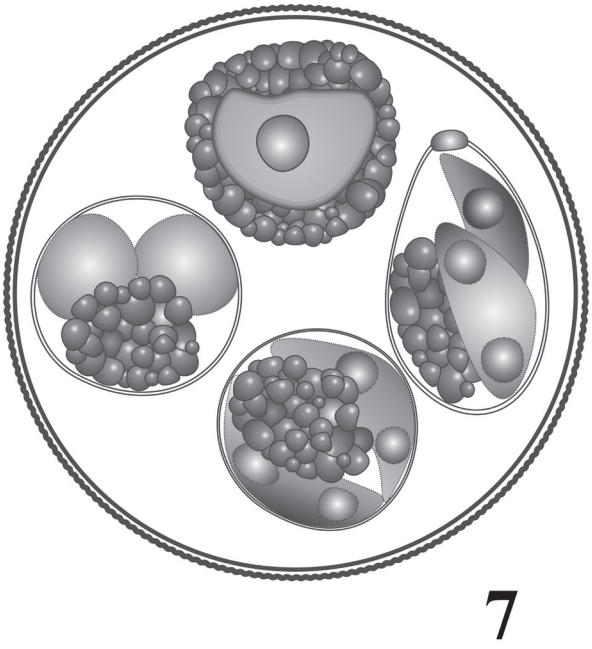
Remarks

E. portovelhensis is essentially different from any other known species of *Eimeria* found in crocodilians. Distinctive aspects are SB apparently double, one at each pole and with one filament curved at each pole.

DISCUSSION

The discovery of *E. paraguayensis* in *M. niger* was an unexpected finding because its host type, the *C. yacare* alligator, does not occur in the Amazon region. However, *C. yacare* and *M. niger* crocodilians occur sympatrically in Bolivia (Aguilera *et al*. 2008), which reinforces the identification of *E. paraguayensis* in *M. niger*. The *E. paraguayensis* oocysts described in this study were slightly smaller than those described by Aquino-Shuster and Duszynski (1989) (30.5 × 23.2 vs. 34.0 × 23.6), with sporocyst sizes of 14.4 × 8.7 vs. 14.0 × 7.1, respectively. All other morphological characteristics were identical.

Brazil has the largest number of crocodilians species in the world, with 6 species in 3 genera (*Caiman*, *Melanosuchus*, and *Paleosuchus*) (Bérnils and Costa 2011). The present study constitutes the first record of coccidia in caimans from the genus *Melanosuchus* and expands the number of hosts for *E. caimani*. It is possible that the diversity of coccidia in crocodilians in Bra-



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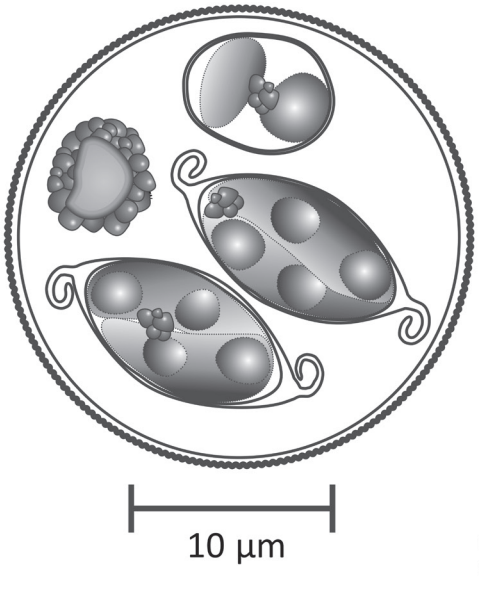

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Figs 7–8. Line drawings of sporulated coccidian oocysts collected from black caiman faeces. **7** – *Eimeria nigeri* n. sp.; **8** – *Eimeria portovelhensis* n. sp.

zil is underestimated because *C. crocodilus, P. palpebrosus* and *P. trigonatus* species are also present in the Amazon (Rebêlo and Lugliv 2001). Therefore, efforts should be carried out to sample the crocodilian species mentioned above for the presence of coccidian parasites.

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REFERENCES

Aguilera X., Coronel J. S., Oberdorff T., Van Damme P. (2008) Distribution patterns, population status and conservation of *Melanosuchus niger* and *Caiman yacare* (Crocodylia, Alligatoridae) in oxbow lakes of the Ichilo river floodplain, Bolivia. *Rev. Biol. Trop.* **56:** 909–929

Aquino-Shuster A. L., Duszynski D. W. (1989) Coccidian parasites (Apicomplexa: Eimeriidae) from two species of crocodilian, *Caiman yacare* Daudin and *Caiman latirostris* Daudin, (Alligatoridae) from Paraguay, South America. *J. Parasitol.* **75:** 348–352

Bérnils R., Costa H. C. (2011) (org.). Brazilian reptiles – List of species. Sociedade Brasileira de Herpetologia. http://www.sbherpetologia.org.br/. [Accessed 13 December 2011]

Campos Z. (2000) The "Zilka", a new device for humane killing of crocodilians. *CSG Newsletter* **19:** 20–21

Carini, A. (1932) *Eimeria pintoi* n. sp. parasita de um jacare. *Sociedad Argentina de Patologie Regional del Norte* **2:** 922–923

Carini A., Biocca E. (1940) Uma nova Isospora do intestino do jacare. *Archiv. Biol.* São Paulo **24:** 252−253

Duszynski D. W. (Org.) (2013) Coccidia (*Eimeria* and *Isospora*) of Crocodilians. In Coccidia of the World. http:// biology.unm.edu/biology/coccidia/home.html [Accessed 25 April 2013]

Duszynski D. W., Gardner, S. L. (1991) Fixing coccidian oocysts is not an adequate solution to the problem of preserving protozoan type material. *J. Parasitol.* **77:** 52−57

Duszynski D. W., Wilber P. G. (1997) A guideline for the preparation of species descriptions in the Eimeriidae. *J. Parasitol.* **83:** 333–336

Horna J. V., Cintra R., Ruesta P. V. (2001) Feeding ecology of black caiman *Melanosuchus niger* in a western Amazonian forest: the effects of ontogeny and seasonality on diet composition. *Ecotropica* **7:** 1–11

McAllister C. T., Upton S. J. (1990) The coccidia (Apicomplexa: Eimeriidae) of Crocodilia, with descriptions of two new species from *Alligator mississippiensis* (Reptilia: Alligatoridae), from Texas. *J. Parasitol.* **76:** 332–336

Rebêlo G. H., Lugliv L. (2001) Distribution and abundance of four caiman species (Crocodylia: Alligatoridae) in Jaú National Park, Amazonas, Brazil. *Rev. Biol. Trop.* **49:** 1095–1109

Ross J. P. (1998) Crocodiles: Status Survey and Conservation Action Plan. 2nd ed. Crocodile Specialist Group IUCN/SSC (eds.). Gland, Switzerland. 96 pp.

Thorbjarnarson J. B. (2010) Black Caiman *Melanosuchus niger*. In: Crocodiles. Status Survey and Conservation Action Plan. 3rd ed. (Eds. Manolis S. C., Stevenson C. and Crocodile Specialist Group). Darwin. pp. 29–39. Available from: http://www.iucncsg.org/ph1/modules/Publications/ActionPlan3/ap2010_06.html

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