

Eimeria lepidosirenis n.sp. (Apicomplexa:Eimeriidae) of the South American lungfish *Lepidosiren paradoxa* (Osteichthyes:Dipnoi) from Amazonian Brazil

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The mature oocysts of Eimeria lepidosirenis n.sp. are described in faeces removed from the lower region of the intestine of a single specimen of the South American lungfish Lepidosiren paradoxa, from Belém, state of Pará, Amazonian Brazil. Oocysts with endogenous sporulation: spherical to slightly subspherical, 30.8 × 30.3 μm (28.1 × 25.9 -33.3 × 31.8), shape-index (ratio length/width) 1.0, n = 25. Oocyst wall a very thin, single layer approximately 0.74 μm thick, smooth, colourless, with no micropyle and rapidly breaking down to release the sporocysts. Oocyst residuum a bulky ovoid to spherical mass of approximately 20.0 × 15 μm, composed of fine granules and larger globules and enclosed by a very fine membrane: no polar bodies seen. Sporocysts 15.5 × 9.0 μm (14.5 × 8.0 - 16.0 × 9.0), shape index 1.7 (1.6-1.8), n = 30, ovoid, with one extremity rather pointed and with a very delicate Stieda body but no sub-Stieda body: sporocyst wall a single extremely thin layer with no valves. Sporocyst residuum a spherical to ovoid mass of approximately 5.0 × 4.0 μm, composed of fine granules and small globules and enclosed by a very fine membrane. Sporozoites strongly recurved at their ends and apparently with only a single refractile body. Site of development in the host uncertain: no evidence of endogenous stages was found in fresh scrapings and stained smears of the intestinal epithelium.

Key words: *Eimeria lepidosirenis* n.sp. - oocysts - *Lepidosiren paradoxa* - lungfish - Brazil

As far as we are aware, only two protozoan parasites have been described from the South American lungfish, *Lepidosiren paradoxa*: namely, *Haemogregarina lepidosirenis* (Adeleina: Haemogregarinidae) in the erythrocytes (Jepps 1927), and *Agarella gracilis* (Myxosporea: Chloromyxidae) in the testes (Dunkerly 1915). We describe here the mature oocysts of a hitherto undescribed species of *Eimeria* in faecal material removed from the lower part of the intestine of a single specimen of *L. paradoxa*.

Lungfishes (Superorder Dipnoi, within the Class Osteichthyes – the bony fishes) are represented by only four living genera. In Australia by the monotypic *Neoceratodus forsteri*; in Africa by a few species of *Protopterus*; and in South America by the single species, *Lepidosiren paradoxa*. All are fresh-water fishes, inhabiting swampy regions that flood in the rainy season and dry up when there is little rainfall. During the latter season, when their habitat may dry up completely, *Protopterus* and *Lepidosiren* hibernate within an aestivation chamber situated at the far end of a tube-like burrow excavated in the drying mud, often for as long as six months, before re-emerging when the area is again flooded during the next rainy season.

MATERIALS AND METHODS

The infected lungfish was one of several caught by baited hook and line in an area known as “Terra Firme” on the outskirts of the city of Belém, state of Pará, north Brazil, and being examined by one of us (LR) during a study on the blood of free-swimming and hibernating *L. paradoxa*.

Immediate microscopical examination of an aqueous suspension of faecal material from the lower part of the intestine revealed fully developed coccidial oocysts containing four sporocysts. Further material was then suspended in 2% aqueous potassium dichromate ($K_2Cr_2O_7$) and maintained in a loosely covered Petri dish, at room temperature, for subsequent study. Scrapings of the epithelium of the intestine were examined in physiological saline, under a coverslip, for evidence of any developmental stages of the parasite, and smears of the same material were fixed in absolute methyl alcohol and stained by Giemsa’s method.

Oocysts and sporocysts were measured using a × 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 in μm and given as means with the range in parentheses, followed by the shape-index (ratio of length/width) and the number measured (n).

RESULTS

Eimeria lepidosirenis n.sp.
(Figs 1-4)

The oocysts - In unsporulated forms the sporont fills the oocyst completely (Fig. 1). Mature oocysts (Figs 2-4) are spherical to very slightly sub-spherical and average 30.8

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$\times 30.3$ ($28.1 \times 25.9 - 33.3 \times 31.8$), shape-index (ratio of length/width) 1.0, $n = 25$. The wall is an extremely thin, single layer which is smooth, colourless, approximately 0.74 thick, and with no micropyle. It is very fragile and rapidly breaks down to liberate the sporocysts. The oocyst residuum is a bulky, ovoid to spherical mass of approximately 20.0×15.0 and composed of a mixture of fine granules and larger globules enclosed in a fine membrane. No polar bodies were seen in the 25 oocysts studied.

The sporocysts - The four sporocysts are ovoid, with one extremity rather pointed and bearing a delicate Stieda body, but no sub-Stieda structure. They average 15.5×9.0 ($14.5 \times 8.0 - 16.0 \times 9.0$, shape index 1.7 (1.6-1.8), $n = 30$, and have a delicate, colourless wall of a single layer and with no dehiscence valves. The two sporozoites of each sporocyst are strongly recurved at their ends, and seemingly with only a single refractile body. The sporocyst residuum, of about 5.0×4.0 , is a spherical to ovoid mass of fine granules and small globules contained in a delicate membrane and usually lying between the two sporozoites.

Sporulation - Oocysts are expelled fully sporulated in the faeces.

Site of development - Uncertain. No developmental stages were detected in the intestine and may be in other tissues, such as those of the testes or kidneys.

Host - The lungfish *Lepidosiren paradoxa* (Osteichthyes: Dipnoi).

Type locality - Terra Firme, outskirts of Belém, Pará, north Brazil.

Type material - Phototypes in the Department of Parasitology, Instituto Evandro Chagas. Due to the rapid disintegration of the oocysts, formalin fixed oocysts are not available.

Pathology - The infected fish showed no signs of disease due to this parasite.

Prevalence - Uncertain. Only two fish were specifically examined for coccidial oocysts and only one was infected.

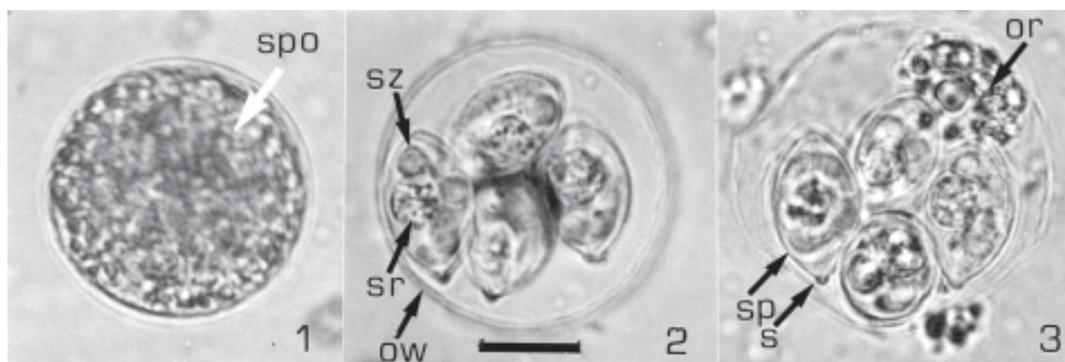
Etymology - The specific name is formed from the generic name of the host lungfish.

DISCUSSION

The oocysts of *E. lepidosirenis* n.sp., exhibit a number of the characteristics which are peculiar to picine coccidia in general, namely their endogenous sporulation and very fragile wall which is without a micropyle and rapidly breaks down to release the sporocysts. Among the named genera possessing four dizoic sporocysts they can readily be separated from the oocysts of *Calyptospora*, the sporocysts of which are covered by a thin "veil" which is held in place by a number of strange knob-bearing projections of the wall, referred to as "sporopodia". The sporocysts of *Goussia* spp., have no Stieda body and the wall is composed of two valves which join in a meridional suture and dehiscence in order to release the sporozoites. Those of the single species of the genus *Crystallospora*, *C. crystalloids*, have a wall which is composed of two valves shaped like hexagonal pyramids: these are joined at their base to give the intact sporocyst the form of a dodecahedron. Sporocysts of *Epeimeria* spp., in true eels of the genus *Anguilla* and the conger eel *Conger conger*, possess sporocysts which are hexagonal when viewed end-on.

In view of these marked differences, and in particular the fact that *E. lepidosirenis* n.sp., has sporocysts with a distinct Stieda body, we have no doubt regarding the parasite's generic status. It is hoped that the examination of further specimens of this lungfish will enable us to locate and describe its endogenous stages of development.

It was once thought that all picine coccidia would utilize direct transmission from one fish to another by way of oocysts expelled in the faeces. However, although some fish coccidia, notable species of the genus *Goussia*, have been shown to be transmitted in this way, experimental attempts to achieve direct transmission of other parasites have failed, leading to the suspicion that perhaps there were intermediate hosts involved in some life-cycles. Landau et al. (1975) increased these suspicions when they showed that sporozoites of an *Eimeria* spe-



Eimeria lepidosirenis n.sp., of the lungfish, *Lepidosiren paradoxa*. Fig. 1: an immature oocyst in faecal material removed from the lower part of the intestine. The sporont (spo) entirely fills the oocyst. Figs 2, 3: mature oocysts in the same faecal sample. Note extremely delicate oocyst wall (ow), and bulky oocyst residuum (or). The sporocyst (sp) has a tiny Stieda body (s), and contains two strongly recurved sporozoites (sz) encircling the sporocystic residuum (sr). Bar = 10.0 μ m for all figures.

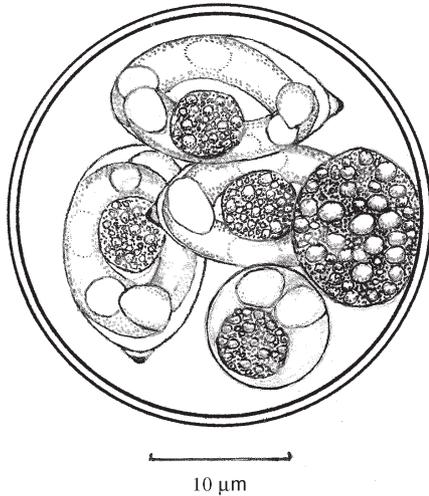


Fig. 4: line drawing of a mature oocyst of *Eimeria lepidosirenis* n.sp. from the lungfish *Lepidosiren paradoxa*.

cies of the moray, *Gymnothorax moringa*, were released in the intestine of pelagic crustaceans of the Order Mysidacea and persisted, in latent form, in the intestinal cells. Final proof of the role of intermediate hosts in the life-cycle of some fish coccidia was furnished by Fournie and Overstreet (1983) and Overstreet et al. (1984), who

showed that only the ingestion of crustaceans harbouring such latent sporozoites permitted transmission of the coccidian *Calyptospora funduli* to another fish. The possibility of intermediate hosts in the transmission of *E. lepidosirenis* clearly needs to be considered.

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