

# *Theileria electrophori* n.sp., a parasite of the electric eel *Electrophorus electricus* (Osteichthyes: Cypriniformes: Gymnotidae) from Amazonian Brazil

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*The name Theileria electrophori n.sp. is proposed for a small parasite described in the erythrocytes of the electric eel, Electrophorus electricus, from Amazonian Brazil. Division of the organism in the erythrocyte produces only four bacilliform daughter cells which become scattered in the host cell, without a cruciform or rosette-shaped disposition. Exoerythrocytic meronts producing a large number of merozoites were encountered in Giemsa-stained impression smears of the internal organs, principally in the liver, and are presumably the source of the intraerythrocytic forms of the parasite. This developmental pattern is characteristic of piroplasms within the family Theileriidae, where the author considers the parasite of E. electricus to most appropriately belong. It effectively distinguishes the organism from the dactylosomatid parasites Babesiosoma Jakowski and Nigrelli, 1956 and Dactylosoma Labbé, 1894 also found in fishes. This appears to be the second report of Theileria Bettencourt, Franca and Borges, 1907 in a fish.*

Key words: *Theileria electrophori* n.sp. - *Electrophorus electricus* - electric eel - *Babesiosoma* - *Dactylosoma* - Brazil

Following Lankester's description in 1882 of *Drepanidium* (= *Dactylosoma*) *ranarum* in erythrocytes of the European water frog *Rana "esculenta"* – now considered to be a complex hybrid derived from crosses of *R. lessonae* and *R. ridibunda* (Zimmermann 1986) – similar, pigmentless, piroplasm-like protozoa were recorded in the erythrocytes of various cold-blooded vertebrates, including fishes. Although Jakowski and Nigrelli (1955) erected a new family, the Dactylosomatidae, in which to place some of these enigmatic organisms, they for long remained among the most poorly understood of all protozoan parasites.

It is generally accepted that the family Dactylosomatidae contains only two genera, *Babesiosoma* Jakowski and Nigrelli, 1956 and *Dactylosoma* Labbé, 1894 (Jakowski & Nigrelli 1956, Barta 1991, Lom & Dyková 1992): both have been recorded in marine and freshwater fishes. Stages of *Babesiosoma* in erythrocytes are distinguished by the division of meronts to produce four small merozoites, characteristically in a cruciform or rosette arrangement. Larger, elongated, and club-shaped forms developing from some of the merozoites are regarded as gametocytes. In contrast, *Dactylosoma* species undergo merogony producing up to 16 merozoites, typically in a fan-shaped arrangement:

larger, elongated forms derived from some of the merozoites are, again, considered to be gametocytes. Lom and Dyková (1992) consider 7 species of *Babesiosoma* and 5 species of *Dactylosoma* to have been described in freshwater and marine fishes.

The record of a single species of the genus *Theileria* Bettencourt, Franca and Borges, 1907 (Piroplasmida: Theileriidae) in a fish further complicated the identification of small intraerythrocytic parasites of fishes. Haiba (1962) first named this parasite, in *Clarias lazera* from the Nile river, as *Cytauxzoon clariae*. Division in the erythrocyte was considered to produce no more than four merozoites. In addition, however, Haiba recorded the presence of exoerythrocytic meronts in the endothelial cells of blood vessels of the liver which gave rise to up to 16 merozoites and, for this reason, Krylov renamed the parasite as *T. clariae* (Haiba 1962) Krylov 1974.

Although Lom and Dyková (1992) included *T. clariae* in the family Theileriidae, as the only representative of the genus in a fish, they clearly had doubts regarding its identity and voiced the opinion that "Further study is required to determine the true nature of this parasite". Negm-Eldin (1998) went even further, and suggested that it was indistinguishable from *B. mariae* Hoare, 1930 which has been encountered in a variety of fishes.

The present author has examined blood films and impression smears of the viscera from a single juvenile electric eel *E. electricus* from the state of Pará, North Brazil, and noted the presence of small, piroplasm-like parasites in the erythrocytes. A description of the organism forms the subject of this communication.

## MATERIALS AND METHODS

Two thin blood films and impression smears of spleen, liver, kidney, and heart tissue were air-dried, fixed in absolute methyl alcohol for 3 min and stained by Giemsa's method.

Measurements of the parasites were made using a x 100 neofluar objective, x 10 eyepieces, and an ocular micrometer. Photomicrographs were prepared using a Zeiss "Photomicroscope III" and Kodak TMX 100 film.

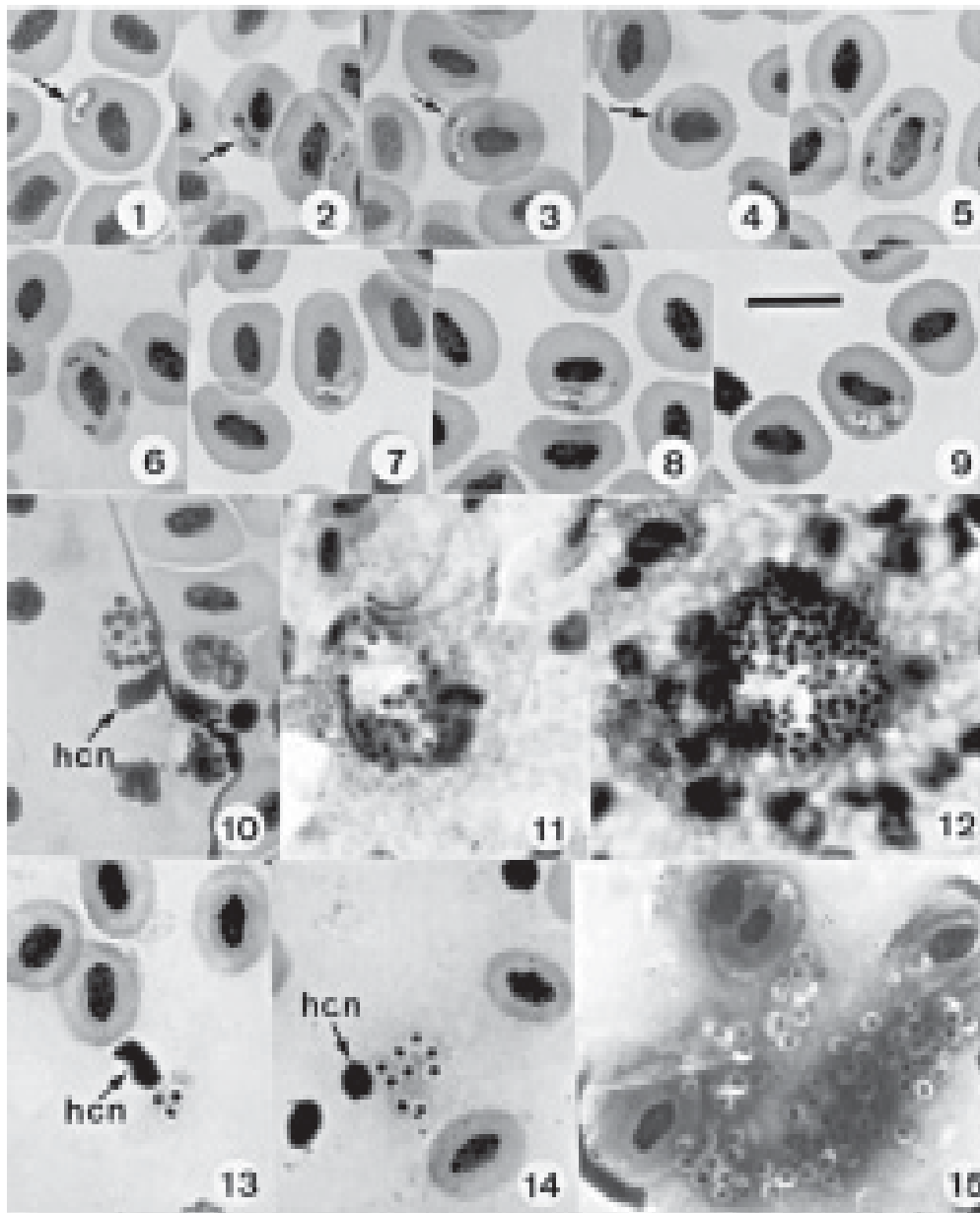
## RESULTS

### *Theileria electrophori* n.sp. (Figs 1-15)

Parasites in the blood films were by no means abundant (5 infected erythrocytes in 100 fields examined using the oil-immersion objective and x 10 eyepieces),

and this necessitated a prolonged search to follow development and establish the number of merozoites produced by the dividing meronts.

*Intraerythrocytic forms* - Undivided parasites were bacilliform, rounded, ovoid, or crescent-shaped, and measured from  $3.0 \times 1.0$  -  $8.0 \times 3.0$   $\mu\text{m}$ . By Giemsa's method the cytoplasm stained very poorly or not at all: the nuclear material was sometimes concentrated at one pole of the elongated parasites or formed a band adhering to one side (Fig. 1). Dividing meronts (Figs 2-6) gave rise to no more than four tiny, curved, bacilliform mero-



*Theileria electrophori* n.sp. in the electric eel, *Electrophorus electricus*. Figs 1-9 - Intra-erythrocytic stages. Fig 1: trophozoite (arrowed), showing nuclear material concentrated at one pole and forming a band adhering to one side. Figs 2-6: division stages (arrowed) producing four bacilliform daughter cells scattered in the erythrocyte. Figs 7-9: larger parasites, possibly developing and mature gametocytes. Figs 10-12: exo-erythrocytic stages in a Giemsa-stained liver smear. Fig. 10: young intracellular meront with 16 nuclei. Figs 11, 12: meronts producing a large number of merozoites. Figs 13-15: Giemsa-stained smear of heart tissue. Figs 13, 14: young division stages, seemingly in lymphocytes. Fig. 15: disrupted meront, producing a large number of merozoites. Bar = 10  $\mu\text{m}$  and applies to all figures; hcn: host cell nucleus.

zoites (Figs 5, 6) which measured approximately  $3.0 \times 1.0 \mu\text{m}$ . They were on no occasion seen to be arranged in cruciform or rosette fashion, and became scattered in the erythrocyte. Larger, broader and elongated parasites measuring up to  $8.0 \times 3.0$ , and often with a hook-like "tail", were considered to possibly represent developing and mature gametocytes (Figs 7-9). Their cytoplasm stained a very faint blue and the nucleus was usually in the form of rather scattered chromatin granules. At no stage were the parasites seen to enlarge the erythrocyte or displace its nucleus.

*Exoerythrocytic forms* - In Giemsa-stained impression smears of the internal organs meronts were found in cells of the lymphocyte series, where they produced up to 50 or more rounded merozoites measuring approximately  $2.0 \times 1.5 \mu\text{m}$  (Figs 10-15). They were most abundant in the liver and heart smears and occasionally were seen in those of the spleen.

*Type host* - The electric eel, *Electrophorus electricus* (Osteichthyes: Cypriniformes: Gymnotidae).

*Type material* - Giemsa-stained blood films and impression smears of the internal organs are held in the author's collection in the Department of Parasitology of the Instituto Evandro Chagas, together with phototypes. Depository number 1983.

*Type locality* - Pará, North Brazil.

*Prevalence* - Unknown. Only one *E. electricus* was available for study.

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

*Etymology* - The specific name is derived from the generic name of the host.

*Vector* - Unknown. As suspected for most piscine blood parasites, transmission of *T. electrophori* is most likely by way of leeches.

#### DISCUSSION AND CONCLUSIONS

The presence of meronts producing only four merozoites in the erythrocytes at first suggested that the parasite might be a species of *Babesiosoma*. The failure to find any cruciform or rosette arrangement of the daughter cells, however, put this diagnosis in doubt, and the finding of exoerythrocytic meronts in cells of the internal organs led to the conclusion that, more likely, the organism was a member of the Theileriidae. By definition species of *Theileria* undergo exoerythrocytic merogony in the lymphocytes, histiocytes, erythroblasts, and other cells of the internal organs, followed by invasion of the erythrocytes by the merozoites, which may or may not reproduce. When division does take place, the parasite produces no more than four daughter cells (Levine 1988). The frequent production of elongate bacillary or "bayonet" forms in the erythrocyte is considered as characteristic of the genus *Theileria* (Barnett 1977).

This was exactly the developmental pattern shown by the parasite of *E. electricus*, and it was therefore concluded that the family Theileriidae was, in fact, the most

appropriate place in which to place the parasite, for which the name *T. electrophori* n.sp. is proposed. The possibility that multiple invasion could account for the presence of the four parasites in a single erythrocyte, such as shown in Fig. 6, was discarded in view of the low parasitaemia, the similar size, and bacilliform shape of all the contained parasites in all such examples examined, and the absence of multiple infections with parasites of variable size such as those shown in Figs 7-9.

The present findings tend to dispel some of the doubts regarding the taxonomic status of *T. clariae* and raise the question as to whether *T. electrophori* might be conspecific with that parasite. This is considered unlikely considering the wide geographic separation and very different hosts of the two parasites. Although I have been unable to compare the morphology of *T. electrophori* with that shown in Haiba's original illustrations of *T. clariae*, his figures reproduced by Lom and Dyková (1992) show the intraerythrocytic meronts to produce four rounded merozoites, whereas those of *T. electrophori* produce daughter cells which are distinctly bacilliform. Haiba's illustration of an exoerythrocytic meronts of *T. clariae*, reproduced by Lom and Dyková (1992), shows an undivided parasite with only 16 nuclei, but continuing division might well have produced a much larger number of merozoites.

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