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# ULTRASTRUCTURAL AND STEREOLOGICAL ANALYSIS OF TRYPANOSOMATIDS OF THE GENUS *ENDOTRYPANUM*

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*Culture forms of four strains of* Endotrypanum (E. schaudinni *and* E. monterogeii) *were processed for transmission electron microscopy and analyzed at the ultrastructural level. Quantitative data about some cytoplasmic organelles were obtained by stereology. All culture forms were promastigotes. In their cytoplasm four different organelles could be found: lipid inclusions (0.2-0.4 µm in diameter), membrane-bounded vacuoles (0.10-0.28 µm in diameter), glycosomes (0.2-0.3 µm in diameter), and the mitochondrion. The kinetoplast appears as a thin band, except for the strain IM201, which possesses a broader structure, and possibly is not a member of this genus. Clusters of virus-like particles were seen in the cytoplasm of the strain LV88. The data obtained show that all strains have the typical morphological features of the trypanosomatids. Only strain IM201 could be differentiated from the others, due to its larger kinetoplast-DNA network and its larger mitochondrial and glycosomal relative volume. The morphometrical data did not allow the differentiation between* E. schaudinni *(strains IM217 and M6226) and* E. monterogeii *(strain LV88).*

Key words: trypanosomatid – *Endotrypanum* – ultrastructure – stereology

Parasites of the genus *Endotrypanum* are flagellate protozoa of the family Trypanosomatidae, a most studied group which includes parasites of vertebrates and invertebrates. The members of this genus are intraerythrocytic parasites of two and three toed sloths, and only a few papers are available dealing with the ultrastructure of these flagellates (Jadin & Creemers, 1969; Shaw & Bird, 1969).

Two species of *Endotrypanum* have been described, based on their morphology within the erythrocytes as well as serological and morphometric data (Shaw, 1969). Isolates of *E. schaudinni* are mostly epimastigotes in the red blood cells (Montero-Gei, 1956), while *E. monterogeii* shows mainly trypomastigote forms (Montero-Gei, 1956; Shaw, 1969). Both species grow in axenic cultures as promastigotes, which are also forms found in their vectors (sandflies) and are morphologically indistinguishable from promastigotes of other trypanosomatids (Jadin & Creemers, 1969).

A biochemical and ultrastructural study showed that several strains of both species of *Endotrypanum* could be grouped in two taxonomic units, by means of DNA bouyant density, isoenzyme analysis and the presence of clusters of virus-like particles in the cytoplasm, although those groups were not in agreement with the original identification of the strains as either *E. schaudinni* or *E. monterogeii* (Croft et al., 1980). More recently, a molecular evolution study using comparisons of nuclear DNA restriction fragment patterns showed four groups among several stocks of both species of *Endotrypanum* (Lopes et al., 1990).

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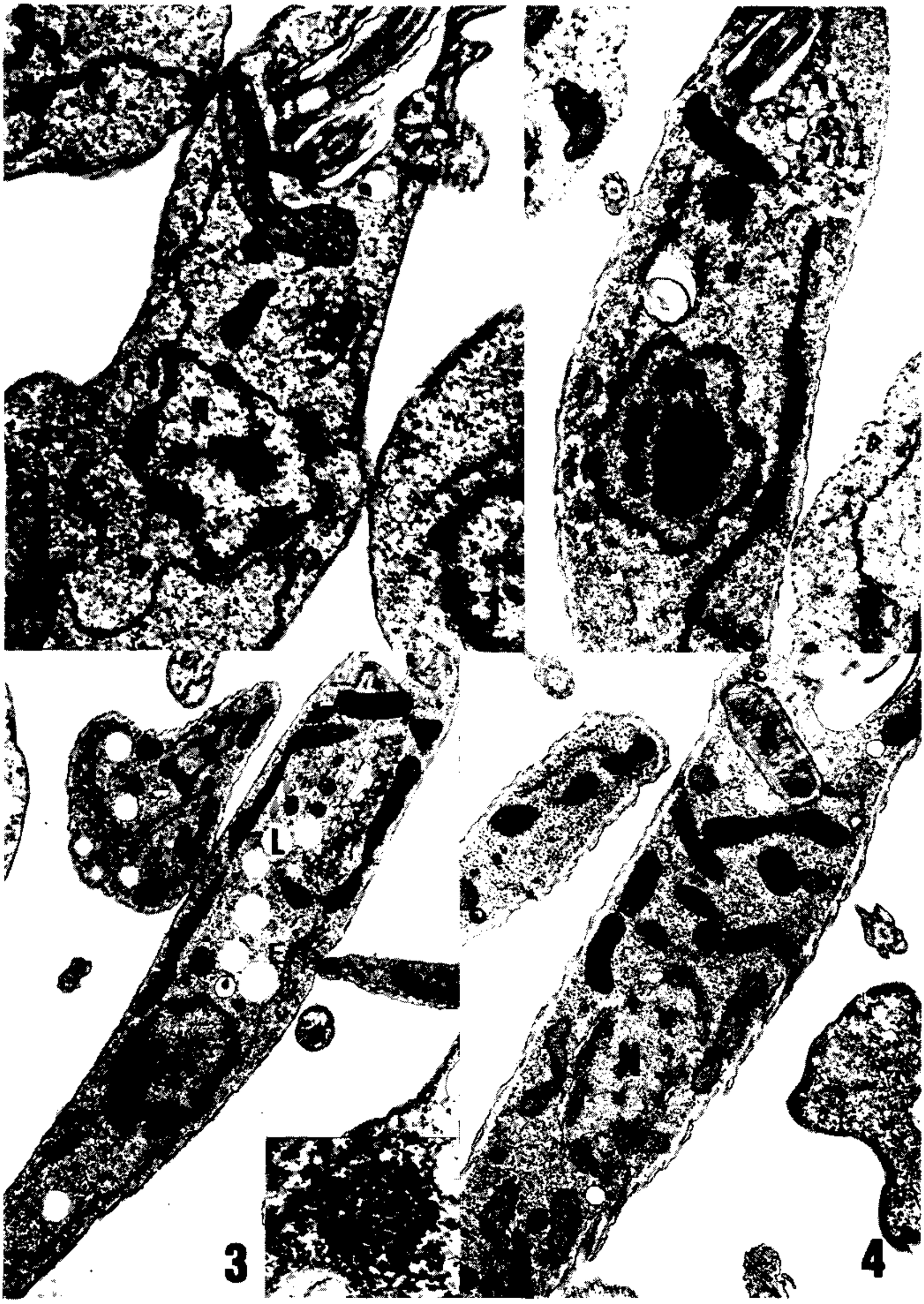


Transmission electron microscopy of ultra-thin sections of culture forms (promastigotes) of *Endotrypanum* sp. Figs 1, 2: *Endotrypanum schaudinni* strains IM217 (Fig. 1) and M6226 (Fig. 2), showing the centrally located nucleus (N), the tubular mitochondrion (M), the kinetoplast (K) and the flagellum (F). Fig. 1: X 16,500; Fig. 2: X 14,500. Fig. 3: *Endotrypanum monterogeii* strain LV88. Several lipid droplets (L) can be seen in the cytoplasm. A cluster of virus-like particles, occasionally found in the cytoplasm, is shown in the inset. ER, smooth endoplasmic reticulum; G, glycosome; K, kinetoplast; M, mitochondrion; N, nucleus. X 12,000; Inset: X 28,000. Fig. 4: *Endotrypanum* sp. strain IM201. The cytoplasm is rich in glycosomes (G). The DNA fibrils of the kinetoplast (K) present a looser organization. M, mitochondrion; N, nucleus. X 16,700.

TABLE I

*Endotrypanum* strains used, with their respective hosts, countries where they were isolated from and donors

| Stock code | Species | Host | Isolated from | Donor |
|---|---|---|---|---|
| LV88 | *E. monterogeii* | *Choloepus hoffmanni* | Costa Rica | 1 |
| IM201 | *Endotrypanum* sp. | *Lutzomyia* sp. | Brazil | 2 |
| IM217 | *E. schaudinni* | *Lutzomyia* sp. | Brazil | 2 |
| M6226 | *E. schaudinni* | *Choloepus didactylus* | Brazil | 3 |

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TABLE II

Mean diameter of the cytoplasmic organelles (in $\mu$m). Values are mean and standard deviation, respectively

| Stock code | Glycosomes | Vacuoles | Lipid inclusions |
|---|---|---|---|
| LV88 | $0.33 \pm 0.10$ (90)[a] | $0.28 \pm 0.11$ (59) | $0.43 \pm 0.12$ (85) |
| IM201 length: | $0.35 \pm 0.14$ (286) | $0.10 \pm 0.02$ (141) | $0.20 \pm 0.07$ (43) |
| width: | $0.23 \pm 0.07$ | | |
| IM217 | $0.21 \pm 0.07$ (107) | $0.24 \pm 0.08$ (55) | $0.21 \pm 0.06$ (45) |
| M6226 | $0.24 \pm 0.10$ (94) | $0.25 \pm 0.08$ (64) | $0.30 \pm 0.07$ (26) |

*a:* numbers in brackets indicate the number of profiles measured.

In the present study, four strains of *Endotrypanum* spp., each one representing one of the above mentioned groups, were processed for transmission electron microscopy and analyzed at the ultrastructural level. Quantitative data about some cytoplasmic organelles were obtained by stereology, according to Weibel (1969), Weibel & Bolenger (1973), and are presented in this paper.

## MATERIALS AND METHODS

*Parasites* – Promastigotes of four strains of *Endotrypanum* spp. were maintained at 26 °C in Schneider's culture medium, supplemented with 20% fetal calf serum. Details of the strains are given in Table I.

*Electron microscopy* – Four-day-old culture forms of all strains were collected by centrifugation at 1,500 g and fixed in 1.5% (v/v) glutaraldehyde and 4% (w/v) paraformaldehyde in 0.05M phosphate buffer, pH 7.2. After washing in the same buffer, the cells were post-fixed in 1% (w/v) $OsO_4$ – 0.8% (w/v) potassium ferricyanide in 0.1M cacodylate buffer, pH 7.2, with 1mM $CaCl_2$ (Forbes et al., 1977), en bloc stained with 2% (w/v) uranyl acetate and embedded in Epon. Ultra-thin sections were observed in a Jeol 100CX electron microscope.

*Stereology* – Electron micrographs were taken at random from ultra-thin sections of all strains and enlarged to 24,000X. A total of 300 to 500 $\mu m^2$ of cell profiles was measured using a digital tracing tablet coupled to a MOP-Videoplan microcomputer (Carl Zeiss, West Germany). The total area of profiles of mitochondria, glycosomes, lipid inclusions and membrane-bounded vacuoles was calculated and expressed in $\mu$m. The total area of profiles of each organelle was compared with the total area of the cell (excluding the flagellum) and the results were expressed as percent of the volume of a certain organelle in relation to the total cell volume ($V_v$ = volume density).

The mean diameter of rounded structures (glycosomes, lipid inclusions and membrane-bounded vacuoles) was also calculated. Glycosomes of strain IM201 were elongated bodies; in this case, measurements were obtained from the length and width of the organelles.

## RESULTS

The culture forms of all *Endotrypanum* strains here analyzed were elongated promastigotes, with a centrally located nucleus. When viewed in the transmission electron microscope, the parasites presented all the characteristic morphological features of the members of the Trypanosomatidae family. In the cytoplasm, four different organelles were found: a) lipid inclusions, not limited by a unit membrane, showing an electron lucent content, 0.2-0.4 $\mu$m in diameter; b) membrane-bounded vacuoles, measuring 0.24-0.28 $\mu$m in diameter, except for the strain IM201, where they measure 0.10 $\mu$m; c) glycosomes (peroxisome-like organelles), membrane-bounded with a granular and electron-dense content, with a diameter of 0.2-0.3 $\mu$m. Glycosomes were abundant and elongated in the strain IM201; elongated glycosomes measured in the average 0.35 x 0.23 $\mu$m; d) the tubular mitochondrion. All these organelles were distributed evenly throughout the cell body of the parasites. Details of the measurements are given in Table II.

TABLE III

Mean length and width of the kinetoplast (in $\mu$m). Numbers are mean and standard deviation, respectively

| Stock code | Length | Width |
|---|---|---|
| LV88 (n = 15) | 1.00 ± 0.02 | 0.15 ± 0.03 |
| IM201 (n = 19) | 0.83 ± 0.20 | 0.30 ± 0.06 |
| IM217 (n = 8) | 0.75 ± 0.19 | 0.12 ± 0.02 |
| M6226 (n = 9) | 0.85 ± 0.21 | 0.14 ± 0.05 |

The kinetoplast-DNA appears as a thin band, except for the strain IM201, which possesses a broader structure, with looser DNA fibers, about two times larger than the others (see Table III). Clusters of virus-like particles (VLPs) were seen in the cytoplasm of the strain LV88. The particles were rounded, with a mean diameter of 52.85 ± 6.87 (standard deviation) nm, and with an electron lucent core. No special array of the particles was seen. The clusters contained usually 30-50 particles.

The stereological data are summarized in Table IV. The relative volume of the tubular mitochondrion ranged from 6.2 to 8.5% and that of the glycosomes from 0.8 to 1.4%. However, in the strain IM201 these values were 11.7 and 6.0%, respectively. Lipid droplets were larger in the strain LV88, with a volume density of 2.8%, while in the other strains lipid inclusions occupy a relative volume of about 0.4-0.5%.

## DISCUSSION

The culture forms of *E. schaudinni* are promastigotes, and have been previously studied by transmission electron microscopy (Jadin & Creemers, 1969). Our results with promastigotes of four strains of *Endotrypanum* spp., previously characterized either as *E. schaudinni* (IM217 and M6226), *E. monterogeii* (LV88), or *Endotrypanum* sp. (IM201), show that culture forms of parasites of the genus *Endotrypanum* have the same basic ultrastructural morphology, with all structures characteristic of the trypanosomatids. The intraerythrocytic forms can be either epimastigotes or trypomastigotes. Shaw & Bird (1969) demonstrated by means of serial sections that the flagellates are indeed found inside the red blood cells. However, ultrastructural studies are lacking to allow a better characterization of the intracellular stages of this parasite.

Lipid inclusions, glycosomes and membrane-bounded vacuoles are cytoplasmic organelles found in all trypanosomatids so far analyzed. The values for mean diameter and relative volume found in this study for these structures are in agreement with those described in other parasites of this family (Soares & De Souza, 1988).

Strain IM201 (*Endotrypanum* sp.) could be differentiated from the others due to: a) its wider kinetoplast-DNA network, which in the other three strains is a long and thin band, and b) its mitochondrion and glycosomes occupy a larger relative volume in the cell. This observation can be explained by the fact that both structures are larger, the glycosomes being also abundant in the cell. The data obtained for the volume density of mitochondrion and glycosomes in the strain IM201 are in accordance to those obtained with other promastigote forms, either of *Leptomonas samueli* (Souto-Padrón et al., 1980), or of *Leishmania donovani* (Brun & Krassner, 1976). Previous results in molecular evolution studies showed that the strain IM201 is as distinct from the remaining isolates of *Endotrypanum* as it is from several

TABLE IV

Volume density of cytoplasmic organelles given as percent of total cell volume

| | Mitochondrion | Glycosomes | Vacuoles | Lipid inclusions |
|---|---|---|---|---|
| LV88 (67)[a] | 8.49 | 1.44 | 0.86 | 2.83 |
| IM201 (50) | 11.73 | 5.96 | 0.33 | 0.48 |
| IM217 (77) | 7.45 | 0.99 | 0.69 | 0.47 |
| M6226 (60) | 6.16 | 0.79 | 0.69 | 0.39 |

*a:* numbers in brackets indicate number of cell profiles measured.

species of *Leishmania, Crithidia, Herpetomonas* or *Leptomonas* (Lopes et al., 1990). The strain IM201 also fails to react with monoclonal antibodies specific for the genus *Endotrypanum* (Lopes & McMahon-Pratt, 1989). Since at least *E. schaudinni* and *L. braziliensis* can infect both the same sloth and the same insect *Lutzomyia* sp. (Christensen & Herrer, 1979), we may not exclude the possibility that the strain IM201 may represent a trypanosomatid other than *Endotrypanum.*

In this study, *E. monterogeii* (strain LV88) could be morphologically differentiated from the other strains due to the presence of great number of large lipid droplets and clusters of virus-like particles (VLPs) in the cytoplasm. Similar VLPs were previously found in this same strain of *E. monterogeii* and in other strains of *E. schaudinni* (Croft et al., 1980), and so can not be considered as a marker for *E. monterogeii.* In previous studies, both species could not be differentiated based either on biochemical aspects (Croft et al., 1980) or using monoclonal antibodies specific for the genus *Endotrypanum* (Lopes & McMahon-Pratt, 1989).

The morphological and stereological data obtained in this study did not provide a distinct morphological marker for *E. schaudinni* alone, and therefore did not allow a distinction between *E. schaudinni* and *E. monterogeii.* Glycosomes and lipid inclusions of *E. monterogeii* were significantly larger (Student's t test, $P = 0.05$) than those structures of *E. schaudinni* strains (IM217 and M6226) and as a result, the relative volume of these organelles was larger. However, since these variations may be related to and influenced by physiological conditions of the parasites, we believe that the small numerical differences found after the stereological analysis are not of relevance in order to discriminate between the parasites. Thus, the differentiation between the two species seems to be based mainly on the intra-erythrocytic stages of the parasites. Therefore, new criteria are needed to distinguish both species in other reliable taxonomic conditions.

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