RESEARCH NOTE

Trypomastigotes in Cultures of *Blastocrithidia culicis* (Novy, MacNeal & Torrey, 1907) (Kinetoplastida: Trypanosomatidae)

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Arthropod trypanosomatids of the genus *Blastocrithidia* Laird, 1959 typically present the epimastigote stage, being largely accepted that their development never goes beyond this, although some species display amastigote stages as flagellar cysts (M Laird 1959 *Can J Zool 37:* 749-752, CA Hoare & FG Wallace 1966, *Nature 212:* 1385-1386, K Vickerman 1976, p. 1-34. In DHR Lumsden & DA Evans (eds), *Biology of the Kinetoplastida* vol. I, Academic Press, New York). Among the species of the genus, *Blastocrithidia culicis* (Novy, MacNeal & Torrey, 1907) has been much studied on account of two related features: its easy cultivation and the presence of bacterium-like symbionts in its cytoplasm (K-P Chang 1975 *J Protozool 22:* 271-276). In our laboratory, a strain of this species (ATCC 30268) isolated from *Aedes vexans* by FG Wallace & A Johnson (1961 *J Insect Path 3:* 75-80) and supplied by Dr EP Camargo (São Paulo University, SP, Brazil) has been routinely maintained in the Yeager's LIT medium (EP Camargo 1964 *Rev Inst Med trop São Paulo 6:* 93-100), either at room temperature or constantly at 27.3 °C. On examining Giemsa-stained smears of these cultures, a trypomastigote stage was found in several opportunities, from the 8th to the 21st cultivation days. Such trypomastigotes occurred at rates ranging from 0.8 to 18.7% (more commonly 2-7%), being considerably smaller than the epimastigotes and generally presenting an inconspicuous undulating membrane and a short flagellum (see Figs). It has been proved that such trypomastigotes in fact belong to *B. culicis* by cloning the parasite culture in a flow cytometer (Coulter's EPICS 751), since they were found in eleven randomly selected clones. Trypomastigotes were also seen in cultures of an aposymbiotic strain of this parasite (ATCC 30257), suggesting that their occurrence is unrelated to the symbiont presence.

Blastocrithidia culicis culture forms (symbiont-bearing strain). Giemsa stain after HCl treatment according to ALM Carvalho (1973 *Rev Pat Trop 2:* 223-274). Fig. 1: epimastigote and trypomastigote side-to-side for comparison. Figs 2-3: transitional forms between epi- and trypomastigote. Figs 4-8: trypomastigotes. All photomicrographs at same magnification (scale bar = 10 μm).

As far as I know, the presence of trypomastigotes in *B. culicis* cultures had not been previously demonstrated. Early descriptions of this species did not include this stage (FG Novy et al. 1907 *J Infect Dis 4:* 223-276, D Mezincesco 1908 *C R Soc Biol 64:* 975-976, Wallace & Johnson *loc. cit.*, FG Wallace 1966 *Exp Parasitol 18:* 124-193). Other reports in culicids of morphological types capable of being identified as epi and trypomastigotes (sometimes both in the same insect) (A Laveran & G Franchini 1920 *Bull Soc Path Exot 13:* 138-147, A Missiroli 1930 *Riv Malariol 9:* 111-119) cannot be surely assigned to *B. culicis* mainly accounting that, in such cases, no symbiont-like organelles (the so called "diplosome" typical of this species) were mentioned and mixed infections were also possible, furthermore regarding that culicids can support the development and multiplication of trypanosomes from different vertebrates (JE

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Bardsley & R Harmsen 1973 *Adv Parasitol 11:* 1-73, JR Baker 1976 p. 131-174. In Lumsden & Evans (eds) *loc. cit.*).

At present, the meaning of such finding has not been determined, but taking into account the ability of *B. culicis* to defferentiate into trypomastigote and its isolation from bloodsucking insects (Novy et al. *loc. cit.,* Mezincesco *loc. cit.*), the possibility of its being a *Trypanosoma* species is one that should be considered. This hypothesis had been already advanced by HM Woodcock (1914 *Zool Anz 44:* 26-33) and even verified by Novy et al. (*loc. cit.*) and Mezincesco (*loc. cit.*), both obtaining negative results throughout inoculations into several vertebrates. However, these experiments cannot be considered conclusive, since these authors did not report the presence of trypomastigotes in the inoculum (the possible infective forms) and could have employed unsusceptible hosts in their experiments. Moreover, as several *Trypanosoma* species only produce scanty parasitemia, their presence cannot be easily evidenced, accounting for negative results (CA Hoare 1972 *The trypanosomes of mammals. A zoological monograph.* Blackwell Sc. Publ., Oxford and Edinburg, V Apanius 1991 *Parasitol Today 7:* 87-90). Then, studies will be undertaken to verify the infectivity to different vertebrates of *B. culicis* cultures presenting trypomastigotes. Furthermore, the occurrence of this stage in experimentally infected culicids will be investigated.

It must me emphasized that the possibility of *B. culicis* being exclusively an insect parasite has not been rejected. This being true, the diagnosis of the genus *Blastocrithidia* proposed by Laird (*loc. cit.*) should be reviewed according to Wallace (*loc. cit.*) that also allowed the occurrence of individuals with postnuclear kinetoplast. However, the species presenting trypomastigotes included by Wallace (*loc. cit.*) in the genus *Blastocrithidia (B. anophelis* and *B. pessoai*) were also found in culicids and likewise the possibility of they being stages of some trypanosome cannot be ruled out. This view had been already advanced by Missiroli (*loc. cit.*) in the case of *"Crithidia" anophelis* (= *B. anophelis*).

Trypomastigotes in monoxenous trypanosomatids were also reported in the genus *Rhynchoidomonas* Patton, 1910, a poorly known group of parasites of the Malpighian tubules and intestine of nonhematophagous Diptera (review by Wallace *loc. cit.*). Despite the presence of trypomastigotes in cultures of *B. culicis,* with the data now at hand, it is not possible to establish any relationship between it and the *Rhynchoidomonas* species.

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