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Short Communication

Amoeboid Stage of *Gigantomonas herculea* (Parabasalia) Attached to the Hindgut o the Termite Host *Hodotermes mossambicus*

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Summary. A light microscopy examination of the luminal surface of the hindgut of *Hodotermes mossambicus* revealed large amoeboid cells attached to the cuticle. Sections observed by transmission electron microscopy showed cells with large finger-like pseudopodia adhering to the cuticle and containing conspicuous bundles of microfilaments. The cuticle surface was covered by these large pseudopodial processes, and the usual bacterial covering was removed. The spiralled rows of the axostyle and the cresta structure in these large amoeboid cells indicated that they belong to *Gigantomonas herculea* which, to date, was only known to live free in the hindgut fluid.

Key words: *Gigantomonas herculea*, hindgut attachment, *Hodotermes mossambicus*, microfilaments, ultrastructure.

INTRODUCTION

Hodotermes mossambicus is a subterranean grass-eating "lower termite" living in East Africa. It harbours a varied fauna of symbiotic protozoa in the hindgut (Dogiel 1916, 1922; Yamin 1979) that I have reinvestigated using immunofluorescence and transmission electron microscopy techniques (Brugerolle and Bordereau 2003, Brugerolle 2005). One of these protozoa, *Gigantomonas herculea*, is a large amoeboid flagellate that presents a flagellate, an amoeboid and a plasmodial stage of development (Dogiel 1916, Kirby 1946). A recent study has dealt with the most numerous amoeboid forms in the hindgut fluid (Brugerolle 2005). These cells have an internalized flagellar apparatus typical of devescovinid parabasalids with a large cresta structure associated with the enlarged recurrent flagellum. More remarkably, these amoeboid cells present a thick cytoplasmic margin with a microfibrillar network and internal bundles of microfilaments similar to those of lobose amoebae. A further examination of the termite hindgut's luminal surface has revealed the presence of large adhering amoeboid cells attached to the cuticle, and the ultrastructural study has allowed me to identify them as *Gigantomonas herculea.*

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MATERIALS AND METHODS

Hodotermes mossambicus termites were collected in Kenya several years ago and maintained in a laboratory terrarium at the Université de Bourgogne in Dijon, France (Brugerolle 2005). The hindgut of a termite was opened with a pair of tweezers into a drop of Ringer's solution. The first hindgut paunch, or P3 segment according to Noirot

(1995) numbering, was fixed in a solution of 1% glutaraldehyde (Polysciences) in 0.1 M phosphate buffer solution, pH 7, for 1 h at room temperature. After two washes in the buffer, the gut was postfixed in 1% osmium tetroxide in the buffer for 1 h. After a water rinse, it was pre-embedded in 1% agar (Difco), stained "en bloc" with saturated uranyl acetate in 70% ethanol for 1 h, completely dehydrated in an alcohol series, and embedded in Epon 812 resin (Merck). Sections were cut with a diamond knife (Drukker) on a Reichert Ultracut S microtome (Leica). Semi-thin sections 1 mm thick were stained for 1 min with a 0.2% blue Azur II solution at pH 8.5, followed by destaining with a 0.1 N NaOH solution and were observed under a Leica DMR light microscope equipped with a G-Fish Light Station. Ultra-thin sections were stained with lead citrate for 15 min and examined under a JEOL 1200 EX electron microscope at 80 kV.

RESULTS

Globular cells attached along the lumen surface of the hindgut were first observed under a stereomicroscope. Semi-thin sections of fixed and stained hindgut showed aligned amoeboid cells adhering to the cuticle, and most of the hindgut surface was covered with these cell processes (Fig. 1). The electron microscopy study revealed that the amoeboid cells adhered to the cuticle via fingerlike pseudopodial processes containing numerous microfilaments (Figs 2, 3). Bundles of microfilaments of variable thickness, often associated with tubular vesicles, joined the pseudopod surfaces to the cell body (Figs 3, 4). Bacteria were present between the pseudopodia, and also inside the food vacuoles of the protozoan. Within the zone of adhesion of these amoeboid cells, the bacteria adhering to the cuticle and usually forming a thick and dense coverage were removed (Fig. 3). The cytoplasm of the cell body of these amoeboid cells contained spread Golgi bodies, hydrogenosomes, and food vacuoles filled with wood particles and with the various bacteria present in the hindgut fluid, including easy-identifiable spirochetes. Through the sections, the cells contain one or two nuclei that are accompanied by the spiralled microtubular rows of the axostyle, parabasal filaments supporting Golgi bodies and the cresta structure (Fig. 5). Sections of flagella, basal bodies and their appendages, atractophores and a paradesmosis were also observed close to the nuclei (not shown).

DISCUSSION

The amoeboid cells attached to the hindgut and having a microtubular spiralled row as an axostyle, a cresta structure, a parabasal apparatus, free Golgi bodies and hydrogenosomes, resemble those described in the fluid content, and have been identified as *Gigantomonas herculea* (Brugerolle 2005). The attached forms of *G. herculea* were not mentioned in the previous studies of Dogiel (1916) and Kirby (1946), who only observed this amoeboid flagellate in the hindgut fluid as the other symbiotic flagellates of this termite. However, Cleveland (1966) using Kirbys' preparations mentioned that "*Gigantomonas* is attached to the chitinous intima of its host" and he drawn an attached cell with a holdfast and a rostellum but did not further comment these observations. A major highlight is the remarkable development of the cell processes and pseudopodia and their microfilament bundles in the attached forms that confirm previous observations in the amoeboid cells collected in fluid content (Brugerolle 2005). Similar conspicuous microfilament bundles in the pseudopodia have not been reported in any other parabasalid flagellates. Parabasalid flagellates living in the hindgut of "lower termites" or in the wood-feeding roach *Cryptocercus* have not been reported as being attached to the gut, as they usually swim in the hindgut fluid. This contrasts with oxymonad flagellates living in the hindgut of the same hosts, which are attached to the cuticle by a finger-like holdfast apparatus containing microfilaments such as *Pyrsonympha* (Hollande and Carruette-Valentin 1970) and a rostellum containing microtubules such as *Oxymonas* (Brugerolle and König 1997). In these oxymonads, the holdfast apparatus is situated at the anterior tip of the cell and is connected to the flagellar apparatus. In the large amoeboid forms of *G. herculea*, the whole cell surface is surrounded by a microfilament margin that transforms into finger-like pseudopodia able to adhere to the gut surface, but there is no microtubular rostellum in *Gigantomonas.* Amoeboid trichomonads, such as *Histomonas meleagridis* (Wenrich 1943) which is parasitic of birds or *Trichomonas vaginalis* which is parasitic of humans, are known to adhere to the host cells in infested organs (Brugerolle et al. 1974, Gonzales-Robles et al. 1995) but they nether contain microfilament bundles as large as those of *Gigantomonas herculea*.

In the largest amoeboid forms of *Gigantomonas*, Dogiel (1916) and (Kirby 1936) generally observed two nuclei linked by a paradesmosis and an internalized flagellar apparatus at variable stages of development. Only Kirby (1946) mentioned a very large amoeboid or plasmodial form containing 36 nuclei without crestas, axostyles or paradesmoses around the nuclei, from the

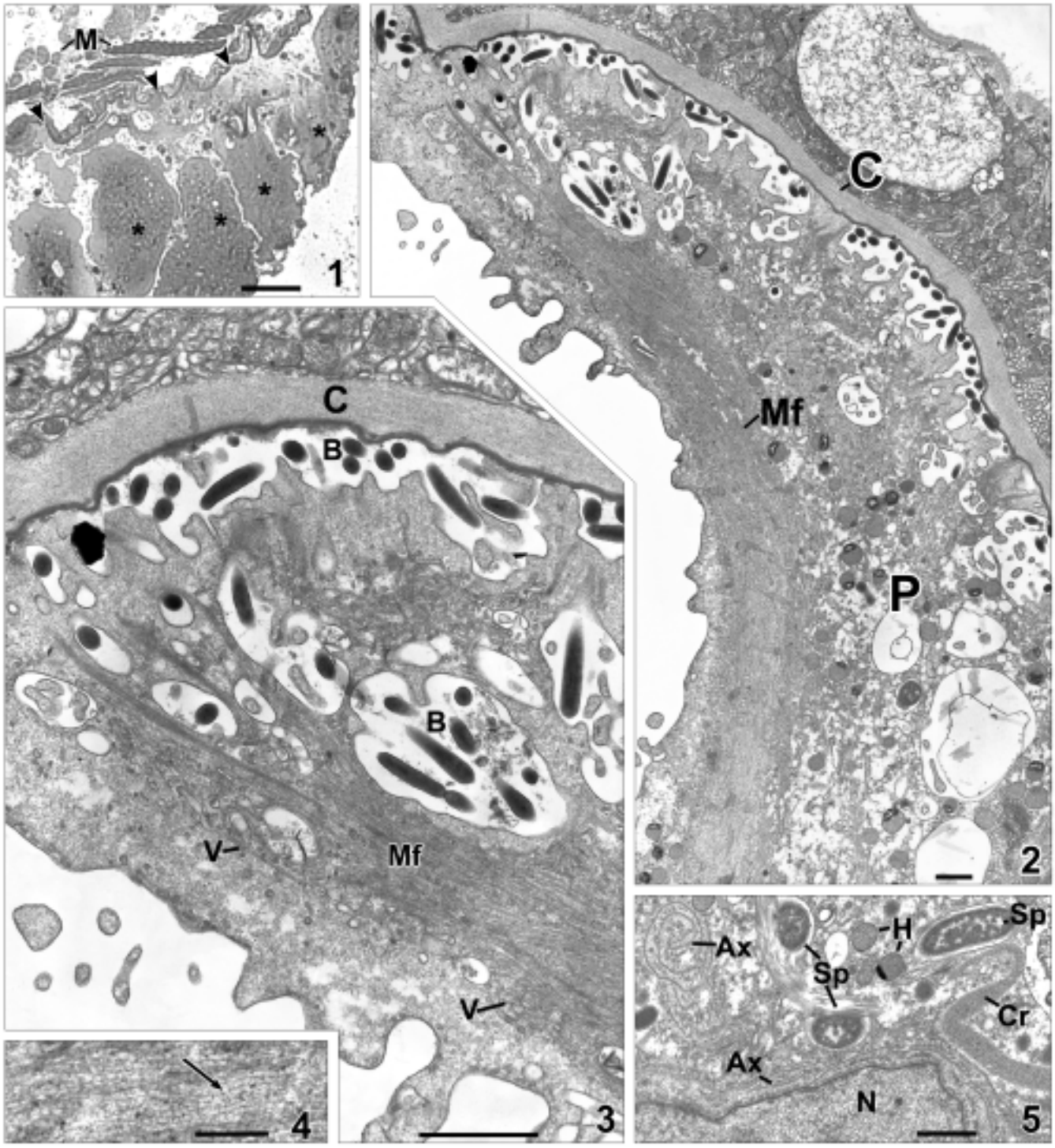

Fig. 1. Light microscopy micrograph of a semi-thin section of the hindgut of *Hodotermes mossambicus* where amoeboid cells of *Gigantomonas herculea* (*) are attached to the cuticle (arrowheads); muscle layer (M). Scale bar 50 µm.
Figs 2-4. Transmission electron micrographs. **2 -** finger-like pseudopod (P) of an amoeboid *Gigantomonas* adherent to the hindgut cuticle (C). **3 -** bundle of microfilaments (Mf) associated with vesicles (V), stretched between the pseudopod surface and the cell body, and numerous bacteria (B) close to the pseudopod surface and inside food vacuoles. **4 -** microfilaments (arrow) at high magnification. Scale bars 1 µm (2, 3); 0.25 µm (4).
Fig. 5. Section of the nucleus (N), showing the microtubular rows of the axostyle (Ax), cresta structure (Cr), hydrogenosome (H) and spirochetes (Sp) of an attached cell. Scale bar 1 µm.

hindgut of *Microhodotermes viator*, a termite which has the same protozoan fauna as *Hodotermes mossambicus*. That was corroborated by Cleveland (1966) who described multinucleate forms that divide by plasmotomy. Two parabasalid flagellates *Kirbinia pulchra* and *Rhizonympha jahieri* do have a plasmodial stage attached to the hindgut of the termite *Anacanthotermes ochraceus* (Grassé and Hollande 1951; Grassé 1952a, b), but unfortunately these flagellates have not been re-examined using modern techniques.

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