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Pseudotrypanosoma elphinstonae sp. n., a Trichomonad Symbiotic in *Schedorhinotermes* (Isoptera: Rhinotermitidae)

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Summary. A new species of *Pseudotrypanosoma*, *P. elphinstonae* sp. n., is described which is symbiotic within the hindguts of the rhinotermitid termites *Schedorhinotermes secundus* and *S. intermedius*. *P. elphinstonae* possesses most of the features of the genus: 4 anterior flagella, prominent costa and recurrent flagellum forming an undulating membrane and simple bean-shaped parabasal body. The mastigont complex is of similar composition and arrangement to other trichomonads but the pelto-axostylar complex is greatly simplified being composed of a single layer of microtubules which do not over lap and cannot be distinguished into separate structures. The undulating membrane is much smaller than in related species; the costa is smaller and simpler and there are no microtubular bundles connecting it to the recurrent flagellum. Comparison of the ultrastructure of *P. elphinstonae* sp. n. with that of *P. giganteum* demonstrated that *P. elphinstonae* sp. n. in addition to being much smaller in size had a correspondingly simper ultrastructural organisation lacking several organelles which characterise the latter species.

Key words: Isoptera, Parabasalida, *Pseudotrypanosoma elphinstonae* sp. n., Rhinotermitidae, *Schedorhinotermes*, Trichomonadida, ultrastructure.

INTRODUCTION

Five genera of trichomonads have been reported to possess 4 anterior flagella plus 1 recurrent flagellum forming part of the undulating membrane (4+R arrangement): *Pseudotrypanosoma* Grassi, 1917, *Trichomitopsis* Kofoid et Swezy, 1919, *Pentatrichomonas* Mesnil, 1914 (4+R+I, I being an independent anterior flagellum), *Tetratrichomonas* Parisi, 1910, and *Trichomonas* Donné, 1836. Additionally 1 species of *Tritrichomonas* Kofoid, 1920 has the 4+R arrangement rather than the usual arrangement of 3+R for this genus. These genera are considered to be phylogenetically unrelated (Gerbod *et al.* 2001), representing multiple independent additions of another flagellum to the basic or privileged basal bodies (3+R) (Brugerolle 1991). Of these, *Pseudotrypanosoma* has one of the narrowest distributions being confined to Australian termites of the genus *Porotermes*.

Pseudotrypanosoma was initially described by Grassi (1917), who assigned one species to the genus: *P. giganteum* which was endosymbiotic in the Austra-

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lian termite, *Porotermes adamsoni*. Descriptions of both the genus and type species was subsequently emended by Kirby (1931), who also made mention of "smaller, more typical trichomonads" in a second termite host, *Po. grandis*, but did not formally describe these forms. *P. giganteum* was described a third time by Sutherland (1933) who also examined the "small trichomonads" from *Po. grandis* and assigned them to a second *Pseudotrypanosoma* species: *P. minimum*. Cleveland (1961) re-examined *P. giganteum* from *Po. adamsoni* describing the cell division cycle of this species in great detail. Cleveland (1961) was of the opinion that *Pseudotrypanosoma* was synonymous with *Trichomonas* as both genera exhibited similar morphology despite the great disparity in size between *P. giganteum* and most species of *Trichomonas*. Despite this, Cleveland (1961) did not make a clear taxonomic statement synonymising *Pseudotrypanosoma* with *Trichomonas* and it has been universally ignored by subsequent treatments of the trichomonads (e.g. Honigberg 1963, Yamin 1979). This has been partly vindicated by recent molecular phylogenetic studies which have suggested that *Pseudotrypanosoma* is distinct from *Trichomonas* and if they were to be synonymised, then *Pentatrichomonoides* and *Trichomitus* must also be synonymised with *Trichomonas* (Keeling *et al*. 1998). Recently, the ultrastructural characterisation of *Pseudotrypanosoma giganteum* has reinforced its distinctiveness from *Trichomonas* particularly in the structure of the costa (Brugerolle 1999). Here we report a third species in the genus *Pseudotrypanosoma* which is the first to be described from a termite other than *Porotermes*.

MATERIALS AND METHODS

Schedorhinotermes intermedius and *S. secundus* colonies were collected by hand from under fallen timber, within dead fallen branches and from within tubular galleries within the bark of living trees. Individual termites representing the worker, major soldier and minor soldier castes were collected from each colony. Voucher collections were made of each colony by preserving 5 of each caste in 70% ethanol and they were used to identify the termite species collected. Nest material was collected along with termites and each colony was provided with tissue paper soaked in water as a moisture and food source to maintain the colony in the laboratory. Workers were examined shortly after collection by dissecting the hindgut into a small drop of Locke's fluid. Some workers were removed directly from the nest and examined but they generally yielded "dirty" preparations with abundant coarse wood fibres and siliceous particles. "Clean" preparations were generated by isolating individual workers from nest material and rearing them on water-soaked tissue paper for at least 4 days to purge them of dirt and coarse wood fibre in their guts.

Light microscopic observations were performed on Giemsa-stained smears made by spreading gut content diluted with a small volume of Locke's fluid on slides. Partially air dried smears were fixed with methanol and stained with Giemsa's stain. Giemsa slides were examined without cover slips by bright-field microscopy under immersion oil. Both "clean" and "dirty" preparations were examined by light microscopy to determine if cleaning cause artefactual changes to the cells. Cells were drawn using a camera lucida, and measurements were made using a calibrated eye piece micrometer for each cell dimension except for cell length, anterior flagellum length and undulating membrane length which was measured from drawings by planometer. Measurements are presented as a range of values followed by the average in parentheses.

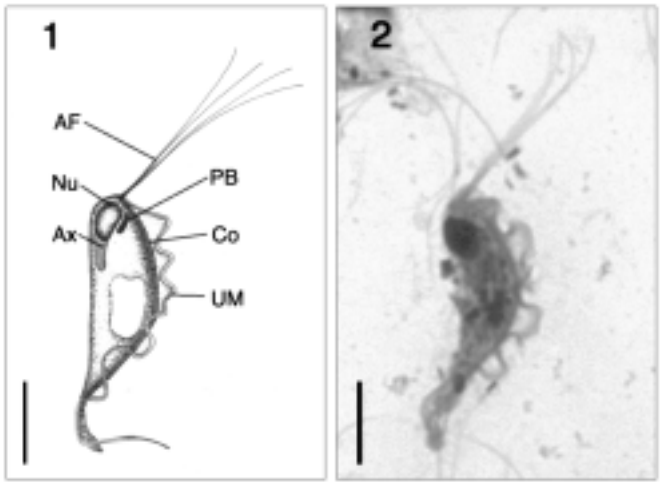
Specimens for transmission electron microscopy were collected exclusively from "clean" termites dissected into Locke's fluid and fixed in at least 10 volumes of 3% glutaraldehyde in 0.066 M cacodylate buffer (pH 7.2) for 30 min. Fixed samples from several termites were pooled and washed three times in Sorenson's phosphate buffer (pH 6.8) for 30 min each. Cells were post-fixed in 4% osmium tetroxide for 1 h and washed 3 times in distilled water (10 min, 10 min and overnight). Specimens were then dehydrated in a graded series of acetone solutions (5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 95, 100 and 100%) for 10 min. each. Cells were gradually infiltrated with Epon resin (25, 50, 75% Epon in 100% acetone for 1 h each, 100% Epon overnight) and embedded in fresh 100% Epon, pelleted by gentle centrifugation and cured for 1 day at 60°C. Semithin survey sections were cut with glass knives, stained with 1% toluidine blue and used to orientate sections. Ultra-thin sections (70 nm and 90 nm) were cut with diamond knives, mounted on formvar-coated copper slot grids, stained with 5% uranyl acetate in 50% methanol for 2 min, washed in distilled water for 30 s and dried. The sections were then counter-stained with Reynold's 2% lead citrate for 1 min, washed in distilled water for 30 s and dried prior to examination. Sections were examined in a JEOL 1010 transmission electron microscope.

RESULTS

Colonies of *S. secundus* were collected from the following localities Brisbane, QLD (8 colonies), Herberton, QLD (1 colony), Nanango, QLD (1 colony) and New Brighton, NSW (1 colony); 1 colony of *S. intermedius* was collected from Brisbane (QLD). All soldiers and workers from every colony examined were host to a small trichomonad species whose characteristics were consistent with the description of the genus *Pseudotrypanosoma* Grassi, 1917. The species appeared to be novel and its morphology and ultrastructure are described below.

Pseudotrypanosoma elphinstonae sp. n.

Type host: *Schedorhinotermes secundus*
Other host: *Schedorhinotermes intermedius*



Figs 1-2. Morphology of *Pseudotrypanosoma elphinstonae* sp. n. **1** - line diagram, **2** - light micrograph. Scale bars 10 µm.

Habitat: termite hindgut.

Type locality: Pinjarra Hills, Brisbane, QLD.

Type material: holotype deposited with the Queensland Museum (Brisbane, Australia), accession number: G463726.

Description: cells long, narrow and sinuous, 10.2-21.6 (16.3) µm long by 2.4-7.8 (4.1) µm wide (Figs 1, 2). Live cells highly flexible, capable of coiling upon themselves but rarely becoming spherical (characteristic of other *Pseudotrypanosoma* spp). Four anterior flagella, 8.7-19.2 (11.9) µm long, emerge just ventral of the cell apex, flagella often grouped into 2 pairs proximally, splitting distally. Nucleus ovoid, 1.8-3.6 (2.8) µm long by 1.2-2.4 (1.9) µm wide, located at anterior of cell immediately beneath the anterior flagella. Axostyle short and rod-like, 1.2-3.6 (2.4) µm long by 0.6-1.8 (1.1) µm wide, projects from nucleus posteriorly through middle of the cell, axostyle is non-emergent. Undulating membrane sinusoidal, 7.5-20.4 (13.7) µm long reaching almost to posterior end of the cell, recurrent flagellum extends freely posterior of the undulating membrane short distance.

Differential diagnosis: *P. elphinstonae* differs from *P. giganteum* Grassi, 1917 on the basis of body size (it is much smaller) and costa morphology (it is narrow and not greatly contractile in the former species). *P. elphinstonae* differs from *P. minimum* Sutherland, 1933 in having a very short, non-projecting axostyle whereas the latter species has a strongly projecting axostyle.

Etymology: *P. elphinstonae* is named after a good friend and great dancer Miss Kara Elphinstone.

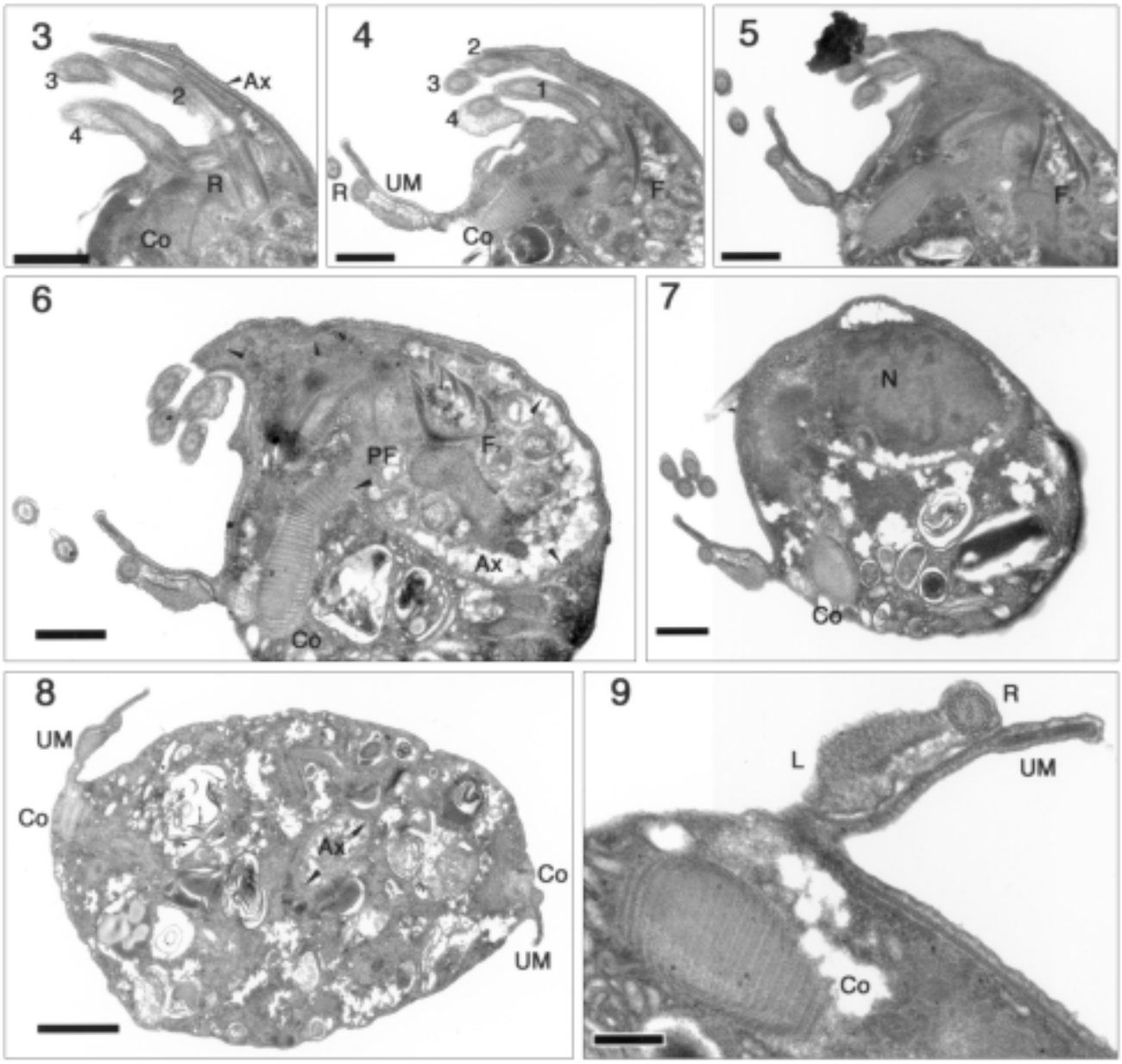
Ultrastructural study

Mastigont system

There are 4 anterior flagella and 1 recurrent flagellum which forms part of the undulating membrane (Figs 3, 4, 10). The anterior flagella are deeply recessed within a ventral pocket; the anterior end of the cell thus forms the periflagellar canal from which the flagella emerge (Figs 3, 10). Flagellum 2 is adherent to the roof of the periflagellar canal and there is a thickening of the cell membrane in this area (Fig. 4). The basal bodies of the anterior flagella are associated to form two pairs - 1,4 and 2,3 (Fig. 6). The basal body of the recurrent flagellum (R) is perpendicular to the other basal bodies and emerges from the centre of the diamond which they form (Fig. 10). There is a small pocket around the base of the recurrent flagellum as it emerges from the cell (Fig. 10). Basal bodies 1 and 3 bear a hooked lamina emerging from the top of the basal body and curving around it clockwise before running posteriorly for a short distance (Fig. 13). The sigmoid fibres (F_2) are located posterio-dorsal to the basal bodies and are attached to the basal body 2 (Fig. 14). F_2 is composed of a dorsal set of 5 fibres which are unbranching and a single ventral fibre which splits into 4-5 fibres before curving dorsally towards the end of the fifth dorsal fibre (Figs 4-6). The whole structure thus has a claw-like appearance in profile. The reticular bodies found in *P. giganteum* by Brugerolle (1999) were absent; a superficially similar structure, the scroll-form body was located dorsal to the basal bodies but was composed of convoluted membranes not microtubules (Fig. 15).

Nucleus and axostyle

The pelto-axostylar complex is simple composed of a single layer of microtubules throughout. There is no pelta distinct from the axostyle. The capitulum of the axostyle instead opens out to form a broad hood overlap the mastigont structures (Figs 6, 12). There is no over or doubling of the microtubules as has been observed in other trichomonads where the pelta overlap the axostyle. The axostyle is a thin sheath wrapped tightly around the nucleus and projecting posteriorly into the main body of the cell (Figs 8, 12). The nucleus is located within the anterior end of the axostyle immediately posterior to the Golgi stack (Figs 11, 12). Nuclear chromatin is densely

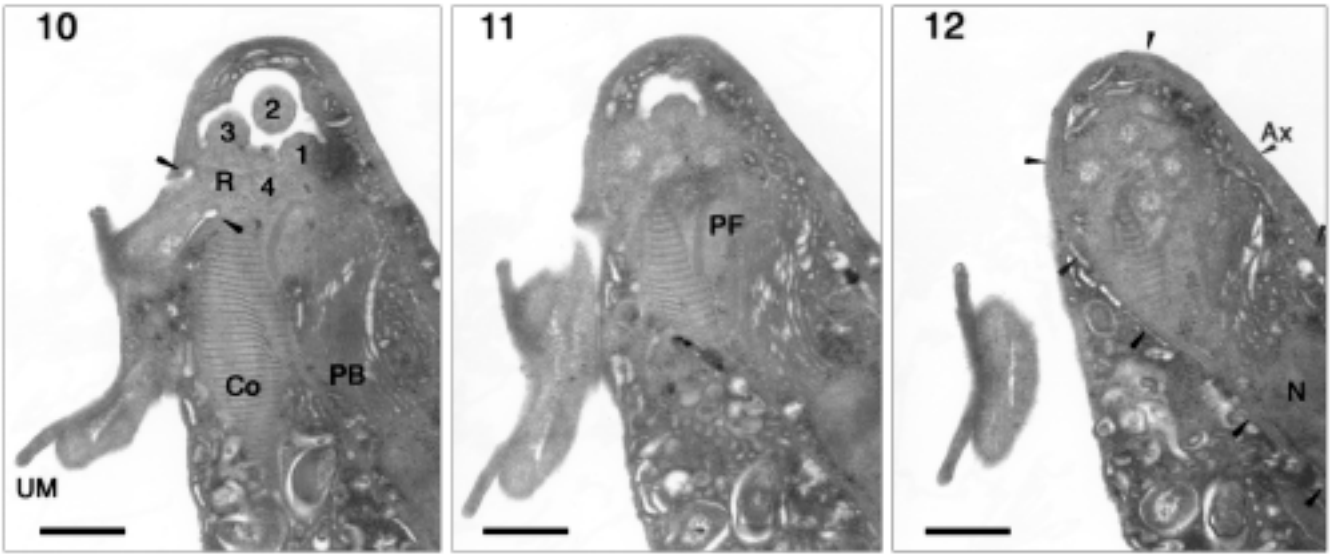


Figs 3-9. Transmission electron micrographs of *P. elphinstonae* sp. n., transverse sections. **3-8** - serial sections through the same cell, anterior to posterior; **9** - costa and undulating membrane. Arrow heads in 6 and 8 show course of axostyle. 1 - flagellum 1, 2 - flagellum 2, 3 - flagellum 3, 4 - flagellum 4, Ax - axostyle, Co - costa, F_2 - sigmoid fibre, L - lamella, N - nucleus, PF - parabasal fibre, R - recurrent flagellum, UM - undulating membrane. Scale bars 10 µm (3-8); 1 µm (9).

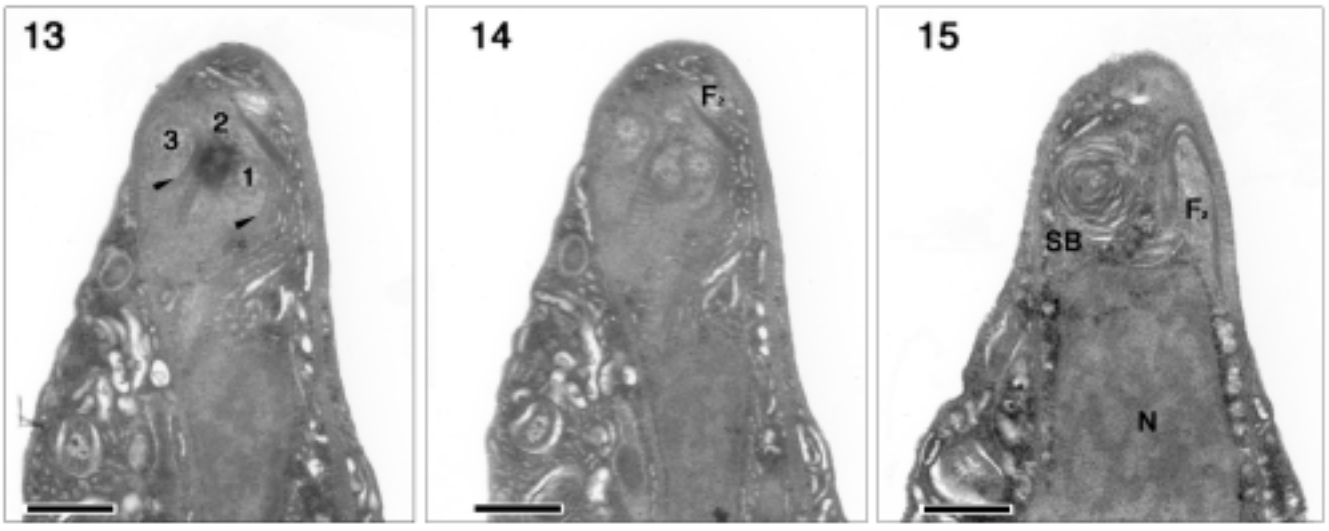
packed but has not condensed into discrete bodies (Figs 7, 15).

Parabasal apparatus

The parabasal apparatus is simple composed of a single parabasal fibre and a single Golgi stack of cisternae (Figs 10, 11). It is not branched as in many other trichomonads and is entirely contained within the pelto-axostylar complex. The parabasal fibre is a short, thick, electron opaque fibre attached anteriorly to basal body 4, runs posteriorly parallel to the costa and joins the Golgi ventrally at the point where the cisternae approach the costa (Fig. 11). The parabasal fibre is attached to the Golgi superficially; it does not penetrate the stack as in *P. giganteum*. The whole parabasal apparatus is small, squeezed between the nucleus and the costa (Figs 10-12).



Figs 10-12. Transmission electron micrographs of *P. elphinstonae* sp. n., sagittal sections I. **10-12 -** serial sections through the same cell, ventral to dorsal. Arrowheads in 10 highlight the sac-like opening at the base of the recurrent flagellum. Arrowheads in 12 show the course of the axostyle. 1 - flagellum 1, 2 - flagellum 2, 3 - flagellum 3, 4 - flagellum 4, Ax - axostyle, Co - costa, N - nucleus, PB - parabasal body, PF - parabasal fibre, R - recurrent flagellum, UM - undulating membrane. Scale bars 1 µm.



Figs 13-15. Transmission electron micrographs of *P. elphinstonae* sp. n., sagittal sections II. **13-15 -** serial sections through the same cell, ventral to dorsal, arrowheads in 13 denote the hooked lamella. 1 - flagellum 1, 2 - flagellum 2, 3 - flagellum 3, F_2 - sigmoid fibre, N - nucleus, SB - scroll-form body. Scale bars 1 µm.

Undulating membrane and costa

The costa is a thick, striated cord wrapped in a loose spiral around the cell in association with the undulating membrane (Figs 8, 9). Anteriorly, it is a narrow cord attached to the posterior of basal body 2 and directed posteriorly between basal bodies 3 and 4 (Fig. 12). It thickens significantly immediately posterior to basal body 4 giving the appearance of also being attached to this basal body (Fig. 11). For much of its length, the costa is of constant width, is roughly circular in cross-section and heavily striated. The undulating membrane is composed

Table 1. Morphometrics of *Pseudotrypanosoma elphinstonae* sp. n., measurements in micrometers.

Character	x	SD	Min	Max	n
Body length (L)	16.31	3.002	10.2	21.6	40
Body width (W)	4.11	1.172	2.4	7.8	40
Shape index (L/W)	4.16	1.010	2.04	6.0	40
Anterior flagellum length	11.94	2.688	8.7	19.2	40
Nucleus length	2.85	0.430	1.8	3.6	40
Nucleus width	1.95	0.330	1.2	2.4	40
Axostyle length	2.4	0.775	1.2	3.6	26
Axostyle width	1.11	0.333	0.6	1.8	26
Undulating membrane length (UM)	13.68	2.922	7.5	20.4	40
Undulating membrane proportion (L/UM)	1.2	0.135	0.95	1.53	40

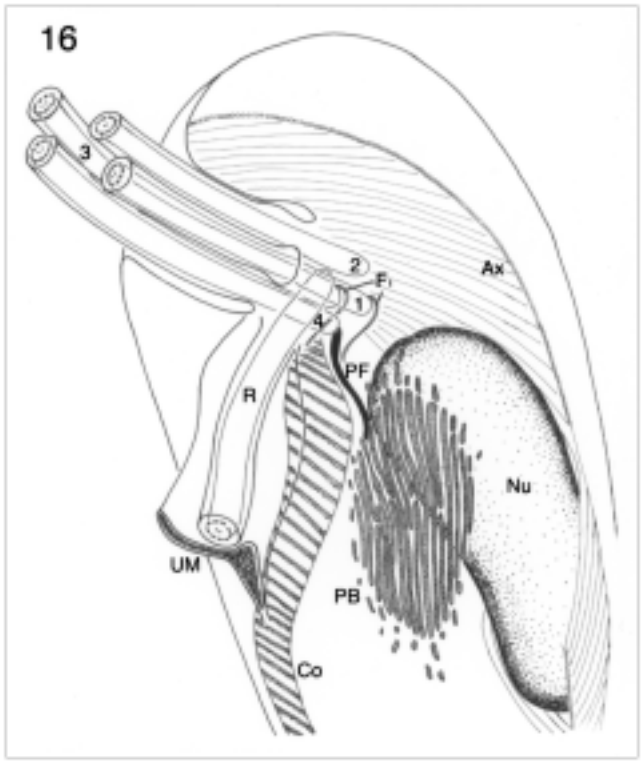


Fig. 16. Cut away view of the mastigont system of *Pseudotrypanosoma elphinstonae* sp. n., sigmoid fibre and scroll-form body omitted in the interests of clarity. 1 - basal body 1, 2 - basal body 2, 3 - basal body 3, 4 - basal body 4, Ax - axostyle, Co - costa, F_1 - hooked lamella, Nu - Nucleus, PB - parabasal body/golgi apparatus, PF - parabasal fibre, R - recurrent flagellum, UM - undulating membrane.

of the recurrent flagellum and lamella (Fig. 9). The recurrent flagellum is dilated in a long thin band which projects back towards the costa, this dilated portion lacks internal structure (Fig. 9). No microtubular bundles were found to connect to the undulating membrane to the costa as has been observed in *P. giganteum*.

DISCUSSION

Whilst superficially similar the trichomonads which possess 4 anterior flagella are readily distinguishable from each other by their distinctive ultrastructural characteristics. *P. elphinstonae* differs from *Trichomonas gallinae* in the arrangement of the fibres associated with the flagellar bases: (1) absence of a shallow periflagellar canal; (2) lack of a pelta which conspicuously overlaps the anterior end of the axostyle; (3) very different structure to the sigmoidal fibre (2 sheets composed of 5 and 1 microtubules in the former species versus a single sheet of 9 microtubules in the latter) (Mattern *et al*. 1967). In addition the structure of the parabasal body of *T. gallinae* is much more complex than that of *P. elphinstonae*. In *Trichomonas* there are 2 parabasal fibres, each the diameter of a kinetosome, PF1 derives from basal body 2 and curves around the basal body 3 to the right and external to the hooked lamina and PF2 has a common origin with the costa, between basal bodies 3 and 4 before running dorsally along the length of the costa; the parabasal body itself is composed of an irregular stack of few discs (Mattern *et al*. 1967). In contrast in *P. elphinstonae* there is a single parabasal fibre derived from basal body 4 which attaches superficially to the dense, regularly stacked Golgi apparatus. The undulating membrane and costa is much less developed in *Trichomonas* than in *Pseudotrypanosoma*. The costa itself is much smaller, about the diameter of a kinetosome whereas in *P. elphinstonae* it is about 3 times the diameter. There is also no contact between the recurrent flagellum and the undulating membrane along its length in *Trichomonas* whereas they are intimately associated in *P. elphinstonae* and *P. giganteum* (Mattern *et al*. 1967,

Brugerolle 1999). These differences challenge the proposal of Cleveland (1961) that *Pseudotrypanosoma* and *Trichomonas* are synonymous and is consistent with molecular phylogenetic results suggesting the distinctiveness of *Pseudotrypanosoma* (Keeling *et al.* 1998, Gerbod *et al.* 2001).

Pentatrichomonas is a second genus with 4 anterior flagella (plus an independent flagellum which gives the cell the appearance of 5 anterior flagella), which has been suggested, is closely related to *Pseudotrypanosoma* on the basis of molecular sequence analysis (Gerbod *et al.* 2001) despite their widely separated hosts (mammals *vs*. termites). Some features of the kinety ultrastructure supports this hypothesis (Honigberg *et al.* 1968); in *Pentatrichomonas* the 4 anterior flagella are grouped in pairs and the sigmoidal fibre splits into several subfibres of similar appearance to that seen in *Pseudotrypanosoma* (Honigberg *et al.* 1968). Most of the other features of the cell are however identical to those found in *Trichomonas* so the significance of the ultrastructural similarities between *Pentatrichomonas* and *Pseudotrypanosoma* are difficult to gauge.

Pseudotrypanosoma elphinstonae is very similar to *P. giganteum* but differs in several features (Brugerolle 1999). The arrangement of the basal bodies and their associated fibres is very similar, basal bodies 1-4, R and the hooked lamina fibres are all arranged identically in the two species. The sigmoid fibre (F_2) has a similar structure (5 dorsal and 1 ventral) and origin (basal body 2) in the two species but is much smaller and divides into fewer secondary fibres in *P. elphinstonae* (4-5 secondary fibres *vs*. 30 in *P. giganteum*). The reticulate bodies found in *P. giganteum* were not present in *P. elphinstonae*. The costa was a much less conspicuous organelle in *P. elphinstonae* than in *P. giganteum*. Even considering the smaller size of the cell, the costa of *P. elphinstonae* was proportionately smaller, less conspicuously contractile in living cells and was never observed independent of lysed cells. The undulating membrane of *P. elphinstonae* is also simpler than that of *P. giganteum*. The dilated recurrent flagellum of the former species is smaller and lacks the internal structure of para-axonemal fibres found in the latter. The most striking difference between *P. elphinstonae* and *P. giganteum* is the absence of a pelta in the former species and its replacement by an elaboration of the axostyle's capitulum. *P. giganteum* has a large pelta which, similar to other trichomonads, is composed of a ribbon of 5 microtubules at it's base where it overlaps the axostyle capitulum (Mattern *et al.* 1967, Honigberg *et al.* 1968, Brugerolle 1999). There is no such overlapping area in *P. elphinstonae* and the hood-like structure similar to the pelta appears to be continuous with the axostyle. For this reason we have chosen to refer to this structure as an expanded axostyle rather than a simplified pelta. Considering the differences in size and structure of the organelles between the two species it is apparent that *P. elphinstonae* is much smaller and simpler in structure than *P. giganteum*. There are few similar examples comparing the ultrastructure of congeneric trichomonads which vary significantly in their cell size. Further investigation of such pairs may greatly improve our understanding of the probable functions of the various components of the mastigont system.

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