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Description of a New Freshwater Heterotrophic Flagellate *Sulcomonas lacustris* Affiliated to the Collodictyonids

Guy BRUGEROLLE

Biologie des Protistes, CNRS and Université Blaise Pascal de Clermont-Ferrand, Aubière Cedex, France

Summary. This new heterotrophic flagellate has two flagella inserted in the subapical region and a conspicuous ventral groove where bacteria are phagocytosed. Several microtubular fibres are connected to the two basal bodies: a dorsal root that plays the role of a MTOC for cortical microtubules, a left and a right ventral root that border the ventral groove, a root on the left side inserted between the Golgi body and the nucleus, and two fibrils that outline the bottom of the groove. Several features suggest a phylogenetic relationship with collodictyonids, i. e. a similar constriction at the base of the flagella, a similar desmose between the basal bodies that includes the origin of the left root, a similar arrangement of flagellar roots, and tubular mitochondrial cristae. Several features are more specific, such as a fibrillar rhizostyle adhering to a mitochondrial lobe and the single, small dictyosome.

Key words: collodictyonid affiliation, freshwater flagellate, *Sulcomonas lacustris* n. sp., ultrastructure.

INTRODUCTION

The widespread application of molecular methods such as the SSU rRNA (small subunit ribosomal nucleic acid) gene sequences to the identification of uncultivated environmental organisms (Dawson and Pace 2002) has led to the discovery of new phylotypes, that are presumably new species and subgroups of protists (Moon-van der Staay *et al.* 2001; Lopez-Garcia *et al.* 2001, 2003; Diez *et al.* 2001; Moreira and Lopez-Garcia 2002; Massana *et al.* 2002, 2004; Berney *et al.* 2004; Scheckenbach *et al.* 2005; Slapeta *et al.* 2005). These methods are highly efficient in estimating the diversity of the organisms in environmental samples but are not free of artefacts such as the occurrence of chimeric sequences (Berney *et al.* 2004). These methods therefore require isolation of the organisms corresponding to the phylotypes for morphological and physiological studies in order to characterize the species and to understand their role in aquatic ecosystems (Fenchel 1987, 2002; Finlay 1990; Finlay and Fenchel 2004). In traditional morphology, light and electron microscopy have proved important and necessary tools for the identification of species and their taxonomic affiliation and for providing insights into their biology (Ragan and

Address for correspondence: Guy Brugerolle, Biologie des Protistes, Université Blaise Pascal de Clermont-Ferrand, 63177 Aubière Cedex, France; Fax: 330473407670; E-mail: guy.brugerolle@univ-bpclermont.fr

Chapman 1978, Margulis *et al.* 1990, Taylor 1999, Lee *et al.* 2001, Hausmann *et al.* 2003, Adl *et al.* 2005). Several free-living flagellates in aquatic environments have yet to be identified (Patterson and Zölffel 1991, Tong *et al.* 1998, Patterson 1999, Bernard *et al.* 2000, Patterson *et al.* 2001, Schroeckh *et al.* 2003, Lee *et al.* 2005). This study describes a protist flagellate not yet reported in the protistology or ecology literature, and which is probably rare in aquatic environments. Several features revealed by electron microscopy suggest that it is affiliated to *Diphylleia (= Aulacomonas*) and the collodictyonids, which are a small group of flagellates with tubular mitochondrial cristae phylogenetically distant from other flagellate groups (Brugerolle and Patterson 1990, Brugerolle *et al.* 2002).

MATERIALS AND METHODS

Origin. This flagellate was isolated from the intestinal content of a tadpole of *Bufo bufo* from Lake Chauvet in the Clermont-Ferrand region (Central France). The gut content was mixed with a few ml of a Ringer's solution in a Petri dish and left for 48 h at room temperature. Several protists developed, among which *Sulcomonas lacustris* was the most abundant and was collected, using a micropipette for light microscopy observations and preparation for electron microscopy. The flagellate population then decreased as ciliates invaded the dish and fed on both the flagellates and bacteria.

Microscopy. Living cells were observed under a phase contrast microscope (Leitz), transformed into drawings, and then photographed after fixation. Electron microscopy was performed by collecting the cells using a micropipette under a stereo-microscope followed by fixation in 1:1 mixture of 1% glutaraldehyde and 2% osmium tetroxide (Polysciences) in 0.1 M phosphate buffer at pH 7 for 1 h. Cells were washed twice in the buffer by centrifugation and then post-fixed in 1% osmium tetroxide in 0.1M phosphate buffer for 1 h. After washing in water, the cells were pre-embedded in 1% agar (Difco) and stained 'en bloc' in saturated uranyl acetate in 70% ethanol for 15 h. After complete dehydration in an alcohol series to 100% alcohol and 1,2-propylene oxide, the cell pellet was embedded in Epon 812 resin (Merck). Ultrathin sections were obtained using a Reichert Ultracut S microtome (Leica), and stained for 15 min with lead citrate before examination under a JEOL 1200EX electron microscope at 80 KV.

RESULTS

Light microscopy

The cells have an ovoid shape with a conspicuous ventral groove, and an average length of 9.8 µm (8.4-11.2 µm), for an average breath of 5.6 µm, from 50 measured cells (Figs 1, 3). The two flagella are inserted in the sub-apical region. The anterior flagellum is shorter, about two times the cell length, and oriented forward, while the posterior flagellum is longer, about three times the cell length, and oriented backwards (Fig. 3). In the living cell, the posterior flagellum generally lies in the ventral groove and sometimes adheres to the substratum, but in the fixed cells both flagella are often deflected forwards. Swimming is not very efficient since the two opposite beating flagella do not propel the cell quickly. The nucleus is situated in the anterior part, the cell cortex appears rigid, and the cytoplasm contains food vacuoles.

Electron microscopy

Flagella. The two basal bodies (1, 2) are arranged in parallel, one above the other in the longitudinal axis of the cell and both oriented toward the ventral side (Figs 2, 4). Basal bodies are of a quite normal length of 0.5 µm, and are interlinked by a dense fibre which caps their proximal section (Figs 2, 4, 8) and is connected to the 'rhizostyle' (see below). The basal body cylinder does not show an evident cartwheel structure and the transitional zone is relatively long at 0.1 µm (Figs 5, 8). Remarkably, the proximal part of the flagellum is occupied by an electron-dense constriction zone about 0.05 µm long where the flagellar membrane is in contact with the doublets of the axoneme (Fig. 5). A transverse section across this constriction shows that the central pair of microtubules is surrounded by a very dense sleeve (Fig. 5c). The flagella do not bear any mastigonemes on their surface.

Flagellar roots. The two flagella arise from an aperture whose dorsal border is supported by a microfibrillar zone (Figs 4, 13). The anterior basal body (1) is connected to a dorsal microtubular root comprising about five microtubules that is oriented toward the dorsal left side (Figs 2, 6, 7, 10). Distantly spaced microtubules arise along this fibre and run under the plasma membrane (Figs 6, 11). They cover the anterior part of the cell cortex, and also accumulate along the two ridges of the ventral groove (Figs 2, 15). These cortical microtubules arise from microfibrillar material present along the dorsal root and close to the anterior basal body that acts as a MTOC (Figs 10, 11). At the right side of the basal body (1) is attached a second root (rvR) of five interlinked microtubules that runs along the right border of the ventral groove under the plasma membrane (Figs 2, 7, 9, 14). The posterior basal body (2) is connected to the anterior basal body by a desmose that includes the left ventral root (lvR) comprising five to six interlinked

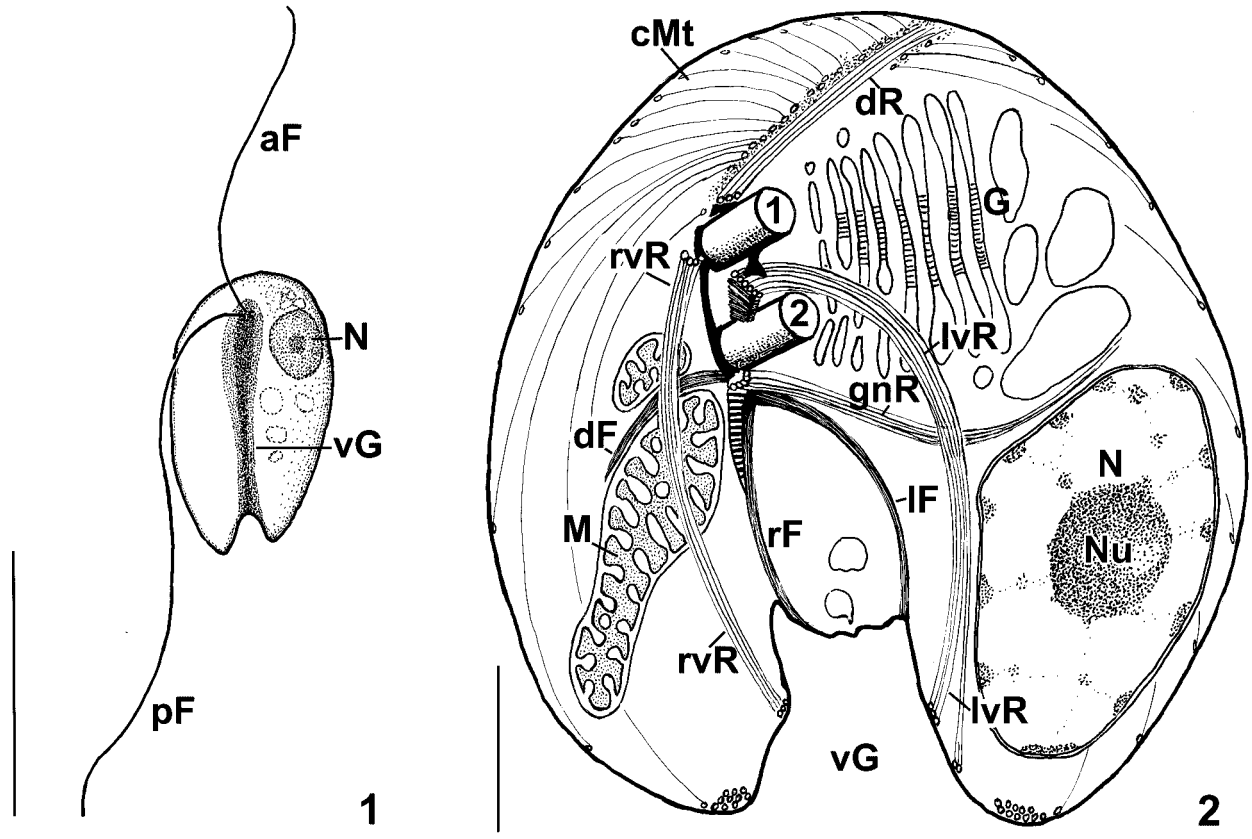
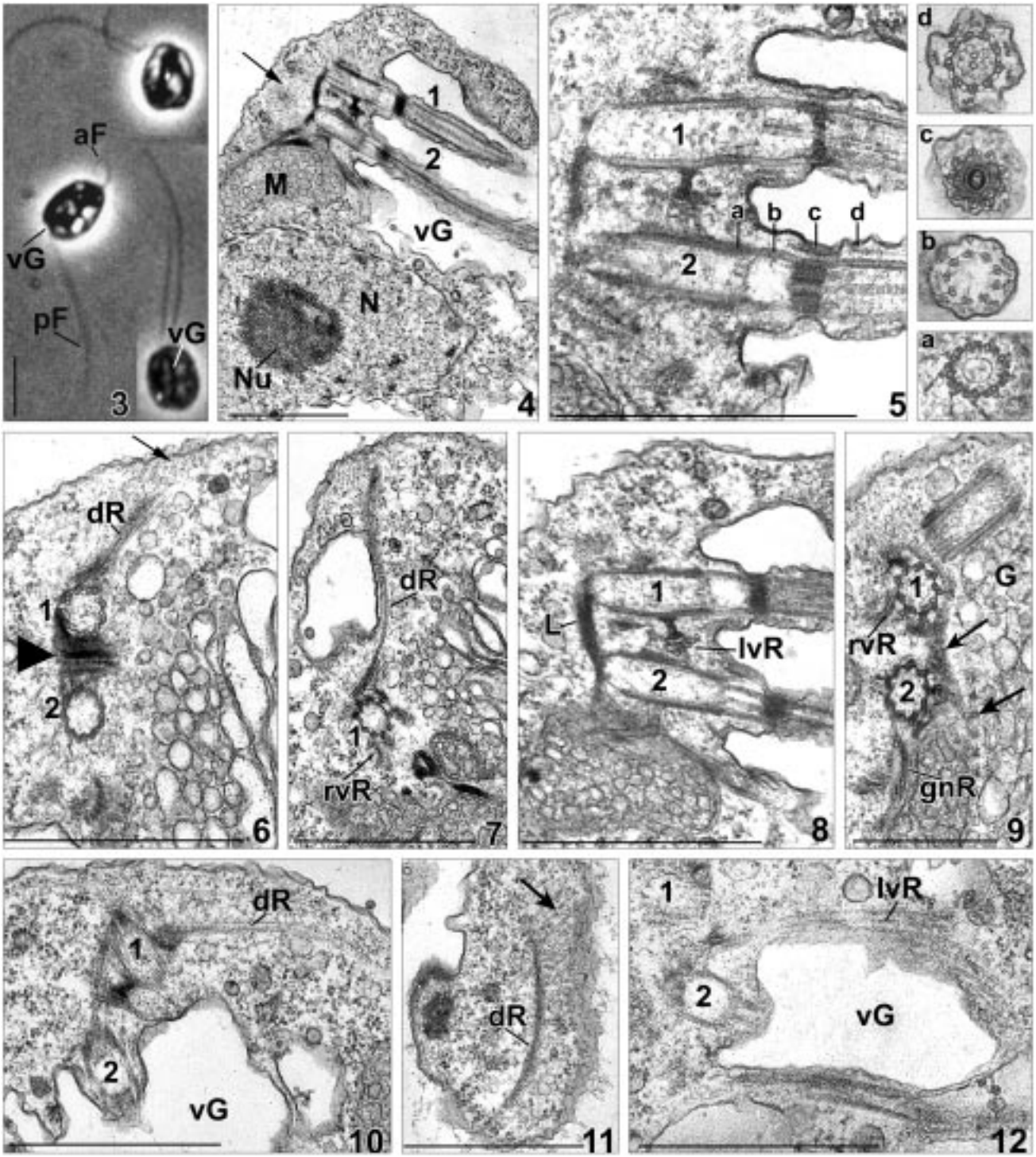


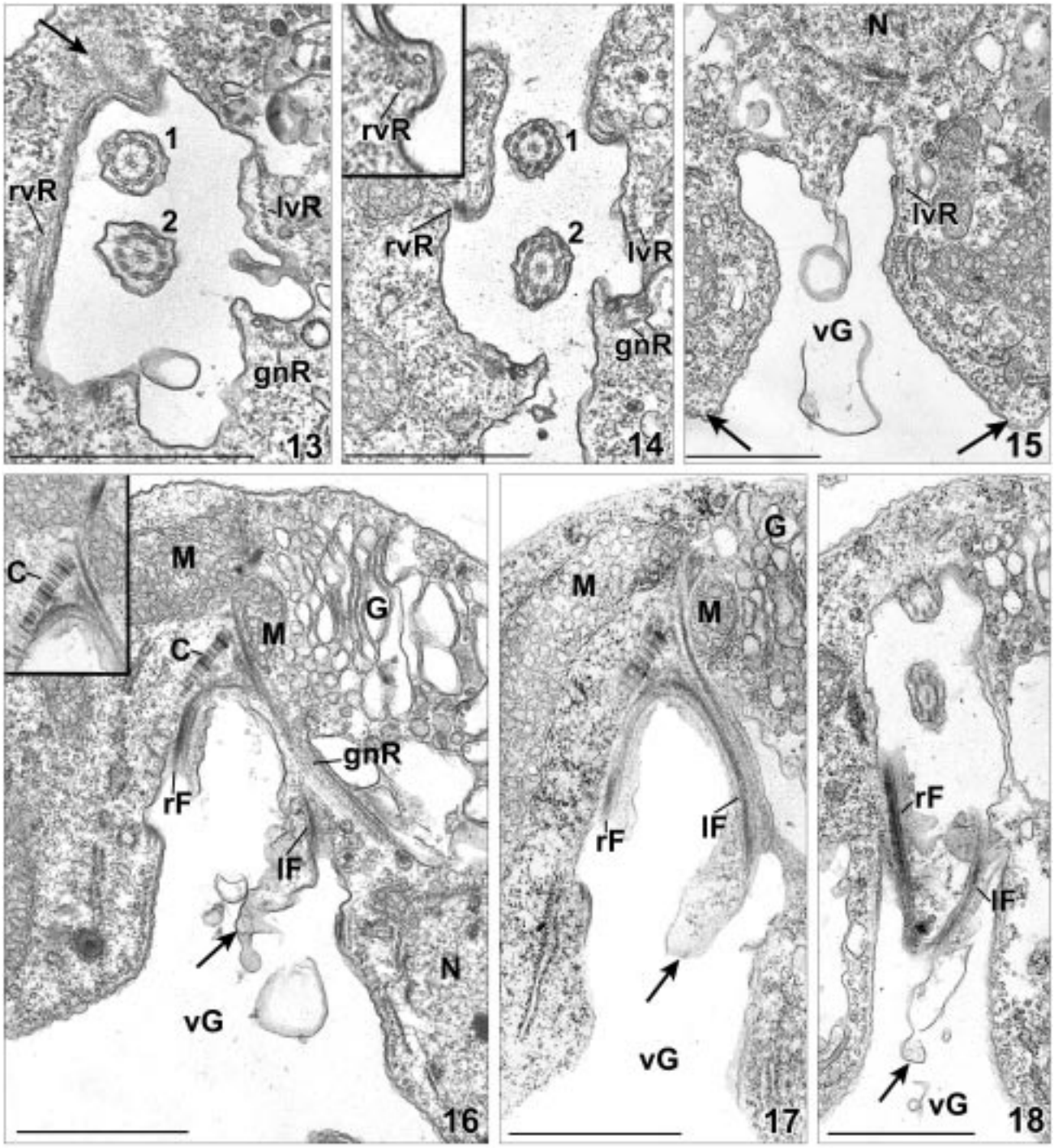
Fig. 1. Light microscopy representation of *Sulcomonas lacustris* showing the two flagella (aF - anterior flagellum, pF - posterior flagellum) apically-inserted, the longitudinal ventral groove (vG) and the nucleus (N). Scale bar 10 μm.
Fig. 2. Reconstruction scheme of the flagellar system of *Sulcomonas lacustris*, view onto the anterior pole. The two basal bodies (1, 2) are connected to the dorsal root (dR) from which arise cortical microtubules (cMt) covering the anterior part, the right ventral root (rvR) oriented to the right side of the groove (vG), the left ventral root (lvR) oriented to the left side of the groove, the Golgi nucleus root (gnR) inserted between the Golgi body (G) and the nucleus (N) with a nucleolus (Nu), the two ventral fibrils (rF, lF) that develop on the upper bottom of the groove and the rizostyle-like dorsal fibril (dF) linked to a mitochondrial lobe (M). Scale bars: 1 μm.

microtubules (Figs 2, 6, 8). This desmose comprises a dense connector attached to the anterior basal body (1) on one side and a microfibrillar striated structure attached to the posterior basal body (2) on the other side (Fig. 8). Distally, this left microtubular root is oriented to the anterior left face of the ventral groove and merges with cortical microtubules of the left border of the groove (Figs 12-15). At the posterior side of the basal body (2) is attached a microtubular root of five microtubules (gnR) oriented to the left side of the groove and distally inserted between the Golgi body and the nucleus (Figs 2, 9, 13, 16). This root shares a common origin with several electron-dense fibrils. The ventral fibril divides into two branches (rF) and (lF) that outline the upper bottom of the groove (Figs 2, 16-18). The base of the right side branch is doubled by a striated cord (Fig. 16). The dorsal fibril (dF) is oriented dorsally and similarly to a small rhizostyle it is attached to a large mitochondrial lobe indenting its surface (Fig. 19). In addition, on the left side of the basal body pair there is a striated structure that links the two basal bodies and continues along the Golgi stack of cisternae (Fig. 9, arrows).

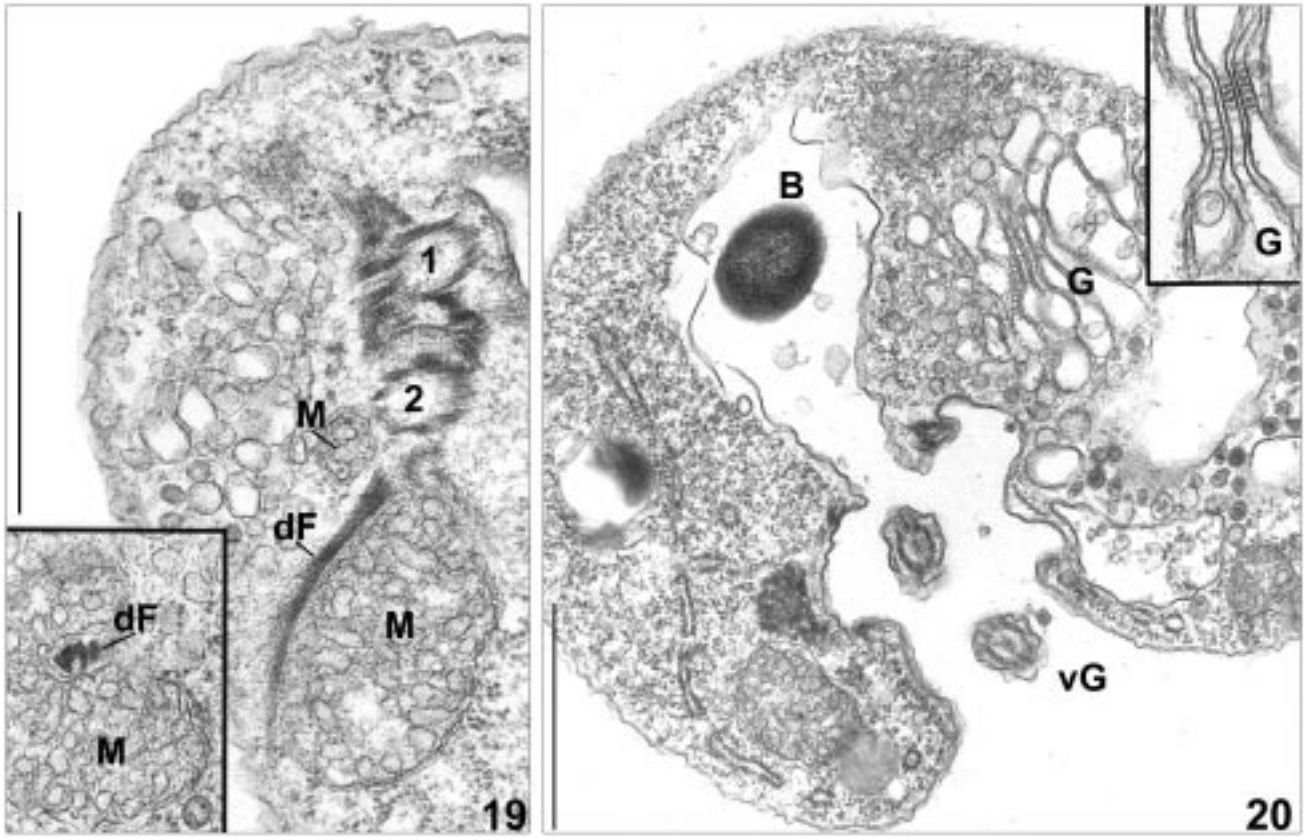
The Golgi body is situated on the left side of the basal bodies with its “trans” face close to the basal bodies and its “cis” face oriented toward the cell surface (Fig. 20). The “cis” face of the Golgi body comprises large vesicles that widen to form vacuoles close to the cell surface in the zone in front of the nucleus (Figs 2, 20). Inside the staggered Golgi cisternae there is a peculiar structure composed of bridges that link the two closely apposed membranes inside a cisterna. Examination of the sections through the cell suggests that the mitochon-



Figs 3-12. Light microscopy micrographs (3) and electron micrographs (4-12) of *S. lacustris*; **3** - phase contrast micrographs showing the two unequal flagella (aF, pF) inserted at the top of the ventral groove (vG); **4** - view of the two basal bodies / flagella (1, 2) inserted subapically in a dorsal microfibrillar zone (arrow) and arising in the ventral groove (vG), the mitochondrion (M), and the nucleus (N) with a nucleolus (Nu); **5** - ultrastructure of the basal bodies/flagella (1, 2) and transverse sections at different levels (a, b, c, d); notice the constriction zone and the special ultrastructure at the # c level; **6**, **7** - sections showing the dorsal microtubular root (dR) attached to basal body (1) from which arise cortical microtubules (arrow), the desmose structure between the basal bodies (arrowhead) and the origin of the right ventral root (rvR); **8** - origin of the left ventral root (lvR) between the basal bodies (1, 2); basal body connecting link (L); **9** - section showing the right ventral root (rvR) attached to the anterior basal body (1), the Golgi nucleus root (gnR) attached to the posterior basal body (2) and a striated structure (arrows) connecting the basal bodies and the Golgi zone (G); **10**, **11** - dorsal root (dR) connected to basal body (1) and the origin of the cortical microtubules close to this root (arrow); **12** - origin of the left ventral root (lvR) between the two basal bodies (1, 2). Scale bars: 10 µm (3); 1 µm (4-12).



Figs 13-18. Electron micrographs of *S. lacustris*. **13**, **14** - transverse sections of the upper part of the groove where the two flagella (1, 2) arise, dorsal microfibrillar border of the groove (arrow), right ventral root (rvR), left ventral root (lvR), Golgi nucleus root (gnR); **15** - transverse section at the nucleus level (N) showing the microtubules (arrows) accumulated in the two borders of the ventral groove (vG) and microtubules of the left ventral root (lvR); **16-18** - oblique section showing the Golgi nucleus root (gnR) inserted between the Golgi body (G) and the nucleus (N), as well as the two fibrils (rF, lF) that outline the bottom of the groove, the striated connecting cord (C) and pseudopodial processes (arrow) at the bottom of the groove (vG). Scale bars: 1 µm.



Figs 19-20. Electron micrographs of *S. lacustris.* **19** - the dorsal fibril (dF) connected to basal body (2) that adheres to a mitochondrial lobe (M) indenting its surface (inset); **20** - transverse section to show the endocytosis of a bacterium (B) at the bottom of the groove (vG) and the peculiar bridges between the membranes within the Golgi (G) (inset). Scale bars: 1 µm.

drion is a mitochondrial network with tubular or ampullar cristae and an electron-dense matrix. The nucleus is situated on the left dorsal part of the cell and contains a central nucleolus (Figs 2, 4). Digestive vacuoles containing bacteria are scattered within the cytoplasm. These digestive vacuoles are formed at the upper bottom of the groove (Fig. 20), a place where small pseudopods develop (Figs 15-18), and this area is a privileged zone of endocytosis of food particles.

DISCUSSION

This flagellate shows common features with *Diphylleia* and *Collodictyon*, two heterotrophic free-living flagellates with an undetermined phylogenetic position among flagellate groups (Brugerolle and Patterson 1990, Brugerolle *et al.* 2002). The basal part of the flagella has a similar constriction and ultrastructure, and the typical desmose between basal bodies including the origin of the left microtubular root is also similar. The other roots that compose the cytoskeleton are also very similar: the dorsal root is associated with an MTOC for cortical microtubules and the ventral roots outline the ventral groove. Comparatively to *Diphylleia* and *Collodictyon* other features are more specific, such as the kind of rhizostyle that links the mitochondrion to the basal bodies and the limited extension of the Golgi body.

The features of the flagellar apparatus did not allow to suggest a close phylogenetic relationship with any other group of flagellates, despite the mitochondrion with tubular cristae (Taylor 1999). From light microscopy observation, the presence of a deep groove could suggest a phylogenetic relationship with a member of the Excavata super-group (Adl *et al.* 2005), but a precise examination of the flagellar apparatus organization by electron microscopy did not support a close relationship with any of the diverse groups composing the Excavata. The use of SSU rRNA sequences of *Diphylleia* to locate this group in the tree of life has not fulfilled its

promise (Brugerolle *et al*. 2002). Unfortunately this flagellate could not be collected again in the nature in order to identify it by SSU rRNA sequencing and use it for further phylogenetic analysis.

The history of the origin of this species did not indicate that it is a free-living species. However, since free-living protozoa such as a ciliate, a euglenid of the genus *Scytomonas* and a volvocid of the genus *Polytoma* developed within the culture dish, this suggests that they were ingested with food by the young tadpoles. To the best of my knowledge, no such flagellate has been thus far been described in the literature. The only flagellate to date that has a similar morphology as revealed by light microscopy is *Protaspis simplex* Vørs, 1992, but this species has a shallow ventral groove and a marine habitat (Tong *et al.* 1998). Unfortunately there is no electron microscopy image of a *Protaspis* species available to compare with. I propose to create a new species name to identify this flagellate.

Diagnosis

Sulcomonas lacustris n. g., n. sp. from the Latin *sulco* that means groove or furrow and *lacustris* from lake.

Cell of about 10 µm length with two sub-apical flagella and a ventral groove extending across the whole cell length. The anterior flagellum is two times the cell length and the posterior flagellum is three times the cell length. The constriction at the base of the flagella and the desmose between the two basal bodies including a microtubular fibre are typical. The cortical surface is sustained by microtubules that originate from a dorsal MTOC and the ventral groove is outlined by three microtubular roots together with dense fibrils. There is a kind of rhizostyle-like fibril that attaches the posterior basal body to a lobe of the mitochondrion that has ampullar cristae. The Golgi body is situated on the anterior left part above the nucleus. This flagellate lives in freshwater and feeds on bacteria.

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