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Protistology

Katabia gromovi nov. gen., nov. sp. – a new soil flagellate with affinities to *Heteromita* (Cercomonadida)

Serguei A. Karpov[1,2], Flemming Ekelund[2] and Øjvind Moestrup[3]

[1] *Biological Faculty, St. Petersburg State University, St. Petersburg, Russia*
[2] *Department of Terrestrial Ecology, Zoological Institute, University of Copenhagen, Copenhagen, Denmark*
[3] *Botanical Institute, University of Copenhagen, Copenhagen, Denmark*

Summary

An unusual flagellate from soil has been investigated in culture using LM and TEM, with special reference to the structure of the flagellar apparatus. Trophic forms have two smooth heterodynamic flagella; cysts are surrounded by a thick translucent wall. Flagellates swim permanently and attach to the substratum only just before encystment. They ingest bacteria by pseudopodia whilst swimming. A preliminary study of the partial sequence of SSU rDNA strongly suggests that its closest relatives are *Heteromita*, *Cercomonas*, and *Cryothecomonas*. In general morphology, cytoskeleton structure, behaviour, and life cycle the new strain revealed close affinity to the cercomonad *Heteromita*. The cell is covered by only a plasmalemma underlain by kinetocysts; the basal body of the posterior flagellum lies at approximately right angles and in a different plane to the anterior one; there is a cylinder connected with the A-tubules of the axoneme in the flagellar transition zone; flagellar rootlet homology is clear; the nucleus has a prominent nucleolus; a microbody with amorphous contents is located near the nucleus; mitochondria have vermiform tubular or vesicular cristae. The flagellate differs from *Heteromita* in behaviour as well as in general morphology (presence of unusual mushroom-like bodies) and in details of the cytoskeleton structure. On the basis of behavioural, morphological and molecular data we establish a new genus and species for our strain: *Katabia gromovi* gen et sp. nov., of the order Cercomonadida.

Key words: *Katabia gromovi*, cercomonads, ultrastructure, cytoskeleton, soil flagellates, partial SSU gene sequence

Introduction

Heterotrophic flagellates are, together with the naked amoebae, the most abundant soil protozoa, and, hence, are among the most important bacterial grazers in soil (Ekelund and Ronn 1994; Ekelund et al., 2001). The information available on the composition of the soil flagellate community is extremely scarce (Ekelund

and Patterson, 1997; Ekelund et al., 2001), and general problems, e.g., whether the soil flagellate community is just a ''diluted'' version of the freshwater community and whether the statement that ''everything is everywhere'' (Finlay and Clarke, 1999) is applicable to it are presently unsolved.

As our present knowledge on the soil flagellate community is very sparse, many soil flagellates probably still need to be described.

In this paper we present data on an unusual soil flagellate, which superficially resembles *Katablepharis*. Preliminary molecular data (F. Ekelund, unpublished) showed its relationship to *Cercomonas*. Ultrastructural investigations often yield very good results permitting us to define the place of the protist among the taxa. We have many recent examples of such successful works (Karpov et al., 1998; Guillow et al., 1998, 1999; Flavin et al., 2000). Here the results of our light and electron microscopical observations, and partial sequence SSU rRNA gene on the flagellate are presented.

Material and methods

Isolation, maintenance and proliferation of cultures

A clonal culture of *Katabia gromovi* was isolated from soil collected in a yew (*Taxus baccata*) plantation in Fælledparken (Copenhagen, Denmark) in April 1995. The culture was maintained on ''modified Neff's amoeba saline'' (Page, 1988) supplied with a sterilised wheat grain, in the culture collection at the Zoological Institute, University of Copenhagen. Prior to the present investigation the cultures were fed with *Pseudomonas chlororaphis* (ATCC, Accession no. 43928) in order to provide dense flagellate cultures.

Light microscopy

Light microscopy on living material was performed using the Olympus BX 50 microscope equipped with a camera and flash, and the inverted microscope Leica DM IRB. Fixation of living material was made with 1-2% water solution of glutaraldehyde for 20 min at room temperature. Prior to fixation the cells were collected by centrifugation on 202 MK (Sigma) centrifuge (rotor diameter 15 cm) at a speed of 9 000 rev/min. Digital images of fixed cells were prepared using a videocamera connected to a LEITZ DM RD (Leica) microscope using the PC program ACDSee.

Electron microscopy

For ultrathin sections, 1.5 ml of cells was mixed with 1.5 ml of 5% glutaraldehyde in the medium. After fixation for 2 hours on ice, the pellet was collected by centrifugation and placed without rinsing on the 1% solution of osmium tetroxide in the same medium for 1 hour at 4°C. After dehydration in an alcohol series and acetone, the pellet was embedded in Spurr's resin. Blocks were serially sectioned with a diamond knife on the Reichert or LKB ultramicrotome, mounted on formvar-coated slot grids, and post-stained with uranyl acetate and lead citrate. Whole mounts and sections were viewed on a JEM 100CX electron microscope operating at 80 kV.

For three-dimensional reconstruction of the cytoskeleton, serial sections of the flagellar apparatus of 20 cells were examined. To exclude potential mistakes with rootlet interpretation, we did not analyse micrographs of predivisional stages, when additional basal bodies appear (e.g., just prior to mitosis, additional rootlets appear in conjunction with the replication of basal bodies).

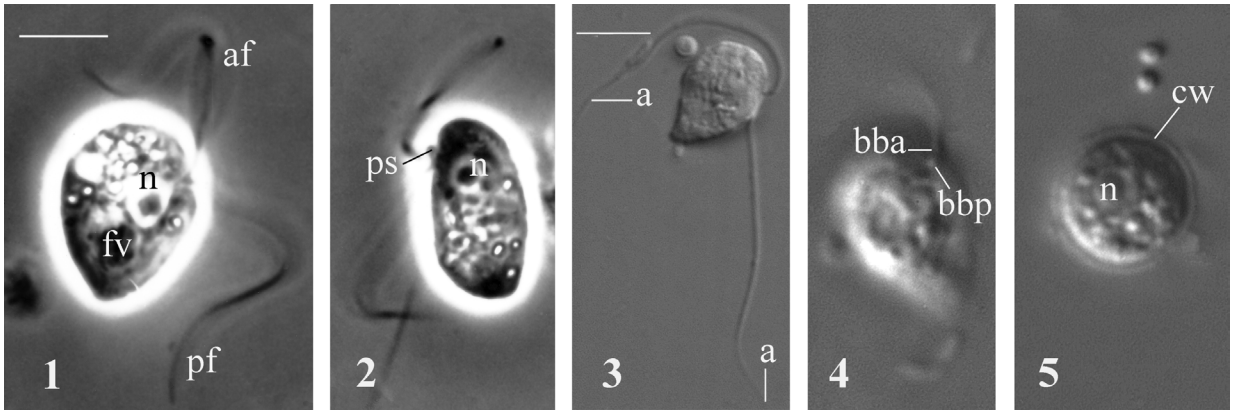
Abbreviations

a - acroneme, af - anterior flagellum, ar - two-stranded microtubular anterior rootlet of BBP, ax - axoneme, bba - basal body of anterior flagellum, bbp - basal body of posterior flagellum, cp - crystal-like protrusion, cr - composite rootlet, 2 microtubules are associated with fibrillar band in its proximal part, cw - cyst wall, d - dictyosome, di - diaphragm-like structure, dm - dense material, dp - dense plate, er - endoplasmic reticulum, fb - fibrillar bridge between kinetosomes, fc - fibrillar cone of BBP, fcr - fibrillar band of CR, fl - flagella, fn - fibrillar network between PB and GR, fr - fibrillar rootlet, fs - fibrillar sheath at the base of basal bodies, fv - food vacuole, gr - girdle rootlet of 3 microtubules, kn - kinetocyst, l - lipid droplet, lr - left rootlet originating from dense plate, m - mitochondria, mb - microbody, mrb - mushroom-like bodies, n - nucleus, ne - nuclear envelope extensions, nu - nucleolus, pb - band of 4-5 microtubules, passing in a posterior direction, pf - posterior flagellum, ps - pseudopodium, sm - secondary microtubules, st - stalk, tc - transitional cylinder, or fibrillar ring, tp - transversal plate, ur - two-stranded microtubular (upper) rootlet of BBA, v- vacuole.

Results

Light microscopy

The trophic cells of our strain move exclusively by swimming. They swim relatively slowly, rotating along their longitudinal axis. The cells are droplet-shaped with a broad and rounded anterior end and a tapering



Figs 1-5. Light microscopical views of *Katabia gromovi*. 1-2 - phase contrast of living flagellates, a short pseudopodium is visible in Fig. 2. 3 - fixed flagellate under DIC, 4 - flagellar insertion (the BBP is directed away from the viewer), 5 - cyst (DIC). For abbreviations see Material and methods.

posterior end (Figs 1, 3, 4). The ventral side[1] is somewhat flattened, while the dorsal side appears more prominent (Figs 3, 4). The cell length is 8–12 μm and the cell width 5–7 μm. The two smooth flagella emerge subapically, approximately 1/4 of the cell length from the anterior end from a small platform on the cell surface (Fig. 3). One flagellum, approximately 2.5 times the cell length, is oriented to the left and backwards and has a rather long acronema (Figs 1, 3, 4). The other flagellum is 1.5 times longer than the cell length, has a more anteriorly located basal body and a slightly anterior-right hand side ventral orientation, and a short acronema (Figs 3, 4). We consider the latter flagellum as the anterior one, because of basal body orientation.

The nucleus, which contains a large nucleolus, is located in the anterior part of the cell (Figs 1, 2). In the middle and posterior part of the cell, one to three large vacuoles are present; sometimes bacteria can be seen inside the vacuoles (Fig. 4). The cytoplasm is densely packed with small particles.

When the flagellates encyst the cells become rounded, and involve the flagella (Fig. 8) before settling on a substrate. They are surrounded by a distinct cyst wall. The cysts measure 7-8 μm in diameter and are covered with a rather thick, translucent mucilage-like wall. The nucleus and various particles can be seen inside the encysted cell, while large vacuoles are absent (Fig. 5).

The trophic cells have a strong superficial similarity to certain species of *Katablepharis/Leucocryptos* (Clay and Kugrens, 1999), especially *Leucocryptos marina* (Vørs, 1992). We have not been able to observe the mode of trophozoite feeding, as the cells were always swimming. However, the cells produce pseudopodia (Figs 2, 7), and bacteria were found in the food vacuoles of the cells.

[1] To describe flagellar orientation and organelle localization within the cell we have designated the cell surface from which the flagella emerge as the ventral side, and the opposite side as the dorsal one.

Electron microscopy

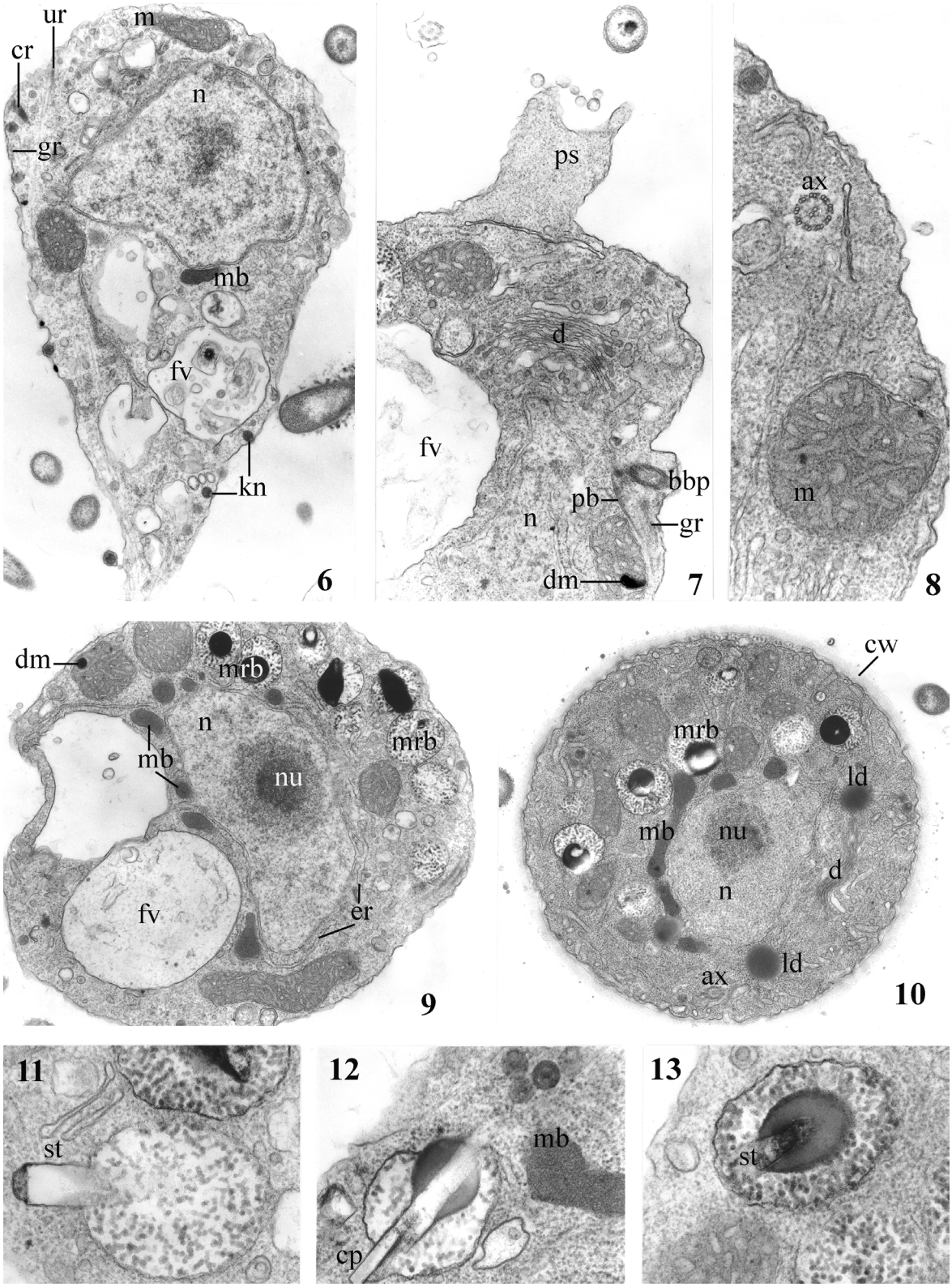
General organisation of trophic cell.

The only visible cell covering of the trophic cells is the plasmalemma (Figs 6-9). The cytoplasm inside the cell is densely packed with organelles, granules and vesicles.

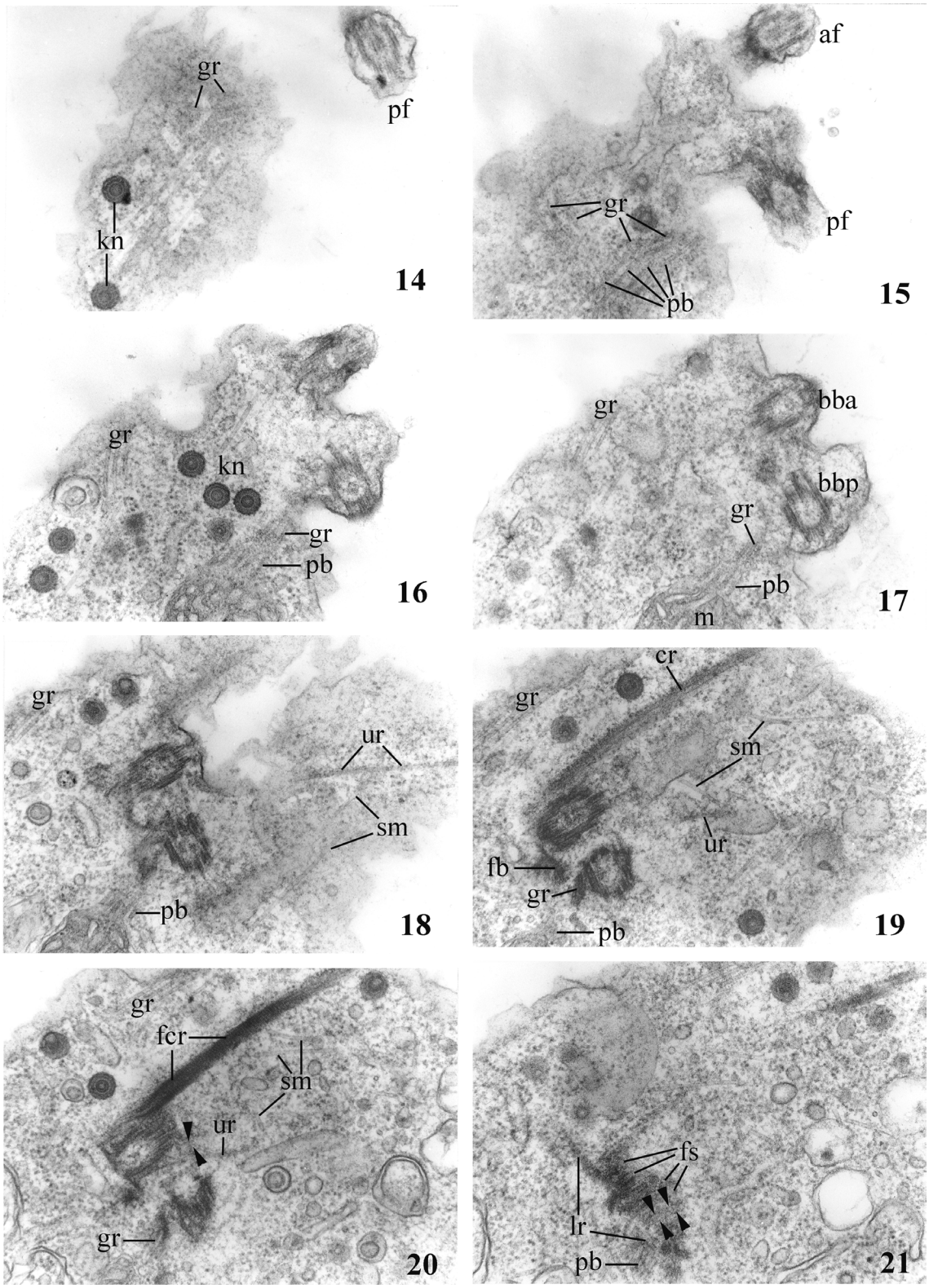
The nucleus is of the vesicular type with a prominent nucleolus and a small amount of heterochromatin (Fig. 9). The perinuclear space is connected to a rough endoplasmic reticulum (ER), which is often visible on the sections (Figs 7-9). The ER cisterns are always present around the nuclear surface. A large branched microbody, which is located in close vicinity to the nucleus (Figs 6, 9, 10), has a single membrane that encloses the rather osmiophilic thin granular contents (Figs 9, 10, 12). Profiles of mitochondria are prominent and scattered throughout the cell. The mitochondria have tubular, worm-like, rarely branching cristae and a slightly electron-opaque matrix (Figs 6-10). Often very dense spots or even bands of dark material are located in the matrix at the periphery of mitochondria (Figs 7, 9).

Two dictyosomes are located at one side of the nucleus (Fig. 7), anterior to flagellar basal bodies.

Large vacuoles are present in the middle and posterior parts of the cell. They have a translucent appearance and normally contain membrane remnants and unrecognisable amorphous material (Figs 6, 7, 9). We consider these vacuoles to be food vacuoles, since they sometimes, though rarely, contain bacteria (Fig.



Figs 6-12. Ultrastructure of *Katabia gromovi*. 6 - longitudinal section of the cell, 7 - pseudopodium at the anterior end, 8 - resorbed flagellum (axoneme) inside the cell prior to encystment, 9 - transverse section of the cell, 10 - cyst, 11-13 - different stages in formation of the mushroom-like bodies. For abbreviations see Material and methods.



Figs 14-21. The flagellar apparatus of *Katabia gromovi*. Serial sections. Cell viewed from the ventral side. The posterior end of the cell is to the left, the anterior end is to the right. For further details, see the text. Arrowheads on fig. 21 show upper rootlet. For abbreviations see Material and methods.

6) and since no other vacuoles containing bacteria were found in the cytoplasm.

Many small extrusomes (kinetocysts) are present just below the plasmalemma (Figs 6, 12, 14-21). Most of them are situated along the ventral side and in the posterior end of the cell. At the anterior end, they are arranged in rows along the cytoskeletal or rootlet microtubules (Fig. 38). The vesicles, which enclose the extrusomes are about 110×150 nm and contain two cylinders of different height and diameter inside each other (Figs 30, 32). This is a feature of kinetocysts in cercomonads.

Vesicles of approximately 800 nm to 1 μm in diameter are also found in the cytoplasm (Figs 9-13). They are filled with small rounded and elongated vermiform particles of 20 nm in diameter, and with a mushroom-like dense body (MRB) inside each vesicle. Some of these vesicles have a stalk protruding into the cytoplasm; they probably represent an early stage during the formation of the organelle (Fig. 11). In others, the stalk is located inside the vesicles, covered with an electron dense cap (Figs 12, 13). In some sections, the internal stalk of the MRB has the appearance of a crystal protruding into the cytoplasm (Fig. 12).

Pseudopodial protrusions are quite rare; they are broad and flat and may be of the lobopodial or lamellipodial type (Fig. 7).

Cyst structure.

The cyst wall is electron-translucent and appears amorphous (Fig. 10). The innermost layer of the wall is more opaque than the outer one, and very delicate, thinner than the plasma membrane. Inside the cyst we found some of the same organelles as in trophozoite: a nucleus with a prominent nucleolus and small amounts of heterochromatin, mitochondria, dictyosomes, microbodies, MRBs and many small vesicles and cisternae of the membrane system (Fig. 10). The cytoplasm of the cysts is normally denser than in trophic cells, lipid droplets are present, and flagellar axonemes may be visible in peripheral cytoplasm; however, there are no large vacuoles, and kinetocysts are rare (Fig. 10). The flagella are resorbed into the cell before the cyst wall formation, and the profiles of the axoneme may be often seen in the cytoplasm of encysting cells (Fig. 8).

Flagellar apparatus.

Both flagella are smooth. The free part of each flagellum includes only an axoneme of usual structure (9+2) surrounded by relatively thick layer of cytoplasm (Fig. 27). Swellings on the flagellar membrane with a translucent content are sometimes present, both at the base and in more distal parts of flagella.

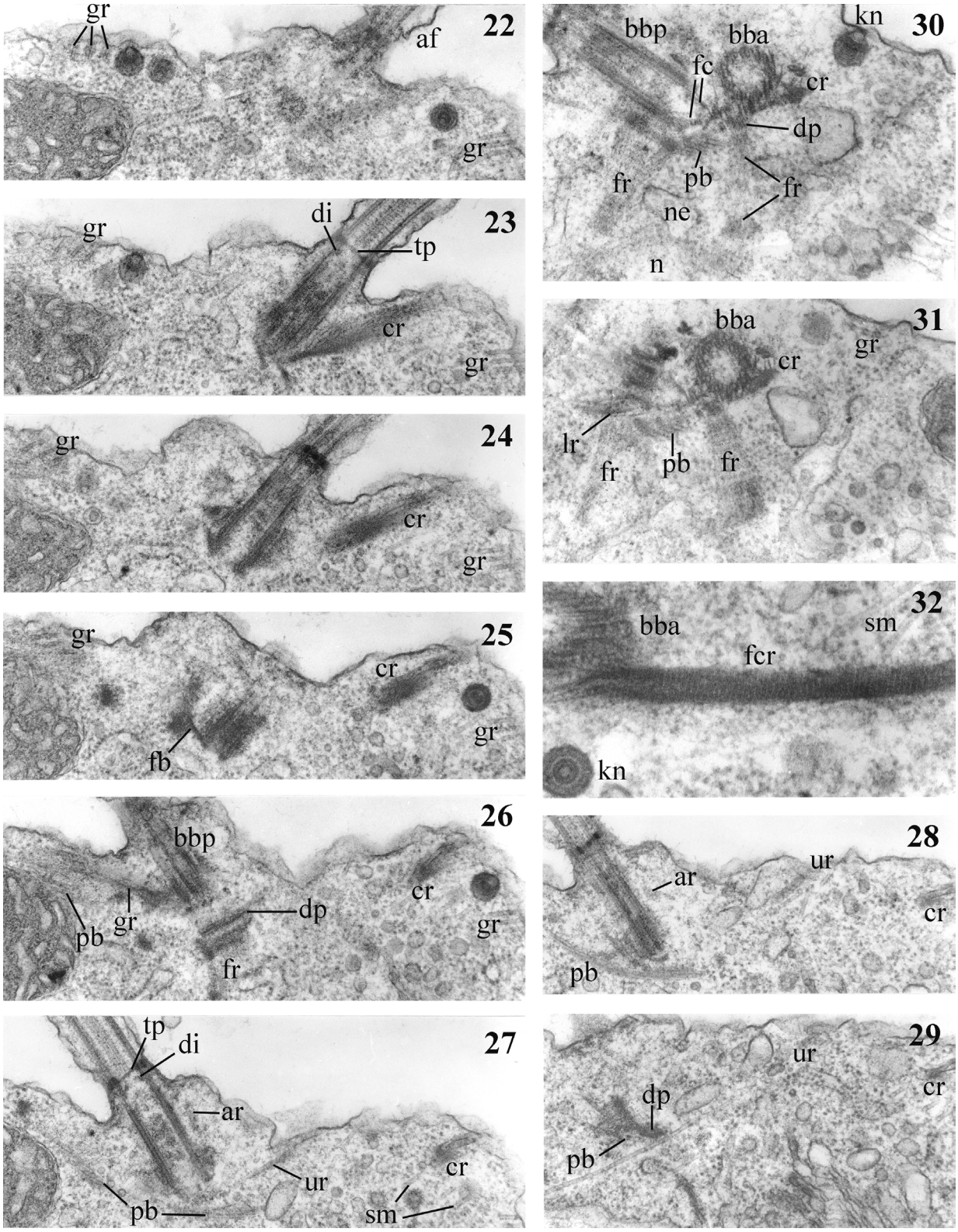
The flagellar transition zone is identical in both flagella and contains a transverse plate located some 150-170 nm above the level of the plasmalemma (Figs 23, 27). There is a short cylinder at the level of transverse plate (Figs 23, 27, 46, 47). It is connected with A-tubules of the axoneme and corresponds to the concentric ring fibres. Slightly underneath the transverse plate, there is a diaphragm-like thickening at the inner surface of the axoneme (Figs 23, 27). The middle part of each basal body is filled with electron dense globules (Figs 24, 27).

The flagellar basal bodies are located in different planes, approximately at right angles to each other (Figs 22-29, 50). We consider the anteriorly oriented basal body to represent the anterior flagellum (BBA), and another basal body turned to the left, to represent the posterior flagellum (BBP). The base of BBP is located approximately opposite to the middle part of BBA (Figs 50-52). The basal bodies are connected by two or three fibrillar bridges (Fig. 53), and produce rather prominent fibrillar material around their proximal ends.

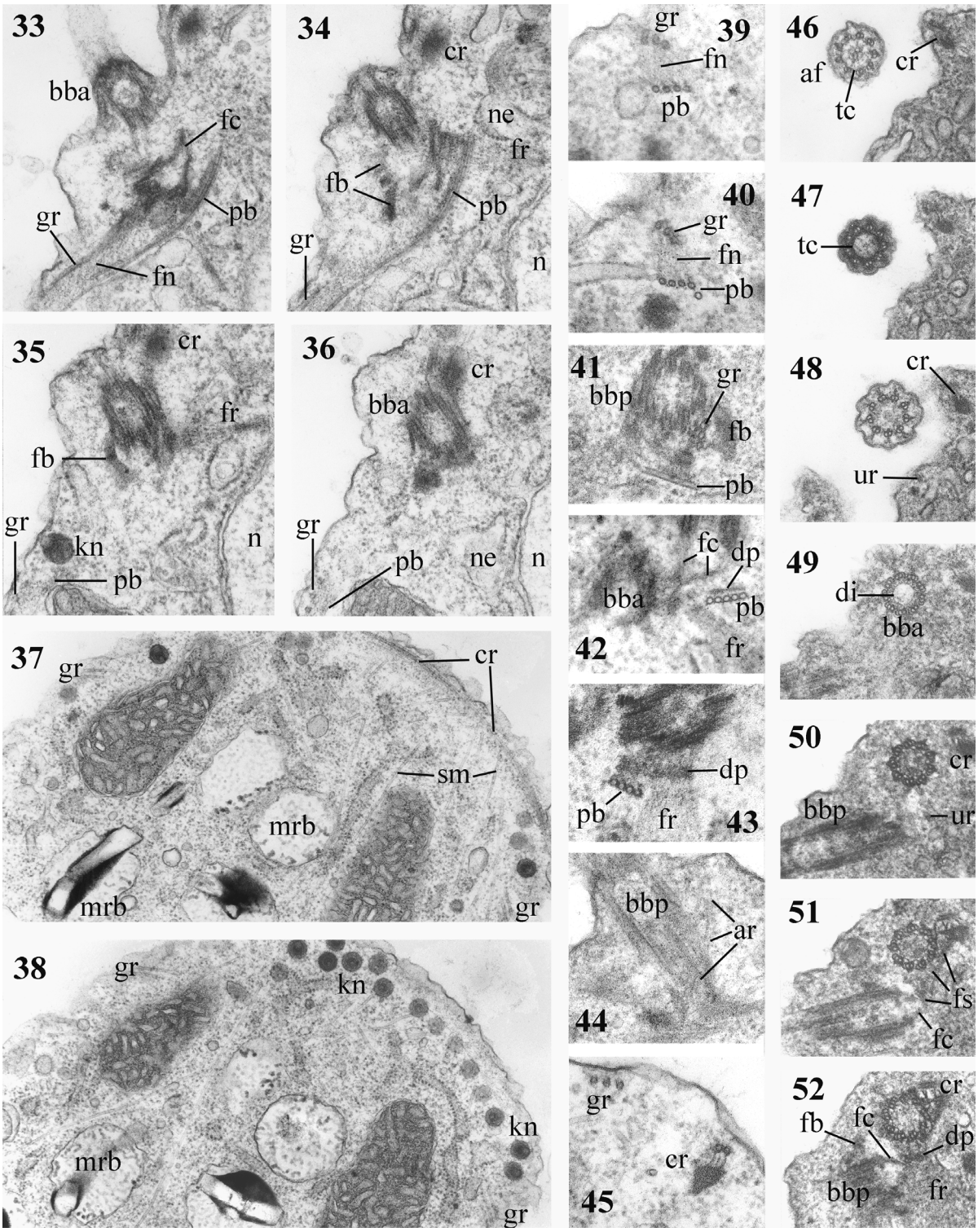
The BBA has two microtubular rootlets. The composite rootlet (CR) originates from the right side of BBA (Figs 19-21, 32, 45). It is composed of two microtubules attached to a rather dense fibrillar striated band via two or three rows of thin filaments. The fibrillar band accompanies the microtubules of the CR for approximately 1 μm. It is about 80 nm thick and shows a transverse striation with a periodicity of 15-17 nm (Fig. 32). This rootlet passes to the right part of the cell, nucleating secondary microtubules in posterior direction (Figs 20, 37).

Another microtubular rootlet arises from the opposite side of BBA, and passes under the plasmalemma in the same general direction as CR a little closer to the anterior end (Figs 18-20, 26-28). It is composed of two microtubules, accompanied with thread-like fibrillar material. Its proximal end is located in fibrillar material produced by BBA on its dorsal side. Leaving the basal body region, this upper rootlet (UR) produces secondary microtubules in a posterior direction (Fig. 6).

There are distinct fibrillar connections near the basal bodies. The proximal part of BBA is covered with a sheet of fibrillar material, which is connected to the CR (Figs 21, 30, 31, 33-36, 51, 52). This material extends deeper into the cell in direction of the nucleus. It looks like a cone ending with the dense plate (Figs 31, 52). Another fibrillar cone passes from the proximal end of BBP and terminates at the same plate (Figs 30, 31, 33, 42, 51, 52). This connection, together with the surrounding fibrillar material, unites the proximal ends of the basal bodies into a common sheet (Figs 21, 25).



Figs 22-32. The flagellar apparatus of *Katabia gromovi*. 22-29 - series of longitudinal sections through both basal bodies. Cell seen in lateral view, the sequence of sections is from right to left. The anterior end of the cell is to the right, the posterior to the left of the figure. 30-31 - two consecutive sections through the fibrillar bands. 32 - cross striation of the fibrillar band of CR. For abbreviations see Material and methods.



Figs 33-52. The flagellar apparatus of *Katabia gromovi*. 33-36 - Serial sections through mid-region of the basal bodies. 37-38 - kinetocysts between GR and CR, from the anterior end of the cell. 39-40 - consecutive cross sections of GR and PB in the direction of the basal bodies. 41 - origin of the rootlet GR near BBP. 42, 43 - different number of microtubules (4 and 5, respectively) near the proximal end of BBP. 44 - Anterior rootlet (AR) of BBP. 45 - transverse section of GR and CR. 46-52 - series of transverse sections through the flagellar transition zone and the basal body of BBA (view from the tip of flagellum). For abbreviations see Material and methods.

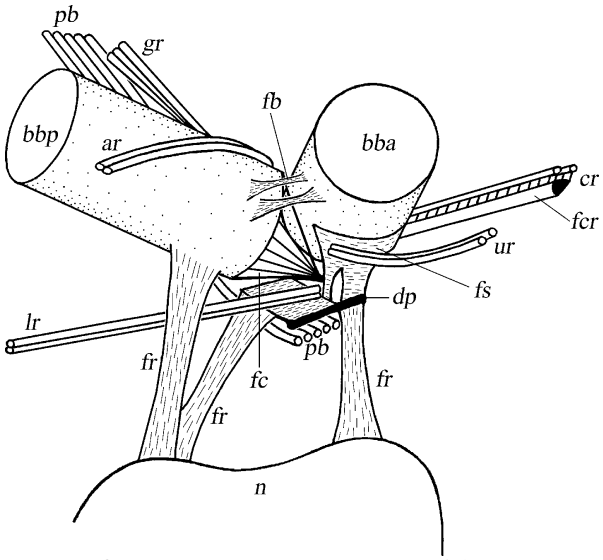


Fig. 53. Diagrammatic representation of the microtubular cytoskeleton of *Katabia gromovi*. The cell is seen from the anterior end side, the dorsal side is towards the bottom of the page. Secondary microtubules are not shown. For abbreviations see Material and methods.

Two other microtubular rootlets are connected with the sheet just mentioned. One rootlet, the posterior band (PB), which consists of four to five microtubules, is associated with the dense plate (Figs 33, 34, 41, 43). It continues in the posterior left direction, opposite to the direction of CR (Figs 15-21). The dense plate follows the ventral surface of PB at its proximal part (Figs 33, 42). The PB passes underneath the proximal end of the basal bodies, then turns to the ventral surface and continues straight to the posterior end of the cell as a band of four microtubules (Figs 15-18, 27, 28, 33-35, 39, 40). This part of PB is normally associated with the mitochondrial surface (Figs 16, 17, 23-27). At the distal part, the PB splits into separate microtubules, and additional microtubules may be present. These microtubules support the shape of ventral cell surface.

Another rootlet connected with the dense plate is composed of two microtubules, and passes perpendicular to PB (Figs 31, 53). This left rootlet (LR) passes to the left side of the cell and also produces secondary microtubules.

Two additional microtubular rootlets associate with the BBP. The girdle rootlet (GR), which consists of three microtubules, starts from the ventral surface of BBP in a plane parallel to its longitudinal axis, associated with PB (Fig. 41). While it passes towards the plasmalemma it turns 90° clockwise along its longitudinal axis, and becomes located in the same plane as PB (Figs 39, 40, 41). On its way, GR is connected to PB by a delicate fibrillar network (Figs 33-36, 39, 40). GR then turns to the right, passing beneath the plasma membrane in a semicircular way around the basal bodies (Figs 14-17, 22-26, 33-36). It eventually becomes parallel to the CR of BBA (Figs 18-20, 22-26, 45). A battery of kinetocysts is located between these two rootlets in the anterior part of the cell (Figs 37, 38).

The second small rootlet of BBP, the anterior one (AR), comprises two microtubules attached to its anterior surface (Figs 44, 53). It arises at the same place as GR, and passes to the left side of the cell, overlapping the anterior surface of BBP.

Each basal body has at least one weakly striated broad fibre extending to nuclear surface (Figs 30, 31, 34, 35, 42, 43, 52, 53). One fibre arises directly from BBP, and two others originate from the opaque plate. All the fibrous bands are connected with the nuclear envelope, which often has special extensions of the perinuclear space in these areas (Figs 34-36).

The arrangement of the flagellar apparatus and its location in the cell are illustrated in Figures 53 and 54.

Preliminary molecular data

An approximately 700-bp-long strand of SSU rDNA from our strain (details concerning the molecular work will be reported elsewhere, F. Ekelund and T.A. Koch, in preparation) was submitted to NCBI

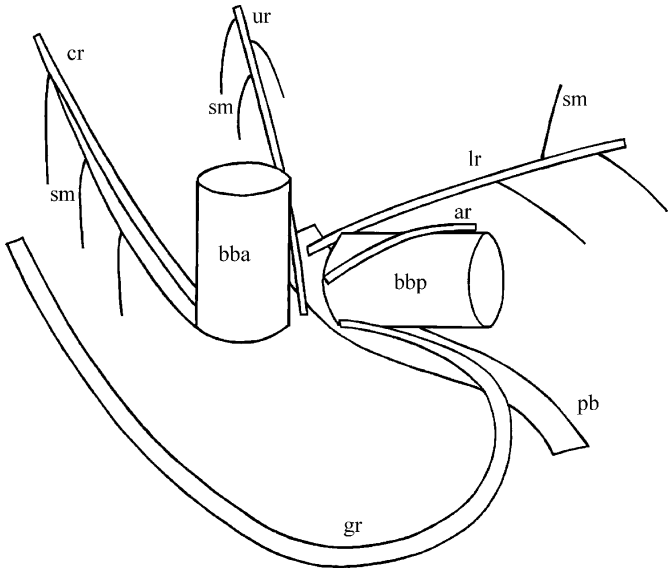


Fig. 54. Diagrammatic representation of the microtubular cytoskeleton of *Katabia gromovi*. The cell is seen from the ventral side of the cell, the anterior end is at the top of the page. For abbreviations see Material and methods.

Blast search (Altschul et al., 1997). The closest match was *Heteromita globosa* (95%), followed by *Cryothecomonas longipes* (94%) and *Cercomonas* ATCC50317 (93%).

Discussion

The main goal of the discussion is to examine the taxonomic position for the new flagellate. All information is considered, including general morphology, cytoskeleton structure, behaviour, life cycle and molecular data.

Molecular data

As mentioned above, the partial sequence of SSU rDNA from our strain strongly suggests that its closest relatives are *Heteromita*, *Cercomonas* and *Cryothecomonas*, indicating that our flagellate is a cercomonad. Below we compare the morphological features of *Katabia gromovi* gen. et sp. nov. with the morphology of the cercomonads in order to evaluate this hypothesis.

General morphology

In its morphology, the organism resembles a cercomonad as it is an amoeboflagellate with a naked cell body, has two smooth heterodynamic flagella and uses pseudopodia for feeding (Mylnikov, 1986, 2000; Karpov, 1997). The latter character is difficult to observe while the organism is swimming. However, a short pseudopodium was found in a living cell, and in thin sections, several cells were seen to produce pseudopodia from the lateral and anterior parts. No structures resembling a cytostome or a cytopharynx were observed, and we surmise that the cells ingest food particles by means of pseudopodia.

Our strain has two stages in the life cycle: the amoeboflagellate and the cyst, as in cercomonads. The latter commonly produce plasmodia, and this is a character of the family Cercomonadidae, which includes the majority of cercomonads (*Cercomonas*, *Massisteria* and *Helkesimastix*). The trophozoites of these genera possess gliding amoeboid cells with two heterodynamic flagella. Pseudopodia are of various shapes, including branched types. The transformation from monad to amoeboid stage only takes a few seconds. The cytoplasm contains microtoxicysts and trichocysts. The microtubular cone usually emerges from the basal bodies to the nucleus. Swimming cells are very rare in the life cycle of cercomonads, which normally glide upon a surface (Mylnikov, 1986). However, some species of *Cercomonas* are also capable of swimming (Mylnikov, pers. comm.).

Another cercomonad family, Heteromitidae, contains only the genus *Heteromita*. Its representatives have gliding cells with a rigid body and two heterodynamic flagella. During movement, the posterior end is constantly shaking, and this is a very distinctive character of the genus. The anterior end of the cell has a small rostrum, and the flagella arise from a small pocket. Cells contain only one kind of extrusome, the kinetocyst. A microtubular cone is absent, and there are no plasmodia in the life cycle.

In these features our strain is more similar to *Heteromita* than to other cercomonads. It has a rigid body similar to *Heteromita*, or *Bodomorpha*[2] . In contrast to *Cercomonas* it lacks plasmodia.

Three species of *Heteromita* have been already investigated: *Heteromita globosa* (MacDonald et al., 1977), *Heteromita* (*Bodomorpha*) *reniformis* (Mylnikov, 1984, 1995) and *Heteromita* sp. (Karpov, 1997). Their ultrastructural features were summarised as follows (Karpov, 1997): the cell is covered only by the plasmalemma; kinetocysts are present beneath the plasmalemma; the basal bodies are inserted at approximately right angles to each other and in different planes; both basal bodies have dense cores in the lumen and are connected to each other by 3-4 fibrillar bridges; a spiral fibre, or cylinder is present, connected to the A-tubules of the axoneme in the transition zone; the nucleus has a prominent nucleolus; a microbody or paranuclear body with amorphous contents joins the posterior part of the nucleus; the mitochondria have vermiform tubular or vesicular cristae.

These features are shared with our species. Our species resembles *H. globosa* in cell dimensions (7×5 μm); it has two flagella of similar length (9 and 14 μm, respectively), and cysts of nearly the same diameter (5 μm), with a thin transparent wall. Ultrastructural similarities include kinetocysts, a well-developed microbody, mitochondria with a dense granule in the matrix, mushroom bodies (which are referred to in *H. globosa* as food vacuoles because of a somewhat inadequate fixation). The flagellar apparatus has not been investigated in detail, but the striated fibrillar rootlet directed to the nucleus is also present in both species. *H. globosa* has acronematic flagella.

While our strain swims, *H. globosa* glides upon the substratum as more typical representatives of the genus. Our organism has a more rigid cell body, and the flagella emerge subapically (not in an apical position as in *H. globosa*). Our strain lacks a collar around the basal

[2] The taxonomic discussion on the validity of the name *Bodomorpha* is outside the scope of this paper, therefore we do not use this name.

bodies and kinetocysts inside the flagellum, features reported in *H. globosa*, although not clearly illustrated in the published figures.

Cytoskeleton structure

The cytoskeleton has been investigated in detail only in *H. sp.* (Karpov, 1997), which is half the size of our organism. The cells of both strains seem to have homologous structures. The posterior band in *H. sp.*, named MLS, passes in the same direction and resembles PB of our strain. It is associated with a small left rootlet of two microtubules oriented perpendicular to the "MLS", similar to the LR of our organism. The ventral rootlet of *H. sp.* is associated with the "MLS", passing in the posterior direction, and resembling the GR in our strain. The dorsal rootlet of *H. sp.* is composed of two microtubules. It arises from BBA and passes forward straight into the rostrum, nucleating secondary microtubules. It may be homologous to the left rootlet of BBA in our strain, which is also ribbed. There is at least one fibrillar rootlet directed towards the nucleus, which terminates in a granule in *H. sp.*, and there are extensions of the perinuclear space near the basal bodies in both species.

However, the cytoskeletal dissimilarities between *H. sp.* and our species are rather substantial. The layered structure of *H. sp.* has eight microtubules, instead of four to five in the PB of our strain. The latter is more similar in this respect to *H. reniformis*, which has only four microtubules (Mylnikov, 1984, 1995). *H. sp.* has only one striated fibrillar rootlet connecting to the nucleus, and a large dense granule is present at the distal end with no direct connection to the nucleus. Our strain has two or even three rootlets connecting to the nuclear envelope. There is no rootlet in *H. sp.* similar to CR of our strain. The posterior basal body of *H. sp.* has a single microtubular rootlet (regarded as the ventral one) associated with fibrillar material. It passes in a ventral direction and then posteriorly under the plasmalemma. This rootlet is not homologous with the CR of our strain as the latter starts from BBA, and is directed towards the anterior end of the cell.

Other ultrastructural characters and conclusion

Our strain is very similar to the investigated species of *Heteromita* in its general morphology and cytoskeleton structure. The mushroom-like bodies have also been found in two species of *Cercomonas* (*C. longicauda* and *C. crassicauda*), and were considered to be immature extrusomes (Mylnikov, 1995). In our species they can not be considered as prekinetocysts, as the kinetocysts are much smaller, have a distinctive structure, and no transitional stages have been observed. Similar structures were found in the cercomonad *Cholamonas cyrtodiopsidis*, termed refractile granules (Flavin et al., 2000). They were of two types: apical (empty relatively small vesicles) and median refractile granules, showing similar appearance and dimensions to the MRB, except that small dots are not visible in the micrographs. The function and the origin of mushroom-like structures remain unexplained and need closer investigations. At present, they have not been observed outside the cercomonads.

The dense dark inclusions in the mitochondrial matrix of *H. globosa* and of our strain have not been reported in other cercomonads, nor in other flagellates. Yet they are rather common in ciliates (K. Hausmann, pers. comm.), which is probably associated with the activity of some enzymes, and production and storage of fatty acids, which results in osmium condensation in the mitochondrial matrix. Thus, the MRB and dark inclusions are characteristic not only of *Heteromita*.

We conclude that our strain differs so markedly from other cercomonads in morphology and behaviour that it should be considered different not only at the species but also at the generic level. This is supported by the comparison of the partial sequences of SSU rRNA gene in which *Heteromita*, *Cercomonas* and our strain form three different clusters.

Katabia **Karpov, Ekelund et Moestrup, gen. nov.**

Cercomonads with two heterodynamic flagella, a drop-shaped cell body with a broad anterior and the tapering posterior end. Trophozoites swim freely rather than glide upon the substrate. The life cycle includes trophozoites and cysts, the latter surrounded by a thick mucilage-like wall. Feeding is by pseudopodia. Cells contain a microbody, refractile granules (mushroom-like bodies), kinetocysts and a well-developed cytoskeleton resembling that of *Heteromita*.

Katabia gromovi **Karpov, Ekelund et Moestrup, sp. nov.**

Soil flagellate, with flattened ventral side and prominent dorsal side. Cell length 8–12 μm, cell width (in the broadest anterior part) 5–7 μm. The flagella emerge subapically, approximately 1/4 cell length from the anterior end. One flagellum, approximately 2.5 times the cell length, is oriented to the left and backward; it has a long acronema. The anterior flagellum has a shorter acronema and is 1.5 times the cell length. The cyst measures 7-8 μm in diameter.

Culture location: Department of Terrestrial Ecology, Zoological Institute, University of Copenhagen, Copenhagen, Denmark.

Type figures: Figures 1-5.

Etymology: the generic name has no meaning; the specific epithet was selected to commemorate the late Prof. B.V. Gromov – a well-known Russian microbiologist and protistologist.

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Address for correspondence: S.A. Karpov. Department of Invertebrate Zoology, Faculty of Biology and Soil Sci., St. Petersburg State University, Universitetskaja nab. 7/9, St. Petersburg, 199034. RUSSIA. E-mail: karpov@SK1027.spb.edu