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Diversity of Free-Living Morphospecies in the Ciliate Genus *Metopus*

GENOVEVA ESTEBAN[1]), TOM FENCHEL[2]) & BLAND FINLAY[1])

[1]) Institute of Freshwater Ecology, Windermere Laboratory, Ambleside, Cumbria, U.K.;
[2]) Marine Biological Laboratory, University of Copenhagen, Helsingør, Denmark

Summary: This is a taxonomic revision of anaerobic free-living ciliates in the genus *Metopus*. It includes a rationalization of all nominal species described in the literature, and the allocation of the survivors to "morphospecies". The revision is based on examination of cultured species together with an exhaustive comparison of the published descriptions of nominal species. All free-living *Metopus* can be allocated to one of five general morphological types. Each type contains several morphospecies (and their synonyms), each with conservative features. The seventy-six nominal species of *Metopus* are reduced to 22 morphospecies, and *M. nivaaensis* n. sp. is described.

Key Words: *Metopus*; Anaerobic ciliates; Symbionts; Morphospecies; Diversity.

Introduction

Ciliates of the genus *Metopus* are characterized by torsion of the anterior part of the cell, and a frontal lobe which overhangs an obliquely ascending adoral zone of membranelles. The anterior twisting often gives *Metopus* a characteristic S-shape. All well-studied *Metopus* species have been shown to be anaerobic organisms, with a variable but always modest tolerance of dissolved oxygen. Although cells of some *Metopus* species may occasionally be found in oxygenated water, these are probably always accidental migrants. Oxygen sensitivity and other physiological features of *Metopus* and other anaerobic ciliates have been described (FENCHEL & FINLAY 1990a; FINLAY & FENCHEL 1992; FENCHEL & FINLAY 1995). Representatives of the genus seem to be ubiquitous filter-feeders of bacteria in sediments, landfills and anoxic water.

The history of the genus can be traced to the description of *Trichoda es* (syn. *Metopus es*) by O. F. MÜLLER in 1786. The current generic name was erected by CLAPARÈDE & LACHMANN in 1858. KAHL (1935) discovered and named most of the currently known *Metopus* species. Thereafter, the genus continued to absorb new species, CORLISS (1961) considered splitting it, and JANKOWSKI (1964) carried out a partial taxonomic revision – allocating some species to the restored genera *Bothrostoma* and *Cirranter*, and others to the newly-created *Brachonella* and *Tesnospira*. Thus the remaining *Metopus* species became morphologically more homogeneous. The current status of the genus is that it contains 76 nominal species, and the obvious question is "why are there so many?". The following factors are probably relevant. Anaerobic ciliates are never very abundant and most species are relatively rare (at least in relation to aerobic ciliates). This means that observations of anaerobic ciliates in natural samples are often isolated sightings and it may be easier to give the different sightings different species names than it is to gain an impression of the spread in morphological variation of a natural population. Furthermore, until relatively recently, no anaerobic ciliates had been cultured, so the variation characteristic of anaerobic ciliate species was rarely known. When we include some other relevant factors: (a) the existence of polymorphic anaerobic ciliates (the most spectacular example being *M. palae-*

formis), (b) species nomenclature based on differences in gross cell morphology rather than the more conservative somatic and oral ciliature, (c) the physical fragility of many anaerobic species and the ease with which aberrant specimens can be produced (and described), and (d) some obvious cases of ignorance of previously-published literature, it becomes clear that too many species have been erected for the variety of *Metopus* that have been observed so far.

How should we deal with this rather inflated collection of nominal species? In the first place we acknowledge the close correlation between form and function in ciliates, and we adopt the morphospecies concept (FINLAY et al. 1996) as a pragmatic approach to defining the entities we call species. We also use information derived from cultured species in various physiological states to determine the range of variation in size and shape we can reasonably expect for a *Metopus* morphospecies. Armed with this information, we attempt to seek out and re-locate synonyms.

This paper includes a taxonomic revision of the free-living *Metopus* species. The revision is incomplete insofar as it has not been possible to culture all *Metopus* species. Some species are very rare indeed. As we cannot know the limits of morphological variation in these, we cannot define the boundaries of these as morphospecies. Nevertheless we have attempted to produce a realistic rationalization of the nominal species, reducing them to about one third of the original number.

Materials and Methods

Sampling sites

Details of the sampling sites are specified below for each *Metopus* species. Ciliates belonging to the nominal species *M. palaeformis* were isolated from several locations, and kept in anaerobic cultures (FINLAY & FENCHEL 1991a). They were isolated from four different municipal landfill sites (FINLAY & FENCHEL 1991a), from a septic tank in Cumbria (England), and from the sediment of a sulphide-rich solution lake in Spain. *M. striatus* was isolated in culture from soft sediment close to the shoreline of the Navalmedio reservoir in Madrid (Spain), and in a lagoon next to the Duratón River (Segovia, Spain). *M. es* was isolated from sediment sampled from a productive freshwater pond (Priest Pot, England), and from the Guadarrama River (Madrid, Spain). All marine *Metopus* described in this paper were isolated from anaerobic marine sediments close to Helsingør (Denmark).

The freshwater species were grown in anaerobic cultures, using soil extract medium enriched with a wheat grain or cereal leaves. The marine species were also grown anaerobically using boiled/sterilised seawater with a wheat grain. In both cases, ciliates were grown in serum vials with an headspace of N_2 and incubation at 20 °C. Cultivation methods are described in detail in FENCHEL & FINLAY (1990b) and FINLAY & FENCHEL (1991a).

Cell sizes

Several hundred cells of cultured species were measured in a variety of physiological stages. The length and breadth of each cell was measured with the aid of an ocular micrometer. Measurements made from photographs of living cells did not differ significantly from those of formalin-fixed (4% formaldehyde final conc.) cells. Cell shape in *Metopus* appears to be very well preserved following formaldehyde fixation. The non-cultured *Metopus* were measured using living cells, fixed cells, and photographs.

Microscopical techniques

Both formaldehyde-fixed cells and living cells were used for microscopic observations using Nomarski interference contrast and phase contrast. Fixed specimens were also used for the observation of autofluorescing endosymbiotic methanogenic bacteria (FINLAY & FENCHEL 1989). Protargol silver staining (TUFFRAU 1967; WILBERT 1975) was used for the study of the infraciliature. Material for transmission electron microscopy was prepared by fixation in glutaraldehyde (2%), followed by OsO_4 (2%) (both buffered with sodium cacodylate, 0.1 M pH 6.8), the dehydration in an ethanol series, embedding in Spurr resin, and thin sectioning. Lead citrate, uranyl acetate, and lead citrate (in that order) were used to stain thin sections prior to examination.

Species records

We have examined all *Metopus* species described by KAHL (1935), DRAGESCO (1968), and FOISSNER et al. (1992), plus all those included in the Zoological Record (1935–1995), as well as our own observations. All the original descriptions were studied, and compared with each other. For each *Metopus* species we then sought to apply the general criteria for ciliate morphospecies (FINLAY et al. 1996). Accordingly, in the present survey each morphospecies groups together those *Metopus* species which have an insufficient previously-published description, and are difficult or impossible to tell apart from other nominal species. Thus the nominal species become synonyms within the new morphospecies.

Results and Discussion

All *Metopus* species described so far and all those we have observed, fall within five broad categories of cell shape (Fig. 1A). KAHL (1935) too divided *Metopus* into five groups of species, according to general cell morphology and the size of the AZM (his Group VI is currently represented by the genus *Brachonella)*. The great morphological variety which characterizes many *Metopus* species, allows them to be placed in groups based solely on cell shape. The groups are as follows:

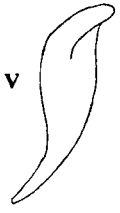
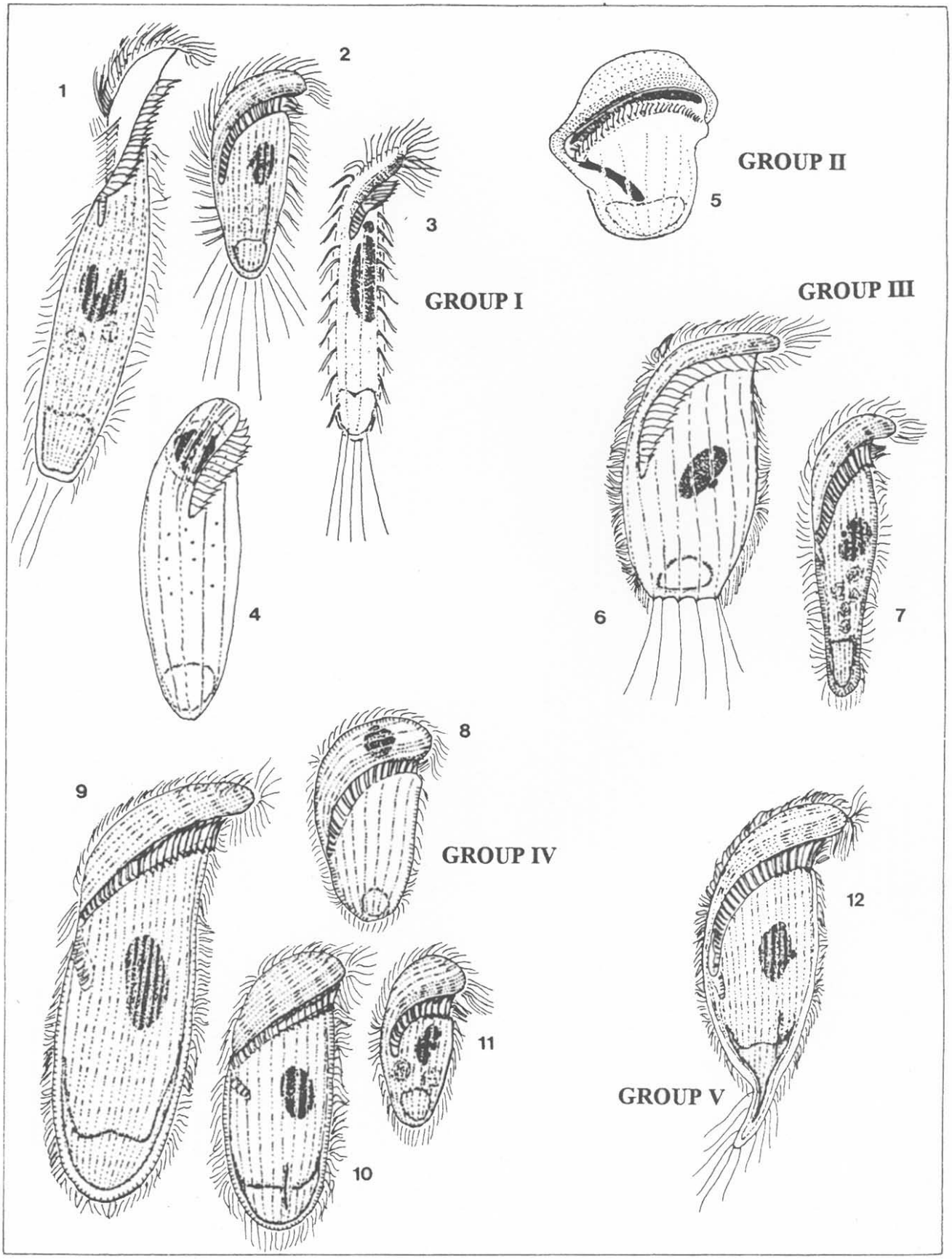
GROUP	MORPHOSPECIES	SYNONYMS	FORMER *METOPUS* SPECIES		TRANSFER
I 	*Metopus palaeformis* Kahl, 1927	*M. tenuis* Kahl, 1927 *M. hyalinus* (Kahl, 1927) Kahl, 1935 *M. rostratus* Kahl, 1927 *Tesnospira alba* Jankowski, 1964	*M. angustus* Kahl, 1927 *M. latus* Kahl, 1927	→ →	*Palmarium latum* (Kahl, 1927) Jankowski, 1964 *Palmarium latum* (Kahl, 1927) Jankowski, 1964
	Metopus hasei Sondheim, 1929	*M. latusculisetus* Tucolesco, 1962 *M. fusus* Vuxanovici, 1962			
	Metopus setosus Kahl, 1927	*M. setifer* Kahl, 1935			
	Metopus laminarius Kahl, 1927 *Metopus micrans* Jankowski, 1964	*M. trichocystiferus* Jankowski, 1964			
II 	*Metopus striatus* McMurrich, 1884	*M. acuminatus* (Stokes, 1886) *M. acutus* Kahl, 1927 *M. bacillatus* Levander, 1894 *M. denarius* Kahl 1927 *M. dentatus* Kahl, 1927 *M. fastigatus* Kahl, 1927 *M.gibbus* Kahl, 1927 *M. minimus* Kahl, 1927 *M. pulcher* Kahl, 1927 *M. pullus* Kahl, 1927 *M. recurvatus* Vuxanovici, 1962 *M. recurvatus* var. *pusillus* Vuxanovici, 1962 *M. violaceus* Kahl, 1927	*M. caducus* Kahl, 1927 *M. campanula* Kahl, 1932 *M. cydonia* Kahl, 1927 *M. darwini* Kahl, 1927 *M. galaetus* Kahl, 1927 *M. intercedens* Kahl, 1927 *M. pyriformis* Levander, 1894 *M. spiralis* Smith, 1897	→ → → → → → → →	*Brachonella caduca* (Kahl, 1927) Jankowski, 1964 *Brachonella campanula* (Kahl, 1932) Jankowski, 1964 *Brachonella cydonia* (Kahl, 1927) Jankowski, 1964 *Brachonella darwini* (Kahl, 1927) Jankowski, 1964 *Brachonella galeata* (Kahl, 1927) Jankowski, 1964 *Brachonella intercedens* (Kahl, 1927) *Brachonella pyriformis* (Levander, 1894) *Brachonella spiralis* (Smith, 1897) Jankowski 1964
	Metopus turbo Dragesco & Dragesco-Kernéis, 1986				
III 	*Metopus acidiferus* Kahl, 1935	*M. entorhipidioides* Jankowski, 1964 *M. mathiasi* Viulleneuve-Brachon, 1940			
	Metopus contortus (Quennerstedt, 1867) Kahl, 1932	*M. bivillus* Tucolesco, 1962 *M. sapropelicus* Tucolesco, 1962	*M. extensus* Kahl, 1926 *M. mirabilis* Kahl, 1927 *M. mucicola* Kahl, 1927 *M. nasutus* Da Cunha, 1915 *M. undulans* Stokes, 1887	→ → → → →	*Bothrostoma extensa* (Kahl, 1926) *Bothrostoma mirabilis* (Kahl, 1927) Jankowski, 1964 *Bothrostoma mucicola* (Kahl, 1927) *Copemetopus nasutus* (Da Cunha, 1915) *Bothrostoma undulans* (Stokes, 1887)
	Metopus es O. F. Müller, 1786	*M. daphnides* Jankowski, 1964 *M. caucasicus* Alekperov, 1984			
	Metopus contractus Penard, 1922 *Metopus nivaaensis* n. sp. *metopus halophila* Kahl, 1925 *Metopus major* (Kahl, 1932) nov. comb.	*M. ridiculus* Kahl, 1927			
IV 	*Metopus barbatus* Kahl, 1927 *Metopus fuscus* Kahl, 1927	*M. fuscoides* Alekperov, 1984 *M. jankowskii* Dragesco, 1968			
	Metopus ovalis Kahl, 1927 *Metopus tortus* (Kahl, 1927) Kovalchuk, 1980	*M. ventrosus* Vuxanovic, 1962			
V 	*Metopus spinosus* Kahl, 1927	*M. curvatus* Kahl, 1927 *M. convexus* Kahl, 1927 *M. attenuatus* Penard, 1931	*Metopus lemani* Dragesco, 1960	→	*Brachonella lemani* (Dragesco, 1960)
	Metopus propagatus Kahl, 1927	*M. strelkowi* Jankowski, 1964 *M. ovatus* Dragesco & Dragesco-Kernéis, 1986 *M. sp.* Dragesco & Dragesco-Kernéis, 1986			
	Metopus vestitus Kahl, 1935 *Metopus verrucosus* Da Cunha, 1915	*M. caudatus* Da Cunha, 1915			

Fig. 1A. Rationalization of the genus *Metopus* (see text).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2
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GROUP I
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GROUP II
GROUP III
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GROUP IV
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GROUP V

Group I: *Metopus palaeformis*-like organisms. The equatorial part of the cell is as wide as or narrower than the posterior part. These ciliates are usually thin, and elongate.
Group II: *Metopus striatus*-like organisms. Bell-shaped organisms, resembling spine-less *Caenomorpha*. Cells are wider at the anterior and equatorial parts of the cell than in the posterior half. With or without caudal protuberance or projection; when present, this projection is not spine-like, but a round short projection (Fig. 1A).
Group III: Cells are wider at the equator than at the anterior and posterior ends. The posterior end can vary between species, from very narrow (e.g. *M. es*) to less so (e.g. *M. contortus*).
Group IV: Approximately ovoid cells (e.g. *M. ovalis*).
Group V: *Metopus* with the posterior part of the cell narrower than both the cell equator and the anterior end, and with a conspicuous and distinctive spine-like posterior extension, e.g. *M. vestitus* (Fig. 1A).
All groups are illustrated in Fig. 1A, where we show a line drawing of the general outline shape for each group, the names of the constituent morphospecies, and the synonyms of the latter. After thorough examination and comparison of all published *Metopus* descriptions, we erected the morphospecies in Fig. 1A with the species which have been properly described in the literature and whose morphological variation embraces species that have been poorly described in the past. Consequently, the external morphologies of any two morphospecies should be relatively easy to tell apart (e.g. *M. contortus* and *M. es*). As some *Metopus* are extremely rare, we have not encountered some of the species mentioned in the literature, and we have left these as morphospecies for the time being (Fig. 1B). This is so for *M. turbo* (Group II) and *M. contractus* (Group III). The J-shaped macronucleus of the former, as well as its geographical location (Cotonou region, Africa; DRAGESCO & DRAGESCO-KERNEIS 1986), justifies its retention as a separate morphospecies, although complementary information about variability (if any) of the macronucleus shape is needed. *M. contractus* was first described by PENARD (1922), KAHL (1935) encountered it only once, and there are no subsequent published records. The ciliate resembles *M. contortus* (Fig. 1B).
Two morphologies of *M. pulcher* are described in KAHL (1935). The larger one, which JANKOWSKI later (1964) observed, is very like *M. striatus*. This *M. pulcher* has been included in Fig. 1A as a synonym of *M. striatus*. However, *M. pulcher* var. *tortus* in KAHL (1935) is retained as the morphospecies *M. tortus*, observed and renamed by KOVALCHUK in 1980 (Fig. 1B). *Tesnospira*

Table 1. Measured dimensions (lengths and breadths) of a large number of cells belonging to four species of *Metopus*. Cells were selected at random from a variety of cultures of different ages; thus the measured cells represent a broad range of physiological states, and probably the maximum size ranges of each species. *M. palaeformis* is polymorphic and only the "typical" troph sizes are directly comparable with the other three species.

Species	n	Length (μm)			Breadth (μm)		
		min	max	**mean**[a])	min	max	**mean**[a])
M. striatus	252	38	71	**51** ± 1	30	60	**44** ± 1
M. contortus	244	89	165	**119** ± 2	26	51	**41** ± 1
M. major	312	113	191	**146** ± 2	26	49	**39** ± 0.5
M. es	291	112	203	**155** ± 2	23	66	**42** ± 1
M. palaeformis	403[b]) 626[c])	70	132	**101** ± 1	8	31	**18** ± 1

[a]) Arithmetic mean with 95% confidence interval for the unknown population mean
[b]) "Typical" trophs
[c]) The total for all cells of the morphospecies

Fig. 1B. The remaining free-living *Metopus* morphospecies accepted in Fig. 1A. All illustrations are after KAHL (1935), JANKOWSKI (1964), FOISSNER (1981), KOVALCHUK (1980), and DRAGESCO & DRAGESCO-KERNEIS (1986). **Group I:** 1. *M. laminarius* KAHL, 1927; 200–260 μm. 2. *M. setosus* KAHL, 1927; 60–90 μm. 3. *M. hasei* KAHL, 1927; 70–100 μm. 4. *M. micrans* JANKOWSKI, 1964; 40–50 μm. **Group II:** 5. *M. turbo* DRAGESCO & DRAGESCO-KERNEIS, 1986; 50–105 μm. **Group III:** 6. *M. contractus* PENARD, 1922; 60–100 μm. 7. *M. acidiferus* KAHL, 1935; 100–120 μm. **Group IV**: 8. *M. tortus* (KAHL, 1927) KOVALCHUK, 1980; about 80 μm. 9. *M. fuscus* KAHL, 1927; 180–300 μm. 10. *M. ovalis* KAHL, 1927; 130–300 μm. 11. *M. barbatus* KAHL, 1927; about 65 μm. **Group V:** 12. *M. propagatus* KAHL, 1927; 150–200 μm.

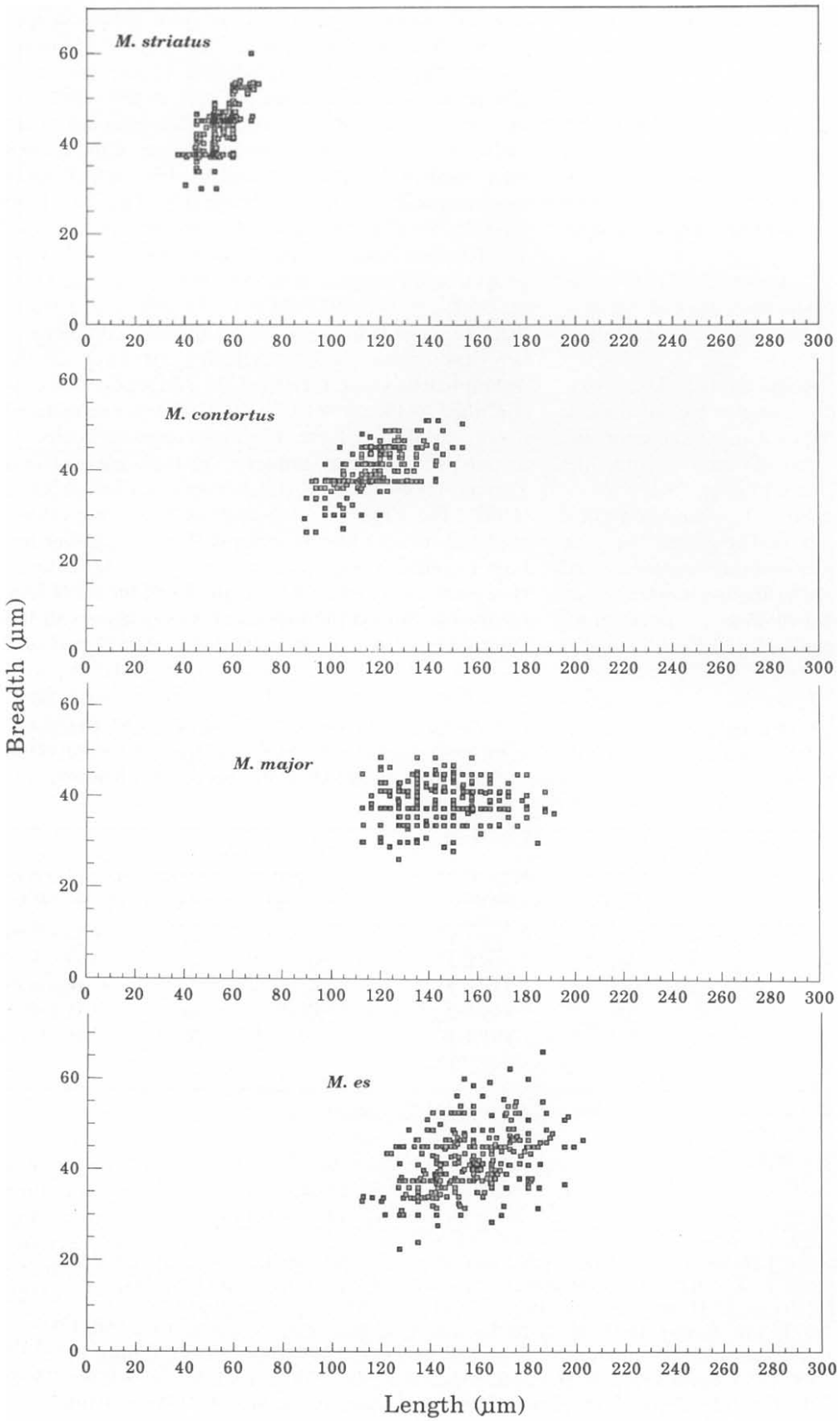


Fig. 2.
Measured lengths and breadths of a large number of cells in four cultured morphospecies. Further details in Table 1.

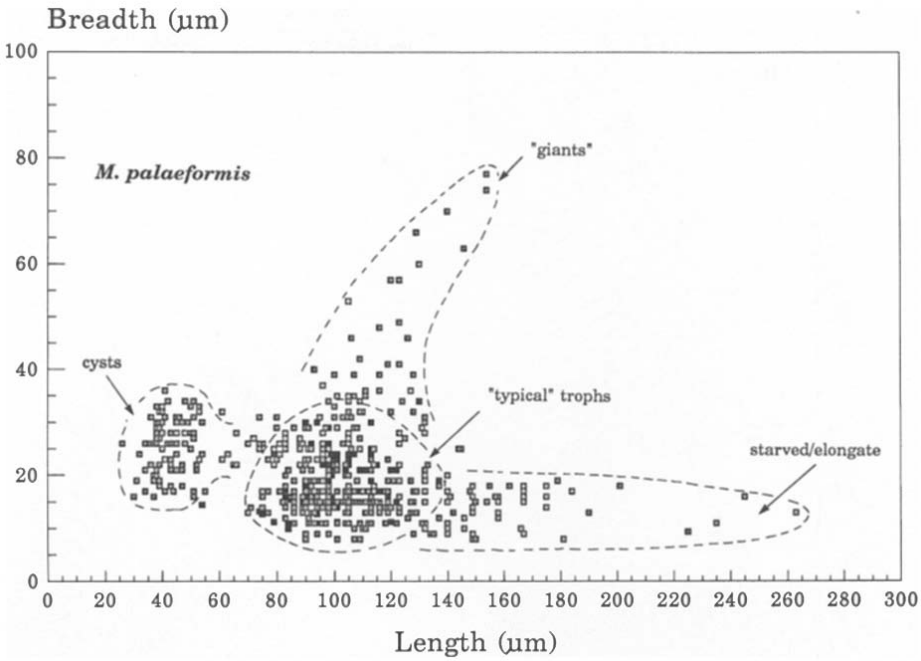


Fig. 3. Measured lengths and breadths in cultured cells of the polymorphic *M. palaeformis*. Further details in Table 1.

alba – included here as a synonym of *M. palaeformis* – is a ciliate with only few membranelles in the AZM, and about 24 somatic kineties (JANKOWSKI 1964). This organism probably corresponds to one of the *M. palaeformis* morphologies (Fig. 46). *M. jankowskii* (DRAGESCO 1968) is almost identical in size and shape to *M. fuscus*, and is considered as its synonym. Again, more information concerning morphological variability of all these ciliates is needed.

The 76 nominal species of *Metopus* have been reduced to 22 morphospecies. The scale of this reduction resembles that in other thorough revisions, e.g. of *Vorticella* (WARREN 1986), and *Stentor* (FOISSNER & WÖLFL 1994). Of the 22, two have been reported only once. These are *M. micrans* (JANKOWSKI 1964), and *M. turbo* (DRAGESCO & DRAGESCO-KERNEIS 1986). Incidentally, these morphospecies, together with *M. nivaaensis* and *M. tortus* (KOVALCHUK 1980), are the only new species we accept since KAHL (1935) and of the 76 nominal species in Fig. 1A no fewer than 45 have been reported only once.

Size and shape variation within morphospecies

We have attempted to discover easily-measured morphological features with which the morphospecies can be readily discriminated. We studied the size and shape variation in five cultured *Metopus* morphospecies: *M. palaeformis, M. striatus, M. contortus, M. es,* and *M. major.* These morphospecies belong to Groups I, Group II, and Group III (Fig. 1A). Results are shown in Table 1, and Figs. 2 and 3. The sizes and shapes of *M. striatus* cells do not overlap with those of any of the other four morphospecies. While parts of the populations in the latter do overlap, it is apparent that measurements of a sufficiently large number of cells will resolve separate morphospecies. Note especially that there is some justification in separating the five morphospecies on the basis of cell length alone, the population means being significantly different from each other in all cases.

Cysts in *Metopus*

We have measured, and probably covered the entire size ranges of the morphospecies we keep in culture. In *M. palaeformis* and *M. es*, we have observed resting cysts. Having observed thousands of *Metopus* spp. cells over several years, we have encountered very few trophs in the process of binary fission (Figs. 54, 55). It is therefore very likely that some *Metopus* species also divide inside cysts.

The organisms

All ciliates in the genus *Metopus* are characterized by a twisted anterior part that includes 5 to 10 kineties, some of them closer to each other at the edge of an overhanging lobe, where they form the so-called perizonal ciliary stripe (JANKOWSKI 1964). The perizonal area runs above the course of the adoral zone of membranelles (AZM), which reaches the equator of the cell in most species. The overhanging lobe is not always prominent, but its presence together with the torsion of the cell, makes most of the ciliates in the genus S-shaped. Most *Meto-*

Fig. 4

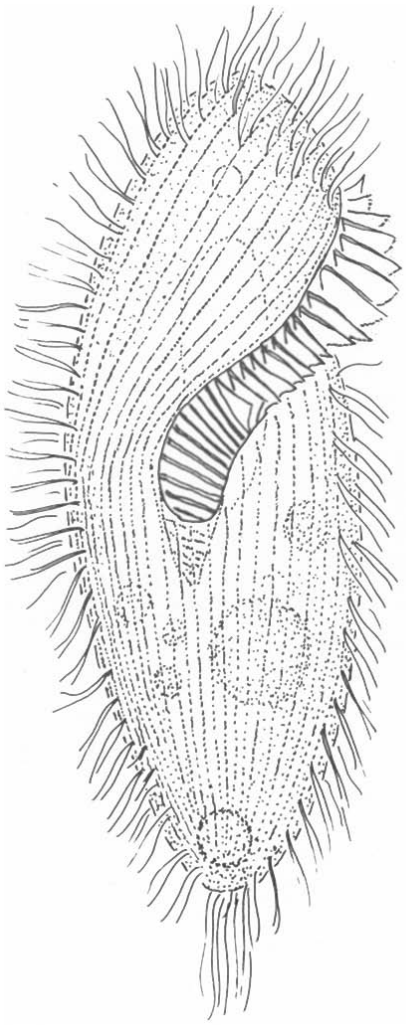
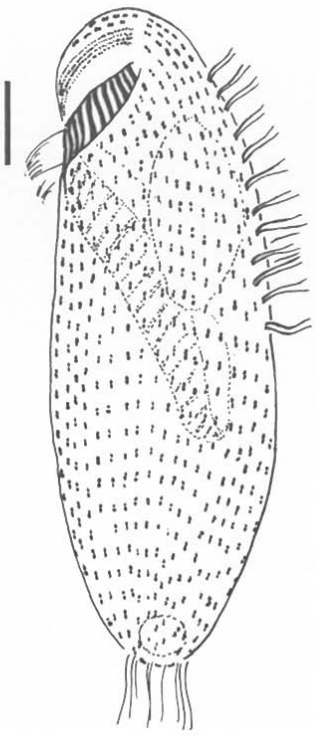


Fig. 5



Figs. 4, 5. *Metopus nivaaensis* n. sp. – **4.** The living organism. Scale bar: 10 µm. – **5.** Protargol-stained cell. Scale bar: 10 µm.

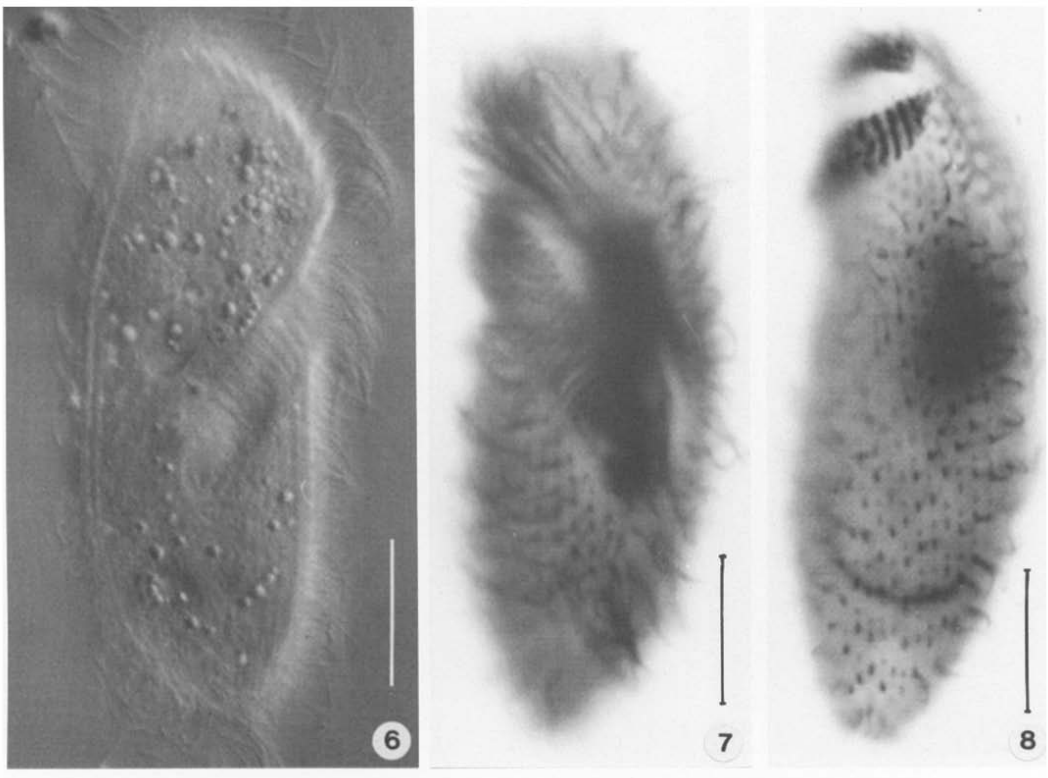
pus species have a group of distinctive intracellular dark particles close to the anterior end of the cell. The size and shape of some *Metopus* species vary greatly with changes in physiological state.

• *Metopus nivaaensis* n. sp.

The ciliate is elongate, ellipsoid, and 80–100 µm long, with a broad perizonal area. The AZM extends from the left anterior part of the cell, twisting rightwards in an S-form to beyond the cell equator. It includes 32–35 membranelles. The anterior part of the cell is wide, compared to other *Metopus* species (Figs. 4, 6). The somatic infraciliature is formed by 50 kineties, all with paired kinetosomes bearing cilia (Figs. 5, 7, 8). Protargol-stained organisms also show at least four kineties closer to each other in the anterior part of the cell, forming the perizonal ciliary stripe (Fig. 5). *M. nivaaensis* has seven to 10 caudal cilia in a group. There is a single contractile vacuole placed close to the posterior end. The single macronucleus is in the centre of the cell, and the micronucleus is placed above it. *M. nivaaensis* is common in anaerobic marine sands and in masses of purple sulphur bacteria. It was isolated from Nivå Bay (Denmark). The ellipsoidal shape, and the wide anterior part make *M. nivaaensis* easily distinguishable from other *Metopus* species.

• *Metopus contortus* (QUENNERSTEDT, 1867) KAHL, 1932

The organisms referred to under this name are identical to those described by QUENNERSTEDT (1867) as *Metopides contorta. M. contortus* has the typical *Metopus* shape: wide at the cell equator, with a narrow peristome in the anterior part (Figs. 9, 10). The living organism is 89–165 µm long, and 26–51 µm wide. The somatic infraciliature is constituted by about 40 kineties (Fig. 11). The kinetosomes in the kineties are paired, and each bears a cilium. The peristome has five kineties, of



Figs. 6–8. *Metopus nivaaensis* n. sp. – **6.** Living organism. Nomarski interference contrast. – **7, 8.** Protargol-stained organism. Scale bars: 20 μm.

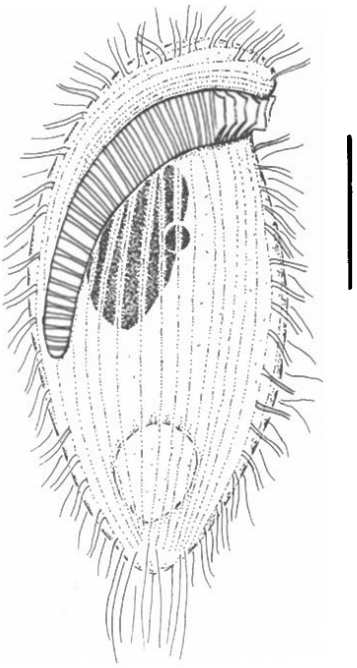
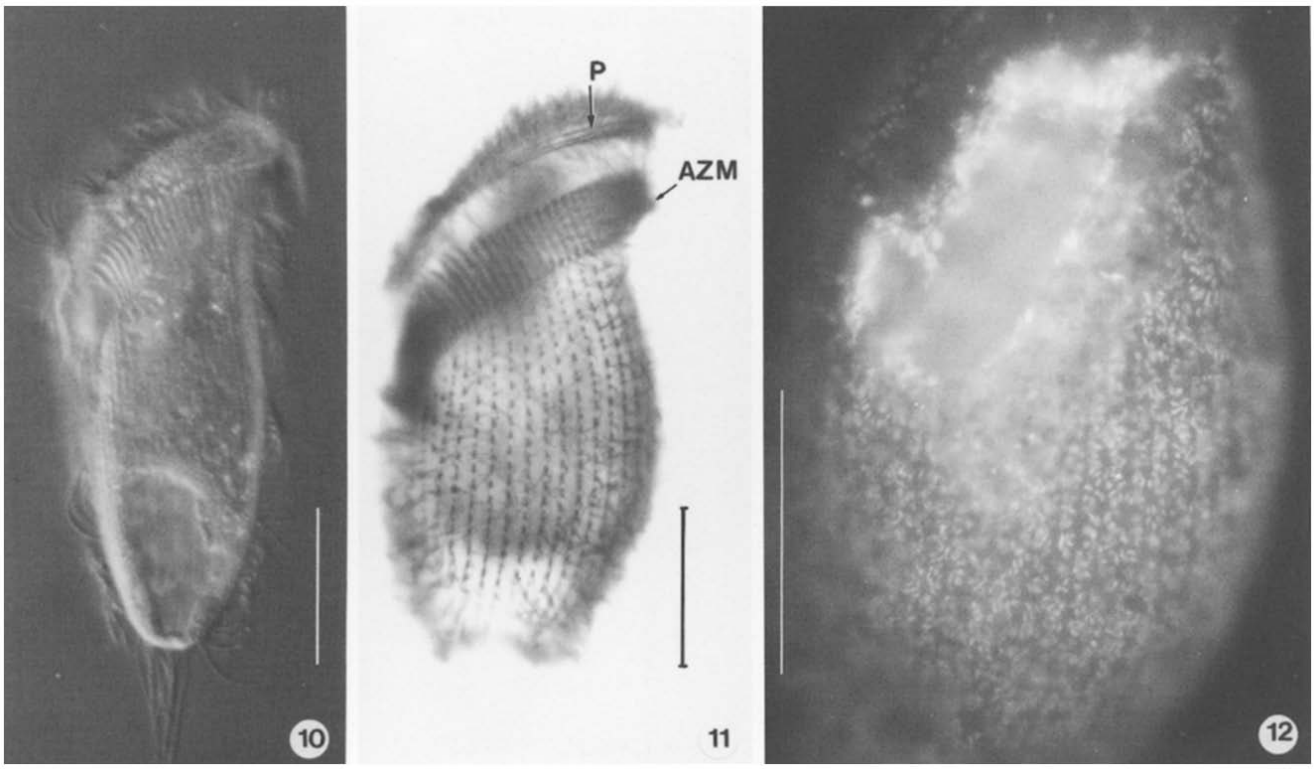


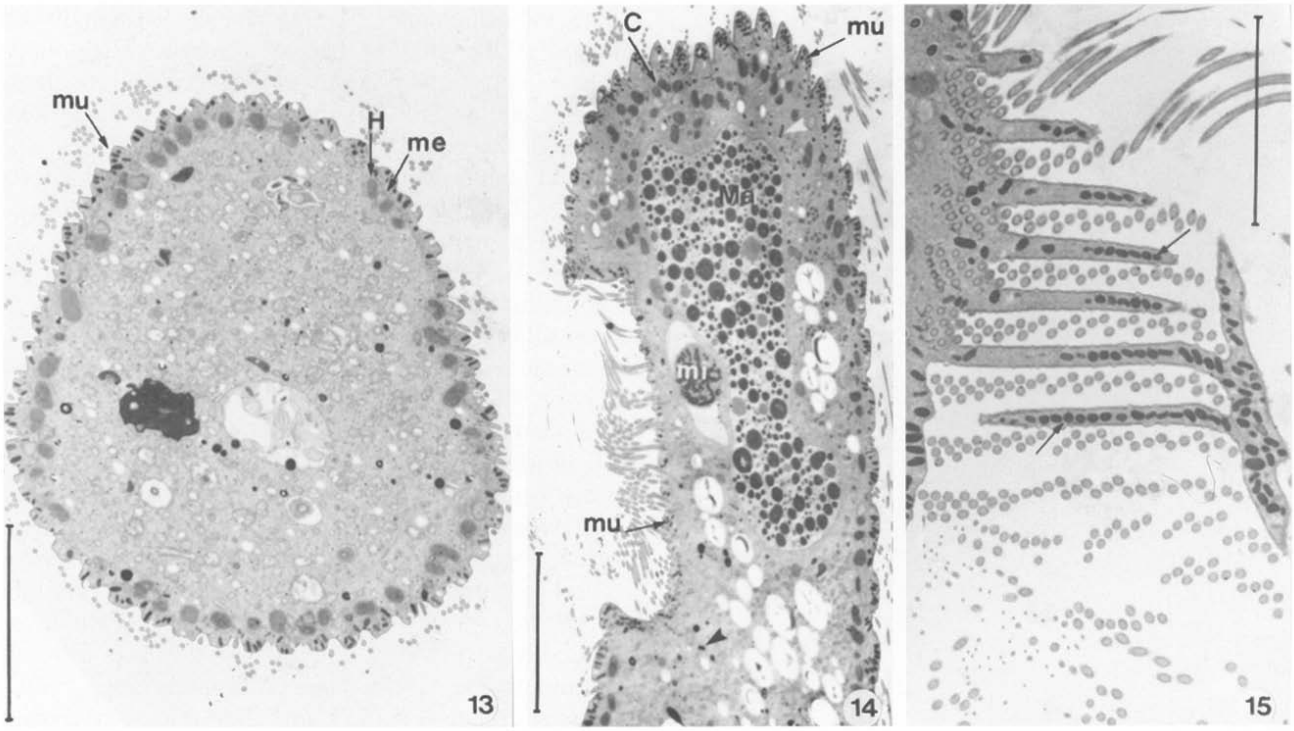
Fig. 9. *Metopus contortus.* Scale bar: 20 μm.

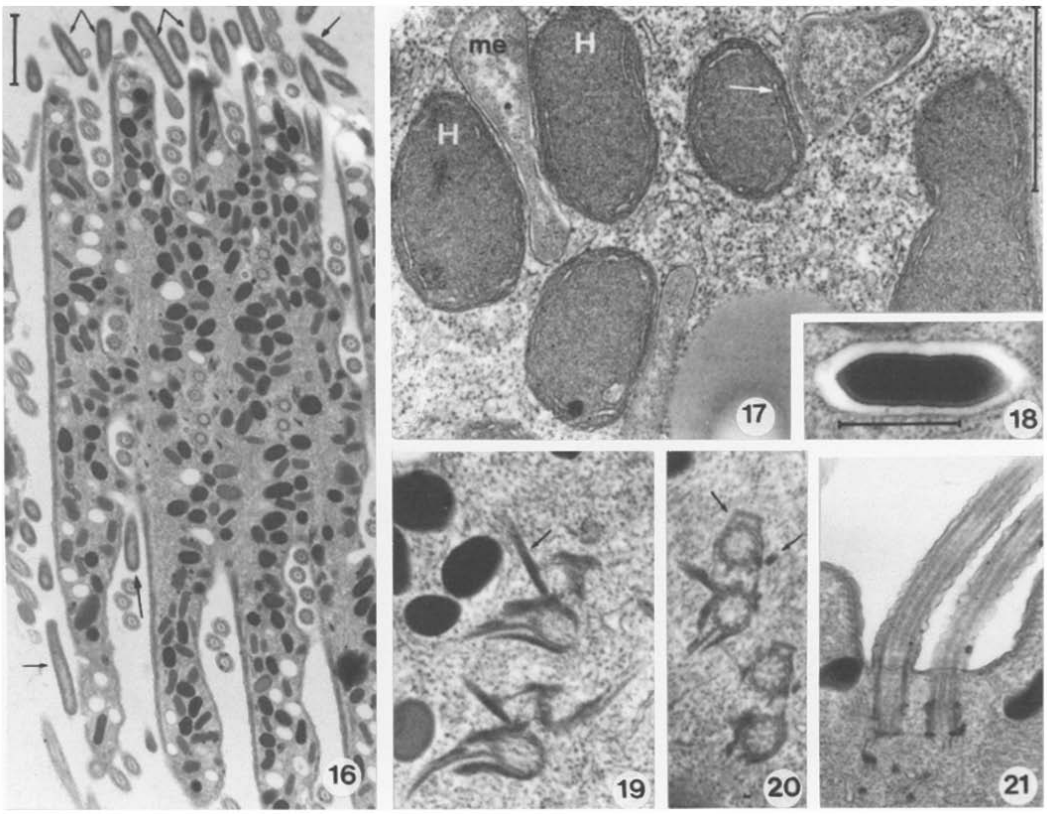
which two or three are closer to each other. The AZM is long, with 35–50 membranelles. The cytostome opens behind the cell equator. The macronucleus is single and situated in the anterior half of the cell. The micronucleus is placed in a depression of the macronucleus. The contractile vacuole is single and close to the posterior end of the cell. Caudal cilia are present. The cytoplasm contains endosymbiotic methanogenic bacteria (Fig. 12). The external surface bears ectosymbiotic bacteria which are probably sulphate reducers (FENCHEL & RAMSING 1992). *M. contortus* is easily isolated in anaerobic culture from marine sediments. It is common in almost all types of anaerobic marine habitats, including the anoxic water column, sediments, bacterial mats and decaying sea weeds.

TEM observations: Figs. 13–21 are TEM images of *M. contortus*. There is a layer of mucocysts lying immediately beneath the cell membrane. These are distributed over the entire periphery of the cell and they tend to group together between somatic kineties and between the oral membranelles (Figs. 13–16). Mitochondria-like microbodies (the hydrogenosomes) lie under the layer of mucocysts (Fig. 13). The hydrogenosomes have an inner membrane, infolded in a fashion resembling mitochondrial cristae (Fig. 17), and they release hydrogen



Figs. 10–12. *Metopus contortus*. – **10.** Living organism. Nomarski interference contrast. – **11.** Protargol-stained. P, perizonal kineties; AZM, adoral zone of membranelles. – **12.** Autofluorescing endosymbiotic methanogenic bacteria in the cytoplasm of the ciliate. Scale bars: 20 µm.





Figs. 16–21. Transmission electron micrographs of *Metopus contortus*. – **16.** Somatic dikinetids, mucocysts, and the ectosymbiotic bacteria (arrows) attached to the cell surface. Scale bar: 1 µm. – **17.** Clusters of hydrogenosomes (H) and transformed methanogens (me). White arrow to cristae inside the hydrogenosomes. Scale bar: 1 µm. – **18.** Untransformed rod-shaped endosymbiotic methanogen in the cytoplasm of the ciliate. Scale bar: 0.5 µm. **Figs. 19–21.** Structure of the somatic dikinetid. – **19, 20.** Two dikinetids and associated fibres. Arrow to the kinetodesmal fibre (Fig. 19), and associated fibres (Figs. 19, 20) (see text). – **21.** Ciliated kinetosomes in a somatic dikinetid.

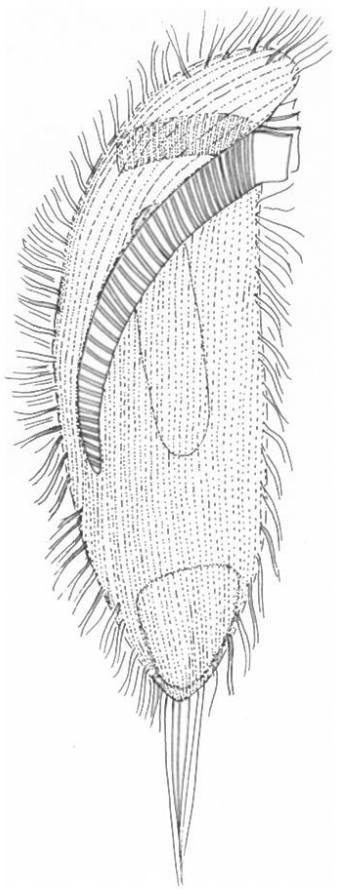
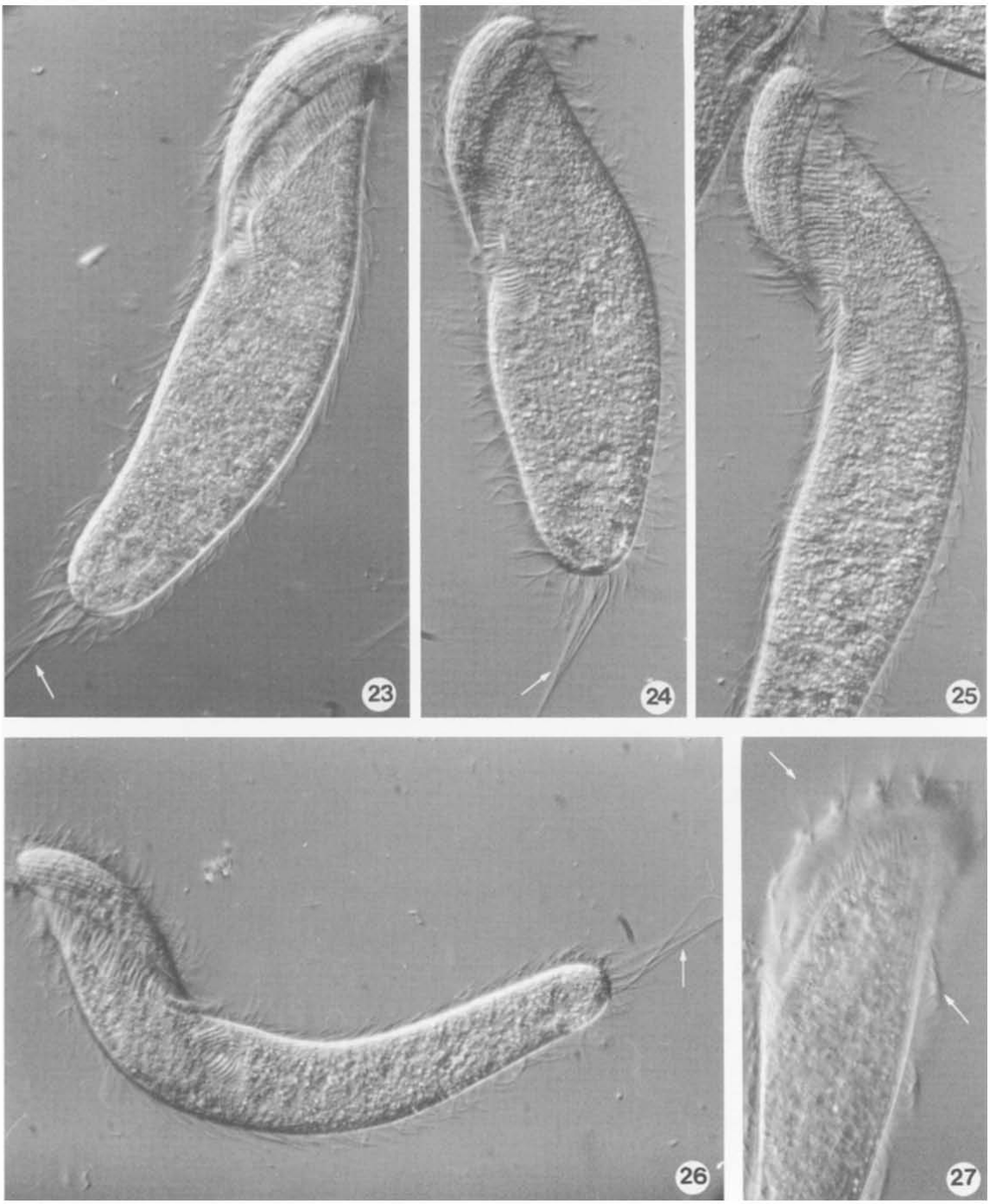


Fig. 22. *Metopus major*. The living organism. Scale bar: 20 µm.

Figs. 13–15. Transmission electron micrographs of *Metopus contortus*. – **13.** Transverse section with the peripheral arrangement (arrows) of hydrogenosomes (H), endosymbiotic methanogenic bacteria (me), and mucocysts (mu, closest to cell membrane). Scale bar: 10 µm. **14.** Longitudinal section. Arrows to the mucocysts (mu) and to hydrogenosome-methanogen clusters (C). Arrowheads to untransformed rod-shaped endosymbiotic methanogens in the cytoplasm (see text). Macronucleus (Ma) and micronucleus (mi) are also shown. Scale bar: 10 µm. – **15.** Thin section at the level of the adoral zone of membranelles. Arrows to the mucocysts in the oral area, located between the membranelles. Scale bar: 5 µm.



Figs. 23–27. *Metopus major:* living organisms. Note the tuft of straight caudal cilia in Figs. 23, 24, and 26 (arrow), and the pattern of the metachronal wave of the somatic cilia (arrow) in Fig. 27.

gas (FINLAY & FENCHEL 1989; FENCHEL & FINLAY 1992). Endosymbiotic bacteria are attached to the hydrogenosomes, forming clusters (Figs. 13, 17). These bacteria show typical methanogen autofluorescence when exposed to ultraviolet light (Figs. 12, 31) and they are polymorphic (FINLAY & FENCHEL 1991b). A complete transformation from rod-shaped bacteria (unassociated with hydrogenosomes) takes place, in which the methanogens lose the outer cell wall, become plastic in shape and associated with hydrogenosomes (Figs. 14, 17, 18). The macronucleus, micronucleus, food vacuoles, and other organelles all lie beneath the peripheral

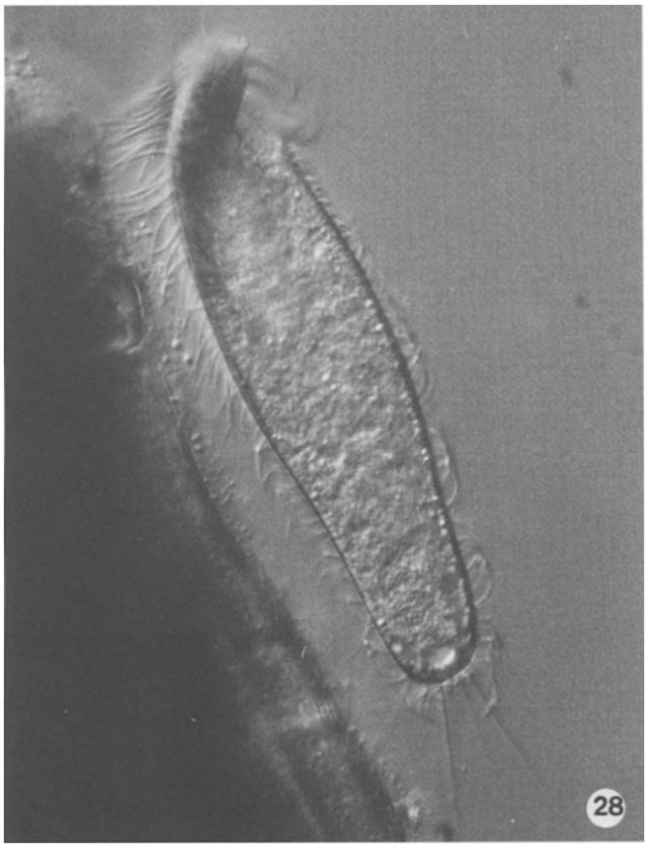
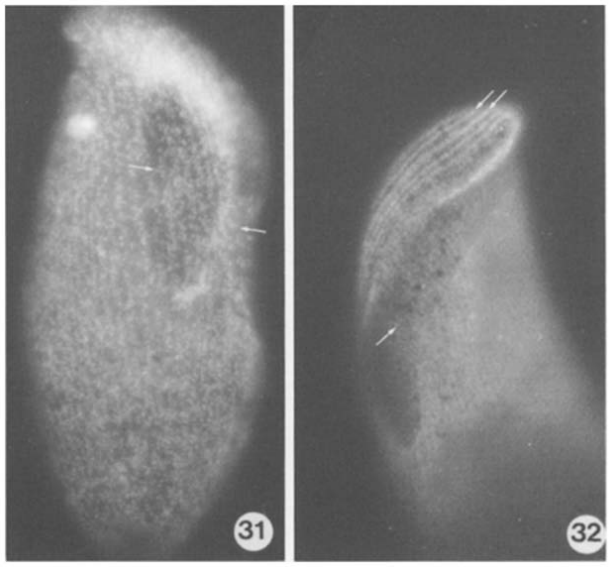
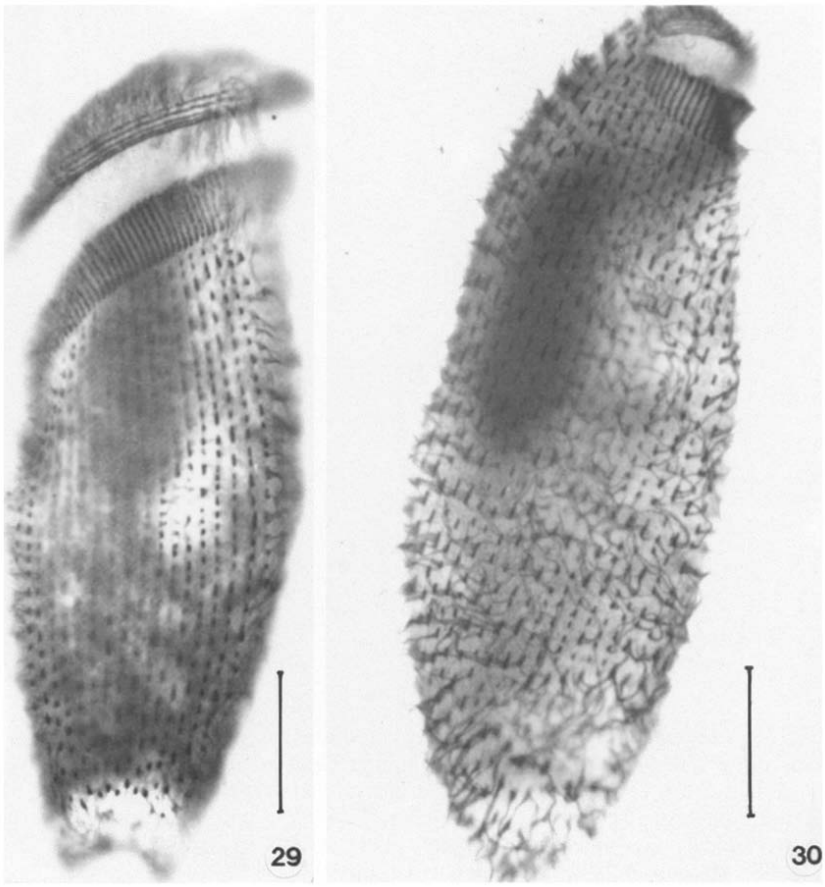


Fig. 28. Living organism of *M. major* attached to a sediment particle by the dorsal cilia.



Figs. 31, 32. Autofluorescing particles in the cytoplasm of *M. contortus* (Fig. 31) and *M. major* (Fig. 32). Fig. 31 shows the small bright endosymbiotic autofluorescing methanogens (arrows) between kineties, whereas Fig. 32 shows the even smaller mucocysts, also lying between the kineties in *M. major*.



Figs. 29, 30. *Metopus major*. Protargol-stained specimens. Scale bars: 20 µm.

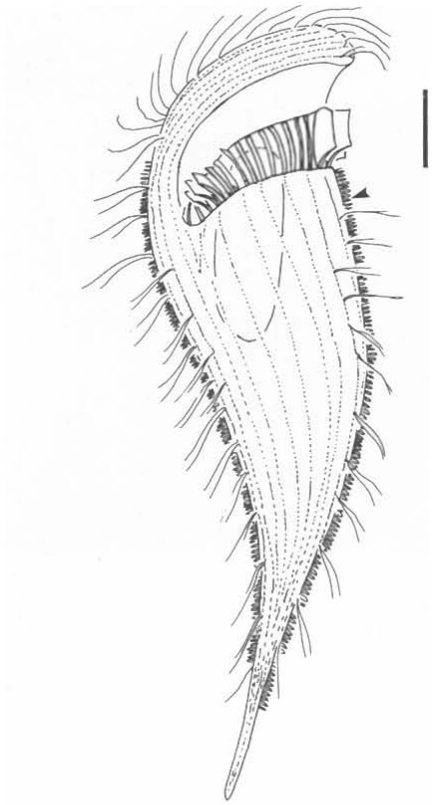


Fig. 33

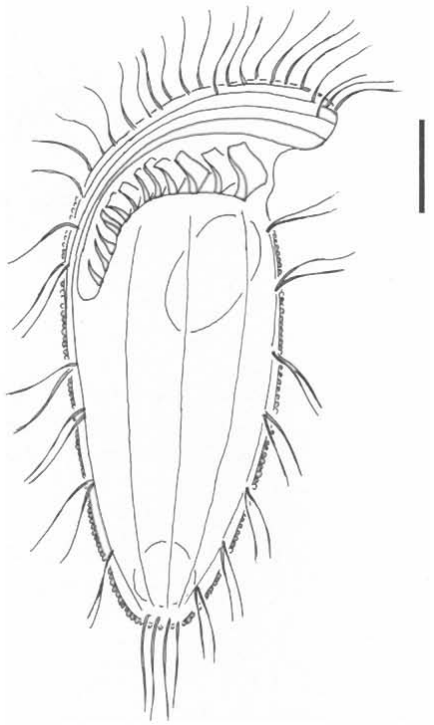
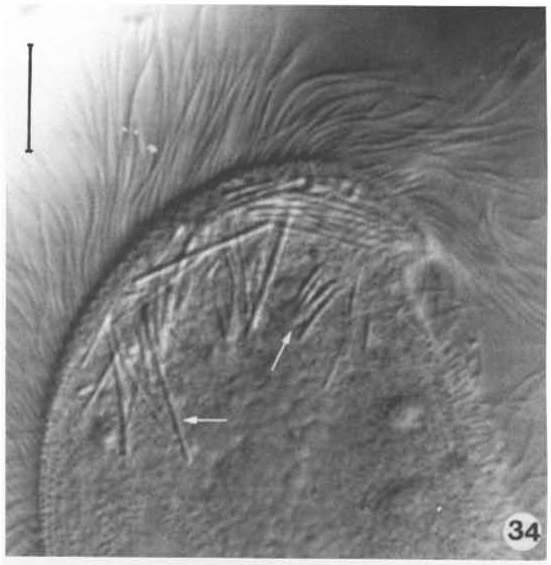
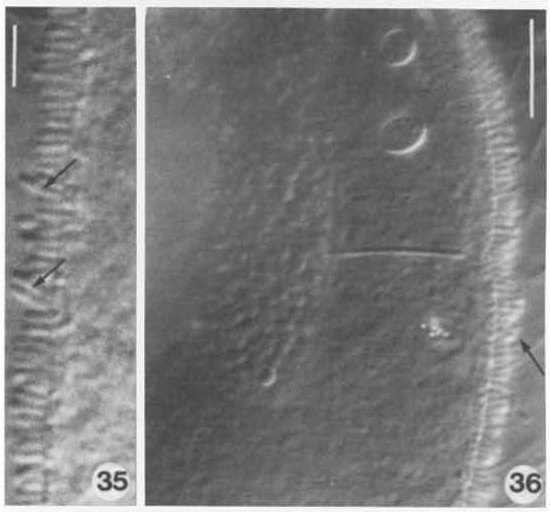


Fig. 38





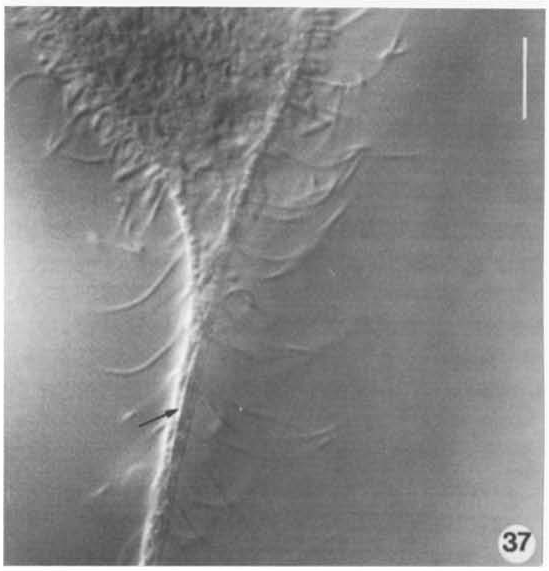
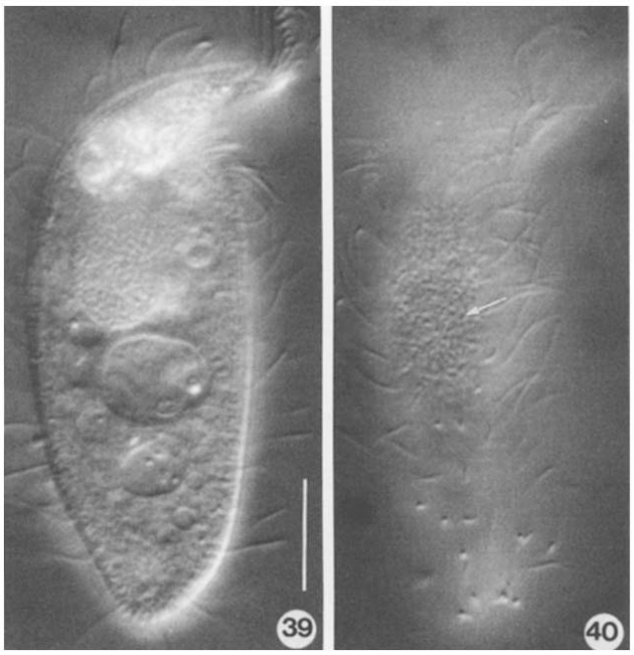


Fig. 33. *Metopus vestitus.* Arrows to the ectobiotic bacteria. Scale bar: 10 μm.
Figs. 34–37. *Metopus vestitus.* Nomarski interference contrast. – **34.** Arrows to the cytoplasmic crystals close to the anterior end. Scale bar: 5 μm. – **35.** Ectobiotic bacteria arranged perpendicular to the cell membrane of the ciliate. Arrows to transversally-dividing bacteria. Scale bar: 2 μm. – **36, 37.** Middle and posterior part, respectively, of the ciliate with ectobiotic bacteria, macronucleus, micronuclei, and crystals in the cytoplasm (arrow). Scale bar: 5 μm.
Fig. 38. *Metopus halophila.* Scale bar: 10 μm.



Figs. 39, 40. *Metopus halophila.* Nomarski interference contrast. – **39.** Living organism. – **40.** Ectobiotic bacteria (arrow). Scale bar: 10 μm.

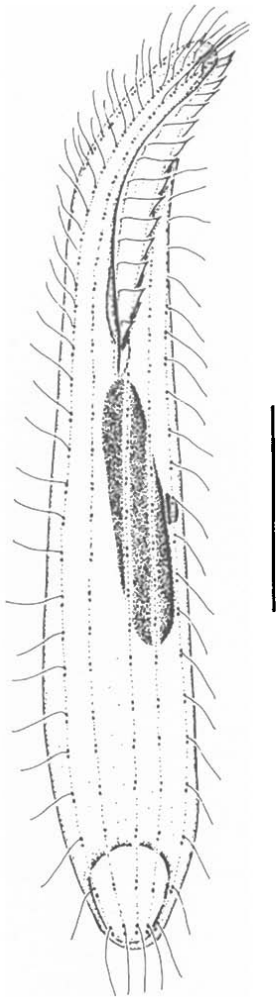


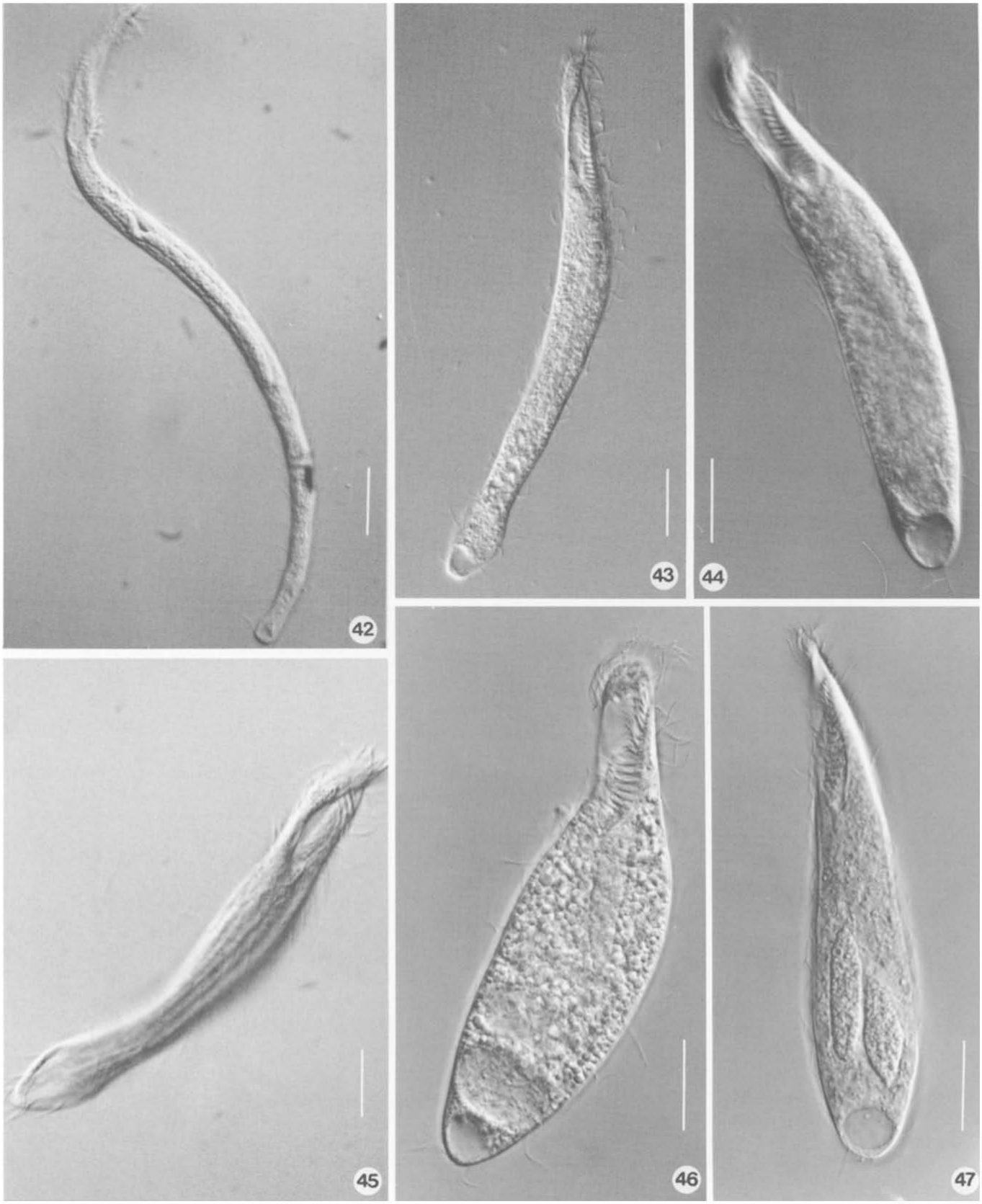
Fig. 41. Diagram of the living *M. palaeformis.* Scale bar: 20 μm.

concentric layers of mucocysts and hydrogenosome-methanogen complexes (Fig. 14). The macronucleus is placed in the anterior half of the cell, and the micronucleus is embedded in the macronucleus (Fig. 14). A normal feature of *M. contortus* is the simultaneous presence of endosymbiotic and ectosymbiotic bacteria. It has recently been shown that the ectosymbiotic bacteria are probably sulphate reducers (FENCHEL & RAMSING 1992). They are arranged on the ciliate cell surface (Fig. 16), lying parallel to the longitudinal cell axis. They rarely cover the entire surface area of the cell.

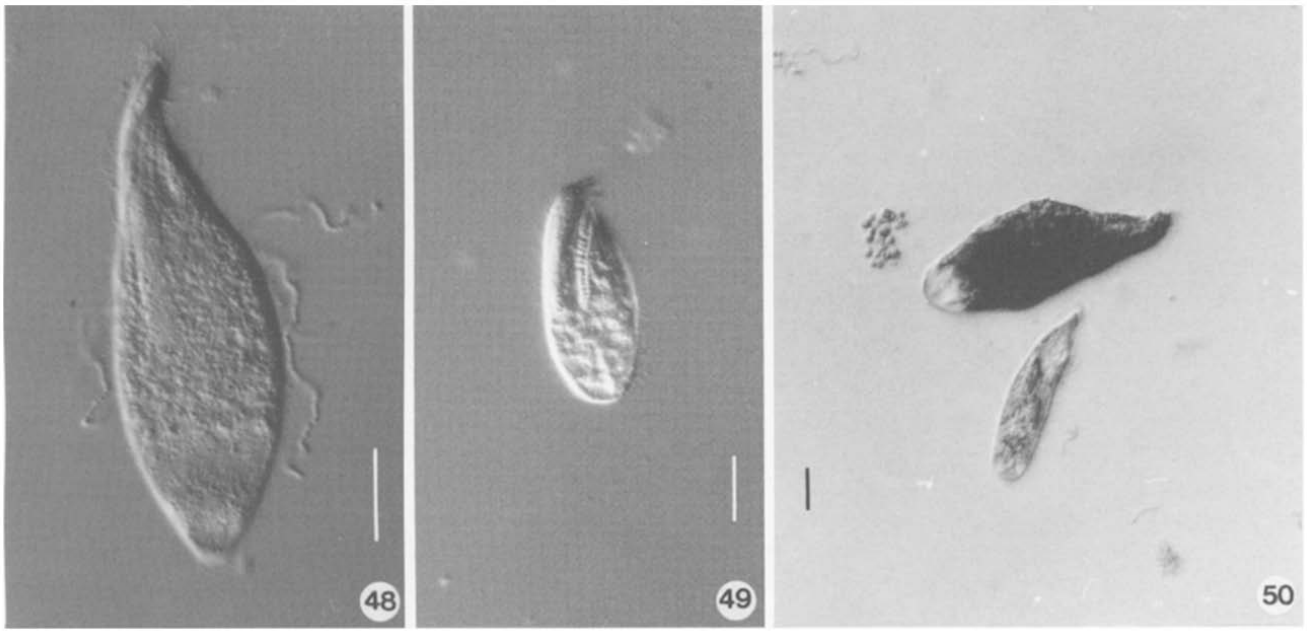
The somatic kineties in *M. contortus* are formed of ciliated dikinetids. The morphology of each dikinetid as seen using TEM is characterized (Figs. 19–21) by : (1) An anterior kinetosome with an angled transverse ribbon formed by about 10 microtubules arranged in two sets. One set is formed by 3 microtubules and starts at the level of triplet 6. The other set includes 7 microtubules and extends over triplets 3 to 5. A dense structure arises from triplets 1 and 2, and connects with triplets 3 and 4 of the posterior kinetosome. A second dense structure is observed at triplet 7 which seems to join triplet 6 of the posterior kinetosome. No kinetodesmal fibre is associated with this kinetosome. (2) A posterior kinetosome with postciliary ribbon, curved and formed from about 15 microtubules. This ribbon extends posteriorly, starting at the level of triplet 9. The kinetodesmal fibre is striated and placed at the level of triplet 6, directed laterally (Figs. 19, 20). Next to the postciliary ribbon there are two accompanying dense structures. One of these starts at triplets 6 and 7, the other at the level of triplets 1 and 2. The interkinetosomal linkages have not been observed with certainty.

• *Metopus major* (KAHL, 1932)

This ciliate is definitely distinct from *M. contortus* as described above. *M. major* is larger (Figs. 22–30, 32): 113–191 μm long and 26–49 μm wide. The somatic infraciliature includes 50 to 55 kineties. The AZM consists of 80 membranelles, and in some specimens it extends far beyond the cell equator (Fig. 29). The perizonal area is formed by at least 5 or 6 kineties lying very



Figs. 42–47. Some of the morphological variations in size and shape observed in *M. palaeformis*. Nomarski interference contrast. Scale bars: 20 µm.



Figs. 48–50. Further morphological varieties of *M. palaeformis*. The dark cell in Fig. 50 is a "giant" next to a "normal" trophont. Scale bars: 20 µm.

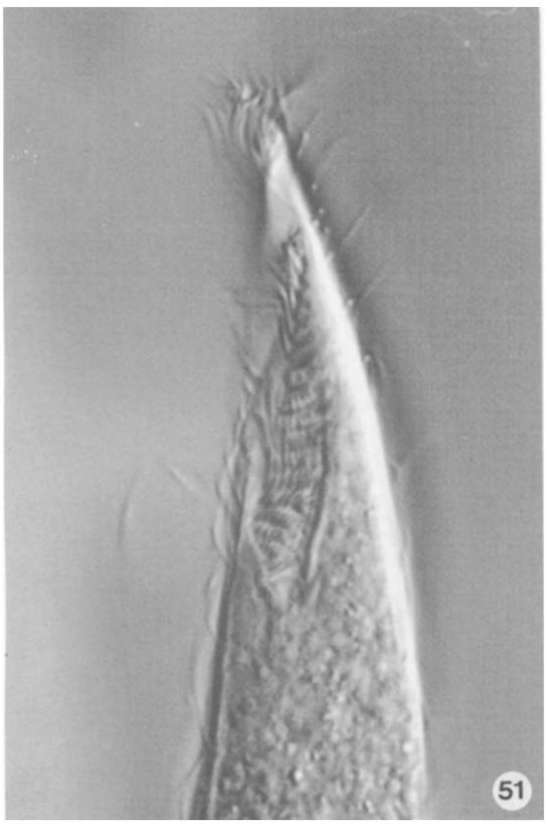
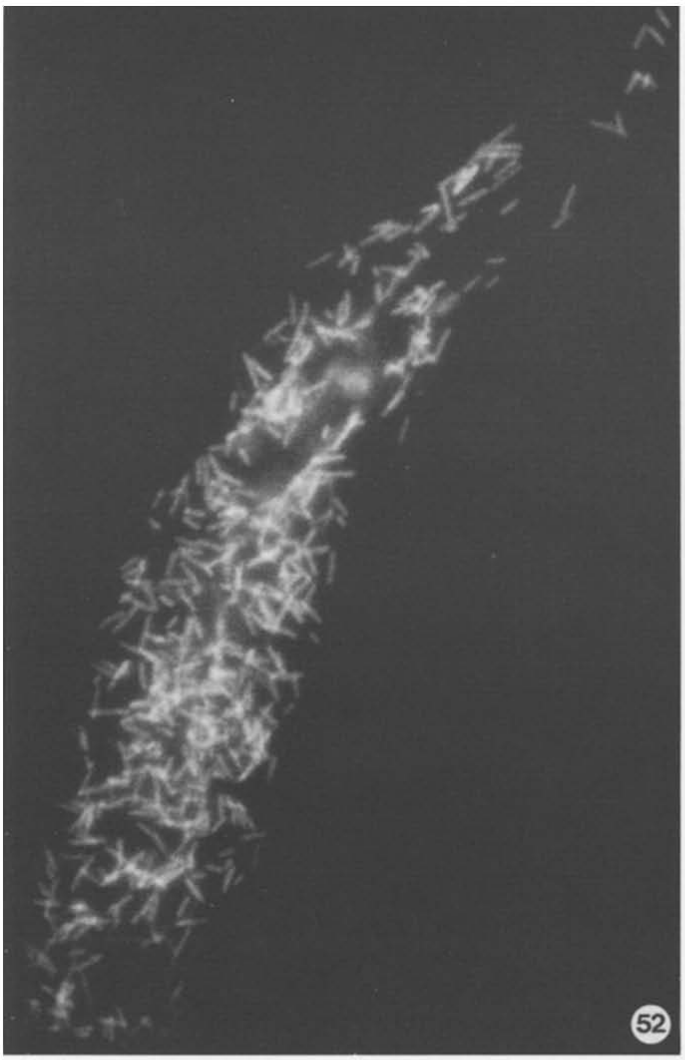


Fig. 51. Close up of the organism in Fig. 47 to show the oral membranelles and twisted anterior part of the cell. Nomarski interference contrast.

close to each other. The contractile vacuole is close to the posterior end of the cell and a group of caudal cilia is also present. In living organisms these caudal cilia are always straight and stiff (Figs. 24, 26, 28). The macronucleus is elongate and placed in the centre of the cell. The micronucleus does not lie next to the macronucleus, but in the posterior half of the cell. *M. major* was isolated from a sulphidic accumulation of decaying eelgrass in Nivå Bay (Denmark) and kept in anaerobic culture.

- *Metopus vestitus* KAHL, 1932

The ciliate is S-shaped, about 70 µm long, with a distinctive, long, spine-like posterior extension (Fig. 33). The ciliates bear a coat of ectobiotic bacteria (Figs. 34–37). These divide transversally (Fig. 35). There are about 25 somatic kineties, with paired kinetosomes. The oral region consists of 20 oral membranelles. The macronucleus is single and elongate, with one or two associated micronuclei. The ciliates bear endosymbiotic methanogens. In the anterior part of the cell and in the posterior projection there are needle-shaped intracytoplasmic structures (Figs. 34, 36, 37), which were observed by TUCOLESCO (1962). These appear to be crystals, and they resemble structures described in other marine ciliates (DRAGESCO 1960). *M. vestitus* is not very common. It occurs in anaerobic layers of marine sand, and sporadically in other marine benthic habitats such as bacterial mats.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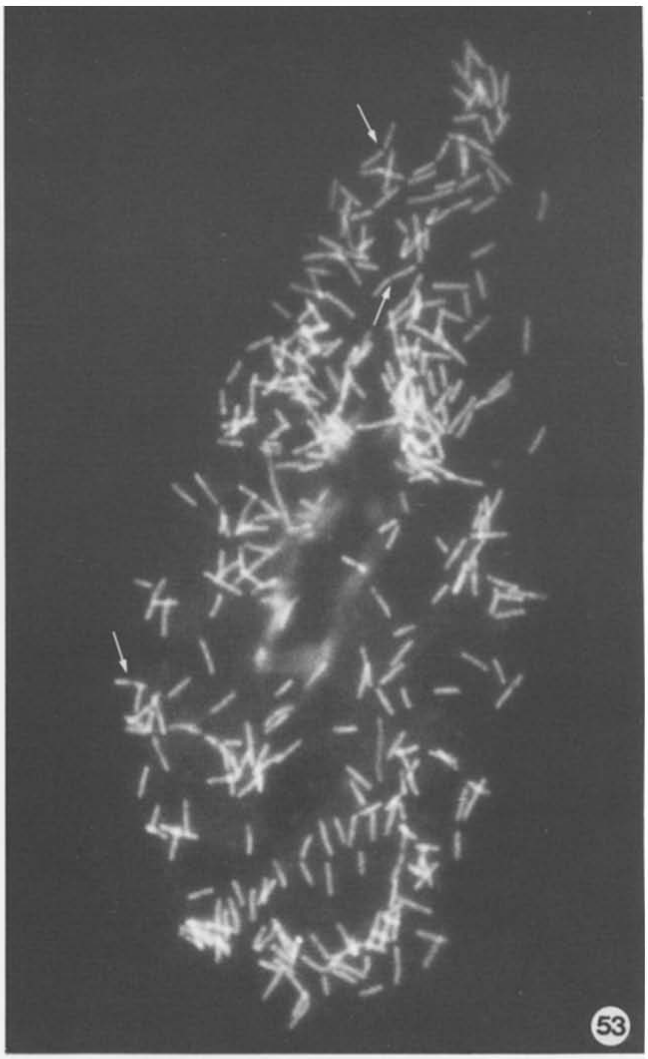
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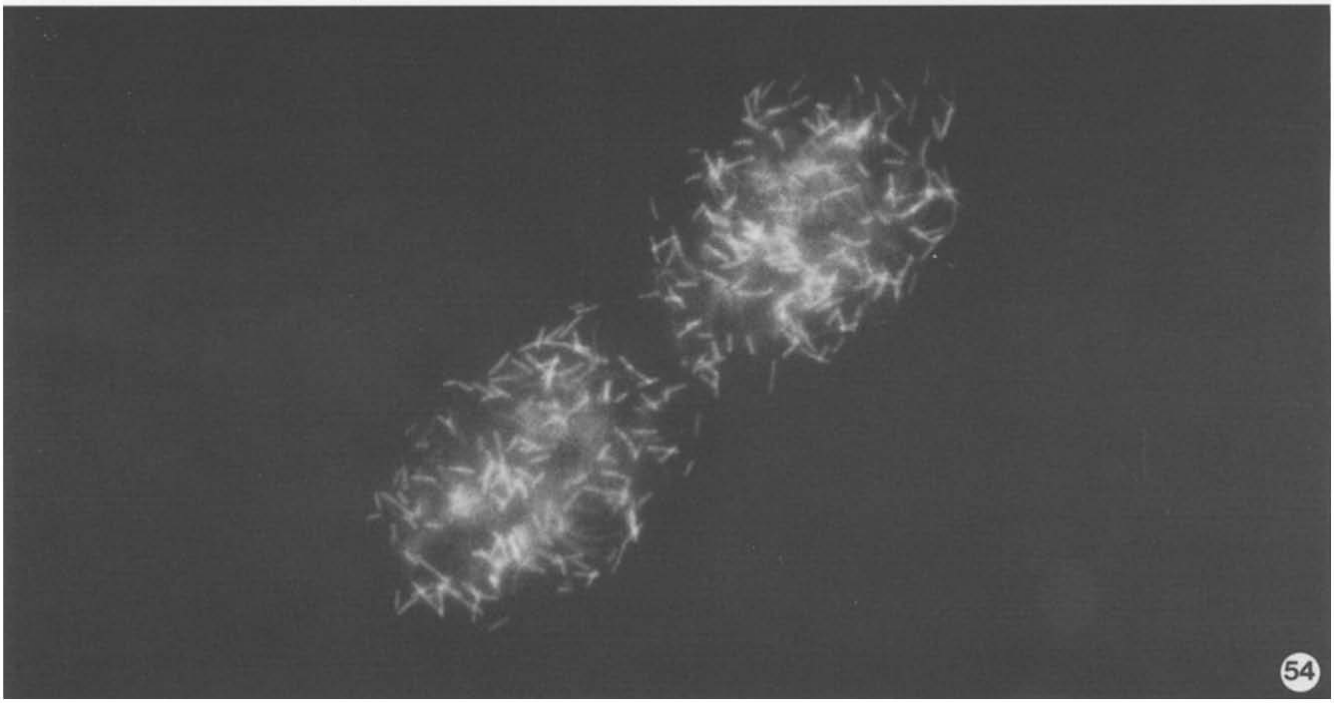
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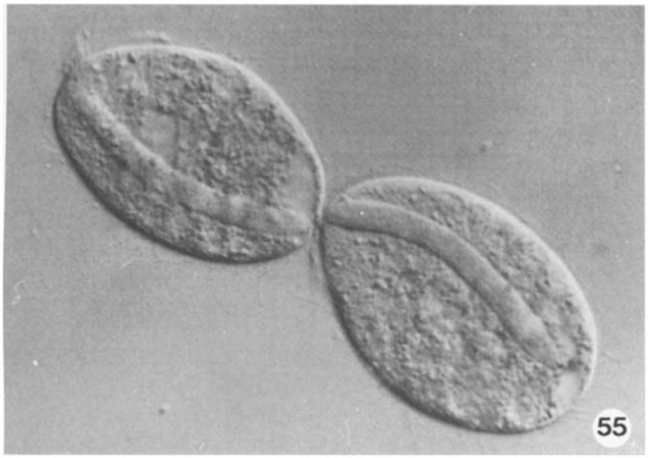

Figs. 55. One of the very few examples we have seen of *Metopus* in binary fission: a dividing *M. palaeformis*. The internal sausage-like structure is the macronucleus.

• *Metopus halophila* (KAHL, 1925) CORLISS, 1960

Metopus halophila is 50–90 µm long, ellipsoidal with the anterior part of the cell projecting forwards, when the cell is seen from the side (Figs. 38, 39). This feature confers a curved appearance, closer to an open C-shape than the typical *Metopus* S-shape. Small ectobiotic bacteria cover most of the external cell surface (Fig. 40). The ciliates have about 20 somatic kineties with paired kinetosomes and every kinetosome bears a cilium. There is also a group of four caudal cilia. The contractile vacuole is terminal and very obvious. The oral region is shorter than in other *Metopus* species, and does not reach the equator of the cell. This oral region is curved towards the dorsal side, following the shape of the protruding anterior part. The AZM includes 12–15 membranelles. *Metopus halophila* is not very common and, along with *M. vestitus,* occurs in the anaerobic layer of marine sand and in bacterial mats.

• *Metopus palaeformis* KAHL, 1927

This ciliate is polymorphic and it is probably the most variable of the *Metopus* spp. in culture (Figs. 42–50). A typical trophic cell is elongate, flattened, and somewhat ribbon-like in shape (Figs. 41, 43, 56). Cell size varies from 70 to 200 µm long (exceptionally, longer), and 8–31 µm wide. The number of somatic kineties is 8 to 14, and there are 10–20 membranelles in the adoral zone (Fig. 51). The somatic kineties are slightly curved in the posterior part of the cell and they are formed by dikinetids (Fig. 67) which have the same ultrastructure as the dikinetids in *M. contortus* (Figs. 19–21). The arrangement of somatic kineties in smaller specimens of *M. palaeformis* resembles that in some species of *Trachelophyllum.*

The organisms used in the present study have been kept in culture for several years, and it has been possible to observe systematic changes in cell size and morphology which seem to be related to starvation (FINLAY & FENCHEL 1991a). The life cycle is shown in Fig. 69. Trophonts start growing when food is supplied. After reaching the stationary phase, and with the depletion of food, growth rate declines and cells become either long and thin, or they form cysts (Figs. 60). When the cells are re-fed, the cycle is repeated. *M. palaeformis* can also produce giant forms, which have at least double the volume of typical trophonts, and a very dark cytoplasm. They do not appear to feed on other ciliates (FINLAY & FENCHEL 1991a).

All forms of *M. palaeformis* (including the cysts) have endosymbiotic methanogenic bacteria distributed throughout the cytoplasm (Figs. 52–54, 59, 66, 68). These endosymbionts do not undergo morphological transformation (Figs. 61–63). They are rod-shaped, and they lie close to the hydrogenosomes (Figs. 62, 64) (FINLAY & FENCHEL 1991a, b). Mucocysts lie beneath the entire cell surface, including the oral region (Figs. 64, 65). *M. palaeformis* is a common freshwater ciliate. It has also been found in samples of anaerobic municipal landfill material (FINLAY & FENCHEL 1991a), in a septic tank, in sulphide-rich solution lakes, in soil from an extinct volcano, and in marine lagoons (ALADRO et al. 1990). Due to the wide morphological variation now known for *M. palaeformis*, we conclude that the species is probably the same thing as the *Tesnospira* described by JANKOWSKI (1964), as well as *Metopus hyalinus* KAHL 1927, *M. rostratus* KAHL 1927, and *M. tenuis* KAHL 1927.

• *Metopus es* MÜLLER, 1776

This is the type species for the genus. It is a very distinctive S-shaped ciliate (Fig. 70). 112–203 µm long, and 23–66 µm wide, with a protruding anterior part and a

Figs. 52–54. Autofluorescing endosymbiotic methanogenic bacteria in the cytoplasm of *M. palaeformis*. Arrows to the recently-divided methanogens. Fig. 54 shows a diving *M. palaeformis.*


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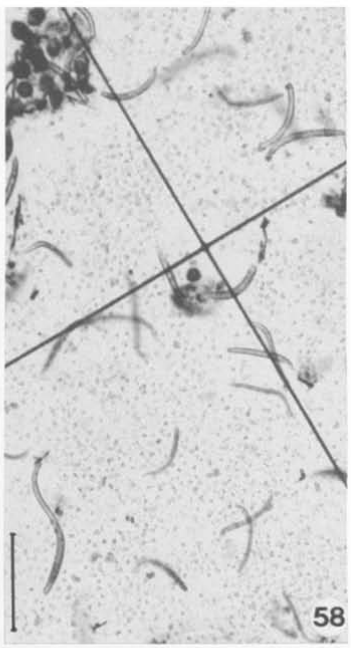
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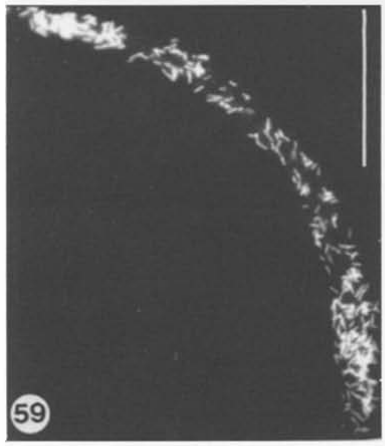
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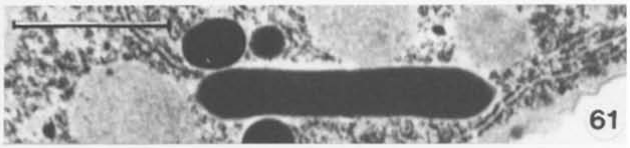
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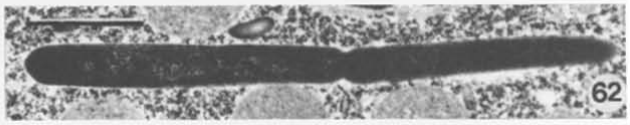
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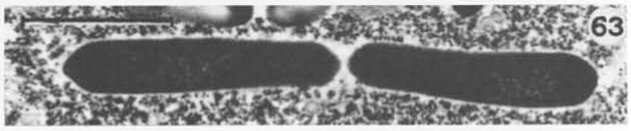
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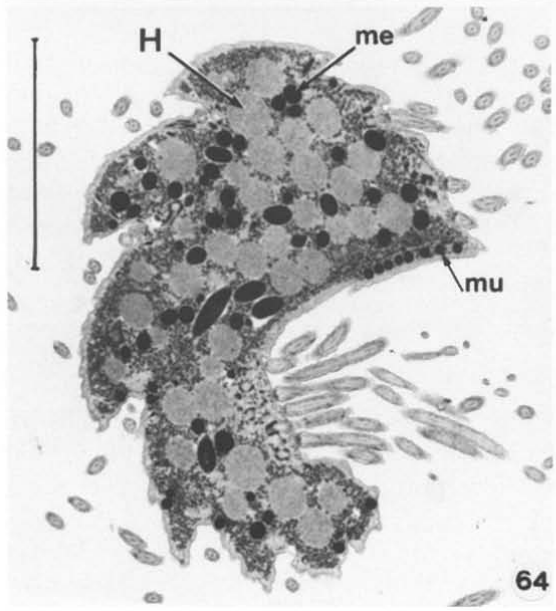
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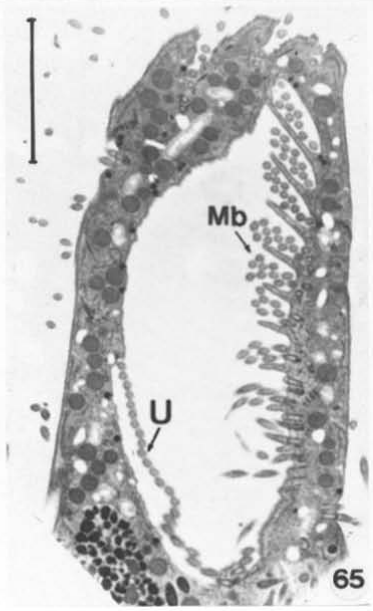
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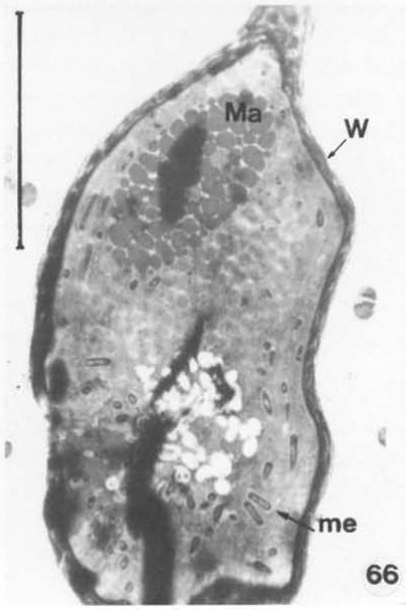
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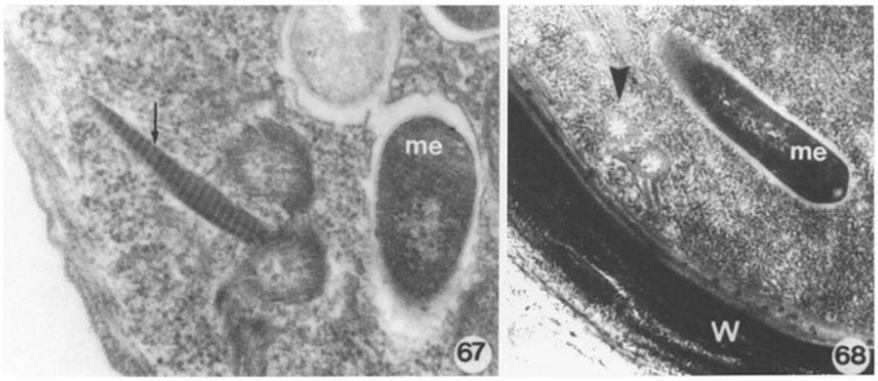


Figs. 67. 68. *Metopus palaeformis.* – **67.** Morphology of a somatic dikinetic and associated fibres. Arrow to the kinetodesmal fibre; me, methanogenic bacterium. – **68.** Dikinetid (arrowhead) in the cyst. W, cyst wall; me, methanogenic bacterium.

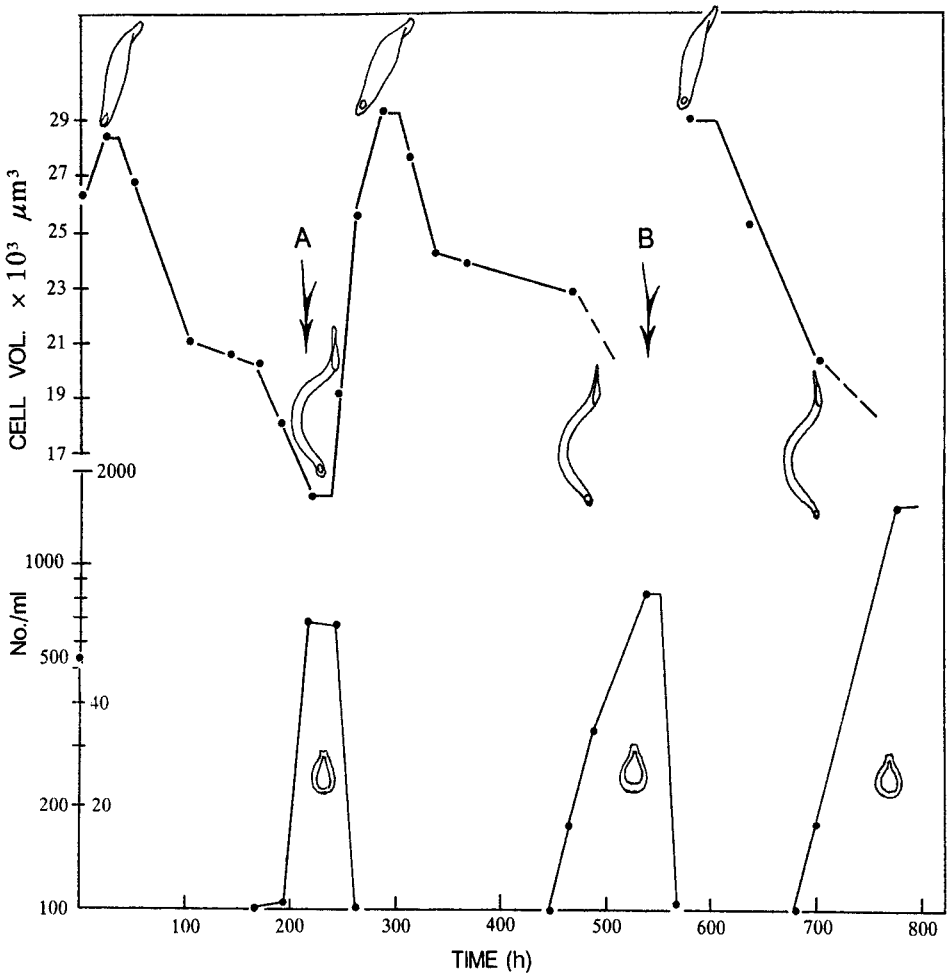


Fig. 69. Polymorphic life cycle of *Metopus palaeformis.* The trophic cells start feeding and they increase in size. As food becomes depleted, they become smaller, and eventually long and thin, or they encyst. Introduction of new food at times A and B induces repetition of the cycle (adapted from FINLAY & FENCHEL 1991a).

Figs. 56–58. *Metopus palaeformis.* – **56.** Organisms after feeding on purple sulphur bacteria (*Chromatium* sp.). Arrows to the sulphur granules in the cytoplasm of the ciliate. – **57.** Culture in exponential growth phase. Scale bar: 200 µm. – **58.** Starved culture. Scale bar: 200 µm.
Fig. 59. Autofluorescing endosymbiotic methanogenic bacteria in the cytoplasm of the ciliate. Scale bar: 20 µm.
Fig. 60. Cysts. Scale bar: 20 µm.
Figs. 61–63. Cell division of the endosymbiotic bacteria. Scale bars: 1 µm.
Figs. 64–66. Transmission electron microscopy. – **64.** Cross section. Cytoplasm of the ciliate packed with methanogens (me) and hydrogenosomes (H). Arrow to a mucocyst (mu). Scale bar: 5 µm. – **65.** Longitudinal section of the oral area. U, undulating membrane; Mb, membranelles in the AZM. Scale bar: 5 µm. – **66.** Longitudinal section of the bottle-shaped cyst. Ma, macronucleus; me, endosymbiotic bacteria; W, cyst wall. Scale bar: 10 µm.

Fig. 70. *Metopus es.* Typical shapes of cells from a culture in exponential growth phase. Nomarski interference contrast. Scale bar: 30 μm.

characteristically elegant motion when swimming (Fig. 70). There are about 35–40 somatic kineties, of which 5 form the perizonal stripe (Fig. 71). The AZM includes about 40–50 membranelles. The endosymbiotic methanogens are found throughout the cytoplasm (Fig. 76). The single macronucleus is more or less ovoid and placed in the anterior part, close to the equator of the cell. The single micronucleus lies next to the macronucleus. There are two short caudal cilia which are only observable on stationary living organisms. This is a very common ciliate in anoxic freshwater habitats.

• *Metopus striatus* MCMURRICH, 1884

This distinctly tear-shaped ciliate (Figs. 72–75) is 38 to 71 μm long, with a pointed posterior end bearing caudal cilia. The number of kineties is about 25, although it may vary with cell size. Five somatic kineties lie closer

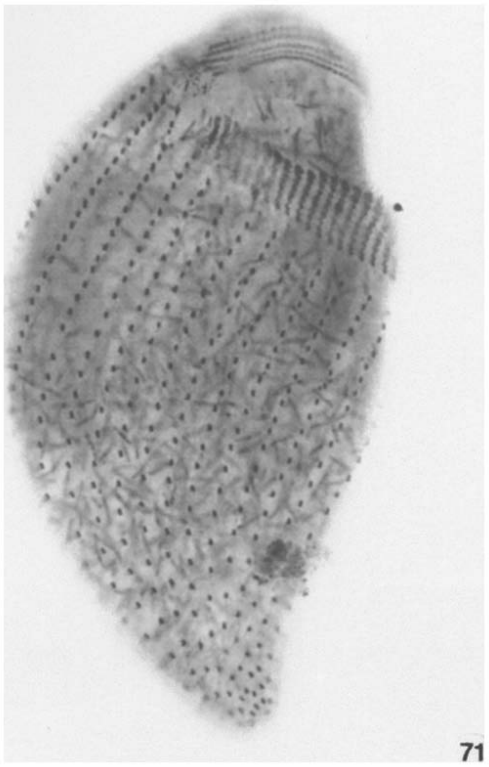


Fig. 71. Protargol-stained *M. es* (photograph courtesy of JL Olmo, University of Madrid).

to each other in the perizonal region. The AZM is formed by about 45 membranelles. The ciliates bear endosymbiotic methanogenic bacteria (Fig. 77). The macronucleus is single, rounded, and located in the centre of the cell, the micronucleus lying close to it. Extrusomes lie beneath the cell membrane (Fig. 73), although they are not always obvious.

When the ciliate is found in nature it has a well-developed caudal protuberance, as explained above. After several weeks in culture, the cells become smaller and more rounded, and they lose the caudal projection. The shape of the macronucleus remains constant. *M. striatus* is quite common in soft sediments in freshwater ponds and lakes as well as in river sediments.

- *Metopus spinosus* KAHL, 1927

These ciliates are characterized by curvature of the cell, which is especially conspicuous in the living organism (Figs. 78–80), and the presence of a caudal prolongation or spine. The latter varies in length and in some individuals it is very short. The curved shape, together with the caudal spine confers a peculiar swimming movement: the posterior half of the cell moves alternately from one side to the other. The ciliates are approximately 70 µm long, and always wider at the cell equator. Our observations agree with those of JANKOWSKI (1964) for *M. caudatus* DA CUNHA, 1915. The contractile vacuole is situated at the cell posterior and is cuboid (Fig. 79) to ellipsoid. In agreement with JANKOWSKI (1964), we have observed long (6–9 µm) intranuclear rod-shaped bacteria (Fig. 80), of unknown identity. The cytoplasm of *M. spinosus* is very transparent, usually containing groups of pink spherical particles – possibly ingested purple bacteria. The cytoplasm bears other rod-shaped bacteria, which we also failed to identify. They did not show typical methanogen autofluorescence. The macronucleus is spherical and situated in the centre of the cell. The AZM includes about 15 membranelles (Fig. 79); the somatic infraciliature is formed by less than 20 kineties, each with a few sparsely-located cilia, and two cilia per dikinetid. There are no caudal cilia. We have encountered *M. spinosus* on several occasions in the anaerobic sediment of freshwater ponds in the U.K.

The caudal prolongation or spine is variable in morphology. We have observed organisms with a completely developed caudal prolongation, as in Fig. 79; other specimens had a little protuberance at the base of the caudal spine, as described by KAHL (1935) for *M. spinosus*, and by JANKOWSKI (1964) for *M. caudatus*. There are also spine-less organisms that look like *M. curvatus* and *M. convexus* described by KAHL (1935). Taking account of all these observations we have decided to name this ciliate *M. spinosus* KAHL, 1927, although the same thing was described by JANKOWSKI (1964) as *M. caudatus* DA CUNHA (1915). DA CUNHA found the organisms he described in marine habitats and they probably correspond to *M. vestitus* (KAHL, 1927). Accordingly, *M. vestitus* should be renamed *M. caudatus*, as the latter was first described. To avoid more confusion we leave *M. vestitus* as it is (KAHL'S description is very precise), with *M. caudatus* as a synonym, and we call our freshwater *Metopus, M. spinosus*, with synonyms *M. curvatus, M. convexus,* and *M. attenuatus.*

- *Metopus verrucosus* (DA CUNHA, 1915) KAHL, 1935

This ciliate is rare and uncommon but it can be found in *Beggiatoa* mats in marine habitats. The ciliate fits the descriptions of DA CUNHA (1915) and KIRBY (1934). KAHL (1935) included this organism *(Spirorhynchus verrucosus)* within the genus *Metopus*. It is 100–140 µm long and about 20 µm wide, elongate and thin (Fig. 81), with both ends tapered, long cilia, and a posterior contractile vacuole. It seems to have three macronuclei and no micronucleus (KIRBY 1934), although variations

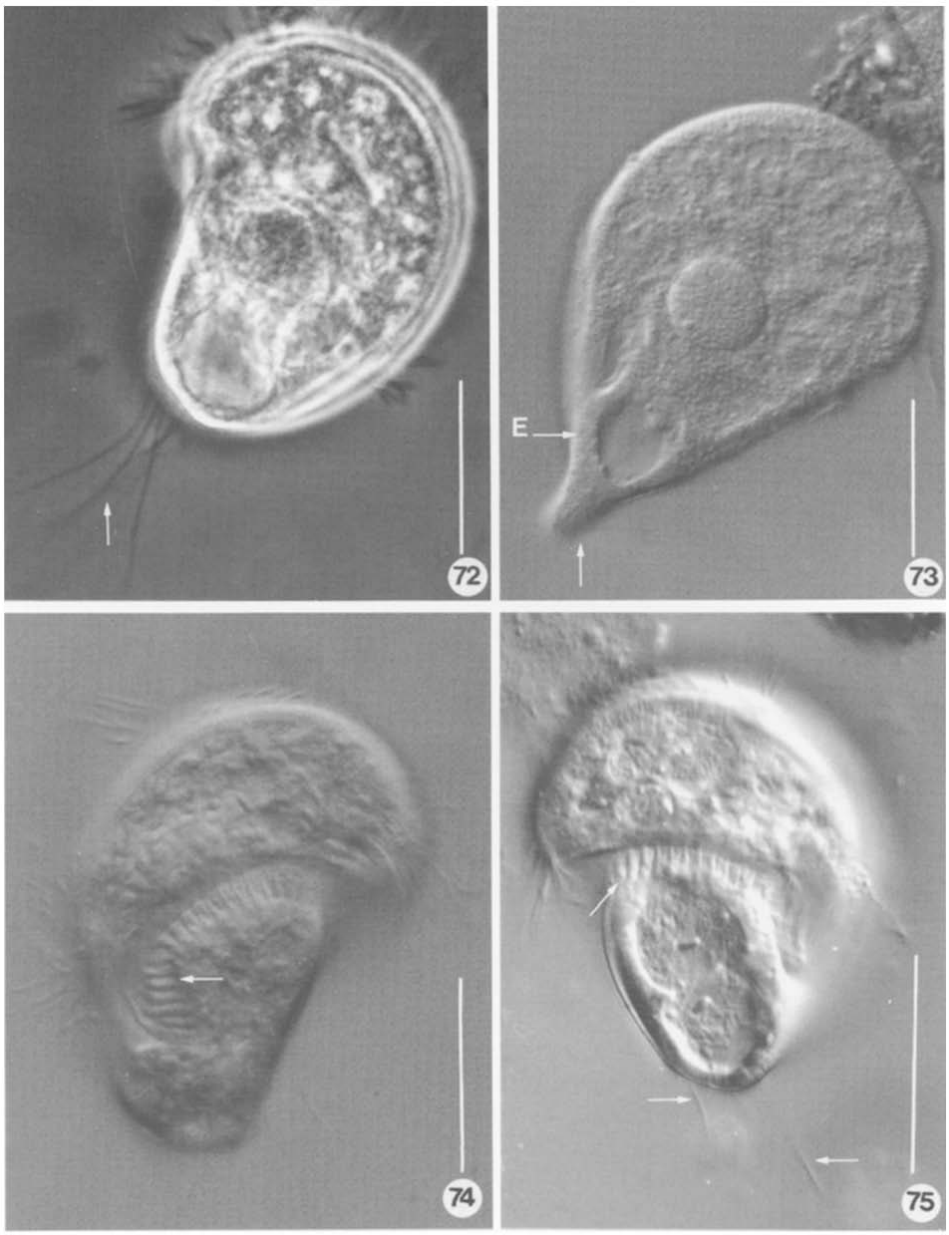
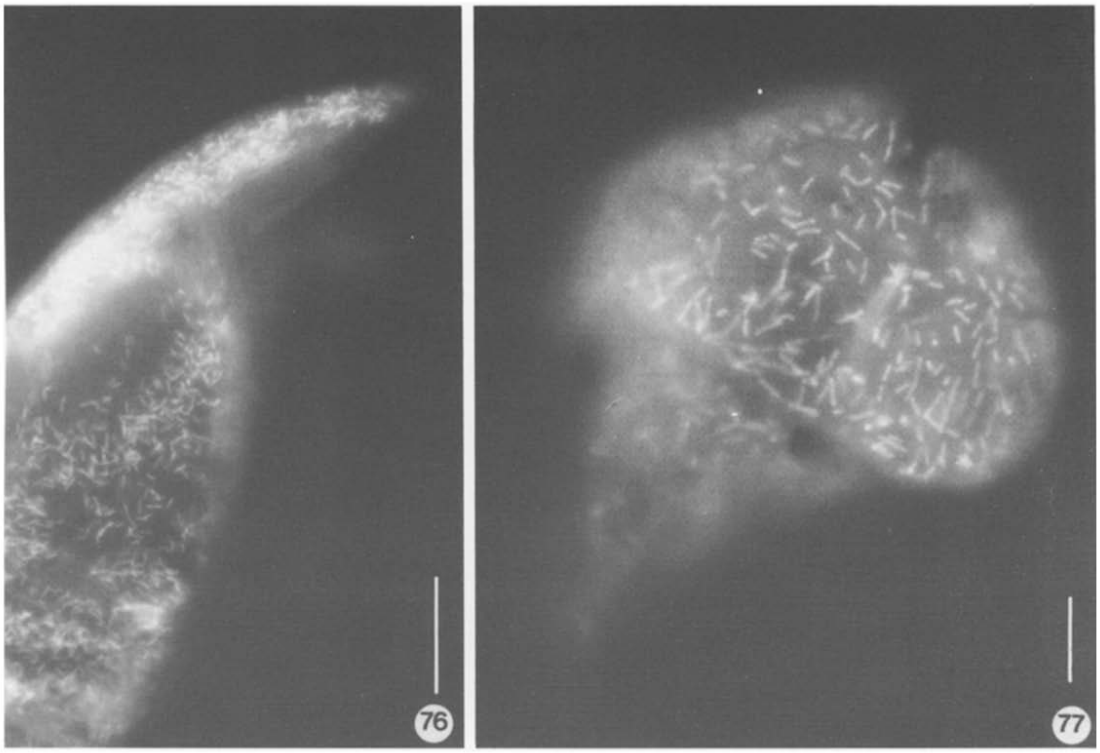


Fig. 72–75. *Metopus striatus.* Cell shape variation. Scale bar: 30 µm. – **72.** Phase contrast. Arrow to the caudal cilia. – **73–75.** Nomarski interference contrast. Fig. 73 showing an organism with caudal protuberance (arrow). E, extrusomes. – **74, 75.** Arrows to oral membranelles and caudal cilia. Note the bacteria inside food vacuoles in Fig. 75.

in this number might be possible (DA CUNHA 1915 did not observe nuclei). The principal feature of this organism are the tufts of ectobiotic bacteria. These are grouped over the surface of the ciliate except in the narrow anterior region. We have encountered it a few times but have failed to photograph it.

In recent years it has become clear that truly anaerobic free-living ciliates do exist, that the majority of them live in symbiotic association with methanogens and/or sulphate reducers, and that together with other free-living anaerobic protozoa, they are the only important predators in the typically short food chains of anaerobic environments. The natural history of some of these ciliate consortia is described in recent reviews (FINLAY & FENCHEL 1993; FENCHEL & FINLAY 1995, and references therein).

While much effort has recently been devoted to describing aspects of the genotype (especially small sub-unit



Figs. 76, 77. Autofluorescing endosymbiotic methanogenic bacteria in the cytoplasm of *M. es* (Fig. 76) and *M. striatus* (Fig. 77). Scale bars: 10 µm.

ribosomal rRNA sequences) of some anaerobic ciliates and their symbionts (EMBLEY & FINLAY 1994), and to describing new species with novel types of symbiotic consortia (e.g. ESTEBAN et al. 1993), very little attention has been directed towards the diversity of anaerobic ciliates as a whole. The taxonomy of these organisms has its foundations in the seminal works of KAHL (1935) and JANKOWSKI (1964), but in the last 10 years or so it has become possible to culture some of these organisms, and we now know a great deal about them. Now we also know something about the distribution and abundance of these organisms in the natural environment (FINLAY et al. 1991; FENCHEL & FINLAY 1995; FENCHEL et al. 1995), and the time is ripe to take a critical look at the diversity of anaerobic ciliates. The current offering has dealt solely with the genus *Metopus*. Further papers will deal with the remaining anaerobic genera.

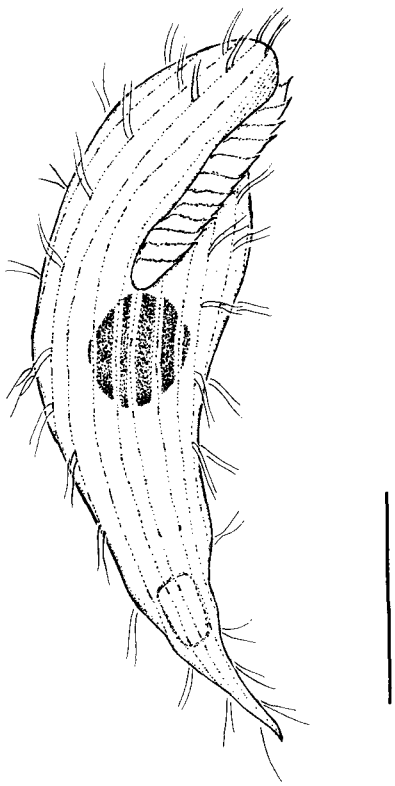
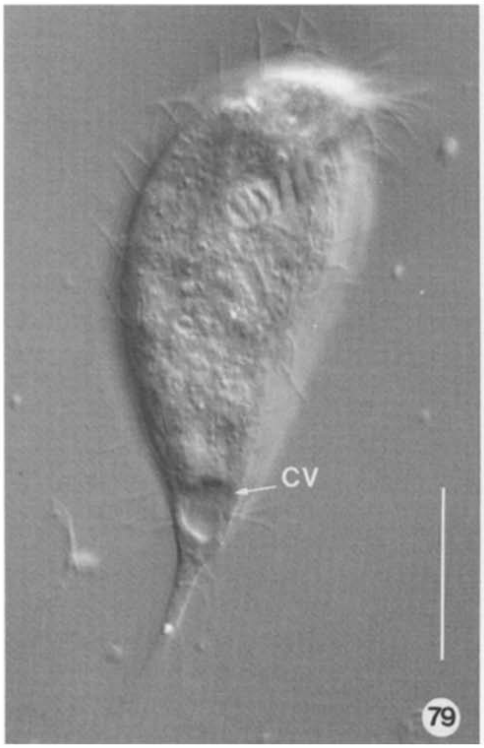


Fig. 78. Diagram of a living specimen of *Metopus spinosus*. Scale bar: 20 µm.



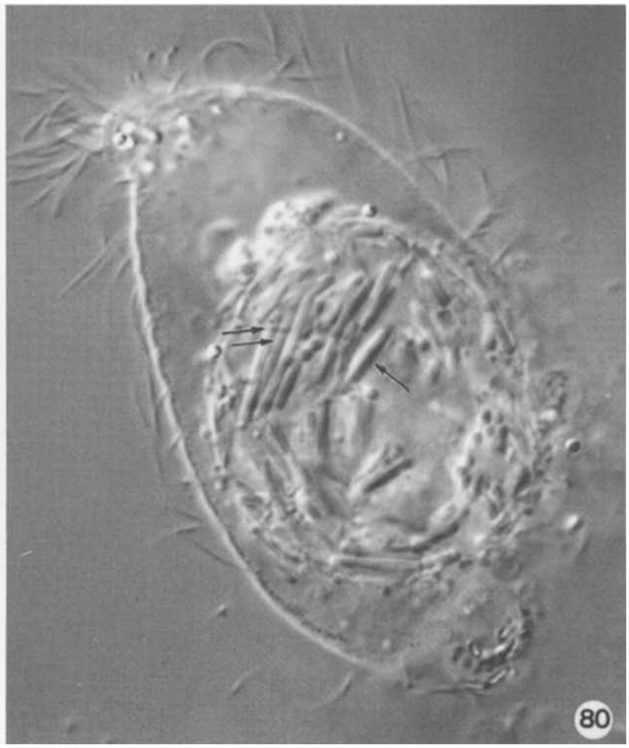


Fig. 79. Nomarski interference contrast photograph of *M. spinosus*. CV, contractile vacuole. Scale bar: 20 µm.

Fig. 80. Arrow to the long rod-shaped bacteria in the macronucleus of *M. spinosus*. The big sphere in the cytoplasm is an enlarged macronucleus filled with these bacteria. Two other types of bacteria are also observed in the cytoplasm, albeit not in the macronucleus. These are small pink spherical bacteria, which have probably been ingested; and small (non-methanogenic) rods. The identities of both need to be investigated.

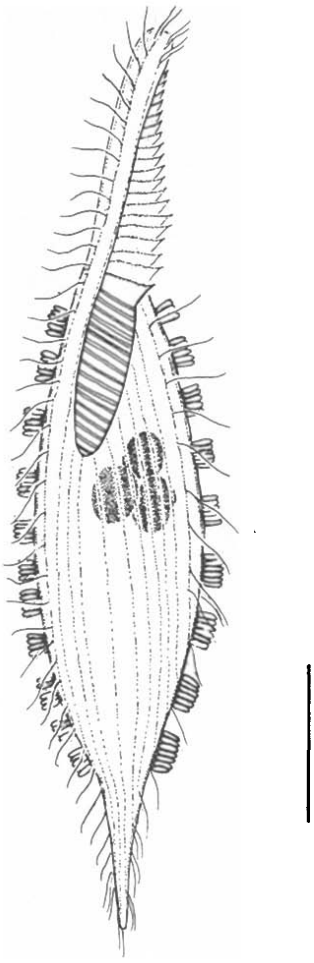


Fig. 81. *Metopus verrucosus*, after DA CUNHA (1915) and KIRBY (1934). Scale bar: 20 µm.

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Authors' addresses: Dr. G. ESTEBAN and Dr. B. J. FINLAY, Institute of Freshwater Ecology, Windermere Laboratory, The Ferry House, Far Sawrey, Ambleside, Cumbria LA22 0LP, U.K.; Prof. T. FENCHEL, Marine Biological Laboratory, University of Copenhagen, DK - 3000 Helsingør, Denmark.