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Kentrophoros (Ciliophora, Karyorelictea) has Oral Vestiges: a Reinvestigation of *K. fistulosus* (FAURÉ-FREMIET, 1950) Using Protargol Impregnation

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Summary: The morphology, infraciliature, and epibiontic bacteria community of *Kentrophoros fistulosus* (FAURÉ-FREMIET, 1950) were studied in live cells, in protargol impregnated specimens, and with the scanning electron microscope. *Kentrophoros fistulosus* is involuted tube-like, except for the body ends; the right side bears many longitudinal ciliary rows, the involuted left side is sparsely ciliated and covered with few, thin spirilla and countless rod-shaped sulphur bacteria which are phagocytised through the cell surface. Thus, *Kentrophoros* has been considered to be secondarily mouthless. The infraciliature consists of dikinetids throughout. The anterior dikinetids of the right side are specialized, i.e. more closely spaced and have both basal bodies ciliated, oblique axes, and nematodesmata-like fibres forming a basket-like structure. These specializations are considered to be vestiges of an oral infraciliature. The posterior end also has specialized dikinetids which give rise to a tuft of caudal cilia whose basal bodies are associated with conspicuous fibres extending into the tail. The left side seemingly has two ciliary rows extending along the cell margins. However, detailed analysis showed that these rows are very likely a single kinety curving around the cell. The bacterial lawn is embedded in a thick layer of mucus, produced by the ciliate to keep the symbiotic kitchen garden in place. The data emphasize the loxodid relationship of *Kentrophoros,* earlier proposed by KAHL and RAIKOV, and suggest synonymizing *K. longissimus* (DRAGESCO) and *K. tubiformis* (RAIKOV & KOVALEVA) with *K. fistulosus* (FAURÉ-FREMIET). Improved diagnoses are provided for the family Kentrophoridae JANKOWSKI and the genus *Kentrophoros* SAUERBREY. The nomenclature of *Kentrophoros* is revised, i.e. correct names, dates, and authorships are given for all species described.

Key Words: Epibiontic Bacteria; Infraciliature; *Kentrophoros*; *Kentrophoros fistulosus*; Loxodida; Sulphur Bacteria.

Introduction

Light and electron microscope studies failed to reveal any oral structures in *Kentrophoros,* a unique psammobiontic ciliate genus carrying a symbiotic kitchen garden of sulphur bacteria on its left side. The bacteria reproduce on the ciliate and are phagocytised through its cell surface (FAURÉ-FREMIET 1950; FENCHEL & FINLAY 1989; KAHL 1935; RAIKOV 1971). However, the general ciliary pattern (infraciliature) of *Kentrophoros* has never been studied in detail, i.e. with silver impregnation methods, which are a powerful means for revealing fine structures usually seen only in the electron microscope. The present study shows that *Kentrophoros* has oral structures, albeit strongly reduced and probably functionless. Furthermore, a detailed redescription of *K. fistulosus* (FAURÉ-FREMIET, 1950) is provided, because previous descriptions are surpri-

singly incomplete and do not meet with the present standard of ciliate alpha-taxonomy. Last not least, I shall clarify the bewildering nomenclature of *Kentrophoros,* i.e. provide a list of the species described with correct names, authorships, dates, and references.

Material and Methods, Type Specimens

Kentrophoros fistulosus occurred in considerable number in the mesopsammon of the French Atlantic coast at Roscoff. Samples were collected and treated exactly as described by FAURÉ-FREMIET (1951), i.e. the specimens were detached from the sand grains by adding about 5 ml of a 12% $MgCl_2$ solution to about 20 ml sand and sea water. The mixture was then gently rotated in a petri dish so that the sand collected in the center and the ciliates could be picked up individually with a capillary pipette from the clear supernatant.

Cells were studied in vivo using a high-power oil immersion objective and differential interference contrast (FOISSNER 1991). The infraciliature was revealed by protargol impregnation (FOISSNER 1991; protocol 2, Wilbert's method), using a special fixative invented by JEAN DRAGESCO (pers. comm.): 5 ml glutaraldehyde (25%), 5 ml saturated, aqueous mercuric chloride, 3 ml aqueous osmium tetroxide (2%) and 1 ml glacial acetic acid are mixed just before use. Specimens are fixed for 15–30 min. and washed three times in distilled water. Preparation for scanning electron microscopy was performed as described by FOISSNER (1991), using the fixative mentioned above.

Counts and measurements on silvered specimens were performed at a magnification of ×1000. In vivo measurements were conducted at a magnification of ×40–1000. Although these provide only rough estimates it is worth giving such data as specimens usually shrink in preparations or contract during fixation. Standard deviation and coefficient of variation were calculated according to statistics textbooks. Drawings of live specimens are based on free-hand sketches and micrographs, those of impregnated cells were made with a camera lucida.

No type slides of *K. fistulosus* are mentioned in the literature. Thus, I have deposited two neotype slides with specimens prepared by the method described in the Oberösterreichisches Landesmuseum in Linz (LI), Austria. Relevant specimens are marked by a black ink circle on the cover glass.

Results

Nomenclature

During the preparation of the manuscript, I noticed a chaotic situation in the species nomenclature of the genus *Kentrophoros*. Thus, I decided to correct the mistakes for the benefit of nomenclatural stability, the present paper, and future workers.

Centrophorus KAHL, 1931 and *Centrophorella* KAHL, 1935 are illegitimate homonyms and synonyms, respectively, of *Centrophoros* MÜLLER & HENLE, 1837 (Pisces) and *Kentrophoros* SAUERBREY, 1928 (CORLISS 1960, 1979).

There is great confusion about the gender of *Kentrophoros* because SAUERBREY (1928) did not fix it. *Kentrophoros* is composed of the ancient Greek words "kentron" (spine) and "phoros" (carrying). The termination "os" can take all genders, however, "phoros" is usually masculine (e.g., phosphoros, Christophoros), rarely feminine, but never neuter. Thus, the neuter termination used by SAUERBREY (1928) for the type species, *K. fasciolatum*, is incorrect and must be emended to *"fasciolatus"*. Later, the genus gender was considered either as neuter or feminine (CAREY 1992; RAIKOV 1971). However, *Kentrophoros* must be treated as masculine according to article 30a (i) of the ICZN ("A genus-group name that is or ends in a noun of variable gender, masculine or feminine, is to be treated as masculine, irrespective of the gender of that noun ...").

The situation of the species names was further complicated by CAREY (1992), who introduced a number of mistakes when he combined the *Centrophorella* species with the legitimate name *Kentrophoros*. He used, for instance, "nomen novum" instead of "combinatio nova" to indicate the transfer. He thus cannot be considered as combining author because a nomen novum replaces a junior homonym, which was not the case. I shall thus combine these species correctly and provide the correct names, dates, and authorships for all *Kentrophoros* species described.

1. *Kentrophoros canalis* WRIGHT, 1982;
2. *Kentrophoros fasciolatus* SAUERBREY, 1928 (nom. em.);
3. *Kentrophoros faurei* (DRAGESCO, 1954a) nov. comb. [basionym: *Centrophorella faurei,* incorrectly dated with 1953 by CAREY (1992)];
4. *Kentrophoros fistulosus* (FAURÉ-FREMIET, 1950) nov. comb. (basionym: *Centrophorella fistulosa*);
5. *Kentrophoros flavus* RAIKOV & KOVALEVA, 1968 (nom. em.);
6. *Kentrophoros gracilis* RAIKOV, 1963 [nom. em.; *graciles* = incorrect second spelling in CAREY (1992)];
7. *Kentrophoros grandis* (DRAGESCO, 1954a) nov. comb. (basionym: *Centrophorella grandis*);
8. *Kentrophoros lanceolatus* (FAURÉ-FREMIET, 1951) nov. comb. (basionym: *Centrophorella lanceolata*);
9. *Kentrophoros latus* RAIKOV, 1962 (nom. em.);
10. *Kentrophoros longissimus* (DRAGESCO, 1954b) nov. comb. (basionym: *Centrophorella longissima*);
11. *Kentrophoros minutus* (DRAGESCO, 1960) nov. comb. (basionym: *Centrophorella minuta*; species inquirenda);

12. *Kentrophoros ponticus* KOVALEVA, 1966 (nom. em.);
13. *Kentrophoros trichocystus* (DRAGESCO, 1954a) nov. comb. (basionym: *Centrophorella trichocystus*);
14. *Kentrophoros tubiformis* RAIKOV & KOVALEVA, 1966 in KOVALEVA (1966);
15. *Kentrophoros uninucleatus* RAIKOV, 1962 (nom. em.).

Redescription of *Kentrophoros fistulosus*

Morphometric data shown in Table 1 are repeated in this section only as needed for clarity. The interpretation of cortical fine structures is partially based on the transmission electron microscope study by RAIKOV (1972a). All observations are from field material. Thus, it cannot be excluded that different species were seen. However, the uniform nuclear configuration and the normal coefficient of variation (10.6%, Tab. 1) of the somatic kinety number indicate that all specimens studied were from the same species despite their high size variability.

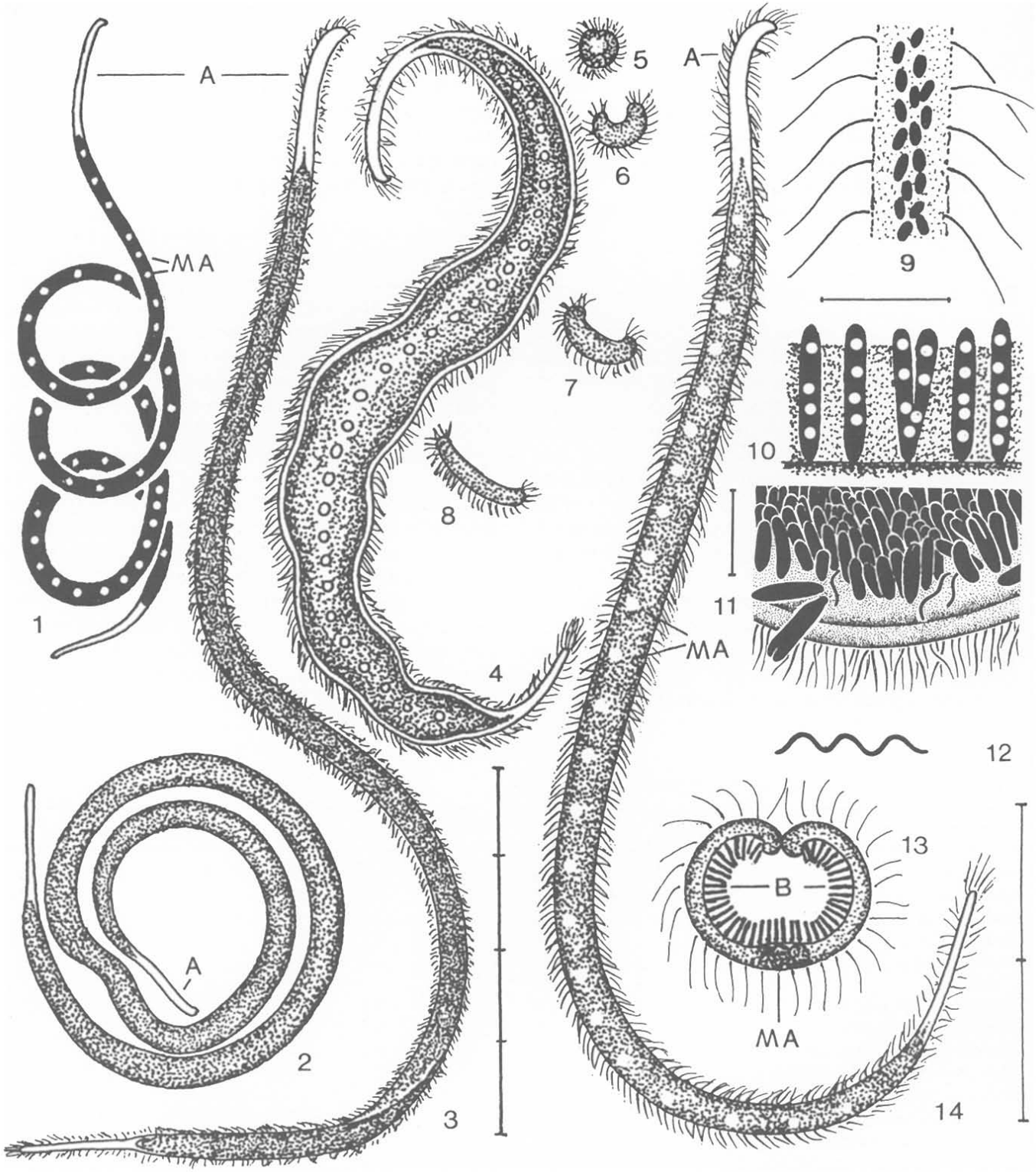
Size in vivo about 500–2000 × 20–30 µm, difficult to measure because specimens were restless and largest individuals, probably up to 3 mm long (cp. RAIKOV 1972b), were always convoluted and only partially preserved in protargol preparations (Figs. 1–3, 33, 37). Very slender, filiform, length/width ratio highly variable, viz. 30:1–70:1 (n = 10). Mid-body region in protargol slides and SEM preparations usually distinctly broader than in live specimens, because tube-shaped portion evolutes and flattens more or less completely due to preparation procedures (Figs. 4–8, 38, 39, 52, Tab. 1).

Body only about 5 µm thick, appears black, except for hyaline and narrowed ends, and flattened ribbon-like at low magnification (< × 50) although tubularly involuted in central region (Figs. 1, 3, 14, 33, 35–37). Blacking caused by dense lawn of sulphur bacteria having refractile granules inside (Figs. 10, 11, 53, 54). Black body portion tube-shaped with epibiontic bacteria lawn inside and ciliated right surface outside (Figs. 13, 19, 43, 52). Disturbed and dying specimens often lose tubular shape, i.e. become 50–100 µm wide, frequently showing bright median ribbon due to regional loss of bacteria lawn (Figs. 4, 34, 38, 39). Anterior body region more or less distinctly narrowed and with short rostrum, flattened up to 2:1, very hyaline and fragile, anterior and lateral margins slightly thickened (Figs. 15, 16, 40). Posterior body portion (tail) very similar to anterior region, but narrower, less distinctly thickened at margins, and evenly rounded at end, which, however, becomes slightly club-shaped in disturbed and prepared specimens (Figs. 19, 24, 50).

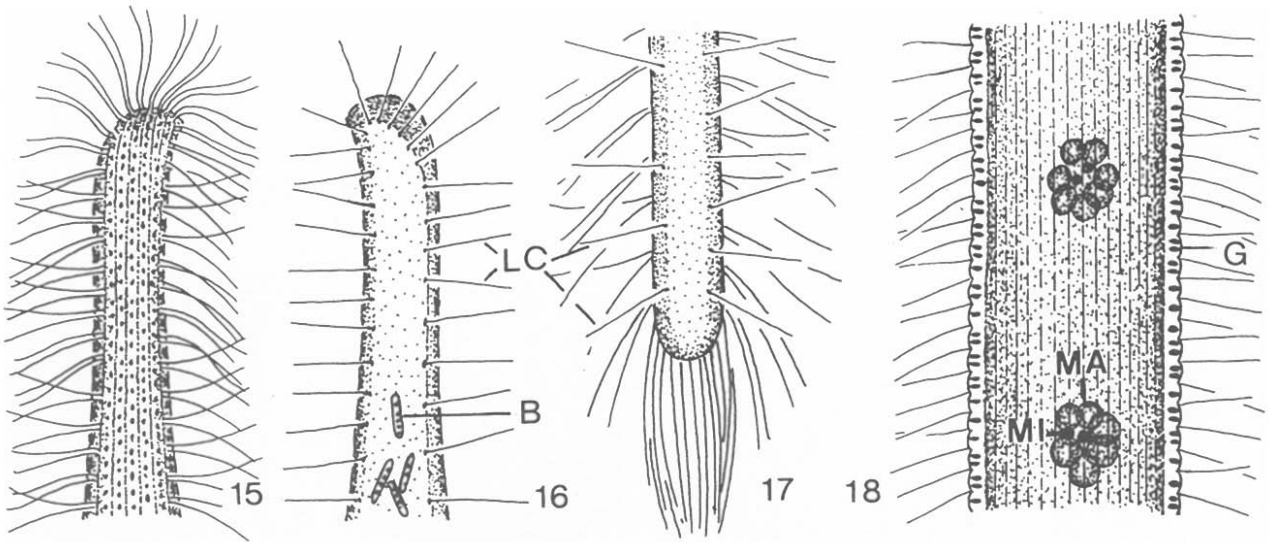
Table 1. Morphometric data from *Kentrophoros fistulosus*[1]).

Character	$\bar{x}$	M	SD	$SD_{\bar{x}}$	CV	Min	Max	n
Body, length	1021.0	1000	363.8	79.4	35.6	450	1700	21
Body, width in anterior region	18.0	18	2.5	0.5	13.8	13	23	21
Body, width in central region	59.1	60	9.7	2.1	16.4	36	76	21
Body, width in posterior region	10.5	10	1.6	0.3	14.9	6	13	21
Anterior end to first nuclear group, distance	168.3	170	32.2	7.0	19.1	110	220	21
Nuclear group, length	11.1	10	3.7	0.8	33.7	7	22	21
Nuclear group, width	8.2	8	2.3	0.5	11.0	6	17	21
Macronuclear nodule, length	4.1	4	0.6	0.1	16.0	3	5	21
Macronuclear nodule, width	3.6	3.5	0.5	0.1	15.0	3	5	21
Micronucleus, largest diameter	2.1	2	–	–	–	2	2.5	21
Somatic kineties in anterior region of right side, number	12.0	12	2.0	0.4	16.6	8	15	21
Somatic kineties in central region of right side, number	36.4	37	3.8	0.8	10.6	30	43	21
Somatic kineties on left side, number	2.0	2	0.0	0.0	0.0	2	2	21
Nuclear groups, number	27.6	25	10.8	2.2	39.2	11	54	25
Macronuclear nodules in nuclear group, number	4.6	4	1.5	0.3	31.9	3	9	21
Micronuclei in nuclear group	2.0	2	0.6	0.1	29.5	1	4	21

[1]) Data based on protargol-impregnated and mounted specimens from field. Measurements in µm. Abbreviations: CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n = number of individuals investigated, SD – standard deviation, $SD_{\bar{x}}$ – standard deviation of the mean, $\bar{x}$ – arithmetic mean.



Figs. 1– 14. *K. fistulosus* from life (Figs. 1– 9, 12–14) and from life and SEM micrographs (Figs. 10, 11). **1, 2.** Spiral specimens. **3, 14.** Typical, gliding specimens. The tube-shaped body portion appears dark due to the symbiotic sulphur bacteria. **4–8.** Disturbed specimen with tube-shaped body evoluted (transverse sections, figs. 5–8). **9.** Surface view showing cortical granules (extrusomes). **10–12.** Lateral and surface view of bacteria covering left body side of ciliate. The bacteria contain sulphur granules (white dots) and are embedded in a mucuous substance. Spirilla (Fig. 12) are found between the proximal ends of the sulphur bacteria, i.e. on the pellicle of the ciliate. **13.** Transverse section in mid-body. The ciliate is involuted tube-like with the symbiotic bacteria inside. A = anterior region of the ciliate, B = symbiotic bacteria, MA = (macro)nuclear groups. Scale bar division 100 µm (Figs. 1–3, 14) and 5 µm (Figs. 10–12).



Figs. 15–18. *K. fistulosus* from life. **15, 16.** Right and left lateral view of anterior body region. The anterior end and the body margins are slightly thickened. The right surface appears rough due to cortical granules (extrusomes) between ciliary rows (cp. Figs. 9, 18). Cilia are paired on the right side and single on the left, where a single ciliary row extends around cell. **17.** Left lateral view of posterior body region showing tuft of slightly elongated caudal cilia. **18.** Mid-body region showing cortical granules (extrusomes), nuclear groups, and right lateral ciliary rows with single cilia. B = symbiotic bacteria, G = cortical granules (extrusomes), LC = left lateral ciliary row, MA = macronuclei, MI = micronuclei.

Morphology and morphometry of nuclear apparatus as described by RAIKOV (1972b), who corrected FAURÉ-FREMIET's (1954) misinterpretations. 10–30 roundish to ellipsoid nuclear groups, each comprising an average of 4 macronuclei and 2 micronuclei, are most common (RAIKOV 1972b, Tab. 1, Figs. 1, 14, 18). Macronuclear nodules usually contain single, large chromocentre (Fig. 30a), easily mistaken as micronucleus if nuclear envelope is weakly stained (FAURÉ-FREMIET 1954; RAIKOV 1972b).

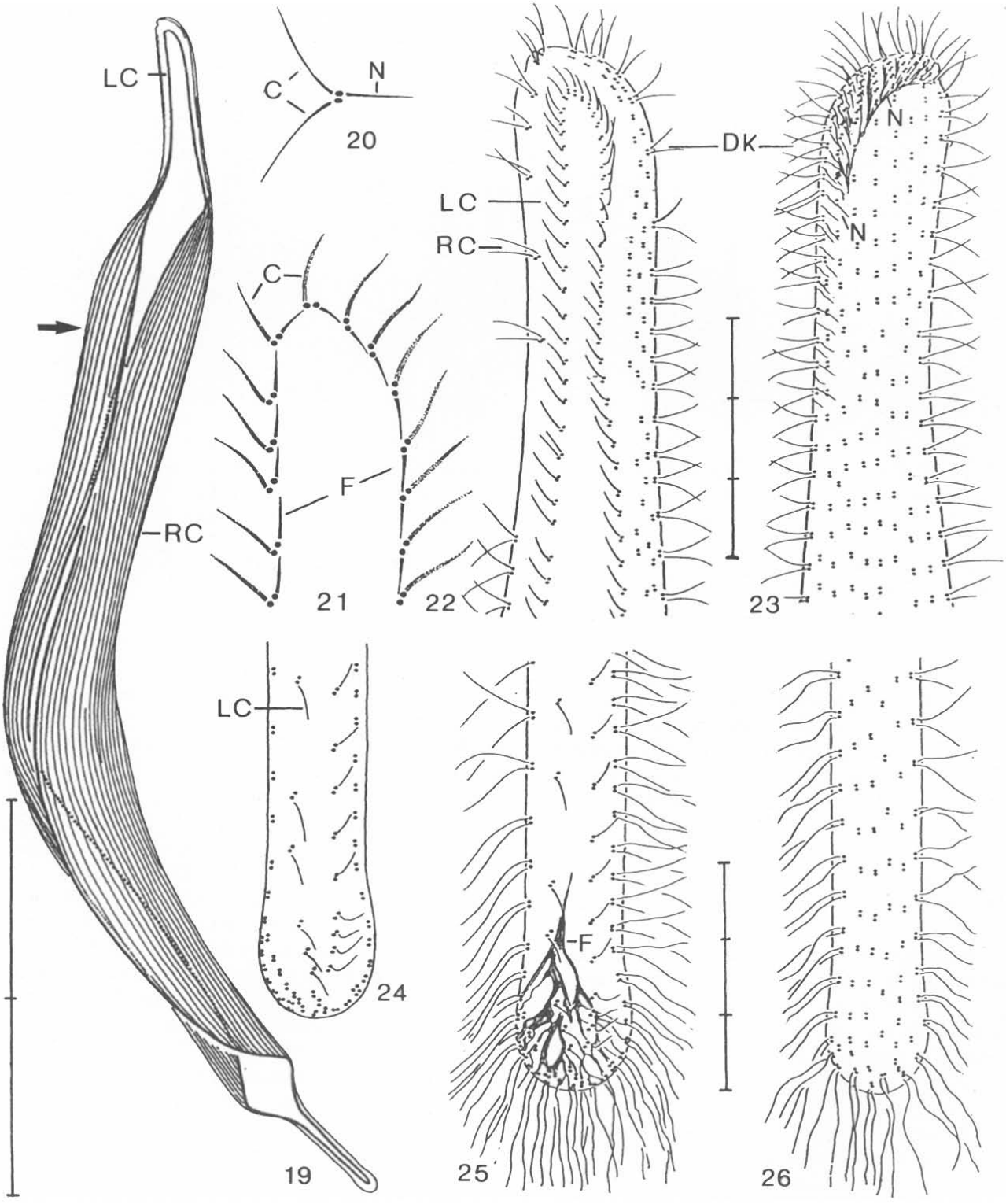
Cortex colourless, gelatinous and very flexible, distinctly striated by refractile granules extending between ciliary rows. Granules – very likely secretory ampullae as in *K. latus* (RAIKOV 1972a, 1974a) – 1–1.5×0.6 µm in size, yellowish, especially conspicuous in hyaline body regions (Figs. 9, 40–42, 44), impregnate occasionally with protargol and explode to 2–3 µm sized blisters when cells are pressed between slide and cover glass.

Cytoplasm rather transparent, contains many 1×0.5 µm sized granules, but lacks food and contractile vacuoles.

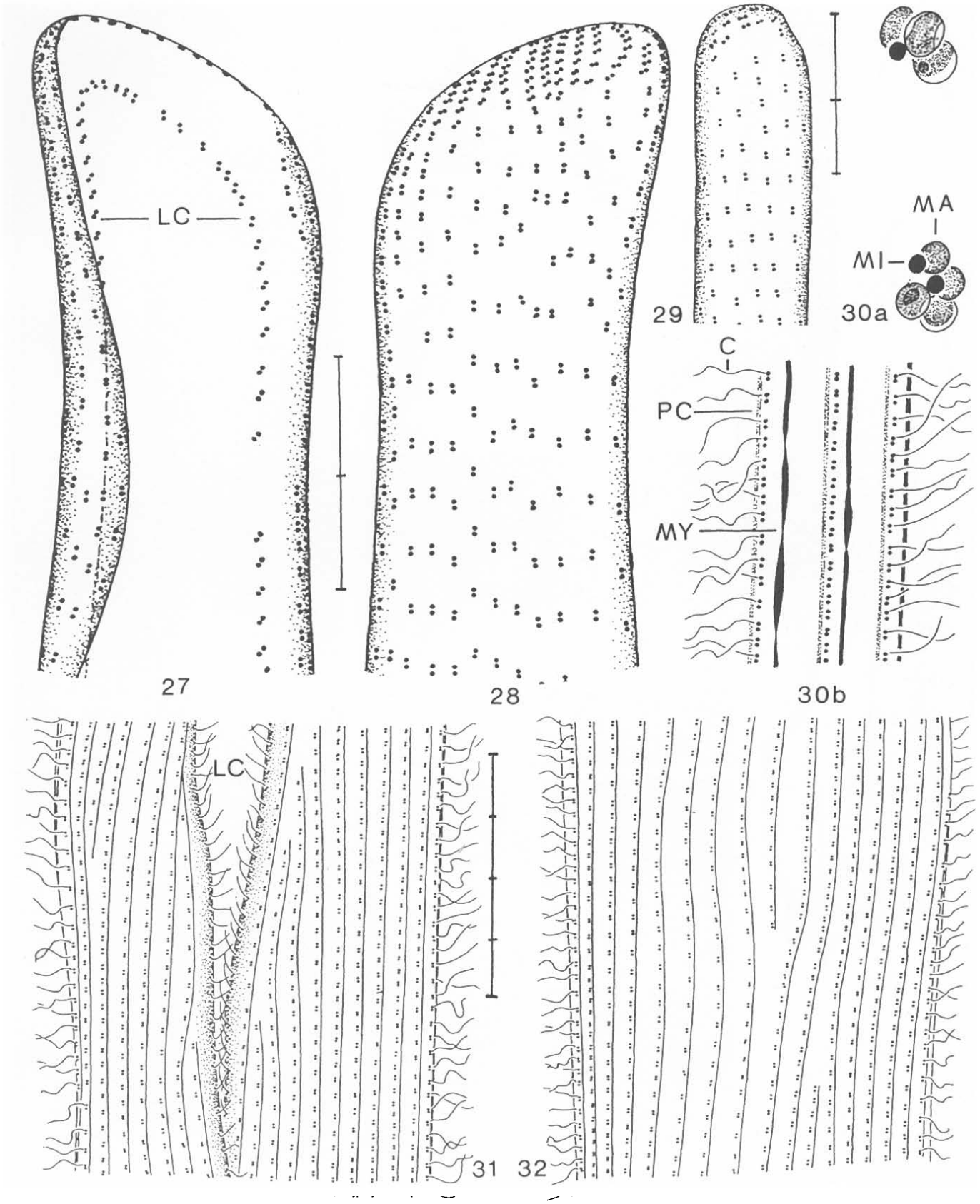
Movement sluggish, winds worm-like between sand grains and glides slowly on petri dish bottom with rostrate end ahead; acontractile but often spiral and/or convoluted (Figs. 1, 2, 35, 37). Conspicuous and beautiful body undulations performed by flat specimens which lost tubular involution (Figs. 4, 38, 39).

Infraciliature composed of dikinetids throughout. Cilia 8–10 µm long, rather stiff, on right surface arranged in equidistantly spaced, longitudinal rows most of which became shortened towards body ends, i.e. abut on left side kinety (Figs. 19, 31, 32, 45). Both basal bodies ciliated in anterior and posterior region of cell, posterior basal bodies barren in tubular body portion (Figs. 15–18, 22, 23, 25, 26, 30–32, 47, 51). Dikinetidal axes parallel to main body axis, except for obliquely oriented and more closely spaced dikinetids at anterior end. Oblique and anterior dorsal dikinetids with conspicuous fibres originating from anterior basal bodies, form basket-like structure right of cell median (Figs. 20, 23, 28, 29, 45–49). Dikinetids also condensed at posterior end of cell, give rise to distinct tuft of slightly elongated caudal cilia and conspicuous bundles of fibres extending about 20 µm into tail (Figs. 17, 24–26, 50). At left side of ciliary rows a distinct, ribbon-like myoneme, at right a faintly stained postciliary microtubule bundle (Figs. 30, 51).

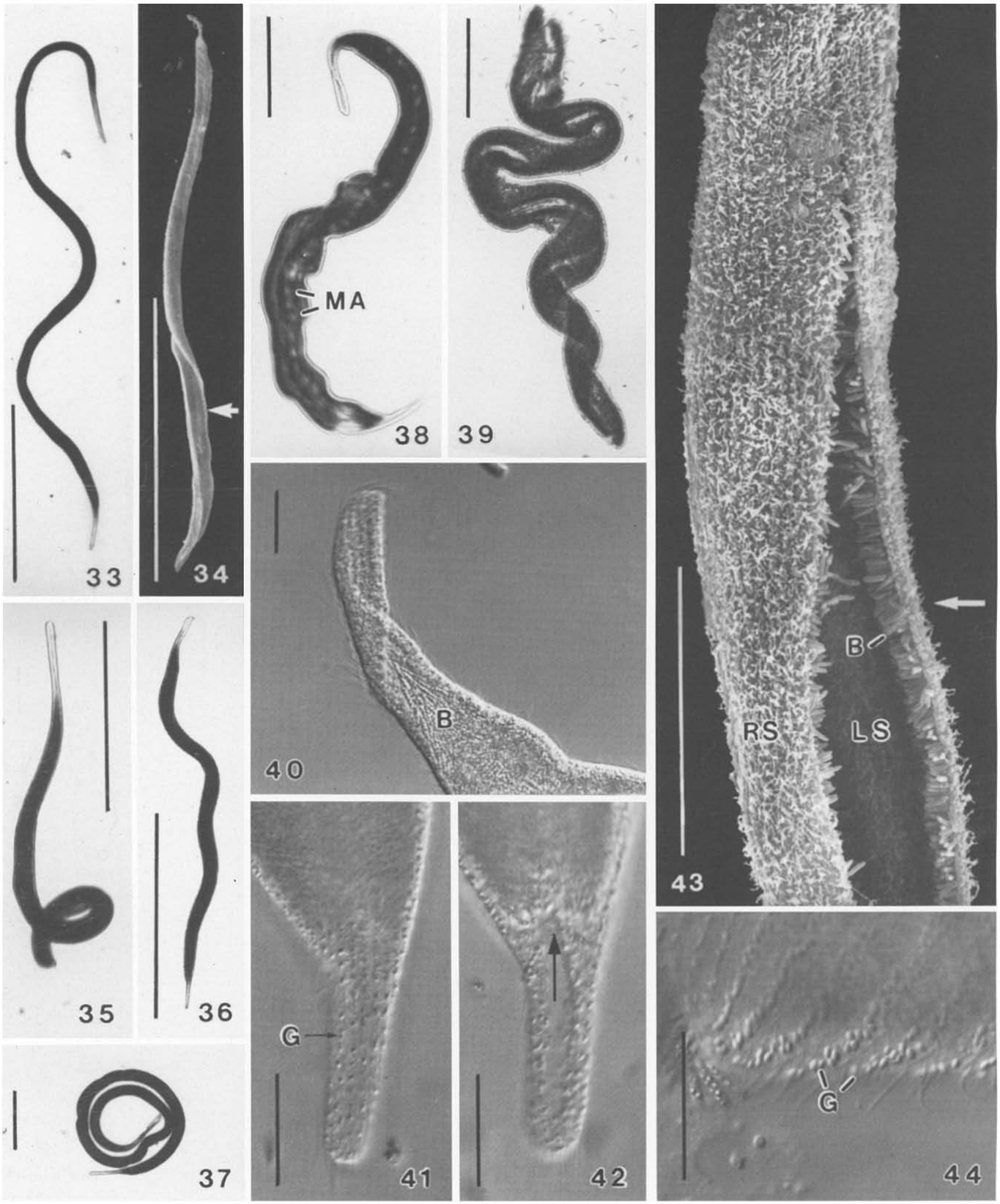
Left side with 2 kineties at margins of cell. Dikinetids of right kinety have the posterior basal body ciliated and an *anteriorly* extending (postciliary?) fibre associated with the anterior basal body; dikinetids of left kinety have the anterior basal body ciliated and a *posteriorly* extending (postciliary?) fibre associated with the posterior basal body (Figs. 21, 22, 24, 25, 45–48). This curious pattern can be reasonably explained if it is assumed that these kineties are in reality a single kinety curving around cell margins (Fig. 21).



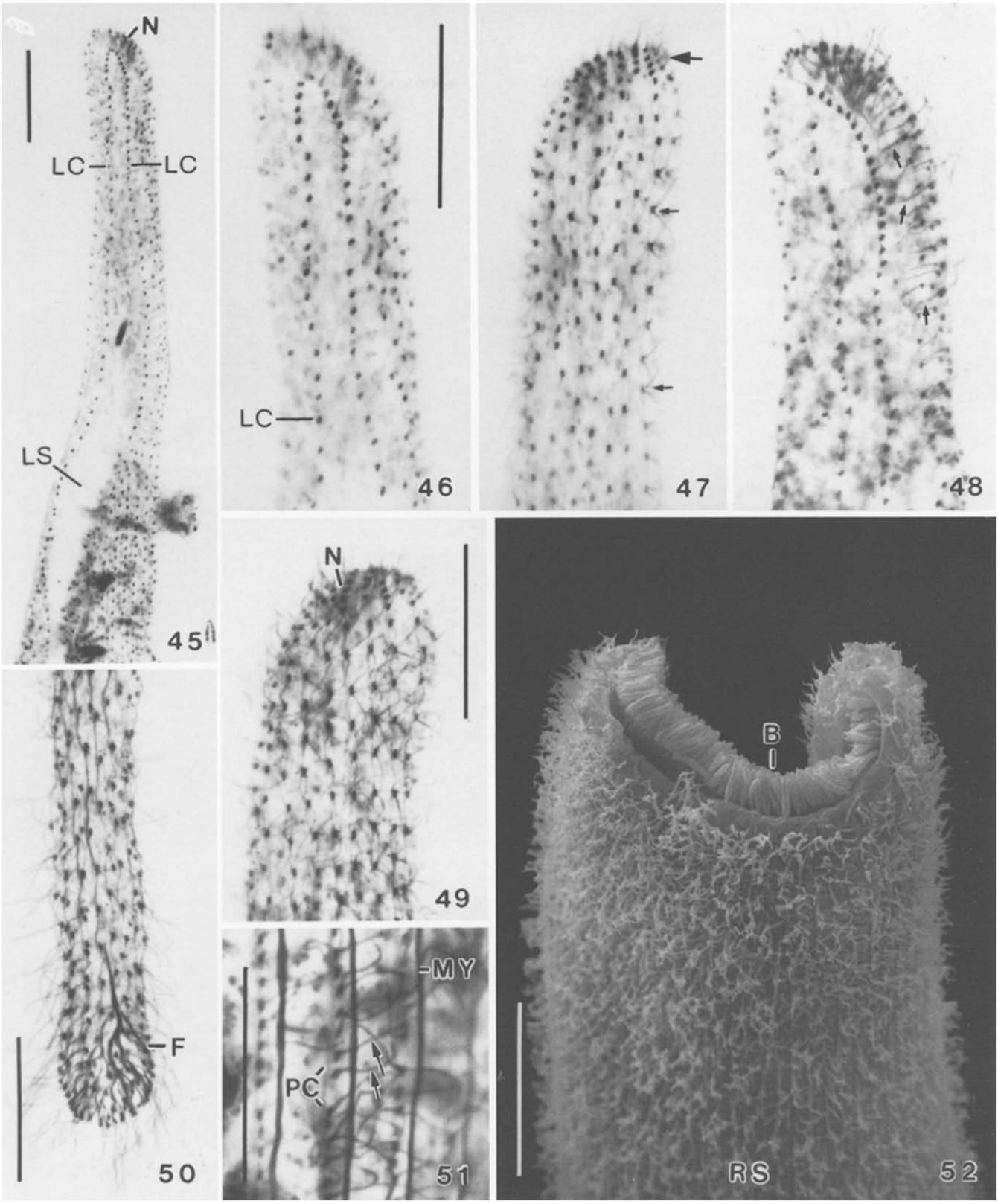
Figs. 19–26. *K. fistulosus*, infraciliature after protargol impregnation. The ciliature consists of dikinetids throughout, but both basal bodies are ciliated only in the anterior and posterior body region (cp. Figs. 31, 32). **19.** Total view of left side. Arrow marks region depicted in figures 31 and 32. **20.** Ventral (oral) dikinetid at high magnification. **21.** Fine structure of the left lateral ciliary row. Note different orientation of fibre associated with dikinetids. **22, 23.** Left and right lateral view of anterior body region. **24–26.** Left and right lateral view of posterior body region. C = cilium, DK = dorsal dikinetids, F = fibres, LC = left lateral ciliary row, N = nematodesmata, RC = right lateral ciliary rows. Scale bar division 100 µm (Fig. 19) and 10 µm (Figs. 22–26).



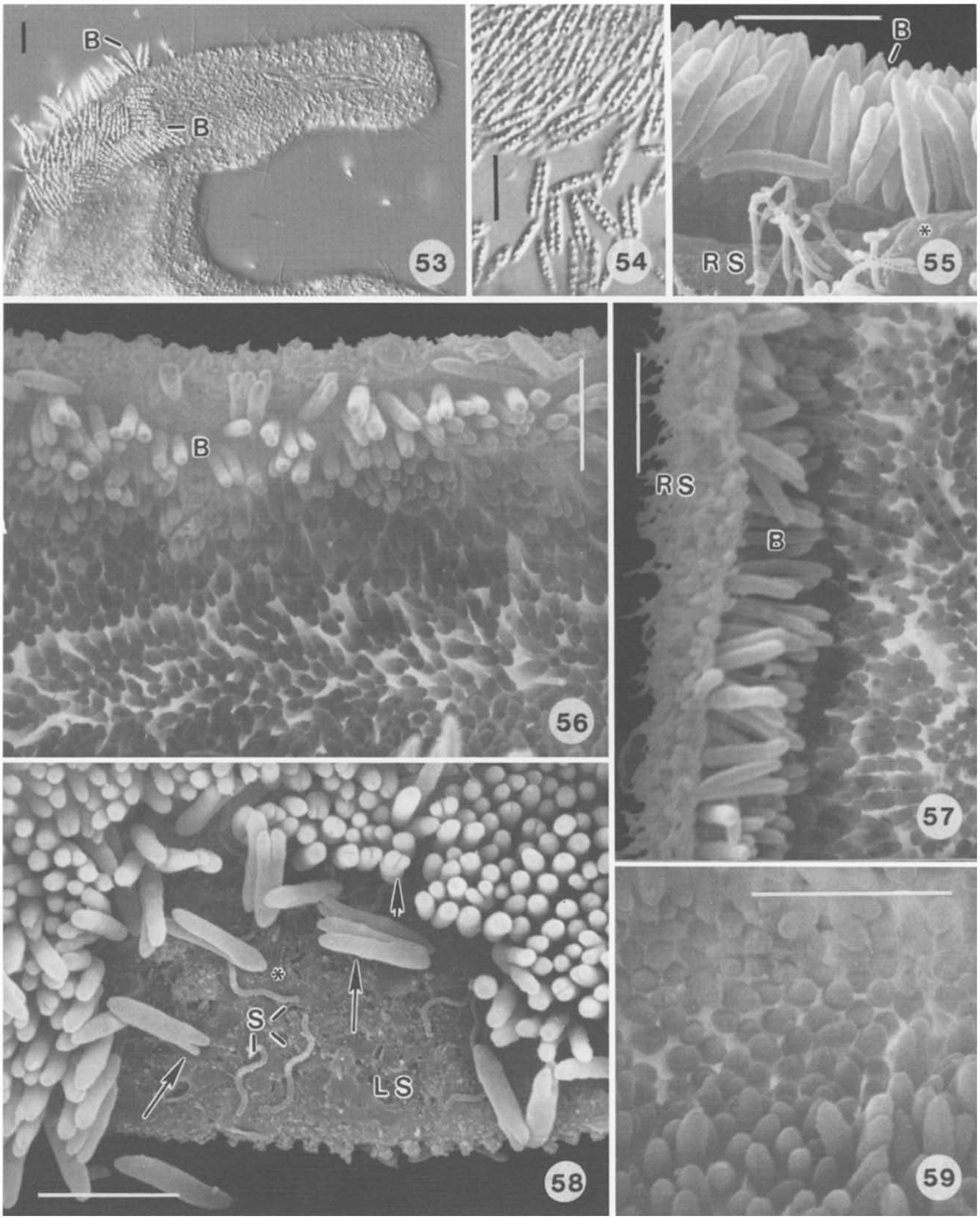
Figs. 27–32. *K. fistulosus,* infraciliature after protargol impregnation. **27–29.** Left and right lateral views of anterior body region of a broad and a slender specimen. Note different orientation of anteriormost (oral) dikinetids and uninterrupted left lateral ciliary row. **30a, b.** Fine structure of nuclear apparatus and of somatic cortex in mid-body. Dikinetids have only anterior basal body ciliated. **31, 32.** Left and right lateral view of mid-body region marked in Fig. 19. C = cilia, LC = left lateral ciliary row, MA = macronuclear nodule, MI = micronucleus, MY = myoneme, PC = postciliary microtubule ribbon. Scale bar division 10 μm.



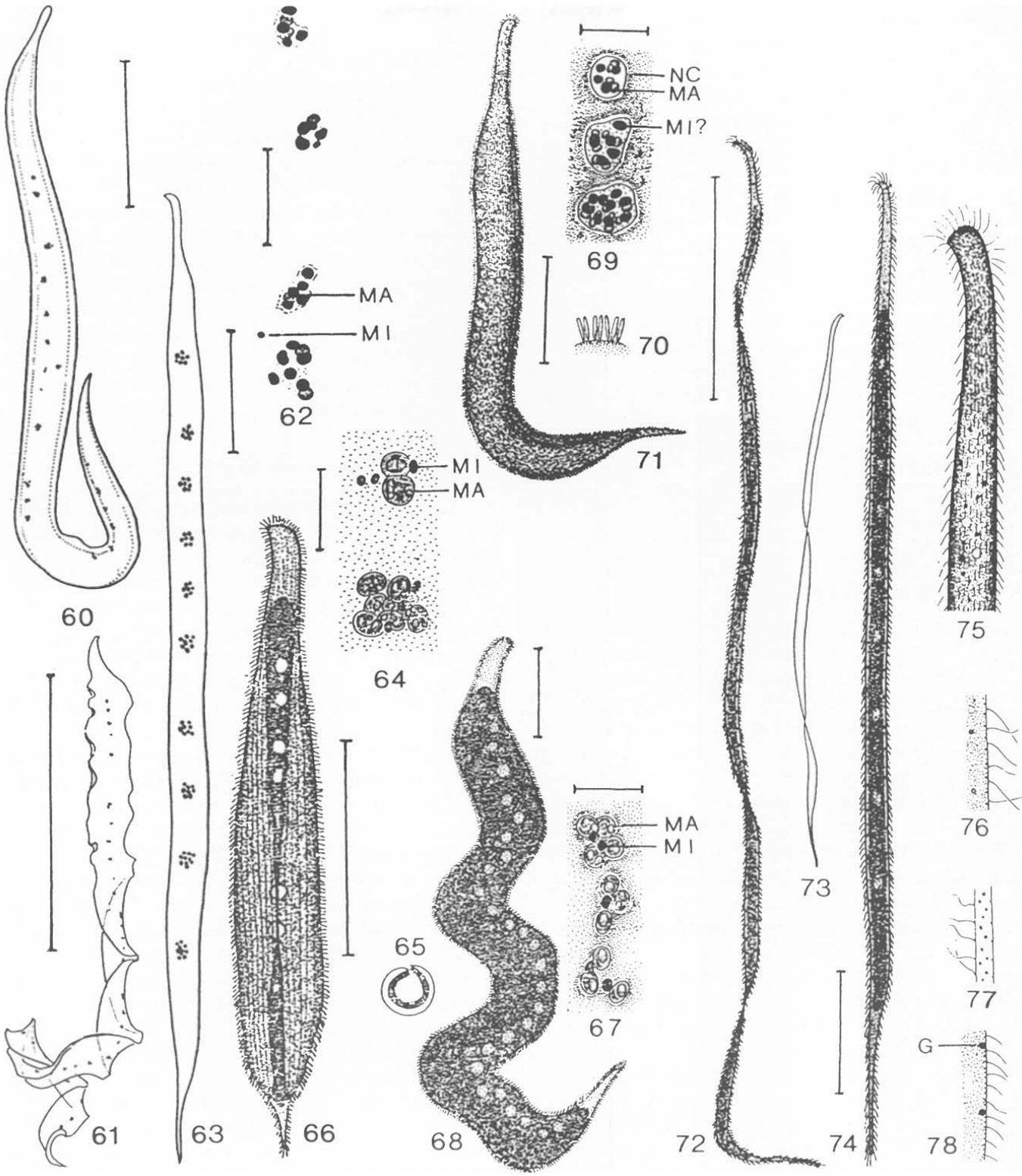
Figs. 33–44. *K. fistulosus* from life (Figs. 33, 35–42, 44) and in the SEM (Figs. 34, 43). **33, 35–37.** Variability in shape and size. Specimens appear black, except for body ends, due to symbiotic sulphur bateria. – **34, 38, 39, 43**. The body is involuted tube-like with the symbiotic bacteria lawn inside. Usually, the involution disappears partially (arrows) or completely (Figs. 38, 39) in disturbed and/or prepared specimens. **40.** Anterior end. **41, 42, 44.** Right and left lateral view of posterior region. Arrow marks commencing involution of body. B = symbiotic bacteria lawn, G = cortical granules (extrusomes), LS = left body surface, MA = (macro)nuclear groups, RS = right body surface. Scale bars 15 µm (Figs. 40–42, 44), 50 µm (Fig. 43), 150 µm (Figs. 37–39), 500 µm (Figs. 33–36).



Figs. 45–52. *K. fistulosus*, infraciliature after protargol impregnation and in the SEM. **45–49.** Anterior left and right lateral views of two specimens. Small arrows mark ciliated dikinetids and nematodesmata, respectively; large arrow indicates obliquely oriented anteriormost dikinetids. **50.** Posterior right lateral view. **51.** Somatic cortex in mid-body. Dikinetids have only the anterior basal body ciliated (arrows). **52.** Mid-body region of broken specimen. B = bacteria, F = fibres, LC = left lateral ciliary row, LS = left side, MY = myonemes, N = nematodesmata, PC = postciliary fibres, RS = ciliated right side. Bars 20 μm.



Figs. 53–59. *K. fistulosus* and its symbiotic bacteria in the light and scanning electron microscope. The bacteria are 5–7 µm long, contain refractile sulphur granules (Fig. 54), and some have a very inconspicuous capitulum at the proximal end (asterisks); many divide (arrows in Fig. 58). The bacteria lawn covers the left surface of the ciliate and is embedded in a mucuous substance (Fig. 59) which appears honycombed if bacteria are lost (Figs. 56, 57). Attached to the pellicle are spirilla (Fig. 58). B = bacteria, LS = left side of ciliate, RS = right side of ciliate, S = spirilla. Bars 5 µm.



Figs. 60–78. *K. fistulosus*, literature data and synonyms [Figs. 60–62, from FAURÉ-FREMIET (1950); Figs. 63, 73–78, from DRAGESCO (1960); Figs. 64–71, from KOVALEVA (1966); Fig. 72, from DRAGESCO (1954b)]. **61– 63.** *K. fistulosus*, schematized. **64–68.** *K. tubiformis*, from life and after FEULGEN'S nuclear reaction. **69–71.** *K. fistulosus*, from life and after FEULGEN'S nuclear reaction. **72–78.** *K. longissimus*, from life. G = cortical granules, MA = (macro)nuclear groups, MI = micronuclei, NC = nuclear capsule. Bars 200 µm (Figs. 60, 61, 63, 66, 68, 71, 72, 74), 10 µm (Figs. 62, 64, 67, 69).

Epibiontic bacteria

My observations on the epibiontic bacteria and spirilla agree with those of FAURÉ-FREMIET (1950), RAIKOV (1971), and HEDIN (1977). I thus restrict the description to a few new observations. The spirilla are 5–15× 0.5 µm in size and found only on the surface of the ciliate, i.e. between the proximal end of the rod-shaped bacteria (Figs. 11, 12, 58). Both, bacteria and spirilla, are embedded in a 4–5 µm thick layer of mucuous material almost equalling the length of the bacteria (Fig. 59). In many specimens patches of bacteria detached due to the preparation procedure, leaving a honeycombed mucus layer (Figs. 56, 57). The mucus was not preserved in all specimens observed (Fig. 55, 58). The contact between bacteria and host is weak and no attachment structures were observed on the ciliate surface (Fig. 58). However, the narrowed proximal extremity of the bacteria was sometimes slightly broadened (Figs. 55, 58), i.e. formed an inconspicuous capitulum as described by RAIKOV (1971).

Discussion

Identification and synonymy

15 *Kentrophoros* species have been described (CAREY 1992). Of these, *K. fistulosus* (FAURÉ-FREMIET, 1950), *K. longissimus* (DRAGESCO, 1954b), and *K. tubiformis* RAIKOV & KOVALEVA, 1966 in KOVALEVA (1966) have a tubular shape, hyaline, narrowed body ends, and 10 or more nuclear groups each comprising about four macronuclei and two micronuclei. Note that CAREY (1992) mixed drawings and diagnostic characters of *K. fistulosus* and *K. fasciolatus*! He thus described *K. fistulosus* as being flattened ribbon-like, while FAURÉ-FREMIET (1950) emphasized its tubular shape ("Toute la surface du corps cyclindrique est ciliée").

Main characteristics of *K. longissimus,* as compared to *K. fistulosus*, are highly refractile cortical granules (extrusomes; Figs. 76–78) and a ribbon-like flattening of the body (DRAGESCO 1954b, 1960). However, refractile extrusomes are present also in *K. fistulosus* (Figs. 9, 41, 42, 44) and the tubular body of this species appears ribbon-like if observed at low (×50) magnification (Fig. 33). Furthermore, DRAGESCO's drawings look ambiguous, i.e. the organism appears strongly flattened in the original description (Fig. 72) but tubular and very similar to the forms observed by me (Fig. 14) in the redescription (Fig. 74).

Size (400–1500 µm) and shape (tubular), as well as number of ciliary rows (25–35) and nuclear groups (7–50) of *K. tubiformis* (Figs. 64–68) are very similar to those reported for *K. fistulosus* (FAURÉ-FREMIET 1950, RAIKOV 1971, 1972b, Tab. 1). RAIKOV & KOVALEVA in KOVALEVA (1966) separated these species by details of the nuclear apparatus, mainly by the lack of an envelope (capsule) surrounding the individual nuclear groups of *K. tubiformis* (cp. Figs. 64, 67, 69). However, RAIKOV (1972b) showed by electron microscopy that *K. fistulosus* also lacks nuclear capsules. RAIKOV & KOVALEVA in KOVALEVA (1966) mentioned also other small differences, viz. that the nuclei are more closely spaced in *K. fistulosus* than in *K. tubiformis,* and that the macronuclei of the former species contain a single, large chromocentre, while those of the latter have several small chromocentres. Such sophisticated differences are usually not considered as species characters and might be staining variations or race differences.

Taking into account the present results and the incompleteness of previous observations, it appears reasonable to synonymize *K. longissimus* and *K. tubiformis* with *K. fistulosus*.

Do kentrophorids have oral structures ?

Previous light and electron microscope studies failed to reveal any oral structures in *Kentrophoros* spp. although FAURÉ-FREMIET (1950), DRAGESCO (1960), and FENCHEL (1968) observed small diatoms in the cytoplasm of *K. fistulosus*, *K. grandis*, and *K. fasciolatus*. More recently, the mouthlessness of *Kentrophoros* spp. was supported by electron microscope investigations providing convincing evidence that they phagocytise their epizoic sulphur bacteria through the cell surface (FENCHEL & FINLAY 1989; RAIKOV 1971).

The results of the present study suggest that *K. fistulosus* has oral structures, albeit strongly reduced and probably functionless. The fibres, which originate from the anterior and subapical dorsal dikinetids and extend into the cytoplasm, are highly reminiscent of oral or oralized somatic nematodesmata found in many ciliates, including *Loxodes* (PUYTORAC & NJINE 1970) and *Remanella* (FOISSNER, unpubl.). Further specializations of the infraciliature in the physiological anterior end of *K. fistulosus* include more narrowly spaced dikinetids having oblique axes and both basal bodies ciliated. Similar peculiarities are found in the oral area of many ciliates from diverse taxonomic assemblages. It is thus reasonable to interpret these specializations as oral remnants. The above mentioned observations of FAURÉ-FREMIET, DRAGESCO, and FENCHEL indicate that these remnants may even be used to phagocytise small food particles although I never observed prey residues in my specimens.

However, the situation is ambiguous because the nematodesmata-bearing dikinetids extend along the anterior and dorsal margin, i.e. are at the wrong place if the loxodid symmetry is applied. Furthermore, *K. fistulosus* has fibre-bearing kinetids not only in the anterior but

also in the posterior body end (Figs. 25, 50). I could understand this curious pattern only when I consulted my unpublished material from *Remanella*, which has not only a normal loxodid oral apparatus with distinct nematodesmata but also, like *K. fistulosus*, nematodesmata-like fibres, originating from a specialized lateral somatic kinety, in the posterior end.

Systematic relationships of *Kentrophoros*

FAURÉ-FREMIET (1950) and DRAGESCO (1960) provisionally placed *Kentrophoros* in the pleurostomatid family Amphileptidae because of the distinct asymmetry of its ciliature and the flattened body. Later, however, FAURÉ-FREMIET (1954) anticipated KAHL'S (1931, 1935) view and stated: "Il ne serait pas absurde de les considérer, provisoirement tout au moins, comme des Loxodidae simplifiés à l'extrême par la réduction des structures buccales et la parte des vésicules de Müller, en même temps qu'ils se caractérisent par un revêtement de Sulfobactéries symbiotiques". Finally, RAIKOV (1972a) confirmed the intriguing predictions of KAHL and FAURÉ-FREMIET by showing distinct similarities in the somatic cortical ultrastructure of *Loxodes* and *Kentrophoros.*

A loxodid relationship of *Kentrophoros* is also evident from the present investigations. The overall pattern of the somatic infraciliature is very similar in *Loxodes*, *Remanella,* and *Kentrophoros*, specifically, all have a peculiar kinety on the left side. Furthermore, *Kentrophoros* has some sort of oral infraciliature in the anterior body region, i. e. where *Loxodes* and *Remanella* have the oral apparatus.

I agree, however, with JANKOWSKI (1980) and SMALL & LYNN (1985) in separating kentrophorids from loxodids at family level, because of their morphological (strongly reduced oral apparatus) and ecological (symbiotic kitchen garden) peculiarities.

JANKOWSKI (1978, 1980) separated *Kentrophoros* at ordinal (Thysanophorida) and subclass (!) level (Symbiophagina) from *Loxodes*, which he assigned to the pleurostomatids in spite of its different nuclear structure (pleurostomatids have a normal, i.e. dividing macronucleus). These hasty proposals are clearly disproved by the present results and literature data (PUYTORAC 1994).

Improved diagnoses

Kentrophoridae JANKOWSKI, 1980: Loxodida JANKOWSKI, 1978 with very likely functionless oral structures reduced to nematodesmata-bearing, condensed dikinetids in anterior body region. Left side covered with mucuous material inhabitated by symbiotic sulphur bacteria phagocytised through cell surface and used as food source. Monotypic: *Kentrophoros* SAUERBREY, 1928.

Remarks: JANKOWSKI mentioned a new family Kentrophoridae already in 1975 without, however, providing any characterization or type genus. Thus, the name is illegitimate, i.e. not in accordance with the rules of nomenclature. I thus date the Kentrophoridae with 1980, when JANKOWSKI characterized them in more detail, albeit vaguely and incompletely. The thoughless actions of JANKOWSKI (1975, 1978) might explain why SMALL & LYNN (1985) gave very ambiguous authorships, viz. "order Loxodida Jankowski, n. ord.", respectively, "Kentrophoridae Jankowski, n. fam."

Kentrophoros SAUERBREY, 1928: With diagnosis of family. Probably diphyletic or polyphyletic as indicated by the different nuclear configurations (two macronuclei with micronucleus in between, many nuclear groups) and body shapes (with or without hyaline ends). Infraciliature of type species, *K. fasciolatus,* not yet known.

Attachment of epibiontic bacteria

Based on transmission electron microscope investigations, RAIKOV (1971) described the epibionts of *Kentrophoros fistulosus* as being attached to the ciliate by an inconspicuous, hemispherical or flat capitulum, either inside a minute cup-like depression of the pellicle or attaching to flat portions of its surface, so that the cell membranes of the host and of the epibiont are in close contact. Furthermore, he noted some fuzzy "cement", but not a mucus layer, in the bacteria-host contact area. However, RAIKOV (1974b) and FENCHEL & FINLAY (1989), investigating two other members of the genus, viz. *K. latus* and *K. fasciolatus,* could not find specializations as described by RAIKOV (1971) in *K. fistulosus.* Thus, FENCHEL & FINLAY (1989) concluded that it is not yet understood how the bacteria are kept in place. They suggested that the epibionts are embedded in a layer of mucus covering the host surface although they failed to demonstrate any mucus.

My data support both RAIKOV (1971) and FENCHEL & FINLAY (1989). Indeed, the bacteria are embedded in a thick layer of mucus, like bee pupae in their honeycombs, and have a capitulum, albeit inconspicuous and often not clearly discernible (Figs. 55–59). RAIKOV (1971) suggested that the lack of pronounced specializations at the contact area of epibiont and host is due to the special morphology of the ciliate, viz. its tube-like involution (Fig. 13), which protects the symbionts from being removed by mechanical forces. However, other members of the genus are not involuted, suggesting the mucus as main adhesive agent.

The mucus is obviously produced by the ciliate since it remains on its surface if the bacteria detach (Figs. 56, 57). Very likely, some mucus covers also the left (ciliated) surface of the ciliate because there was always

fuzzy material on and between the cilia, which made it difficult to obtain "nice" micrographs from the ciliated side. The mucus is produced in small sacks (RAIKOV 1972a).

The thick mucus layer covering the epibiont-carrying side of *Kentrophoros* is apparently uncommon in protozoan episymbioses, according to the reviews by KIRBY (1941), RAIKOV (1971), and RADEK et al. (1992). However, the mucus is obviously not easily preserved with conventional fixatives and also practically invisible in the light microscope, even if advanced techniques such as interference contrast are used. It is thus likely, that such mucus layers are more widespread than hitherto recognized. In another psammophilic ciliate, *Sonderia* spp., the mucus layer is obviously more compact and thus easily seen with the light microscope (KAHL 1931, KIRBY 1934).

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