Check for updates

J. Euk. Microbiol., 45(1), 1998 pp. 64–70
© 1998 by the Society of Protozoologists

Epibiotic Bacteria on Several Ciliates from Marine Sediments

SLAVA S. EPSTEIN,*[1] DENNIS A. BAZYLINSKI,** and WILLIAM H. FOWLE***

*Marine Science Center, Northeastern University, East Point, Nahant, Massachusetts 01908, USA, and
**Department of Microbiology, Immunology, and Preventive Medicine, Iowa State University, Ames, Iowa 50011, USA, and
***Electron Microscopy Center, Department of Biology, Northeastern University, Boston, Massachusetts 02115, USA

ABSTRACT. Bacterial epibionts were observed on the surface of the marine sediment ciliate *Geleia fossata*. Rod-shaped bacteria, from 2–10 $\times 10^3$ per ciliate, were universally positioned in ciliated grooves, in apparent spatial association with dikinetids. SEM and TEM examination of the ciliates confirmed that a tight affiliation exists between the epibiotic bacteria and ciliate cortex ultrastructures. These observations, as well as the distinct bacterial distribution pattern over ciliate surface, suggest that there is a close epibiont/host physiological integration. Epibiotic bacteria were also observed on the surfaces of other sediment ciliates from the genera *Loxophyllum, Trachelorapbis, Geleia, Paraspathidium,* and *Cyclidium.* These findings indicate that the bacterial/protozoa associations are widespread in the marine benthic environment. The potential benefits for both epibionts and their hosts are discussed.

Supplementary key words. Benthos, chemolithotrophs, oxic-anoxic interface, prokaryotes, protozoa, symbiosis.

CLOSE associations between Protozoa and bacteria have been recognized for over a century [5, 6] and include examples of parasitism, commensalism, and mutualism. In many of these associations, the protozoan hosts are inhabited by ecto- and/or endobiotic bacteria. The most studied examples are the cytoplasmic and endonuclear bacteria occurring in *Paramecium* and other Protozoa (reviewed in [32]). Some cytobionts, termed xenosomes [42], are infectious and parasitic. Other associations appear to be more mutualistic in nature, such as those between methanogenic and other anaerobic bacteria and anaerobic ciliates [24, 47]. These symbioses elegantly resolve some of the physiological constraints of the above organisms imparted by the nature of their habitat [25]. In the anaerobic environment, be it marine sediments [26, 45], freshwater chemically-stratified systems [16, 17], the reticulo-rumen of some mammals [29, 47], the hind gut of insects [28, 33], or landfill sites [24], Protozoa-bacteria synergism and symbiosis seem to be the rule rather than exception. In most cases, the close relationship is critical for the energy metabolism for both organisms [25].

Although the most-studied examples of protozoan-bacterial synergism/symbiosis involve anaerobic microorganisms, there is no a priori reason why functionally important associations between bacteria and Protozoa should not exist in well-oxygenated or microaerophilic environments as well. In the latter situation, examples are largely limited to two bacterial-protozoan relationships, both involving epibiotic bacteria, possibly sulfur-oxidizers, and two marine ciliates, *Kentrophoros* [23] and *Zoothamnium* [2]. In this paper, we present evidence to support the idea that such associations are common in the marine benthic environment. We describe associations between what appear to be ectosymbiotic bacteria and several marine sediment ciliates and provide SEM and TEM ultrastructural evidence for close integration of epibiotic bacteria and their hosts' cortical structures.

MATERIALS AND METHODS

Source of organisms. Ciliates were obtained from sediment samples taken at various times of the year from a marine sandy tidal flat, near the Marine Science Center, Northeastern University, Nahant, MA, 10 km north of Boston. The study site is described elsewhere [14, 15].

Identification of ciliates. Sediments were preserved with Bouin's fixative (37% buffered formaldehyde, saturated with picric acid, with glacial acetic acid added to a 1% final concentration prior to fixation; these and other chemicals were, unless otherwise specified, from Sigma, St. Louis, MO). Ciliates were extracted by repetitive sample resuspension and decantation. Extracted ciliates were concentrated on 1- to 2-μm pore size Millipore nitrate cellulose filters and were silver-impregnated with Protargol (Roboz Surgical Instrument Co., Rockville, MD) as described in [36]. Microscopic observations were made using Zeiss Standard or Axiophot microscopes. Original species description and recent reviews [7, 11, 19, 30, 37, 40, 41] were consulted for identification and taxonomy.

Epifluorescence observations. Sediment samples were preserved with 2% glutaraldehyde (final concentration in the sediment). The ciliates were extracted, purified, stained with DAPI (4′,6 diamidino-2-phenylindol) or dual stained with DAPI/FITC (fluorescein isothiocyanate), and concentrated on 2-μm pore size black polycarbonate membranes (Polysciences, Warrington, PA) after Epstein [13]. Observations and microphotography were carried out using Zeiss Axiophot microscope equipped for epifluorescence (Zeiss filter blocks 9108460194 for DAPI (365/400/450 nm excitation filter/beam splitter/barrier filter, respectively) and 9108460019 for FITC (485/505/>530 nm filters)).

SEM. Ciliates were extracted using a sea ice technique [44], collected and transferred to a vial with aid of a micropipette, preserved with EM-grade glutaraldehyde (2% final concentration), concentrated on 2-μm pore size polycarbonate membranes, and post-fixed in 1% osmium tetroxide. Following post-fixation, the polycarbonate membranes were dehydrated using a graded series of ethanol and critically point dried from CO_2. Membranes were then mounted on a specimen stub with a carbon adhesive tab, sputter coated with 15 nm gold-palladium and examined using an AMR-1000 scanning electron microscope.

TEM. Sediments were preserved as for epifluorescence observations. Organisms were separated from the sediment as for the identification of ciliates. Target ciliates were collected by a micropipette and transferred to a vial containing 4.0% glutaraldehyde, 0.3M sucrose, and 0.1M cacodylate buffer (pH 7.4) for 2 h at 4° C. The ciliates were then washed twice with 0.1M cacodylate buffer containing 0.3M sucrose, post fixed for 1 hour in cacodylate buffered 1% osmium tetroxide, dehydrated through a graded series of ethanol, and infiltrated with increasing ratios of Spurrs resin to 100% ethanol. After infiltration in 3 one-hour changes of pure Spurrs resin, individual ciliates were embedded in flat embedding molds to allow for orientation, and polymerized at 60° C for 24 hours. Thin sections cut on a diamond knife were collected, stained with uranyl acetate and lead citrate, and examined in a Zeiss EM-10 transmission electron microscope.

RESULTS

Identification of ciliates. The ciliates examined in this study were identified as follows: *Loxophyllum setigerum, Trachelorapbis primitarum, Trachelorapbis sp., Geleia fossata, Geleia*

[1] To whom correspondence should be addressed. Telephone: 617-581-7370; Fax: 617-581-6076; Email: sepstein@lynx.dac.neu.edu

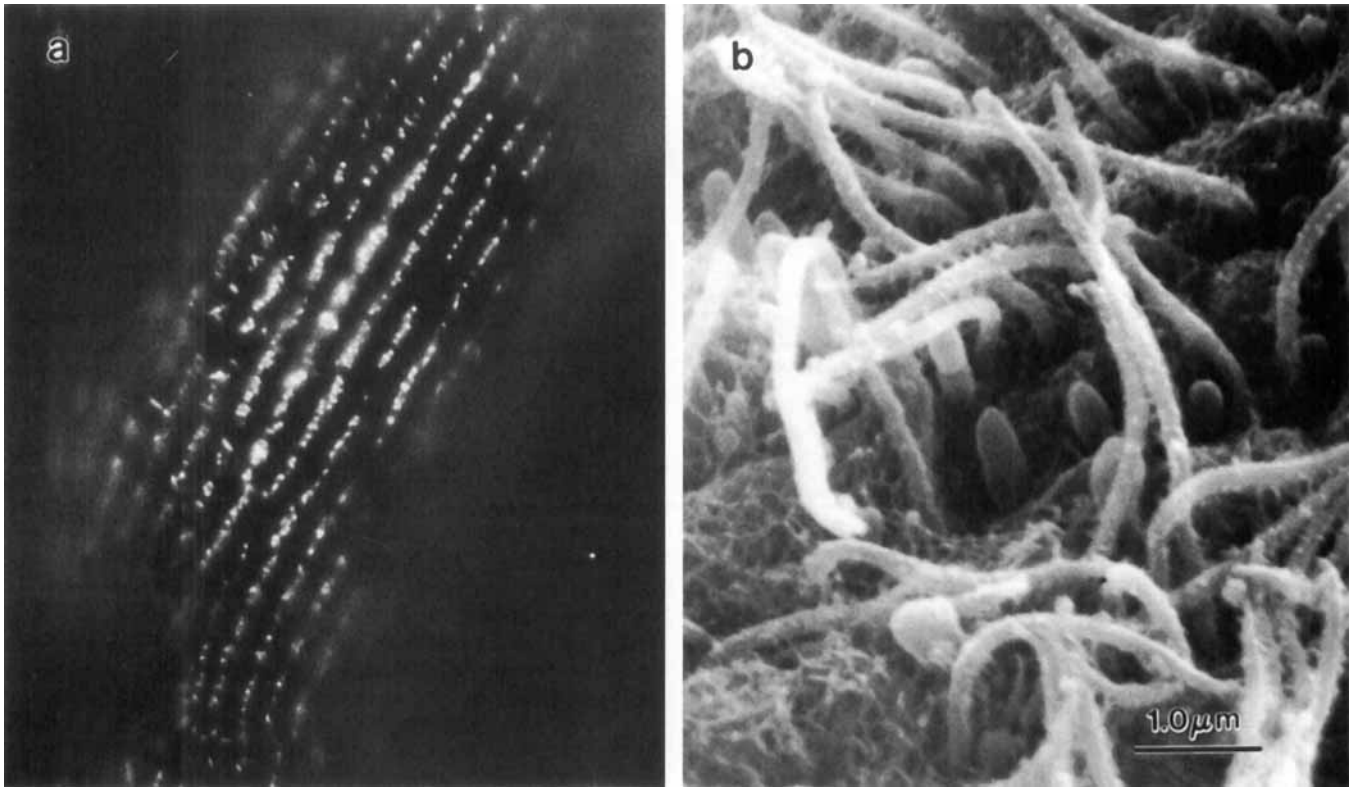

Fig. 1. Bacteria-sized bodies on the surface of *G. fossata*. **a**. DAPI staining, ×1,250. **b**. SEM view at ×17,000.

murmanica, Geleia vacuolata, Geleia sp., Paraspathidium fuscum, and Cyclidium sp.

Our primary focus was *G. fossata*. The identification was based primarily on general morphological characteristics, which corresponded well to the earlier descriptions [11, 19, 30, 37]. The specimens of *G. fossata* we collected were relatively small (190 ± 41 μm fixed; live specimens attained 300 to 500 (contracted) to 700 μm (full extension)), vermiform, with a pointed tail and a curved anterior, and had a distinct brown color. The nuclear apparatus consisted of two macronuclei and a single intercalated micronucleus. The number of somatic kineties varied between 27 and 33 (31 average). The buccal region, surrounded by a circular lip, contained a large field of oblique kineties on the (ciliate) left and a much narrower field of oblique kineties on the (ciliate) right. The buccal ciliature was essentially the same as that described and illustrated in detail by Nouzarède [37]. The similarity extended to the details of the first and second somatic kineties (the kineties immediately adjacent to the buccal cavity) and to the appearance and placement of the single postbuccal kinety. The fossa was easily distinguishable.

Epifluorescence observations. All the ciliates examined had bacteria-sized DAPI-stained bodies on their surface. The largest number of DAPI-stained bodies were found on *G. fossata*. These bodies were observed exclusively in or along the ciliated grooves, uniformly distributed over their entire length (Fig. 1a). Their total number was crudely estimated to be between 2,000 and 10,000. DAPI-stained bodies of similar appearance, though of lower abundance and of less regular spatial pattern, were also observed on cells of other *Geleia* species.

In both the *Tracheloraphis* species and *P. fuscum,* the DAPI-stained bodies were short rods, placed exclusively along the kineties, and concentrated at the anterior end of the ciliates (Fig. 2a). On some specimens of *Tracheloraphis,* the DAPI-stained objects had a secondary place of concentration along the kineties at the organisms' posterior end (Fig. 2b). Usually, a few more individual bodies were observed scattered irregularly, though always in the grooves, over the ciliates' surface. In *L. setigerum,* similar bodies were concentrated around the cytostome, but with no apparent association with the kineties. In all cases, the DAPI-stained objects looked very similar to typical sediment bacteria. Their total number per cell was crudely estimated to be from 1,000–3,000, from 1,000–2,000, and from 500–1,500 for *Tracheloraphis, Paraspathidium,* and *Loxophyllum,* respectively.

The bacteria-like bodies on *Cyclidium sp.* (SEM images are given in Fig. 3) were long rods, up to 3–4 μm in length. The epibionts were present in a varying but universally large number.

SEM. Close examination of the ciliated grooves of *G. fossata* revealed bacteria-like structures, always in or immediately adjacent to the ciliated grooves, protruding to the outside of the ciliates' surface (Fig. 1b). These objects were considerably thicker than cilia and thus could not be broken or underdeveloped cilia. The per ciliate abundance of these bodies corresponded well to the per ciliate abundance of DAPI-stained bodies.

TEM. Several specimens of *G. fossata* were thin-sectioned both longitudinally and transversely. The bacteria associated with the ciliates' surface were immediately apparent (Fig. 4 a–c). All the bacteria observed were morphologically similar short rods, approximately 1.1–1.8 by 0.4–0.7 μm. Usually, the details of the plasma membrane of the cell wall were poorly seen, but occasionally an outer membrane was clearly distinguishable (Fig. 5). This feature and the lack of a thick peptidoglycan layer suggest that these bacteria are Gram-negative.

15507408, 1998, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1550-7408.1998.tb05071.x by Swedish University Of Agricultural Sciences, Wiley Online Library on [10/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

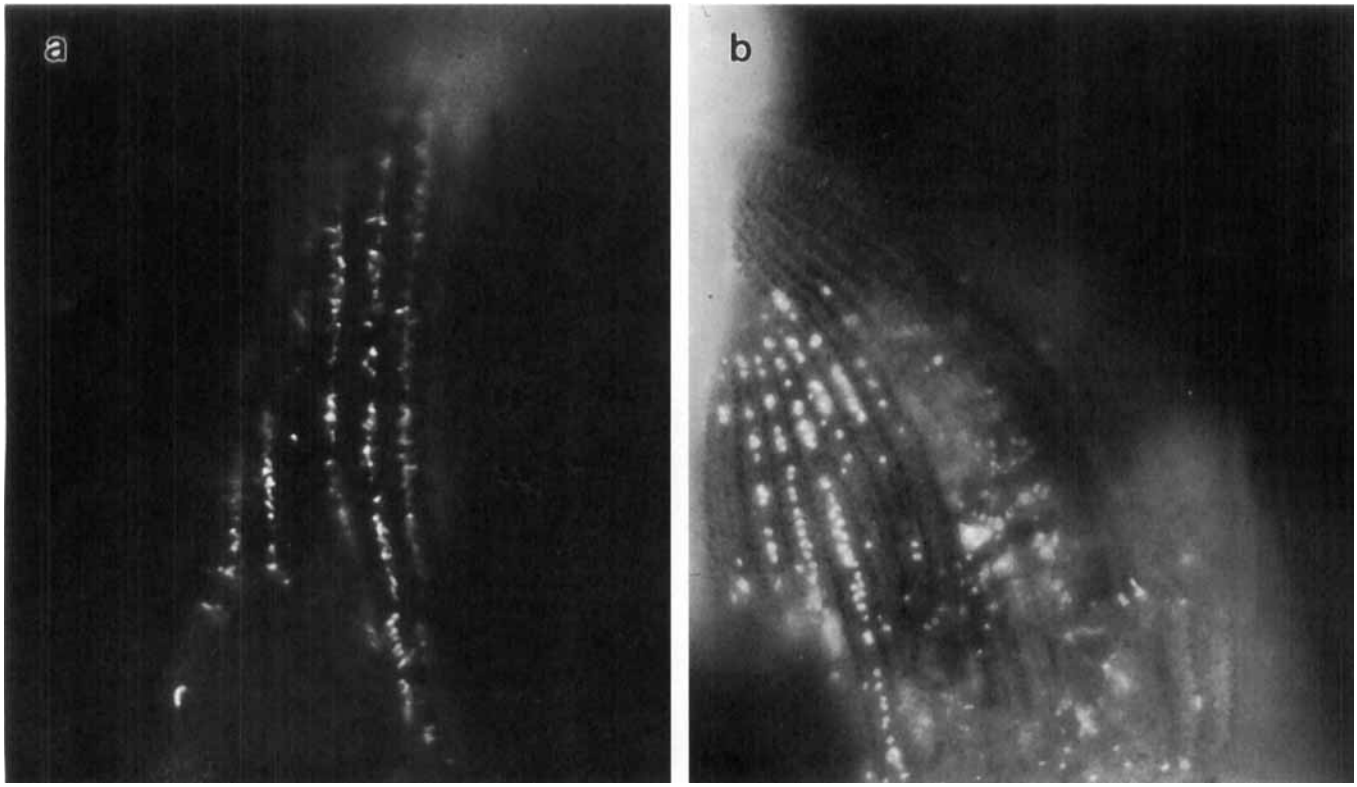

Fig. 2. DAPI-stained bodies positioned along the kineties at the anterior (**a**, ×1,100) and posterior (**b**, ×1,250) end of *Tracheloraphis sp.*

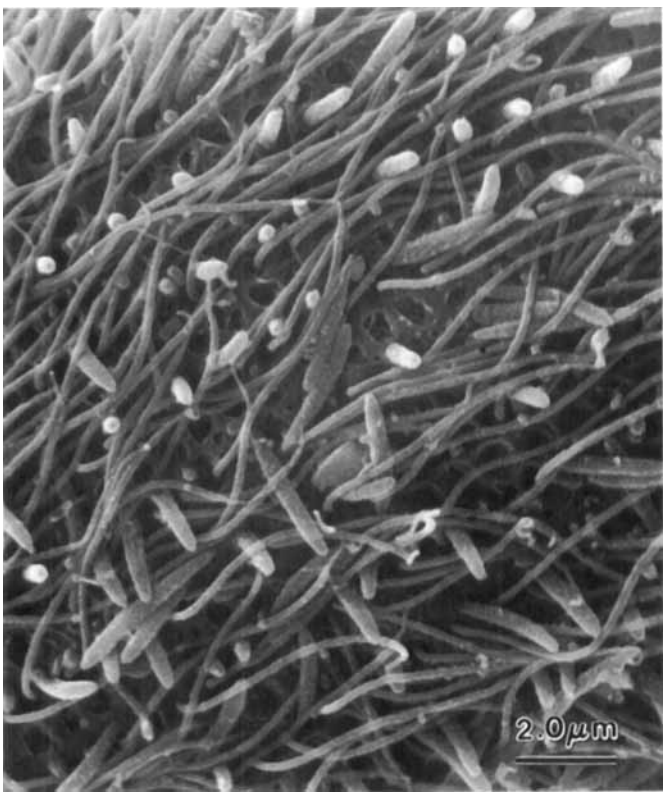

Fig. 3. SEM image of the surface of *Cyclidium sp.* with epibionts of apparently bacterial nature, ×6,500.

The bacteria were always found inside the ciliated grooves and in apparent spatial association with individual kinetids, so that either a single bacterium, or a group of 2 to 3 bacteria, were seen in the same transverse plane as the kinetosome. In cases where there were more than one bacterium per kinetid, they appeared to form imperfect vertical rows (Fig. 4c). The uppermost bacteria were almost universally placed in deep cell membrane invaginations, lined with alveoli, so that over half of the bacterium, if not the entire cell, was below the ciliate surface (Fig. 4b, c). These uppermost bacteria were typically positioned at approximately 45° to both the cilium axis and cell surface (Fig. 4a–c). Other bacteria in the group seemed to be inside unit membrane bound vesicles, each containing a single bacterium. These vesicles had no obvious connection to the surface, but that was not checked by serial sectioning. Some bacterial cells appeared to be dividing (Fig. 4b).

TEM revealed several other morphological features of *G. fossata.* Typical mitochondria were absent; instead, microbodies of unclear nature were observed. These could be either modified mitochondria, hydrogenosomes, or endobiotic bacteria. These microbodies were heavily concentrated in the ectoplasm and were only occasionally seen elsewhere in the cell.

The cortex structures of *G. fossata* were complex (Fig. 4a–c). They were dominated by three extensive longitudinal sheets of myonemes which ran longitudinally, parallel to each other, along non-ciliated ridges. Just below them, a number of transverse myonemes constituted a series of flexible rings, apparently one under each kinetid, possibly adding to the ability of *G. fossata* to move and contract. Curiously, though, these ciliates did not exhibit pronounced contraction upon fixation.

The somatic kinetids of *G. fossata* were dikinetids. Only the anterior kinetosome, at which transverse ribbon originated, appeared to be ciliated. The posterior kinetosome exhibited both

15507408, 1998, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1550-7408.1998.tb05071.x by Swedish University Of Agricultural Sciences, Wiley Online Library on [10/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

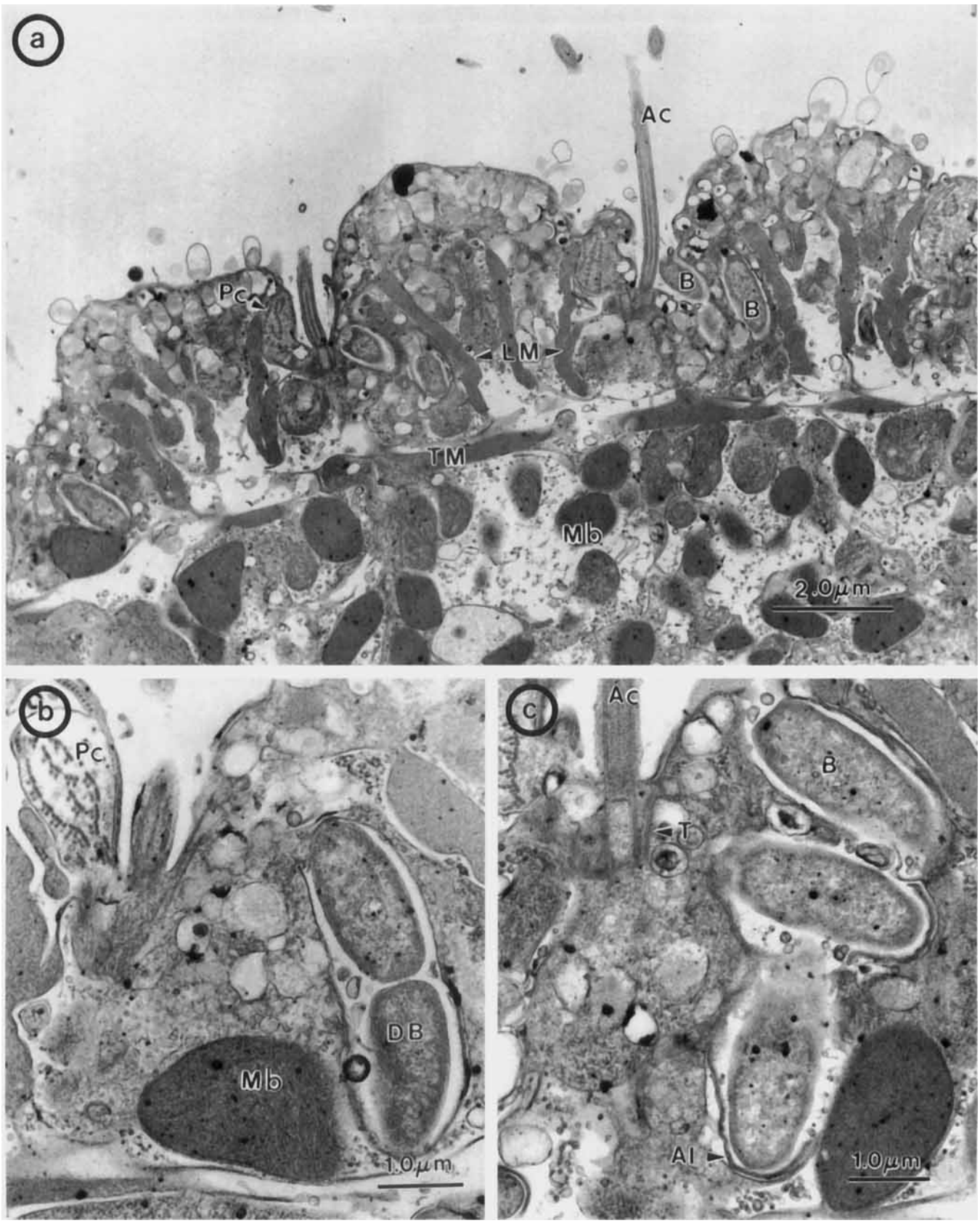

Fig. 4. TEM images of thin sections of *G. fossata*. **a**. Cross section in the mid-body region showing three ciliated grooves, 3 interkinetal ridges, and bacteria positioned near kinetids, ×11,000. **b** and **c**. Close view of single kinetids and associated bacteria, ×30,000. Ac, anterior cilium; Aks, anterior kinetosome; Al, alveolus; B, bacteria; DB, dividing bacterium; LM, longitudinal myoneme; Mb, unidentified microbodies; Pc, postciliary ribbon; T, transverse ribbon; TM, transverse myoneme.

15507408, 1998, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1550-7408.1998.tb05071.x by Swedish University Of Agricultural Sciences, Wiley Online Library on [10/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Fig. 5. Close view (TEM) of epibionts of *G. fossata*. Cell wall structure suggests that the bacteria were Gram-negative, ×33,000.

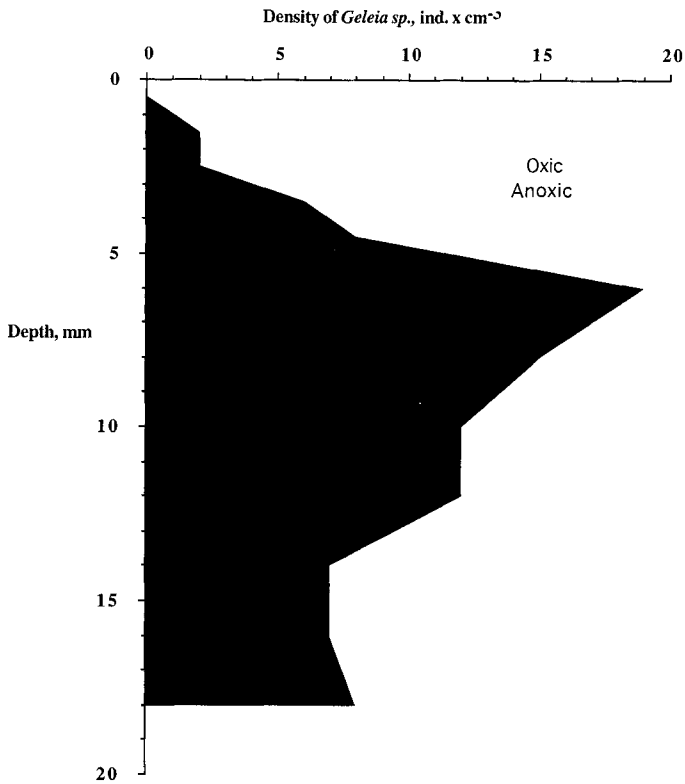

Fig. 6. Vertical distribution of *Geleia sp.* in marine sandy sediments in relation to the position of the oxic/anoxic interface (Epstein & Berninger, unpubl.; for methods, see [4]).

a kinetodesmal fibril and postciliary ribbon, the latter having an unusual microtubule pattern (Fig. 4a–c).

DISCUSSION

Bacterial symbiosis has received much attention since the discovery of unique associations between chemolithoautotrophic and methanotrophic bacteria and animals living at hydrothermal and cold seep sites [8, 9, 10, 43]. Anaerobic Protozoa, including flagellates and ciliates, are also known to harbor a number of ecto- and endosymbiotic bacteria and in many cases, these symbioses have been extensively researched (reviews in [24, 25, 32]). Ectosymbiotic bacteria have been repeatedly observed on the surface of aerobic species of Protozoa as well [1, 3, 18, 20, 23, 30, 31, 35, 38, 39] but few of these associations have been well-studied [2, 23, 46]. In this paper, we extend the list of ciliated Protozoa by including microaerophilic species that form close associations with ectobiotic prokaryotes and suggest that these associations are potentially important for the metabolism of both the bacteria and their unicellular eukaryotic hosts. Moreover, we suggest that such bacterial associations with microaerophilic ciliate species are common in nature.

We examined several marine sediment ciliates. The most notable association with ectobiotic bacteria occurred with *G. fossata*. All specimens of this species had thousands of epibiotic bacterial cells on their surface. The biovolume of the ciliates and the bacteria associated with them were estimated as 70,000–100,000 μm^3 and 0.15–0.25 μm^3 per cell, respectively, so that, at the observed abundance, the bacteria constituted an equivalent of about 1 to several per cent of the ciliate biovolume. The bacteria appeared to be distributed on the surface of the ciliates in clusters almost universally associated with dikinetids. The mechanics of attachment could not be resolved. The positioning of the bacteria in ciliate membrane invaginations and the presence of surface material, revealed by SEM and TEM, on ciliate cortex might both help to secure bacteria on the ciliate surface. The distinct pattern of bacterial distribution on ciliates suggests that a close physiological relationship exists between the ectobionts and their host.

The present study is a phenomenological report and the physiological and ecological interpretation of the reported data is speculative. A large number of mass interstitial ciliates, including another species of *Geleia* closely related to *G. fossata,* and *Kentrophoros,* a close relative to *Geleia,* are known to exist just below the oxic/anoxic interface in sediments (Fig. 6). *Kentrophoros* is known to "garden" chemolithoautotrophic bacteria [23, 38, 39]. Both species share the same microhabitat and both possess large numbers of apparently ectosymbiotic bacteria on their surface. It is tempting to hypothesize that their symbionts are metabolically similar especially since the potential benefits for chemolithoautotrophic bacteria are immediately apparent. If the hosting ciliates prefer to be present at the sediment layer close to the oxic/anoxic interface and followed this interface during the course of its daily migrations, chemolithotrophic ectosymbionts would be constantly exposed to their reduced energy source (e.g. sulfide) and oxygen without expending a great deal of energy on motility. We did not prove that these ectosymbionts were capable of chemolithoautotrophy, and the ultrastructure of the bacteria gave no clue as to their physiological type. Neither internal membranes typical of methanotrophs and nitrifiers nor the sulfur granules typical of sulfide-oxidizing bacteria were observed.

The ciliates might benefit in a way similar to that proposed for *Kentrophoros* [38, 39]. The latter seems to fulfill part of its nutritional requirements by gradual phagocytosis of its ectosymbionts. We observed a number of bacterial cells in apparently membrane-bounded vesicles in *G. fossata* that were po-

15507408, 1998, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1550-7408.1998.tb05071.x by Swedish University Of Agricultural Sciences, Wiley Online Library on [10/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

sitioned below those bacteria still in contact with the outside of the cell (Fig. 4a–c). However, we did not observe bacterial cells in stages of progressive digestion and were not able to show that the apparently membrane-bounded vesicles were completely internal. In addition, the role of epibiotic bacteria in the nutrition of *G. fossata* is likely to be of less significance than in the case of Kentrophoros due to the smaller populations of epibiotic bacteria per unit of ciliate biovolume.

Although we focused mainly on *G. fossata,* we also observed DAPI-stained, bacteria-sized structures on the surfaces of other microaerophilic ciliates including both *Tracheloraphis* species, *Loxophyllum* and *Paraspathidium.* Though the bacterial nature of these structures remains to be confirmed by TEM, the data presented herein indicate that close bacterial-protozoan associations or symbioses may be quite widespread in oxic/microaerobic environments as well as in anaerobic systems.

ACKNOWLEDGMENTS

We thank Drs. D.H. Lynn (University of Guelph, Ontario, Canada) and E. Small (University of Maryland, MD) for helpful discussions, Dr. S. Sokol (Harvard University/ Beth Israel Hospital, Boston, MA) for use of his light microscope, and three anonymous reviewers for helpful suggestions and constructive criticism. This research was supported in part by US National Science Foundation grant OCE-9217250 to SSE and US NSF grant MCB-9696027 and US Office of Naval Research grant ONR N00014-91-J-1290 to DAB. This is contribution number 225 of the Marine Science Center of Northeastern University. This is Journal Paper No. J-17449 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project No. 3370, and is supported by the Hatch Act and State of Iowa Funds.

LITERATURE CITED

1. Ball, G. H. 1969. Organisms living on and in protozoa. *In:* Chen, T.-T. (ed.), Research in Protozoology, Pergamon Press, London and New York. **3**:565–718.

2. Bauer-Nebelsick, M., Bardele, C. & Ott, J. A. 1996. Redescription of *Zoothamnium niveum* (Hemprich & Ehrenberg, 1931) Ehrenberg, 1838 (Oligohymenophora, Peritrichida), a ciliate with ectosymbiotic, chemoautotrophic bacteria. *Europ. J. Protistol.*, **32**:18–30.

3. Berger, J. & Lynn, D. H. 1984. Somatic and oral cortical ultrastructure of the plagiopylid ciliates *Lechriopyla mystax* Lynch, 1930 and *Plagiopyla minuta* Powers, 1933. *J. Protozool.*, **31**:433–443.

4. Berninger, U.-G. & Epstein, S. S. 1995. Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea Sediment. *Aquat. Microb. Ecol.,* **9**:229–236.

5. Bütschli, O. 1886. Beiträge zur Kenntnis der Flagellaten und einiger verwandter Organismen. *Morphol. Jahrb.*, **11**:78–101.

6. Calkins, G. N. 1902. Marine protozoa from Woods Hole. *Bull. US Fish. Comm.*, **1901**:413–468.

7. Carey, P. G. 1992. Marine interstitial ciliates. Chapman & Hall, London. Pp. 1– 351.

8. Carey, S. G., Fisher, C. R., & Felbeck, H. 1988. Mussel growth supported by methane as sole carbon and energy source. *Science,* **240**: 78–80.

9. Cavanaugh, C. M. 1985. Symbioses of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. *Bull. Biol. Soc. Wash.*, **6**:373–388.

10. Childress, J. J., Fisher, C. R., Brooks, J. M., Kennicutt II, M. C., Bidigare, R., & Anderson, A. E. 1986. A methanotrophic marine molluscan (Bivalvia, Mytilidae) symbiosis: mussels fueled by gas. *Science,* **233**:1306–1308.

11. Dragesco, J. 1960. Ciliæes mesopsammiques littoraux. *Trav. Stat. Biol. Roscoff (N.S.),* **12**:1–356.

12. Epstein, S. S. 1994. A New Marine Interstitial Ciliate from Northwest Atlantic, *Tracheloraphis primitarum* sp. n. (Ciliophora, Kinetofragminophora, Karyorelictida, Trachelocercidae). *J. Euk. Microbiol.,* **41**:500–503.

13. Epstein, S. S. 1995. Simultaneous enumeration of protozoa and micrometazoa from marine sandy sediments. *Aquat. Microb. Ecol.*, **9**: 219–227.

14. Epstein, S. S. 1997. Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. (in press).

15. Epstein, S. S. & Rossel, J. 1995. Enumeration of sandy sediment bacteria: search for optimal protocol. *Mar. Ecol. Prog. Ser.*, **117**:289–298.

16. Esteban, G. & Finlay, B. J. 1994. A new genus of anaerobic scuticociliate with endosymbiotic methanogens and ectobiotic bacteria. *Arch. Protistenk.*, **144**:350–356.

17. Esteban, G., Finlay, B. J. & Embley, T. M. 1993. New species double the diversity of anaerobic ciliates in a Spanish lake. *FEMS Microbiol. Lett.*, **109**:93–100.

18. Fauré-Frémiet, E. 1950a. Caulobacteriés épizoiques associées aux *Centrophorella* (ciliés holotriches). *Bull. Soc. Zool. Fr.*, **75**:134–137.

19. Fauré-Frémiet, E. 1950b. Ecologie des ciliés psammophiles littoraux. *Bull. Biol.* **84**:35–75.

20. Fauré-Frémiet, E. & Tuffrau, M. 1955. *Sonderia labiata* n. sp., cilié trichostome psammobie. *Hydrobiologia,* **7**:210–218.

21. Fenchel, T. 1969. The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. *Ophelia,* **6**:1–182.

22. Fenchel, T. & Bernard, C. 1996. Behavioural responses in oxygen gradients of ciliates from microbial mats. *Europ. J. Protistol.*, **32**: 55–63.

23. Fenchel, T. & Finlay, B. J. 1989. *Kentrophoros*: a mouthless ciliate with a symbiotic kitchen garden. *Ophelia,* **30**:75–93.

24. Fenchel, T. & Finlay, B. J. 1991. The biology of free-living anaerobic ciliates. *Europ. J. Protist.*, **26**:201–215.

25. Fenchel, T. & Finlay, B. J. 1995. Ecology and evolution in anoxic worlds. Oxford Series in Ecology and Evolution. Oxford Univ. Press, NY. Pp 1–276.

26. Fenchel, T., Perry, T. & Thane, A. 1977. Anaerobiosis and symbiosis with bacteria in free-living ciliates. *J. Protozool.*, **24**:154–163.

27. Finlay, B. J. & Fenchel, T. 1991. An anaerobic protozoon with symbiotic methanogens, living in municipal landfill material. *FEMS Microb. Ecol.*, **85**:169–180.

28. Gijzen, H. J., Broers, C. A. M., Barughare, M. & Stumm, C. K. 1991. Methanogenic bacteria as endosymbionts of the ciliate *Nyctotherus ovalis* in the cockroach hindgut. *Appl. Environ. Microbiol.*, **57**: 1630–1634.

29. Hungate, R. E. 1975. The rumen microbial ecosystem. *Ann. Rev. Ecol. Syst.*, **6**:39–66.

30. Kahl, A. 1930–1935. Wimpertiere oder Ciliata. Die Tierwelt Deutschland, **21, 25, 30**:1–862.

31. Kirby, H. 1941. Organisms living on and in protozoa. *In:* Calkins, G. N., Summers, F. M. (ed.), Protozoa in Biological Research. Columbia University Press, NY. Pp. 1009–1113.

32. Lee, J. J., Soldo, A. T., Reisser, W., Lee, M. J., Jeon, K. W. & Görtz, H.-D. 1985. The extent of algal and bacterial endosymbioses in Protozoa. *J. Protozool.*, **32**:391–403.

33. Lee, M. J., Schreurs, P. J., Messer, A. C. & Zinder, S. H. 1987. Association of methanogenic bacteria with flagellated protozoa from a termite hindgut. *Curr. Microbiol.*, **15**:337–341.

34. Lloyd, D., Hillman, K., Yarlett, N. & Williams, A. G. 1989. Hydrogen production by rumen holotrich protozoa: effects of oxygen and implications or metabolic control by in situ conditions. *J. Protozool.*, **36**:205–213.

35. Lynn, D. H. & Frombach, S. 1987. The ultrastructure and systematics of *Schizocaryum dogieli,* a ciliate endocommensal in Pacific echinoids. *Can. J. Zool.,* **65**:3133–3143.

36. Montagnes, D. J. S. & Lynn, D. H. 1987. A quantitative protargol stain (QPS) for ciliates: method description and test of its quantitative nature. *Mar. Microb. Food Webs,* **2**:83–93.

37. Nouzaréde, M. 1976. Cytologie fonctionelle et morphologie expérimentale de quelque protozoaires ciliés mésopsammique géants de la famille des Geleiidae (Kahl). *Bull. Stat. Biol. Arcachon (N.S.),* **28**: (Suppl. 1: 1–315, Suppl. II: Plates I–C).

38. Raikov, I. B. 1971. Bactéries épizoiques et mode de nutrition

15507408, 1998, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1550-7408.1998.tb05071.x by Swedish University Of Agricultural Sciences, Wiley Online Library on [10/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

du ciliés psammophile *Kentrophoros fistolosum* Fauré-Frémiet (étude du microscope électronique). *Protistologica,* **7**:365–378.

39. Raikov, I. B. 1974. Etude ultrastructurale de bactéries épizoiques et endozoiques de *Kentrophoros latum* Raikov, ciliés holotriche mésopsammique. *Cah. Biol. Mar.*, **15**:379–393.

40. Small, E. B. & Lynn, D. H. 1981. A new macrosystem for the phylum Ciliophora Doflein, 1901. *BioSystems,* **14**:387–401.

41. Small, E. B. & Lynn, D. H. 1985. Phylum Ciliophora Doflein, 1901. *In:* Lee, J. J., Hutner, S. H. & Bovee, E. C. (ed.), An Illustrated Guide to the Protozoa. Society of the Protozoologists, Lawrence, KS. Pp. 393–575.

42. Soldo, A. T., Godoy, G. A. & Brickson, S. A. 1974. Infectious particles in a marine ciliate. *Nature,* **249**:284–286.

43. Southward, E. C. 1987. Contribution of symbiotic chemoautotrophs to the nutrition of benthic invertebrates. *In:* Sleigh, M. A. (ed.), Microbes in the Sea. Ellis Horwood Ltd., Chichester, England. Pp. 83–118.

44. Uhlig, G. 1964. Eine einfache Methode zur Extraktion der vagilen mesopsammalen Mikrofauna. *Helgol. Wissen. Meeresunter.*, **11**: 178–183.

45. Van Bruggen, J. J. A., Zwart, K. B., Hermans, J. G. F., van Hove, E. M., Stumm, C. K. & Vogels, G. D. 1986. Isolation and characterization of *Methanoplanus endosymbiosus* sp. n., an endosymbiont of the marine sapropelic ciliate *Metopus contortus* Quennerstedt. *Arch. Microbiol.*, **144**:367–374.

46. Verni, F. & Rosati, G. 1990. Peculiar epibionts in *Euplotidium itoi* (Ciliata, Hypotrichida). *J. Protozool.*, **37**:337–343.

47. Vogels, G. D., Hoppe, W. & Stumm, C. K. 1980. Association of methanogenic bacteria with rumen ciliates. *Appl. Environ. Microbiol.*, **40**:608–612.

Received 4-14-97, 8-25-97; accepted 8-29-97

15507408, 1998, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1550-7408.1998.tb05071.x by Swedish University Of Agricultural Sciences, Wiley Online Library on [10/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License