Marine Biology (1998) 132: 349–355

K. R. Buck · W. N. Bentham†

A novel symbiosis between a cyanobacterium, *Synechococcus* sp., an aplastidic protist, *Solenicola setigera*, and a diatom, *Leptocylindrus mediterraneus*, in the open ocean

Received: 5 January 1998 / Accepted: 22 May 1998

Abstract The abundance, biomass and distribution of *Solenicola setigera*, a colonial heterotrophic protist found only with the centric chain-forming diatom *Leptocylindus mediterraneus*, are reported for four major ocean basins. The distribution is cosmopolitan, and abundances and biomass are usually low (< 500 colonies l^{-1}); however, in the summer of 1993, we observed a major biomass component (range = 5 to 31 μg C l^{-1}) in the surface waters of the North Atlantic attributable to *S. setigera*. These colonies of *S. setigera* were exceptionally large, and unusual in possessing high abundances of *Synechococcus* sp., a normally solitary cyanobacterium, embedded in the matrix covering the cells. We hypothesize that this relationship was mutually beneficial for both *Solenicola setigera* and *Synechococcus* sp.

Introduction

Solenicola setigera is an aplastidic protist with an unusual flagellum-like protuberance (Taylor 1982; Patterson and Zölffel 1991), and is reported as occurring in small groups of cells around the circumference, on the girdle bands, of the centric diatom *Leptocylindrus mediterraneus* (Taylor 1982). There appears to be a covering secreted by *S. setigera* over these girdle bands (Taylor 1982). The colonized diatom cells do not appear to possess protoplasm (Hasle 1975; Taylor 1982). *S. setigera* has been infrequently reported since the early part of this century (Gran 1908) as a component of the plankton from a variety of locales (Cupp 1943; Hasle 1959; Taylor 1982), but only in association with *L. mediterraneus*. Little information has been published on the quantitative or qualitative role of this enigmatic organism in the foodwebs of the upper water column of the marine environment. Although *S. setigera* was initially described as a member of the Xanthophyceae, the latest review (Patterson and Zölffel 1991) placed it in the *incertae sedis*, reflecting the lack of information about this protist.

Synechococcus spp. are small, coccoid, solitary cyanobacteria that are found in all but polar waters and are a substantial component of the phytoplankton biomass and primary productivity in the upper water column (Waterbury et al. 1986).

During an oceanographic cruise in the North Atlantic Ocean in 1993, a high concentration of *Leptocylindrus mediterraneus*/*Solenicola setigera* was observed, and many of the colonies of *S. setigera* had *Synechococcus* sp. embedded in the extracellular matrix that surrounds them. There are several recent reports that document symbioses between coccoid cyanobacteria and thecate dinoflagellates from tropical environments (Lucas 1991; Janson et al. 1995). In some of these associations the symbiotic cyanobacteria are ingested and digested (Lucas 1991). We report here on the abundance, biomass, distribution, morphology and ultrastructure of the symbiosis between *Solenicola setigera* and *Synechococcus* sp. that we observed occurring on the cell walls of *L. mediterraneus*.

Materials and methods

The samples studied were collected on several cruises undertaken as part of the U.S. National Oceanic and Atmospheric Administration Global Climate Change Program: (1) the North Atlantic in summer 1993 (Buck et al. 1996); (2) the southern and equatorial eastern Pacific in spring 1994; (3) the Indian Ocean in fall 1995; (4) the subantarctic and temperate western Pacific in spring 1996.

† Deceased January 1995

Communicated by M.F. Strathmann, Friday Harbor

K.R. Buck (✉)
Monterey Bay Aquarium Research Institute,
P.O. Box 628, Moss Landing, California 95039,
USA
Fax: +(0) 831 775 1645

W.N. Bentham
University of California, Electron Microscope Laboratory,
26 Giannini Hall, Berkeley, California 94720-3330,
USA

We used blue light-excitation epifluorescence light microscopy (EFM) to enumerate glutaraldehyde-preserved organisms on polycarbonate filters through which we passed surface seawater samples (Buck et al. 1992a). For relatively rare and large organisms we typically filtered 100 to 200 ml seawater through 5 μm pore-sized filters. Scanning electron microscopy (SEM) was conducted on critical point-dried specimens according to the protocols described in Buck and Newton (1995), and transmission electron microscopy (TEM) was used on thin sections according to the protocols in Buck et al. (1992b). The samples used for electron microscopy preparations were from non-quantitative 35 μm mesh-size net tows taken at the surface, preserved in unbuffered glutaraldehyde (2% final concentration) and kept cold until processing.

Results

We found *Solenicola setigera*, as previously reported (Taylor 1982), in small groups or colonies around the intercalary region of the chain-forming diatom *Leptocylindrus mediterraneus*. The colony size was ≃10 cells (Figs. 1a–d; 2a,b). The individual, hourglass-shaped colonies were separated by relatively long lengths of uncolonized *L. mediterraneus* girdle (Fig. 2a). Occasionally, cells resembling *S. setigera* were observed distributed irregularly, but not in colonies, along the entire length of *L. mediterraneus* (Fig. 2b). Long flagellum-like protuberances emanate from each cell (Fig. 2a). The extracellular polymeric matrix reported by earlier workers (Taylor 1982) was present between *S. setigera* and the diatom girdle bands (Fig. 1b). Seventeen was the maximum number of colonies observed on a single chain of *L. mediterraneus*. No chains of *L. mediterraneus* were observed without colonies of *S. setigera*.

At the seven northernmost stations in the north Atlantic, however, we found a different arrangement. Large numbers of *Synechococcus* sp. were associated with some *Leptocylindrus mediterraneus*/*Solenicola setigera* (Figs. 1c–f; 2d, e; 3). *Synechococcus* sp. was identified by its characteristic phycoerythrin-derived yellow-orange autofluorescence when excited by blue light in epifluorescence preparations, and by cell size (∼1 μm) and the peripherally located thylakoids (Fig. 3d) (Waterbury and Ripka 1989). The number of cells in an individual *Solenicola setigera* colony was greater and the colony size was substantially larger in both diameter and length when *Synechococcus* sp. was present (Table 1; Fig. 1c–e). On some diatom chains the distinction between adjoining colonies all but disappeared (Fig. 1c) due to this increase in the size of the colonies. The *Synechococcus* sp. associated with the *Solenicola setigera* were larger than the free-living individuals. Many of the *Synechococcus* sp. associated with *Solenicola setigera* were elongated and possibly dividing (Figs. 2d, e; 3d). The *Synechococcus* sp. cells were embedded in the (at times thick) extracellular polymeric matrix that is associated with *Solenicola setigera* (Figs. 2c; 3). In colonies of *S. setigera* with associated *Synechococcus* sp., the matrix was thicker than in colonies without *Synechococcus* sp., and covered cells of *Solenicola setigera* completely (Fig. 3).

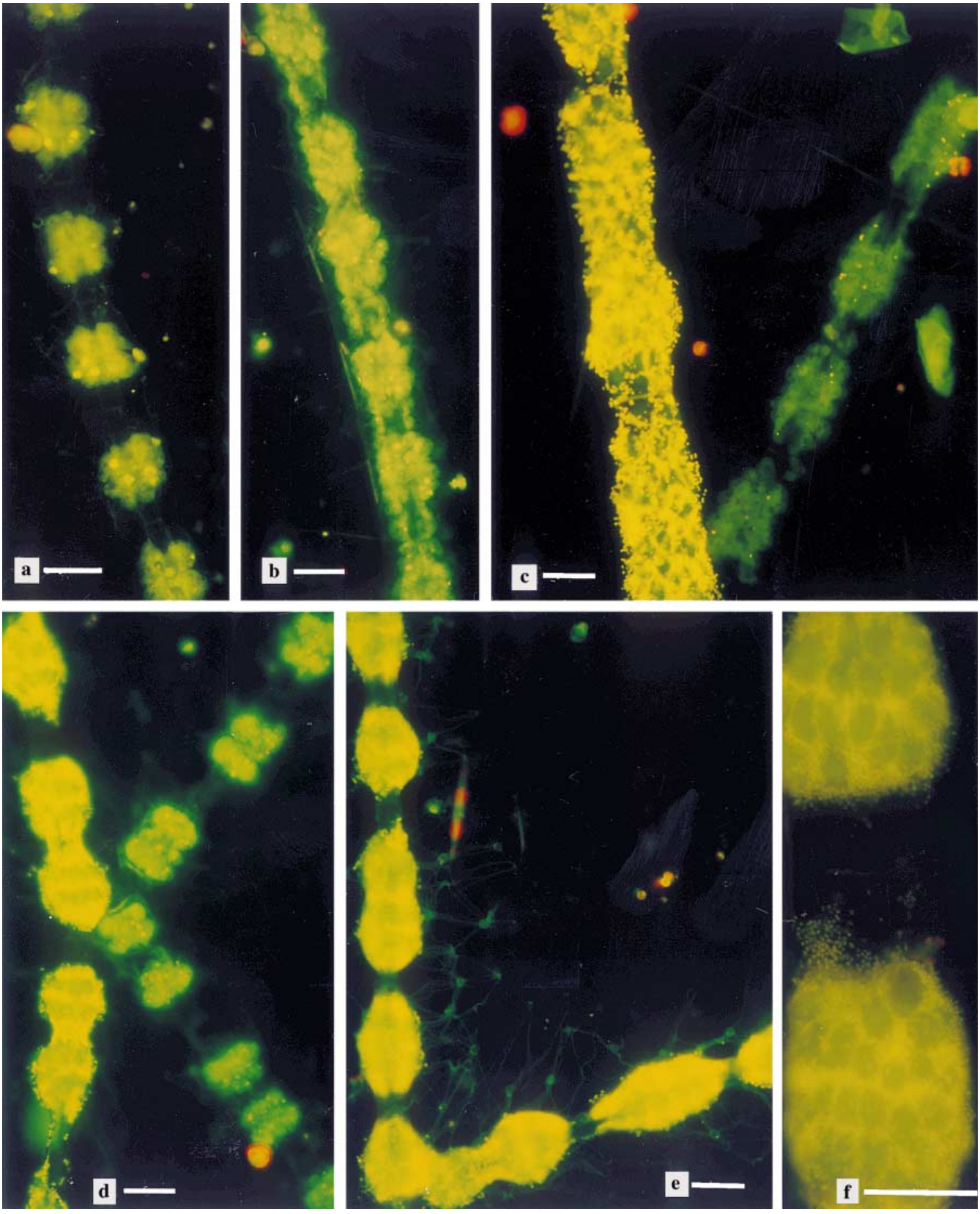
Fig. 1 *Leptocylindrus mediterraneus* and *Solenicola setigera* with and without associated *Synechococcus* sp.: epifluorescence micrographs; cells of *Solenicola setigera* fluoresce green, those of *Synechococcus* sp. bright yellow. **a** Five colonies of *Solenicola setigera* on single chain of *L. mediterraneus*, showing standard morphology in absence of *Synechococcus* sp. **b** Single chain of *L. mediterraneus* with five colonies of *Solenicola setigera* and cells of *S. setigera* between discrete colonies. **c** Two chains of *L. mediterraneus* with *S. setigera*, one with and one without associated *Synechococcus* sp.; colonies with associated *Synechococcus* sp. are larger than those without. **d** Two chains of *L. mediterraneus* with *Solenicola setigera*, one with and one without associated *Synechococcus* sp. **e** Single chain of *L. mediterraneus* with ≃6 colonies of *Solenicola setigera* with associated *Synechococcus* sp.; two of the colonies appear to be dividing. **f** Higher magnification of two colonies of *Solenicola setigera* with associated *Synechococcus* sp.; note that *Synechococcus* sp. are outside *Solenicola setigera* cells. (All scale bars = 25 μm)

None of the chains of *Leptocylindrus mediterraneus* we observed by epifluorescence specific for autotrophic pigment detection revealed the presence of chlorophyll. We observed mitochondria inside the diatoms in the TEM preparations typical of those found in diatoms and almost all other protists; however, chloroplasts, or remnants thereof, were not found. This could be an artifact of the TEM sections; however the diatoms we assayed with TEM were mostly devoid of cell protoplasm (Fig. 3). Although we could identify gross ultrastructural features such as thylakoids of *Synechococcus* sp., cell membranes and vacuoles of *Solenicola setigera* (Fig. 3b–d) and mitochondria inside *L. mediterraneus* (Fig. 3a–c), the fixations did not preserve ultrastructural detail.

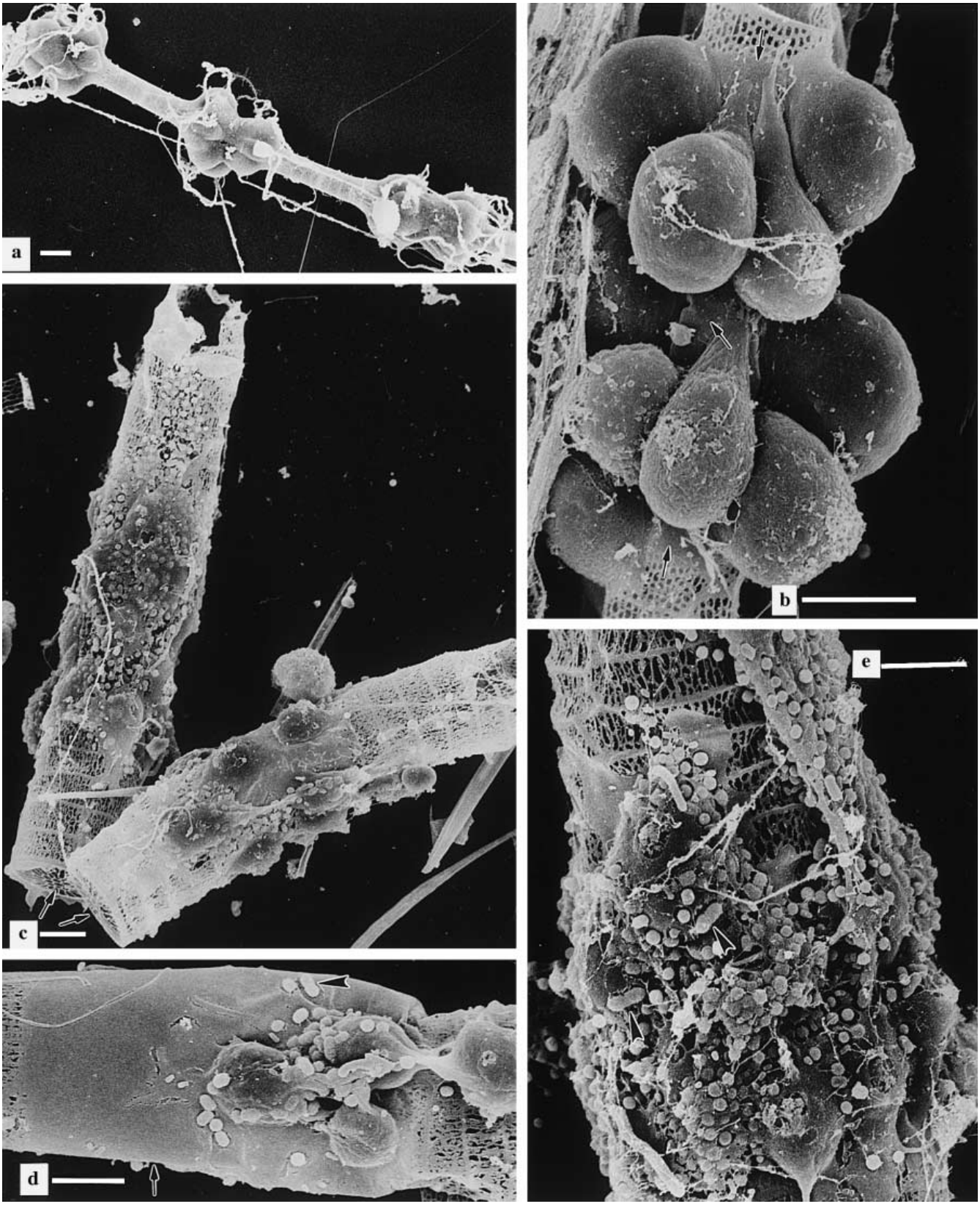
The abundance of all *Solenicola setigera* colonies at these northernmost stations in the North Atlantic ranged from 2300 to 12 000 colonies l^{-1} and the maximum biomass was 31 μg C l^{-1} (Table 2). At these northern stations more biomass resided in the *S. setigera* colonies with *Synechococcus* than in those without (Table 2). The percent of the total heterotrophic protistan biomass that was composed of *Solenicola setigera* at these stations ranged from 14 to 93% (Table 2; and Buck et al. 1996). Outside the North Atlantic, the abundance of *S. setigera* was lower (Table 3). At only four stations in the Indian Ocean was *S. setigera* observed with associated *Synechococcus* sp., and these associations were dissimilar to the North Atlantic symbioses in that there was no increase in colony size.

Discussion

A number of aplastidic protists colonize the siliceous structures of diatoms (Taylor 1982; Patterson and Zölffel 1991), presumably benefiting from this association through grazer avoidance, increased clearance rates and proximity to sources of exuded dissolved organic material and other nutrients. The *Leptocylindrus mediterraneus* and *Solenicola setigera* association has to be one of the more unusual in this category. Some earlier works indicated that the colonized chains of *L. med-*



iterraneus were devoid of cellular content (Hasle 1975; Taylor 1982). In the presence of *S. setigera*, *L. mediterraneus* may be apochlorotic, existing on the exudations of the aplastidic protist for nutrition. There are a limited number of apochlorotic diatoms documented (Li and Volcani 1987) but these are usually epiphytic on



seaweeds. *S. setigera* might be parasitizing the diatom. This would explain the repeated observations of the absence of cell content (e.g. chloroplasts). Clearly, samples with better fixation for TEM or cultures are necessary to resolve the nature of the relationship between *L. mediterraneus* and *S. setigera*.

Fig. 2 *Leptocylindrus mediterraneus* and *Solenicola setigera* with and without associated *Synechococcus* sp.: scanning electron micrographs. **a** Specimens from Monterey Bay, California; **b–e** specimens from North Atlantic. **a** Three colonies of *Solenicola setigera* without *Synechococcus* sp. that display characteristic form in intercalary region of the centric diatom; long flagellum-like protuberances are present. **b** Colony of *Solenicola setigera* without associated *Synechococcus* sp.; reticulate nature of girdle bands of *L. mediterraneus* and extracellular polymeric matrix overlying them (*arrows*) is visible. **c** Two cells of *L. mediterraneus* with colonies of *Solenicola setigera* located in intercalary region and with associated *Synechococcus* sp.; valves of diatom are indicated by arrows. **d** Small colony of *Solenicola setigera* with few associated *Synechococcus* sp., some of which are dividing (*arrowhead*); matrix laid down by *Solenicola setigera* is evident (*arrow*). **e** Individual colony of *S. setigera* with many associated *Synechococcus* sp., some of which are dividing (*arrowheads*). (All scale bars = 5 μm)

The symbiosis between *Solenicola setigera* and *Synechococcus* sp. is also unusual in several aspects. Not all the chains of *Leptocylindrus mediterraneus* that are colonized by *Solenicola setigera* in a sample are in symbiosis with *Synechococcus*. All colonies of *Solenicola setigera* on a particular *L. mediterraneus* chain are consistent in this respect, however. Both *S. setigera* and *Synechococcus* sp. are widely distributed, but we only observed the high frequency of the symbiosis in one locale. Other symbioses between coccoid cyanobacteria and eukaryotes (e.g. tropical thecate dinoflagellates)

Fig. 3 *Leptocylindrus mediterraneus* with colonies of *Solenicola setigera* and associated *Synechococcus* sp.: transmission electron micrographs. **a** Slightly tangential section through cells of *L. mediterraneus*, *Solenicola setigera* and *Synechococcus* sp. **b** Longitudinal section of *L. mediterraneus* showing siliceous diatom cell wall with enclosed mitochondria (*M*), several *Solenicola setigera* cells, numerous cells of *Synechococcus* sp., and matrix in which *Solenicola setigera* and *Synechococcus* sp. are embedded. **c** Detail of mitochondria inside diatom, showing presence of vacuole (*V*) inside one of *Solenicola setigera* and several *Synechococcus* sp. embedded in extracellular matrix. **d** Two cells of *Solenicola setigera* and embedded *Synechococcus* sp. (All scale bars = 5 μm)

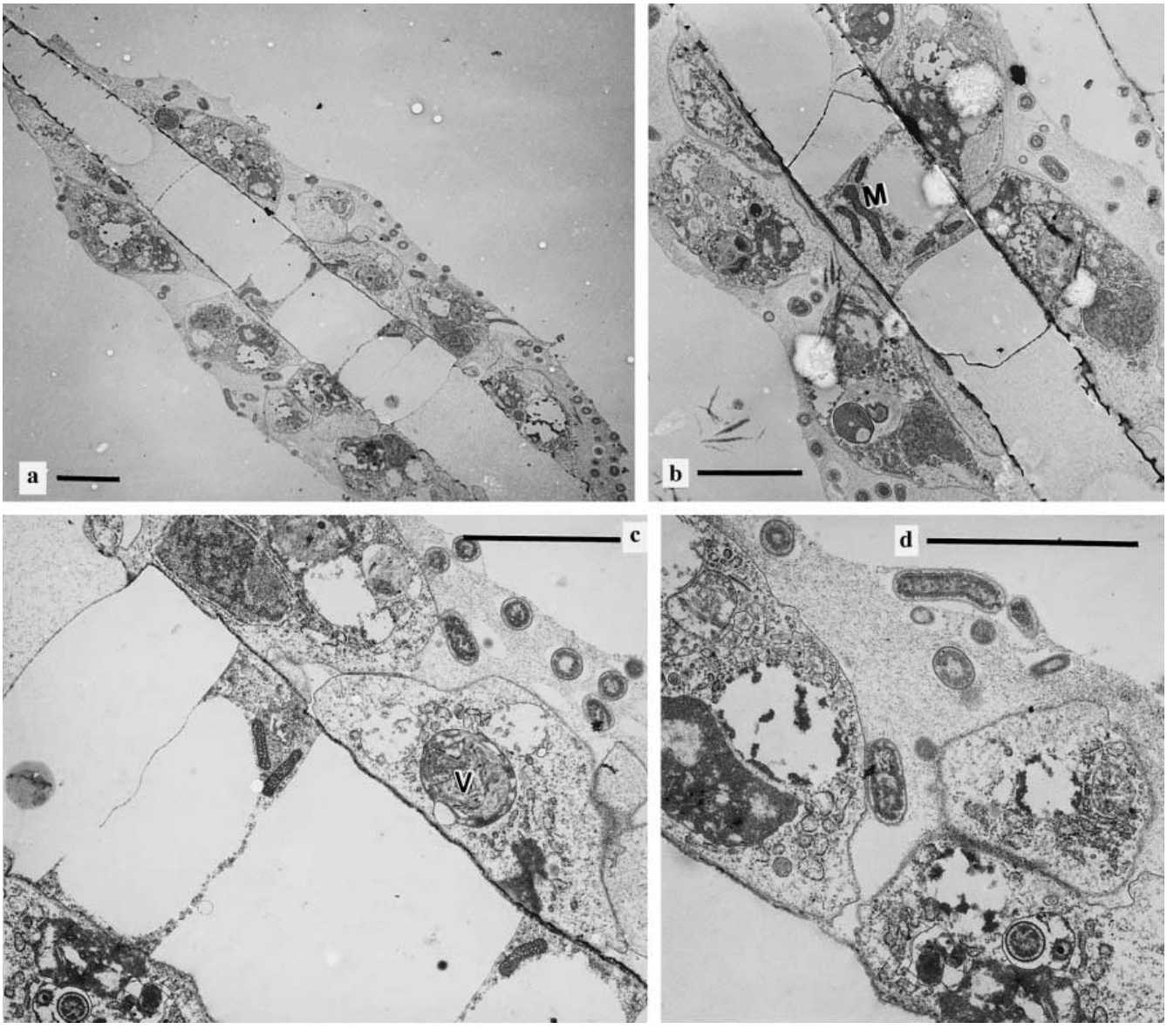


Table 1 *Solenicola setigera*, with and without associated *Synechococcus* sp. Mean (±SD) volume ($\times 10^4$ μm^3) of colonies from surface at seven stations in North Atlantic Ocean in 1993. At all stations, differences in measured volumes were significant at the listed *p* value when means were compared using a Students *t*-test assuming unequal variance in the two samples (*n* number of colonies measured; *na* not applicable

Station	With *Synechococcus* sp.	(*n*)	Without *Synechococcus* sp.	(*n*)	*p*
1	4.9 ± 2.6	(15)	3.2 ± 1.8	(17)	0.046
2	1.7 ± 0.6	(19)	0.7 ± 0.2	(11)	< 0.001
3	2.7 ± 1.7	(14)	0.9 ± 0.4	(17)	0.001
4	5.2 ± 3.3	(22)	1.3 ± 0.5	(14)	< 0.001
5	3.4 ± 1.1	(25)	1.2 ± 0.4	(12)	< 0.001
6	5.7 ± 2.3	(22)	1.2 ± 0.4	(5)	< 0.001
7	4.7 ± 2.5	(22)	na		na

(Lucas 1991; Janson et al. 1995) are obligate, to the point of the dinoflagellates evolving specialized morphology to accommodate the cyanobacteria (Lucas 1991). This is not the case with *L. mediterraneus* and *Synechococcus* sp., in which the relationship seems more facultative. Kaltenbock and Herndl (1992) reported an analogous situation of enhanced abundance and growth rate of *Synechococcus* sp. on the mucus surfaces of marine snow above that observed in the water column for free-living individuals.

One of the globally important attributes of some cyanobacteria is their ability to fix nitrogen. If nitrogen fixation were occurring in the *Synechococcus* sp. embedded in the extracellular matrix of the *Solenicola setigera*, then the association of *Synechococcus* sp. with the *Solenicola setigera* colonies would be a case of consortial symbiosis (Paerl 1996). The proximity of the *Synechococcus* sp. to *Solenicola setigera* and the fact that it is embedded in the same extracellular matrix suggest the existence of a permanently O_2-depleted microzone. The existence of microzones of O_2 depletion are important to cyanobacteria that are actively engaged in N_2-fixation (Paerl 1996). However, there is no evidence that oceanic *Synechococcus* sp. have the capability to fix nitrogen, that is, no cultures of oceanic *Synechococcus* sp. have been shown to possess nitrogenase (Waterbury and Rippka 1989). Reports to the contrary (Rippka et al. 1979; Mitsui et al. 1986; Waterbury et al. 1986) have since been shown to be not *Synechococcus* sp. or to be a sessile species of that genus (Waterbury and Rippka 1989). While we cannot rule out the possibility that the symbiont *Synechococcus* sp. in this case might be PCC 7335, the one strain shown to possess nitrogenase (Waterbury and Rippka 1989), it seems more likely that it is one of the locally abundant oceanic strains and, as such, is not a nitrogen-fixing organism. A definitive answer to this question would require properly preserved samples assayed for either nitrogenase activity (Capone 1993) or the presence of the *nif*H gene (Ben-Porath and Zehr 1994). Immunolabelling of the cyanobionts of the dinoflagellates *Ornithocercus* spp. for nitrogenase activity was negative (Janson et al. 1995).

The high abundance, generally large size and presence of dividing cells of *Synechococcus* sp. embedded in the extracellular matrix of *Solenicola setigera* and the increased size of the colonies of *S. setigera* with *Synechococcus* sp. argue for a mutually beneficial symbiosis. The advantages to *Synechococcus* sp. may be a decrease in grazing pressure by becoming, in effect, a larger particle, and the increased availability of nutrients

Table 2 *Solenicola setigera*, with and without associated *Synechococcus* sp. Abundance, biomass and relative biomass at surface of seven northern stations in the North Atlantic Ocean in 1993 (*Relative biomass* percent *Solenicola setigera* constituted of total heterotrophic protistan biomass; *n* number of colonies counted)

Station	Abundance (colonies l^{-1})	(*n*)	Biomass (μg C l^{-1})		Relative biomass (%)
			with *Synechococcus* sp.	without *Synechococcus* sp.	
1	3660	(360)	7.6	6.1	43
2	12040	(373)	13.7	3.6	33
3	6000	(600)	4.8	3.4	14
4	6170	(617)	15.7	4.0	72
5	6130	(613)	14.3	2.0	22
6	2340	(234)	11.5	0.5	48
7	6845	(1369)	30.9	0	93

Table 3 *Solenicola setigera*. Mean (±SD) and range (colonies l^{-1}) of abundance at stations in North Atlantic (excluding seven northern stations), Eastern Pacific, Indian and Western Pacific oceans (*n* number of stations where *S. setigera* was observed; *N Sta* total number of station/depths sampled)

Locality	Date	Depth (m)	Mean (±SD)	Range	(*n*)	*N* Sta
North Atlantic	July–Aug. 1993	0	337 ± 337	44–642	(14)	44
Eastern Pacific	Mar.–Apr. 1994	0	58 ± 116	5–125	(27)	46
Indian Ocean	Sep.–Oct. 1995	0	225 ± 340	5–1590	(24)	28
Indian Ocean	Sep.–Oct. 1995	> 0	158 ± 158	20–555	(27)	27
Western Pacific	Jan.–Feb. 1996	0	118 ± 142	10–400	(6)	15

in the immediate vicinity of the heterotroph. Presumably *Solenicola setigera* would be able to avail itself of some of the photosynthetically produced organic carbon much in the same way as ciliates with sequestered chloroplasts (Stoecker et al. 1987), or it may be harvesting the cyanobacteria directly. Vacuoles were observed in the *S. setigera*, some possessed cyanobacteria as well as eukaryotes. The thick extracellular matrix surrounding *S. setigera* would make capture and phagocytocysis of particles in the water impossible. Janson et al. (1995) conclude that the cyanobionts of *Ornithocercus* spp. may provide energy to the host dinoflagellate via release of dissolved organic carbon or may be directly phagocytized.

The contribution that nano-/microplankton made to the heterotrophic and total living carbon pools of the North Atlantic was highest at the northern stations and was due to the high abundance and increased size of *Solenicola setigera* (Buck et al. 1996) in this area. This contribution would not have been possible without the symbiosis. Unanswered questions regarding the enigmatic nature of the relationships between *Leptocylindrus mediterraneus* and *S. setigera* and between *S. setigera* and *Synechococcus* sp. remain. The nature of the association between *L. mediterraneus* and *Solenicola setigera* may be relatively easy to resolve given the cosmopolitan distribution of this symbiosis. Fresh samples, properly fixed and sectioned for TEM, would probably resolve the nature of the relationship between this heterotrophic protist and diatom. Even in the absence of nitrogen fixation by *Synechococcus* sp., this three-organism symbiosis is an interesting one, and along with the few other similar reports, it indicates the capacity of coccoid cyanobacteria to exploit potentially beneficial niches.

Acknowledgements We appreciate the assistance and help of the crew and officers of the National Oceanic and Atmospheric Administration's research vessels "Malcolm Baldrige" and "Discoverer". D. Garbary, J. Waterbury and two anonymous reviewers provided useful suggestions. This research was supported by NOAA award NA36GPO269 to F. Chavez.

References

Ben-Porath J, Zehr JP (1994) Detection and characterization of cyanobacterial nifH genes. App envirl Microbiol 60: 880–886

Buck KR, Ærtebjerg G, Larsen L, Thomsen HA (1992a) Biomass and abundance of pelagic cyanobacteria and protists from the transition area between the Baltic and North Sea, July 1990. In: Bjornestad E, Hagerman L, Jensen K (eds) Proceedings of the Twelfth Marine Biologists Symposium. Olsen & Olsen, Fredenborg, Denmark, pp 35–42

Buck KR, Bolt PA, Bentham WN, Garrison DL (1992b) Dinoflagellate cysts associated with Antarctic sea ice communities. J Phycol 28: 15–18

Buck KR, Chavez FP, Campbell L (1996) Basin-wide distributions of living carbon and the inverted trophic pyramid of the central gyre of the North Atlantic Ocean, summer 1993. Aquat microb Ecol 10: 283–298

Buck KR, Newton J (1995) Fecal pellet flux in Dabob Bay during a diatom bloom: contribution of microzooplankton. Limnol Oceanogr 40: 306–315

Capone DG (1993) Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Aquatic microbial ecology. Lewis Publishers, Boca Raton, pp 621–631

Cupp EE (1943) Marine plankton diatoms of the west coast of North America. Bull Scripps Instn Oceanogr (New Ser) 5: 1–238

Gran HH (1908) Diatomeen. In: Brandt K, Apstein C (eds) Nordisches Plankton: botanische. Lipsius & Tischer, Kiel, Germany

Hasle GR (1959) A quantitative study of phytoplankton from the equatorial Pacific. Deep-Sea Res 6: 38–59

Hasle GR (1975) Some living marine species of the diatom family Rhizosoleniaceae. Beih Nova Hedwigia 53: 99–140

Janson S, Carpenter EJ, Bergman B (1995) Immunolabelling of phycoerythrin, ribulose 1,5-biphosphate carboxylase/oxygenase and nitrogenase in the unicellular cyanobionts of *Orntihocercus* spp. (Dinophyceae). Phycologia 34: 171–176

Kaltenbock E, Herndl GJ (1992) Ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea. IV. Dissolved nutrients and the autotrophic community associated with marine snow. Mar Ecol Prog Ser 87: 147–159

Li C, Volcani BE (1987) Four new apochlorotic diatoms. Br phycol J 22: 375–382

Lucas IAN (1991) Symbionts of the tropical Dinophysiales (Dinophyceae). Ophelia 33: 213–224

Mitsui A, Kumazawa S, Takahashi A, Ikemoto H, Cao S, Arai T (1986) Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. Nature, Lond 323: 720–722

Paerl HW (1996) Microscale physiological and ecological studies of aquatic cyanobacteria: macroscale implications. Microscopy Res Tech 33: 47–72

Patterson DJ, Zölffel M (1991) Heterotrophic flagellates of uncertain taxonomic position. In: Patterson DJ, Larsen J (eds) The biology of free-living heterotrophic flagellates. Clarendon, Oxford, pp 427–476

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J gen Microbiol 111: 1–61

Stoecker DK, Michaels AE, Davis LH (1987) Large proportion of marine planktonic ciliates found to contain functional chloroplasts. Nature, Lond 326: 790–792

Taylor FJR (1982) Symbioses in marine microplankton. Annls Inst océanogr, Paris (NS) 58: 61–90

Waterbury JB, Ripka R (1989) The order Chroococcales Wettstein 1924, emend Rippka et al. 1979. In: Kreig NR, Holt JB (eds) Bergey's manual of systematic bacteriology. Williams & Wilkens, New York, pp 1728–1746

Waterbury JB, Watson SW, Valois FW, Franks DG (1986) Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. Can Bull Fish aquat Sciences 214: 71–120