

## The Consortium of the Protozoan *Solenicola setigera* and the Diatom *Leptocylindrus mediterraneus* in the Pacific Ocean

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**Summary.** The consortium of the colonial protozoan *Solenicola setigera* and the frustule of the diatom *Leptocylindrus mediterraneus* is common and wide-spread in the Pacific Ocean between 41°N and 34°S. In the oligotrophic waters of the open-ocean, the consortia tend to appear near the deep chlorophyll maxima with a low abundance (< 20 frustules l<sup>-1</sup>). These *Solenicola–Leptocylindrus* consortia comprised single or a few frustules, with the protozoan colony restricted to the central section of the frustule. In more eutrophic waters, the consortia reached densities up to 8000 frustules l<sup>-1</sup>, with chains of up to 2000 µm length and with a high percentage of the protozoan colonies spread along the entire frustule. No free-living specimens of *L. mediterraneus* were observed in this study. The frustule was devoid of cell contents and exhibited an abnormal morphology in its central section with the occurrence of certain structures, namely the “convex walls” and “growth bands.” The morphology of protozoan cells attached to the growth bands was highly flattened and differed from that in the rest of the diatom frustule. These features suggest that the protozoan maybe able to control the growth of the frustule, used by it as a substrate.

**Key words:** *Solenicola setigera*, *Leptocylindrus mediterraneus*, *Rhizomonas*, protist, protozoan, diatom, plankton, symbiosis, parasitism, Pacific Ocean.

### INTRODUCTION

Earlier studies on marine plankton have reported colonies of a protozoan with an unusual flagellum-like structure growing as an epiphyte in the empty frustule of a diatom (Gran 1908, Mangin 1912). Later Pavillard (1916) described the colonial protozoan as *Solenicola setigera* Pavillard and the empty frustules were identified as the centric diatom *Leptocylindrus* (= *Dactyli-*

*osolen*) *mediterraneus* (H. Peragallo) Hasle. No studies have ever reported the presence of either chloroplasts or protoplasm in the diatom frustule (Hasle 1975, Taylor 1982). Based on transmission electron microscopy, Buck and Bentham (1998) observed mitochondria inside the frustule, although it was uncertain whether they belonged to the protozoan or the diatom. Hasle (1975) observed that the flagellum-like structures of *Solenicola* rendered motility to the consortium. Based on scanning electron microscopy, Taylor (1982) described a mucous matrix covering the frustule. Buck and Bentham (1998) reported the cyanobacteria *Synechococcus* sp. and picoeukaryote cells to be embedded in the matrix of some of the larger chains of *Solenicola setigera*, presenting

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this consortium as a three-partner association. Buck and Bentham (1998) hypothesized that *Solenicola* could be favoured by the association with a nitrogen-fixing cyanobacteria.

Hasle and Syvertsen (1997) reported that the taxonomic position of *L. mediterraneus* was questionable. Patterson and Zölffel (1991) reported that the systematic position of the *S. setigera* is still unclear at the division or class level. Patterson *et al.* (1993) transferred *S. setigera* to the genus *Rhizomonas* Saville-Kent. Saville-Kent (1880) described the genus *Rhizomonas* as cells with one flagellum, pseudopodium and adherent to submerged objects in freshwater environments. The morphology of *Solenicola setigera* is well documented by light, scanning and transmission electron microscopy (Hasle 1975, Taylor 1982, Buck and Bentham 1998). In contrast, nothing is known about the species of *Rhizomonas*, never reported since the original description by Saville-Kent (1880). No morphological or phylogenetical study of the type of *Rhizomonas* exists. *Solenicola setigera* lacks the flagellum *sensu stricto* and pseudopodium and live exclusively attached to the same type of frustule in the oceanic water column. The transfer of *Solenicola setigera* to the genus *Rhizomonas* in this context is considered risky. The combination proposed by Patterson *et al.* (1993) is not adopted in this study.

The consortium of *Solenicola–Leptocylindrus* is ubiquitous in the world's oceans in the equatorial to Arctic and Antarctic waters (Fryxell 1989). Despite its ubiquitous distribution, there is a paucity of information on the basic biology of the *Solenicola–Leptocylindrus* consortium. This study fills this gap in knowledge by reporting the abundance and spatial and vertical distribution patterns of the consortium in several contrasting regions in both hemispheres in the Pacific Ocean between sub-arctic to the equator. The morphology of the frustule and the cells of *Solenicola* have been investigated to determine the nature of association and the mechanisms associated with growth and distribution of this successful consortium.

## MATERIALS AND METHODS

**Collection.** Samples were collected during 11 cruises in the Pacific Ocean. Two cruises were carried out on board R/V *Soyo Maru* (13–20 May and 3–10 July 2002) along the meridian 138°E in the vicinity of the Kuroshio Current (Fig. 1). Nine stations were sampled from 30°30'N to 34°15'N in May and 10 stations from 30°00'N to

34°20'N during the July cruise. At each station, 15 depths from 5–200 m were sampled. Sampling on board R/V *Hakuho Maru* in the Celebes, Sulu and South China Seas was carried out from 7 November to 18 December 2002. Samples were collected from 10 stations covering six depths between 0–150 m. A cruise on board R/V *Mirai* (15–28 January 2003) along the equator from 160°E to 160°W covered 14 depths between 0–200 m at each of the 9 stations sampled. Nine samples were collected off Oshima Island in Sagami Bay (34°39'N, 139°31'E) on board R/V *Seiyo Maru* (7 June 2003) between 5 to 100 m depth. Six cruises on board R/V *Oshoro Maru* and R/V *Wakataka Maru* were carried out respectively, at stations H (41°30'N, 145°47'E) and A7 (41°30'N, 145°30'E), in the Oyashio Current area during spring and summer of 2003. A cruise in the SE Pacific Ocean on board R/V *L'Atalante* from the Marquesas Islands Archipelago to the Chilean coast (26 October – 12 December 2004) involved sampling 14 stations at six depths ranging from 5–270 m (Fig. 1).

**Analysis.** Water samples at each station collected using Niskin bottles, were preserved with acidified Lugol's solution and stored at 5°C. Samples of 500 ml were concentrated by sedimentation in glass cylinders. During a six day settling period, the top 450 ml of the sample was progressively and slowly siphoned off with small-bore tubing. Fifty ml of the concentrate representing 500 ml whole water sample was settled in composite settling chambers. The entire chamber was scanned at 200× magnification under a Nikon or Olympus inverted microscope equipped with a digital camera.

The morphology of the *Solenicola–Leptocylindrus* consortium can be best described as a colony composed of a cluster of the protozoan cells occurring as an epiphyte on the frustule. A chain is normally composed of one to more than 20 frustules. The protozoan tends to appear in the central part of the girdle, occasionally extending to the entire frustule thus making it difficult to differentiate one frustule from another. The *Solenicola–Leptocylindrus* consortia were categorised as 'covered' and 'non-covered' frustules during enumeration. Frustules with a *Solenicola* colony restricted to the central section were considered as 'non-covered', while, those with the protozoan cells dispersed beyond the central section of the frustule or entirely covering it were classified as 'covered' frustules. In the first two cruises in the Kuroshio Current, the 'non-covered' and 'covered' frustules were not enumerated independently, although photomicrographs taken during enumeration revealed trends that were similar to those observed during subsequent cruises.

## RESULTS

### Morphology

**The frustule.** Morphologically, a *Solenicola–Leptocylindrus* consortium is composed of a cluster of *Solenicola* cells attached to the outer wall of a porous tube of silica considered as a diatom frustule. Based on light microscopy, no chloroplasts or other cell contents were observed inside the frustule. The frustule is subdivided into 3 sections. The central section, usually covered by the colonial protozoan, is delimited by two thin convex internal separations in the valvar plane here named

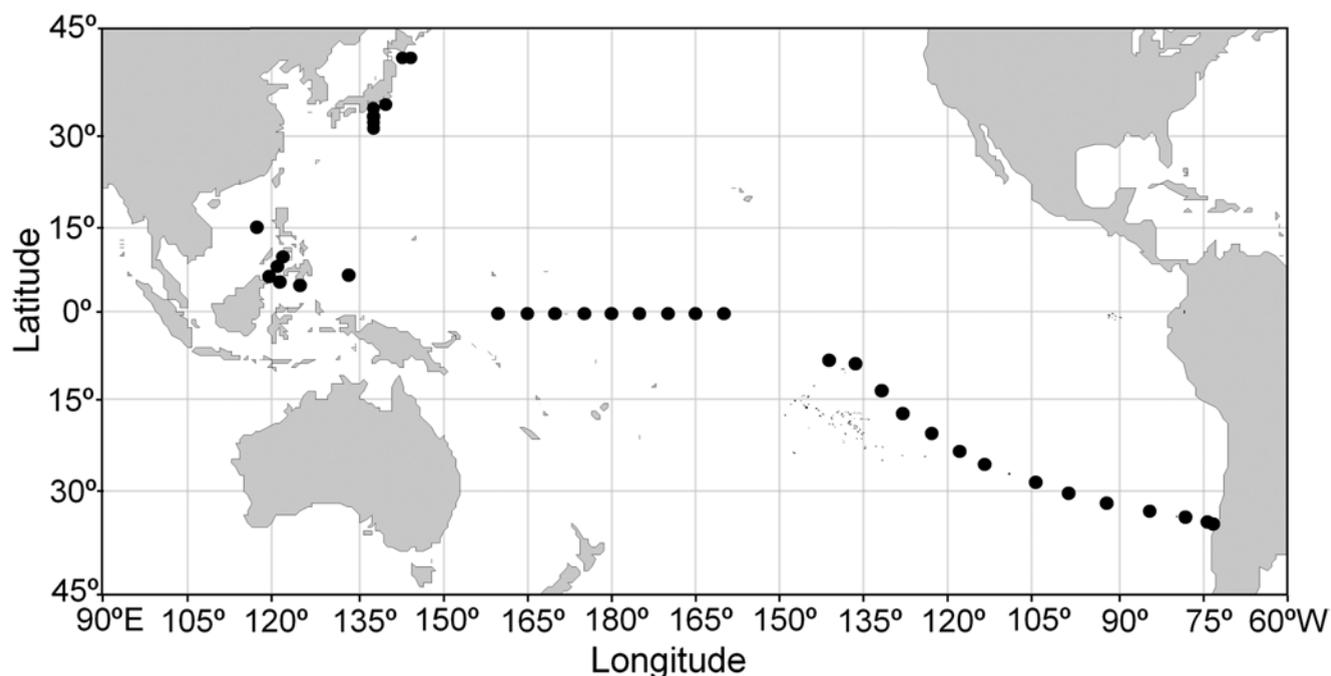


Fig. 1. Distribution of sampling stations (full circles) in the Pacific Ocean.

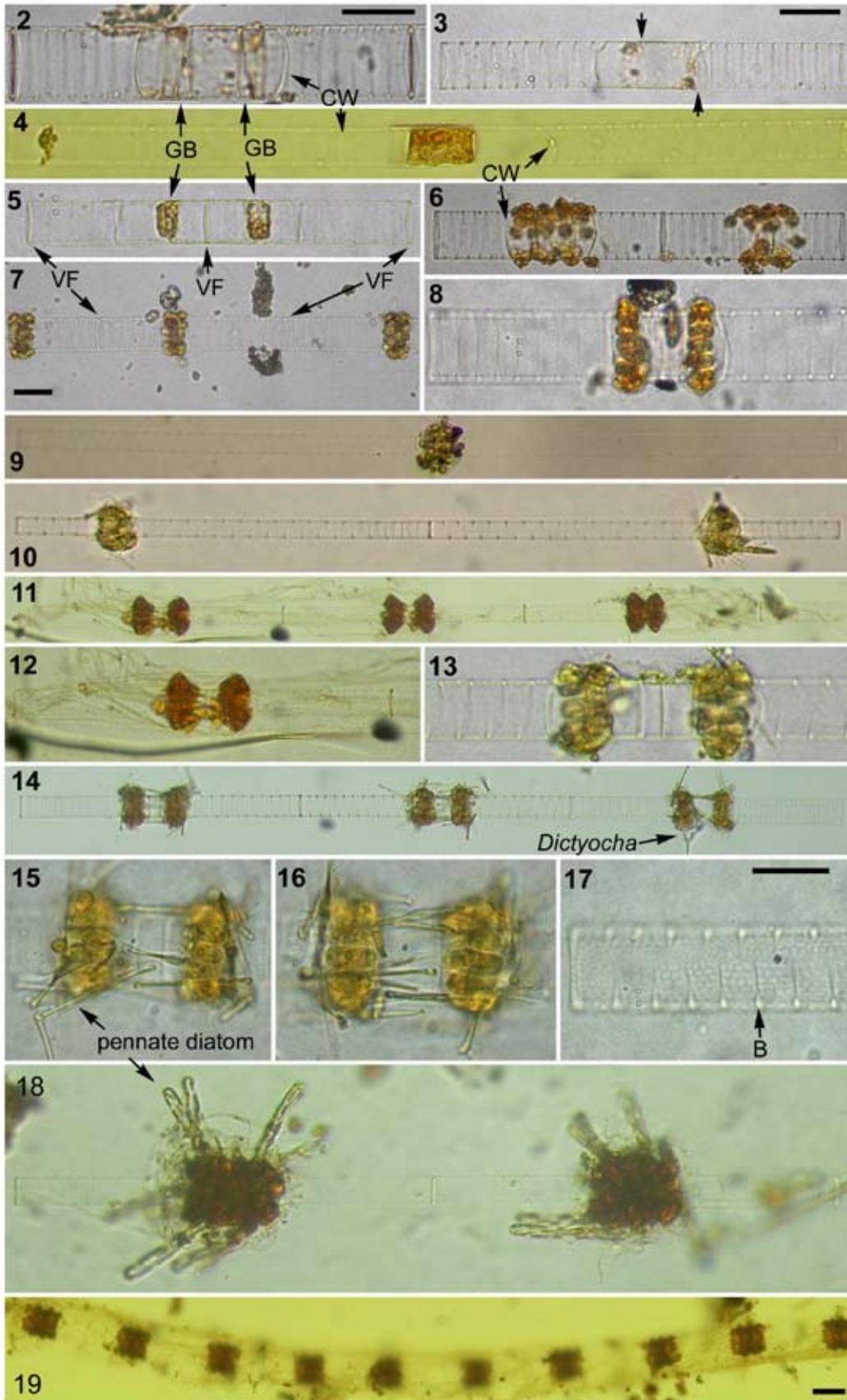
“convex walls” (Figs 2–6). The convex walls delimited a compartment inside the frustule. The inner positions of the central section had two thick modified band-like structures that encircled the frustule. These structures, named “growth bands”, are not visible because they are always masked by the protozoan that formed a crown of cells (Figs 2, 5–6). Rarely, when the usual protozoan cells are absent, it is possible to observe other highly flattened protozoan cells attached exclusively to the growth bands (Figs 4–5, 23).

The other two sections of the frustule extended beyond each convex wall. The frustule in the two lateral sections was perforated and composed of girdle bands (Fig. 13). The ends of the bands were wedge shaped (Fig. 17). The girdle bands in one lateral section were at 180 degrees with respect to the other frustule section. The frustule diameter ranged from 8–35  $\mu\text{m}$ , while the length was highly variable (Figs 2–19). Chains may be formed of several tens of frustules with lengths of up to 2000  $\mu\text{m}$  (Figs 19, 27). The valve faces of each frustule were either flat or slightly concave (Fig. 6).

**The protozoan.** *Solenicola setigera* is a colonial protozoan, growing epiphytically on the outer wall of the frustule. Colour of the Lugol-fixed cells is yel-

lowish (Figs 13, 24) or reddish (Figs 12, 26). Morphologically, the cells are either tear shaped globules (Figs 20–22, 25) or barrel-shaped (Figs 15–16). In some colonies, the protozoan is restricted to a single spot on the growth bands (Figs 2–3) or flattened cells covering the growth bands (Figs 4, 5). The cells are normally dispersed along the longitudinal axis of the frustule over the entire colony (Fig. 22), whereas the flattened cells oriented perpendicularly to the frustule axis (Fig. 23).

The protozoan cells had motile flagellum-like structures, apparently absent in other colonies (Figs 6–8). The flagellum-like structures were directed in the same direction, forming a tube covered by a tuft of hair-like structures (Figs 19, 27). The flagellum-like structures were more apparent in highly dense colonies (Fig. 24), with the exception of some curved colonies that were devoid of these structures (Fig. 29). Higher abundance of *Solenicola* coincided with longer chains and higher percentage of covered frustules. Red inclusions observed might be unicellular cyanobacteria attached to the mucous surface of the colony (Fig. 28). Also, pennate diatoms (Figs 14–18) and the silicoflagellate *Dicthyocha* sp. (Fig. 14) were found adhering to the protozoan colony.



After observing numerous specimens, certain patterns in the division of the *Solenicola–Leptocylindrus* consortium can be inferred. The protozoan cells attach to the outer surface of the frustule, between the convex walls forming a crown around the central section (Figs 6, 7). In dividing frustules, the protozoan clusters were observed to divide into two crowns and the new frustule appeared in the space between the two growth bands (Figs 8, 12–16, 43).

### Vertical and spatial distribution

**Kuroshio Current and north Philippine Sea.** The Kuroshio Current is one of the world ocean's prominent warm currents. Based on physical variables along the 138°E meridian to the south of Japan, it is spatially distinguished into three major regions (Figs 30–32). The slope waters, the Kuroshio Current and the offshore subtropical waters of the north Philippine Sea. The hydrographic conditions differed during the two cruises. In May, a cyclonic gyre in slope waters made the Kuroshio Current narrower than in the July cruise (Fig. 30). Chlorophyll *a* fluorescence was higher in the shallow waters and the surface waters of the slope, with chlorophyll *a* maxima at 70–80 m depth in the offshore subtropical waters (Fig. 30). In July, the Kuroshio Current was wider and warmer (Fig. 32). Fluorescence values were lower in the slope waters, when compared with that in May. South of the Kuroshio Current, the fluorescence profile showed deeper and thinner maxima at 90–100 m depth (Fig. 32).

In May, relatively higher fluorescence values in the slope waters were associated with higher densities of *Solenicola* (up to 1600 frustules l<sup>-1</sup>) (Fig. 31). Samples of the consortium collected showed frustules covered entirely with the protozoan. In the open-ocean oligotrophic waters, cell abundance decreased significantly (< 20 cells l<sup>-1</sup>), coinciding with the subsurface maxima (Fig. 31). In July, oligotrophic conditions prevailed and the abundance of *Solenicola* did not exceed 20 frustules l<sup>-1</sup>,

with the highest abundance below the deep chlorophyll maxima at 100–150 m depth. *Solenicola* was nearly absent in the surface waters (Fig. 33). Photomicrographs revealed dominance of 'non-covered' frustules in open waters.

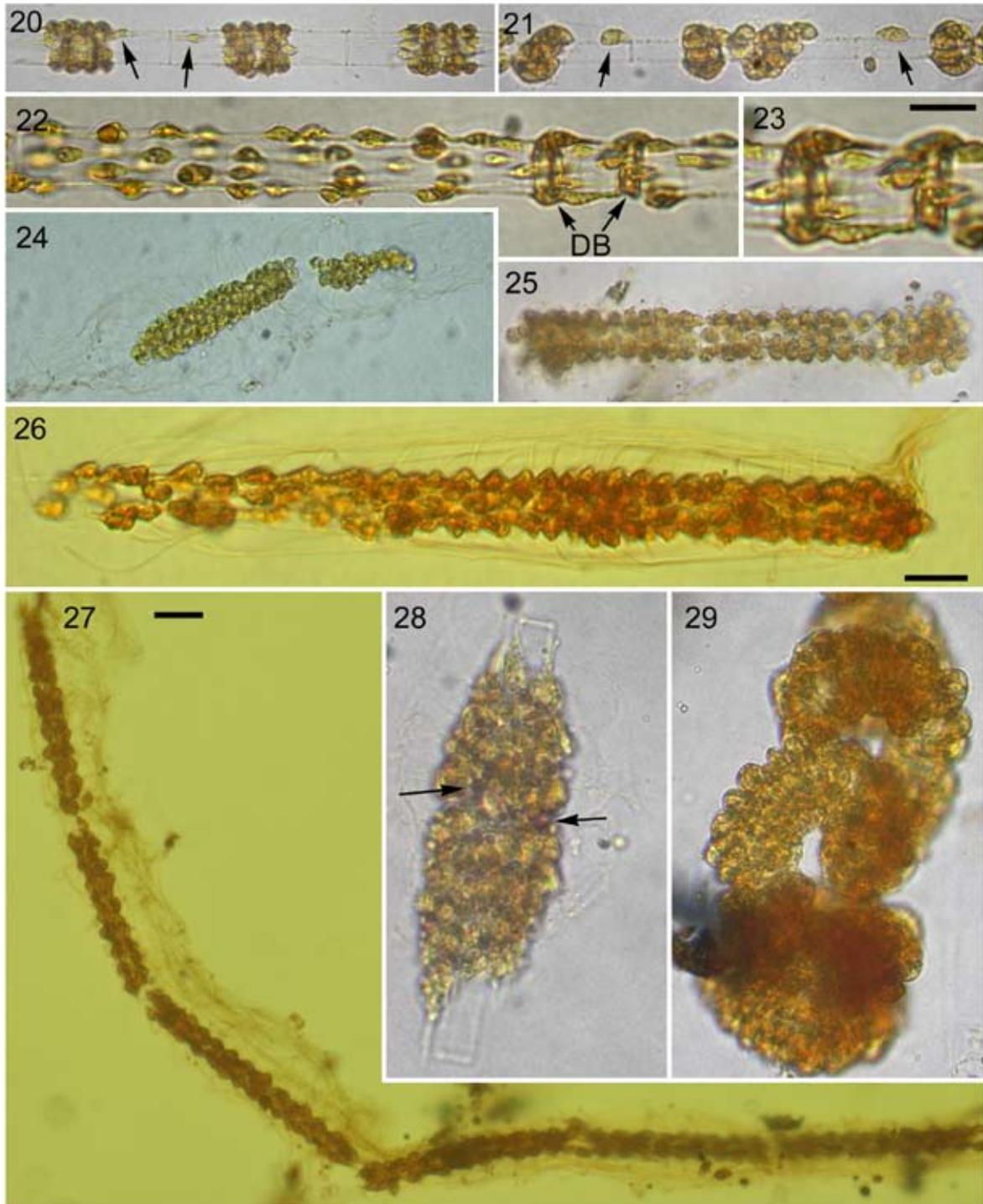
**Oyashio Current off Hokkaido.** Two stations in the subarctic waters of the Oyashio Current were sampled during the six cruises from spring to summer. The abundance of the *Solenicola–Leptocylindrus* consortia was low (< 8 frustules l<sup>-1</sup>), dominated by the 'non-covered' type.

**Celebes, Sulu and South China Seas.** The Celebes and Sulu Seas are one of the least investigated regions of the world ocean. Oligotrophic conditions prevailed in the open waters of the regional seas of the western Pacific Ocean. In the Celebes Sea, upwelling is induced by wind-driven surface water circulation through the Sibu-tu Strait that connects the Sulu and Celebes Seas. The ascent of the 28°C isotherm was associated with an increase in fluorescence at the surface (Fig. 34). In the northern Celebes Sea, surface fluorescence maximum coincided with the higher abundance of the 'covered' frustule of *Solenicola–Leptocylindrus* (80 frustules l<sup>-1</sup>) (Fig. 35). The 'covered' frustules also dominated the subsurface fluorescence maxima in the Sulu Sea. The 'non-covered' frustules were encountered between 50 and 150 m depth with densities not exceeding 50 frustules l<sup>-1</sup> (Fig. 36).

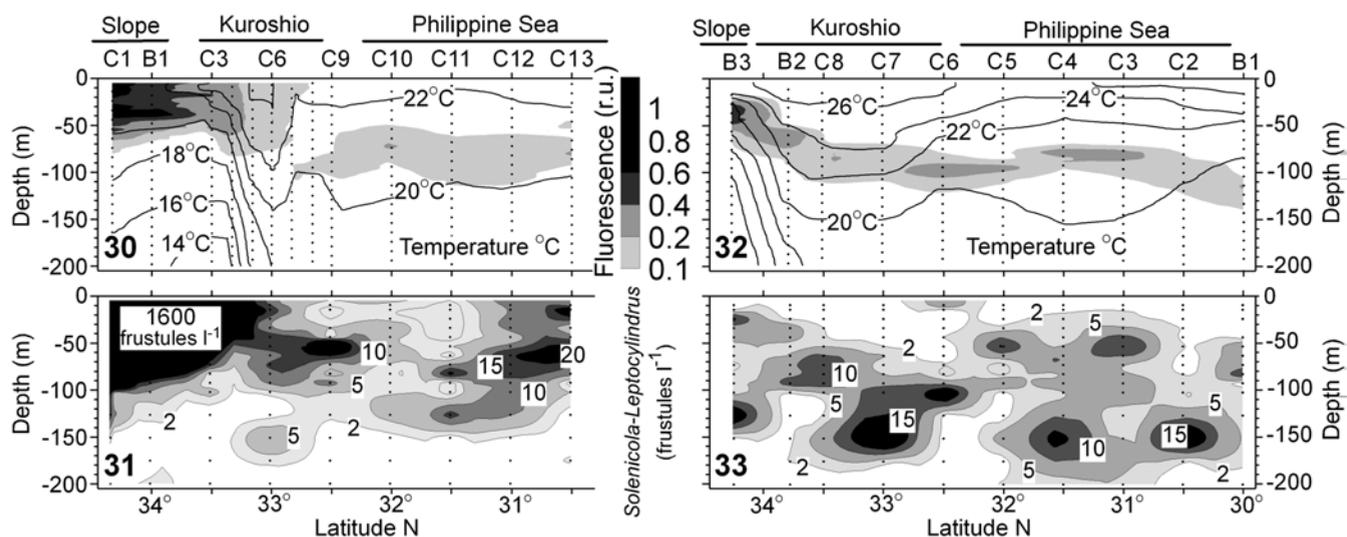
**Western and central equatorial Pacific Ocean.** A longitudinal transect of 4400 km along the equator between 160°E and 160°W was investigated coinciding with the El Niño–Southern Oscillation conditions. The Western Pacific Warm Pool showed a deep fluorescence maximum (90–120 m depth) (Fig. 37). Fluorescence values were higher in the shallow equatorial upwelling region (Fig. 37). The 'covered' frustules were absent in the western and central equatorial Pacific (Fig. 38), while the 'non-covered' frustules were encountered at all stations from 100 to 200 m depth with densities that did not exceed 15 frustules l<sup>-1</sup> (Fig. 39).

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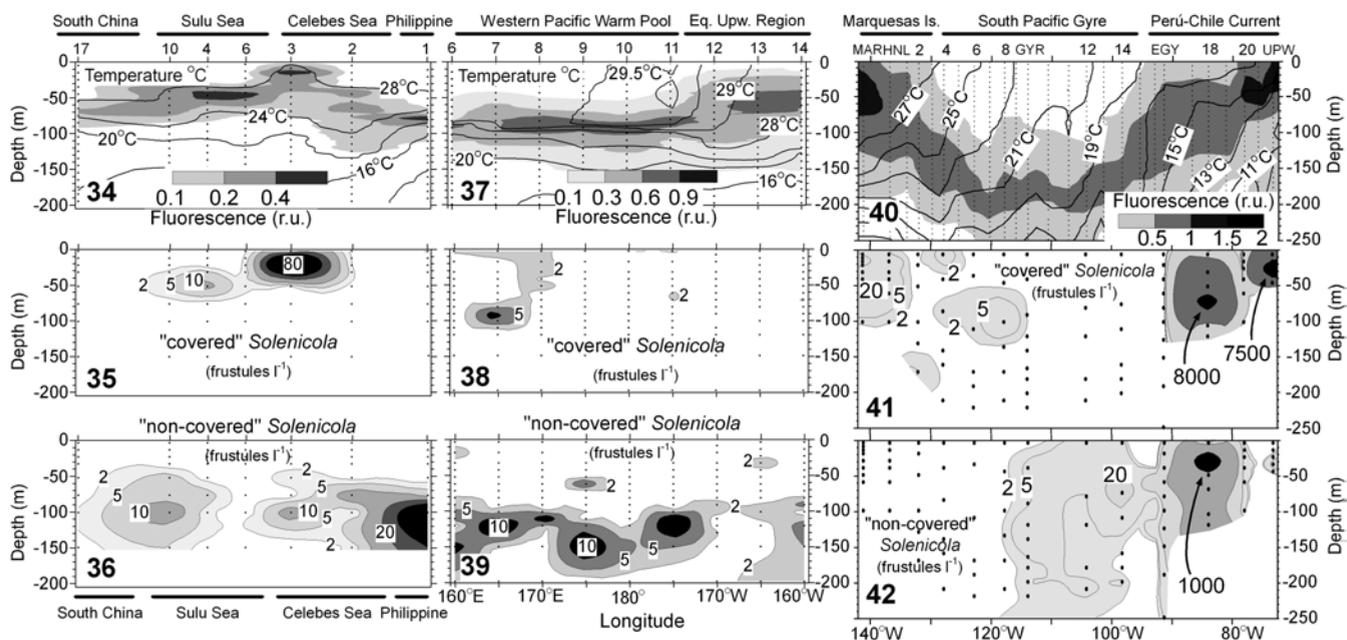
**Figs 2–19.** Photomicrographs of 'non-covered' *Solenicola–Leptocylindrus* frustules, bright field optics. **2** – See one frustule formed of convex walls and growth bands. **3** – The arrows indicate the reduced body of the protozoans. **4, 5** – The protozoan was flattened around the growth bands. **6, 7** – Protozoan colonies around the growth bands. **8** – Colonies separated in a frustule in division. **9, 10** – long frustules. **11–17** – Colonies in frustules under division. **14–18** – *Solenicola* colonies with pennate diatoms or the silicoflagellate *Dictyocha* sp. as epiphytes. **17** – See the girdle bands in the perforated frustule. **19** – Several tens of frustules. The flagellum-like structures surrounding the entire frustules. Geographic coordinates (latitude, longitude); depth (in meters) of the records: **2** – 7°N, 129°59'E, 100 m. **3** – 14°30'N, 118°E, 50 m. **4** – 0°, 180°, 70 m. **5** – 33°45'N, 138°E, 10 m. **6** – 7°N, 129°59'E, 100 m. **7** – 30°N, 138°E, 70 m. **8** – 30°N, 138°E, 80 m. **9** – 0°, 165° W, 150 m. **10** – 0°, 160°W, 150 m. **11, 12** – 0°, 160°E, 110 m. **13** – 0°, 175°W, 120 m. **14–17** – 0°, 165°E, 120 m. **18** – 34°39'N, 139°31'E, 75 m. **19** – 32°42'S, 84°04'W, 105 m. CW – Convex Wall, GB – Growth Band, B – Band, VF – Valve Face. Scale bars: 20 μm.



**Figs 20–29.** Photomicrographs of covered *Solenicola–Leptocylindrus* frustules, bright field optics. **20, 21** – Colonies of *Solenicola*. The arrows indicate several specimens that spread along the frustule. **22, 23** – *Solenicola* cells disperse along the entire frustule. The cluster of *Solenicola* in the central section of the frustule is lacking. The growth bands are covered with flattened protozoan cells. **23–27** – Frustules entirely covered of *Solenicola*. Note the development of the flagellum-like structures. **28** – A *Solenicola* colony with numerous reddish inclusions in the surface that may correspond to coccoid microalgae. **29** – A dense colony with curved axis lacking the flagellum-like structures; Geographic coordinates (latitude, longitude) and depth (in meters): **20** – 32°30'N, 138°E, 60 m. **21** – 32°30'N, 138°E, 70 m. **22–23** – 31°N, 138°E, 90 m. **24** – 0°, 170°E, 15 m. **25** – 5°N, 121°E, 30 m. **26** – 0°, 180°, 40 m. **27** – 32°42'S, 84°04'W, 120 m. **28** – 32°30'N, 138°E, 5 m. **29** – 33°45'N, 138°E, 20 m. GB – Growth Band. Scale bars: 20 µm.



**Figs 30–33.** Section plots of **30** – temperature (°C) and **32** – fluorescence (as relative units) in the grey scale. **31, 32** – Abundance of *Solenicola-Leptocylindrus* (frustules l<sup>-1</sup>). Left and right panels correspond to the distributions along the meridian 138°E in the south of Japan in May and July, respectively.



**Figs 34–42.** Section plots of **34, 37, 40** – temperature (°C) and fluorescence (as relative units) in the grey scale. **35, 38, 41** – Abundance of ‘covered’ *Solenicola-Leptocylindrus* (frustules l<sup>-1</sup>). **36, 39, 42** – Abundance of ‘non-covered’ *Solenicola-Leptocylindrus* (frustules l<sup>-1</sup>). Left panel: Celebes, Sulu and South China Seas; Central panel: western and central equatorial Pacific; Right panel: SE Pacific Ocean.

**South East Pacific Ocean.** The ~8000 km transect in the SE Pacific included several contrasting regions. Waters surrounding the Marquesas Islands Archipelago are influenced by high-nutrient, low-chlorophyll condi-

tions of the equatorial upwelling region. Central part of the transect along the South Pacific Gyre is principally oligotrophic, with a fluorescence maxima at a depth of 180–200 m (Fig. 40). Eastwards, the region influenced by

the Perú-Chile Current was characterised by a fluorescence maxima in the shallow depths, progressively increasing towards the region of upwelling off the Chilean coast (Fig. 40). The abundance of the 'covered' frustules was higher in the eutrophic waters surrounding Marquesas Islands Archipelago (25 frustules  $l^{-1}$ ). In the northern limit of the South Pacific Gyre, only a few 'covered' frustules were encountered. However in the central and southern limit of the gyre 'non-covered' frustules were found distributed in the entire water column. In the eutrophic waters of the Perú-Chile Current, the abundance of both, 'covered' (Fig. 27) and 'non-covered' (Fig. 19) frustules reached densities of 8000 and 1000 frustules  $l^{-1}$ , respectively. This abundance was dominated by long chains of several tens of frustules (Figs 41, 42) with long flagellum-like structures (Figs 19, 27). Data on picoplankton distribution from the cruise showed highest abundance of the *Solenicola*–*Leptocylindrus* consortia coinciding with peak abundances of *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryote cells numbering 110, 28 and 8 million of cells  $l^{-1}$ , respectively (O. Ulloa, personal communication).

## DISCUSSION

### Where are the live diatoms?

The precise identity of the *Solenicola*–*Leptocylindrus* consortium in the literature is still unresolved. The protozoan grows as an epiphyte on the empty frustule of a diatom, initially identified as *Dactyliosolen mediterraneus* and transferred to *Leptocylindrus* by Hasle (1975). However, Hasle and Syvertsen (1997) reported that the taxonomic position of *L. mediterraneus* was questionable. The frustules showed an abnormal morphology with unusual structures in the central section, here named convex walls and growth bands (Fig. 2), that are unknown in congeneric species or other diatoms. In addition, a mucous matrix is found covering the frustule (Taylor 1982, Buck and Bentham 1998). This mucous layer could hinder the exchange of gases and other substances in the case of a living diatom cell inside the frustule. All previous studies reported that the frustules were devoid of cell protoplasm (Hasle 1975, Taylor 1982). Based on the transmission electron microscopy, Buck and Bentham (1998) observed mitochondria inside the frustule, although it was uncertain

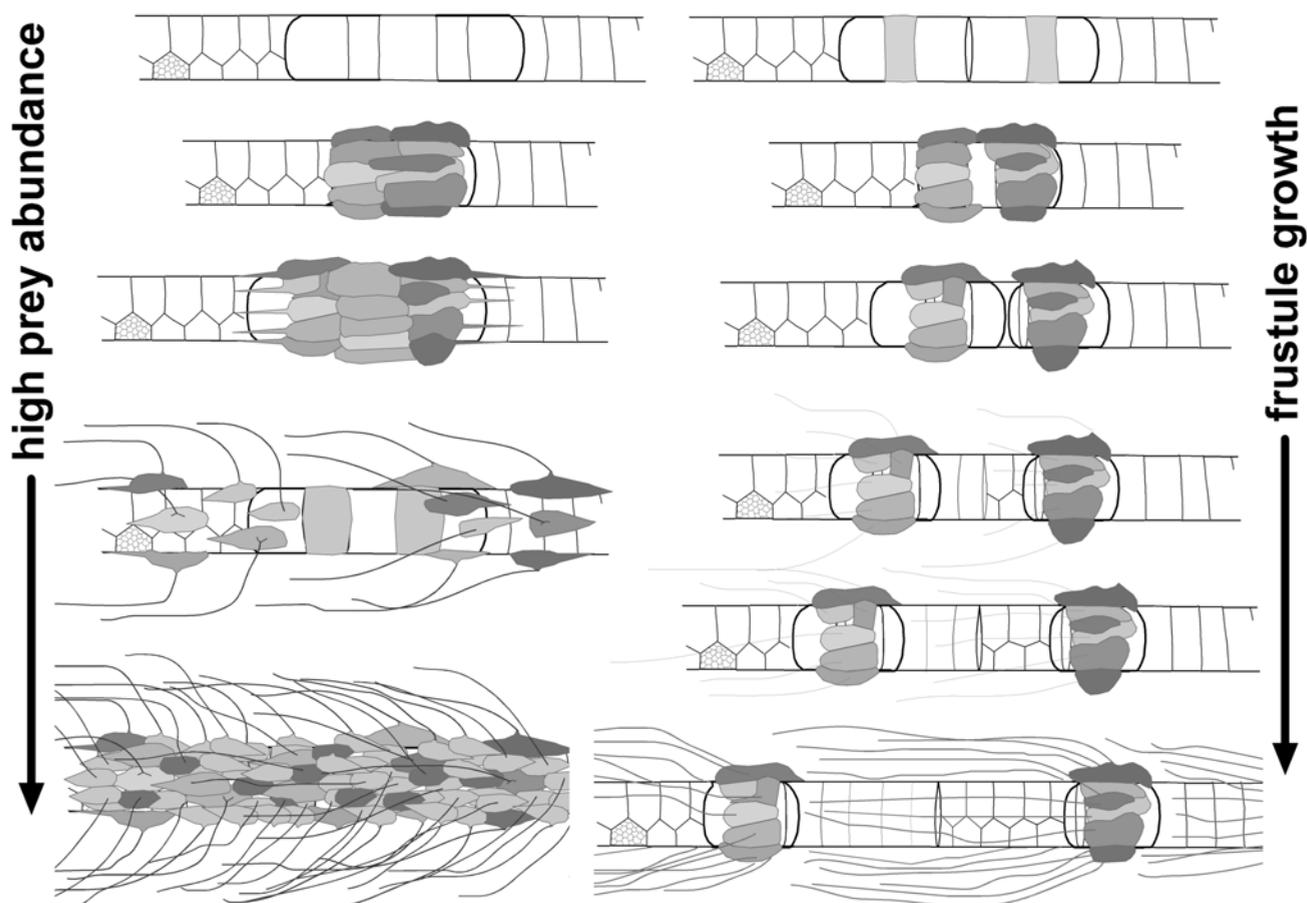
whether the mitochondria belonged to the protozoan or the diatom. In the present study, no free-living specimens of *L. mediterraneus* were recorded, while intact empty frustules of *L. mediterraneus* with *Solenicola* were encountered at nearly all the stations from the sub-arctic to the equatorial waters (Figs 30–42). A parasitic or symbiotic association requires both members to be viable at some stage of the life cycle. In this case, the diatom was never observed to be alive, either as a free-living cell or in consortium.

The frustule morphology seems to be modified to harbour the protozoan (Figs 2, 3). In the region of the growth bands the protozoan showed a flattened cell that differed from the morphology of the protozoan in other parts of the frustule (Figs 4, 5, 22). This modified morphology of the protozoan is often masked by the typical protozoan cells.

### The protozoan

*Solenicola setigera* did not show chlorophyll *a* under epifluorescence microscopy (Buck and Bentham 1998). The movement of the flagellum-like structures renders motility to the entire *Solenicola*–*Leptocylindrus* consortium as observed by Hasle (1975). Taylor (1982) and Buck and Bentham (1998) reported a mucous matrix covering the frustule. Buck and Bentham (1998) reported that the protozoan had vacuoles containing *Synechococcus* and picoeukaryotic cells. In the present study, the inclusions observed on the surface of the protozoan might have been captured microalgae (Fig. 28). These observations suggest that *Solenicola setigera* is a heterotrophic organism that captures pico- and small nanoplanktonic microalgae. It is unknown whether *Solenicola* uses the flagellum-like structures to create a feeding current towards the sticky mucous surface along the frustule. The frustule could provide a substrate for *Solenicola* to expand the mucous layer.

The *Solenicola*–*Leptocylindrus* consortia showed some trends in its distribution and morphology. The higher abundances appeared to be under eutrophic conditions, such as slope waters near Japan (Fig. 31). Highest abundance of the consortium was also found 1300 km off the coast of Chile. At this location, the abundance of large phytoplankton was relatively low, whereas the picoplankton (*Synechococcus* spp., *Prochlorococcus* spp. and picoeukaryote cells) showed a peak in abundance. Unicellular diazotrophic cyanobacteria can be found in the picoplankton (Zehr *et al.* 2001) and these may constitute part of the diet of *Solenicola*.



**Fig. 43.** Morphologies of the *Solenicola–Leptocylindrus* consortium. The left line drawings illustrate the tentative evolution towards “covered” frustules in high prey density environments. The right line drawings illustrate the evolution of the frustule growth.

Buck and Bentham (1998) presented this consortium as a three-partner association and hypothesized that *Solenicola* could be favoured by the association with a nitrogen-fixing *Synechococcus* sp. Symbiotic relationships of plankton with nitrogen-fixing cyanobacteria are favoured in nitrate-limited surface waters (Gómez *et al.* 2005). The *Solenicola–Leptocylindrus* consortia showed higher abundance in nutrient rich waters, or at subsurface depths in oligotrophic waters, generally unfavourable for the plankton-nitrogen fixing cyanobacterial associations.

Morphology of the colonies may vary with prevailing environmental conditions. In open-ocean oligotrophic waters, the consortia were formed of one or two frustules with the protozoan restricted to the central

section of the frustule. Higher abundance was found near the deep chlorophyll maxima where the availability of microalgal prey is expected to be higher. In eutrophic waters with tentatively higher abundance of picoplanktonic prey, *Solenicola–Leptocylindrus* formed larger chains and the protozoan exhibited a tendency to spread along the entire frustule (Figs 19, 27, 41–43). Later, under unfavourable conditions, the chains are expected to decompose. Smetacek (1985) described this mechanism as a seeding strategy that facilitates the dispersal of the colonial diatom species.

The mechanism of growth of the frustule in absence of the diatom contents remains an unsolved riddle. It is suggested that the protozoan might somehow be able to control the construction of the frustule, used by it as a

substrate. It is apparent that little is known about one of the most ubiquitous organisms of the world's oceans.

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