The nature of the diatom *Leptocylindrus mediterraneus* (Bacillariophyceae), host of the enigmatic symbiosis with the stramenopile *Solenicola setigera*

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**ABSTRACT**: The consortium between the colonial stramenople *Solenicola setigera* and the centric chain-forming diatom *Leptocylindrus mediterraneus* is cosmopolitan throughout the world ocean yet rarely abundant. However, the nature of the association remains enigmatic. A mutualistic symbiosis requires a live diatom host, but the frustule of *L. mediterraneus* is apparently empty, lacking protoplasm and plastids. The parasitism requires free-living host cells to be infected, but there is no evidence of populations of the free-living diatom. During experiments attempting to culture the heterotrophic *S. setigera*, we successfully obtained a strain of the host diatom. In the small-subunit (SSU) rDNA phylogeny, *L. mediterraneus* was closely related to *Dactyliosolen blivyanus* and to a new sequence of *Dactyliosolen* sp., while distantly related to the genus *Leptocylindrus*. We proposed the reinstatement of *L. mediterraneus* in the genus *Dactyliosolen* as *D. mediterraneus*. Even under optimal growth conditions, the frustule was nearly empty with reduced protoplasm concentrated in the middle. When compared with congenic species, *D. mediterraneus* showed a double-layered structure that acts as substrate for *Solenicola*. The free-living diatom lacked the convex walls that *D. mediterraneus* showed as host of *Solenicola*. The diatom in consortium with *Solenicola* maintained the photosynthetic machinery to eventually proliferate as a free-living organism. The ecological and morphological observations suggested that the diatom was successfully adapted to the mutualistic symbiosis with *Solenicola*. We discarded a parasitic relationship in this exceptional example of symbiosis.

**KEY WORDS**: Bacillariophyta, *Dactyliosolen*, DNA bar coding, Epibiosis, Epibiont, Molecular phylogenetics, Phoresy commensalism, Plankton, Parasitism, *Rhizomonas*, SSU rDNA, Symbiont, Symbioses

**INTRODUCTION**

The surface water of warm oceans, usually depleted in nutrients, is an unfavourable environment for the proliferation of diatoms. Regardless, several diatoms can proliferate in mutualistic symbioses with other plankton organisms. The diatoms *Rhizosolenia*, *Guinardia*, *Hemiaudus* and *Chaetoceros* are hosts of diazotrophic filamentous cyanobacteria (Vilarreal 1992; Gómez et al. 2005; Hilton et al. 2013). Diazotrophic coccolid cyanobacteria are found in tropical diatoms such as *Climacodium* (Carpenter & Janson 2000). Other diatoms have modified morphologies to host ciliates in a phoresy relationship (transport of one organism by another without physiological or biochemical dependence between them), such as *Chaetoceros tetrasichon* Cleve and *C. dadayi* Pavillard and the tintinnid *Eutintinnus inquinillus* (O.F. Müller) Schrank (Gómez 2007a) or *Chaetoceros coarctatus* Lauder and the peritrich ciliate *Vorticella oceanica* Zacharias (Nagasawa & Warren 1996).

The nature of the consortium of the centric, chain-forming diatom *Leptocylindrus mediterraneus* (H.Péragallo) Hasle and the colonial heterotrophic flagellate *Solenicola setigera* Pavillard [= *Rhizomonas setigera* (Pavillard) D.J.Patterson, K.Nygard, G.Steinberg & C.M.Turley] remains enigmatic. The mutualistic symbiotic consortia of diatoms with cyanobacteria or ciliates allow those to proliferate in the surface oligotrophic waters of the warm ocean. In contrast, the consortium of *Solenicola*–*Leptocylindrus* is widespread from polar to equatorial zones, from coastal to oceanic waters, and often reaches high abundances (Hasle 1976; Taylor 1982; Fryxell 1989; Buck & Bentham 1998; Gómez 2007b; Padmakumar et al. 2012). The most accepted view is that *Solenicola* is a highly adapted epizoic or parasitic organism; although, others have speculated that *Solenicola* is a stage of the diatom life cycle (Margalef 1969; Scott & Thomas 2005). With the aid of molecular biology, *S. setigera* has been reported as the first morphologically identified member of a ubiquitous and diverse clade of environmental sequences known as MAST3 (Marine Stramenopiles III) (Gómez et al. 2011). A second member of this clade was further identified, confirming the nature of this group as represented by unicellular heterotrophic organisms with a flagellum used to capture picoplankton and small nanoplanktonic prey (Cavalier-Smith & Scoble 2013).

The host diatom was first described as *Lauderia mediterranea* H. Péragallo 1888 from the Bay of Villefranche sur Mer, France, later proposed as *Dactyliosolen mediterraneus* (H.Péragallo) H.Péragallo 1892 and more recently as *L. mediterraneus* (H.Péragallo) Hasle 1975 (Péragallo 1888, 1892; Hasle 1975). The diatom is a widespread species in the world ocean (Hasle 1975, 1976; Fryxell 1989), and several studies have investigated its morphology using scanning electron microscopy (Hasle 1976; Taylor 1982; Buck & Bentham 1998; Gómez et al. 2011). The frustule possesses an...
unusual double-layered structure (Hasle 1975). Transmission electron microscopy revealed that the frustule was nearly empty and that the protoplasm with mitochondria occupied a very small part of the cell (Buck & Bentham 1998). However, it is uncertain whether the mitochondria belonged to Solenicola or the diatom. Based on light microscopy, the central section of the frustule, usually covered by Solenicola, is limited by two thin convex internal separations in the valvar plane that were named convex walls (Gómez 2007b). The convex walls enclosed the protoplasm inside the frustule when the diatom was colonised by Solenicola (Buck & Bentham 1998). These features, together with a double-layered structure, are unusual in diatoms, leading Hasle & Syvertsen (1997) to report that the taxonomic position of L. mediterraneus was questionable. Nanjappa et al. (2013) revised the genus Leptocylindrus from isolates of the Gulf of Naples. Leptocylindrus mediterraneus was found in field samples; although cultures were not established because only empty frustules colonised by Solenicola were observed (Nanjappa et al. 2013). Observations by epifluorescence microscopy of the Solenicola–Leptocylindrus consortium do not reveal the red fluorescence of the plastids as usual in diatoms (Buck & Bentham 1998; Gómez et al. 2011). Scott & Thomas (2005) reported free-living cells of L. mediterraneus. However, their images, based on light and scanning electron microscopy, correspond to another diatom. Padmakumar et al. (2012) cited the presence of live cells of L. mediterraneus but without reporting any evidence, such as a picture of the live cells, their protoplasm or plastids.

To the best of our knowledge, there is no information on the organelles (i.e. chloroplasts) or protoplasm of L. mediterraneus as a free-living organism or as host of Solenicola. Cultures have not been established, and there is no molecular information on this widespread diatom. This is an essential step to elucidate the nature of the relationship between Solenicola and Leptocylindrus. The parasitism requires the occurrence of a free-living host to be colonised or infected by Solenicola. However, there is no evidence of living individuals of L. mediterraneus. A mutualistic symbiosis requires a benefit for the diatom, but apparently L. mediterraneus is not alive when colonised by Solenicola.

In this study, we successfully established a culture of the host, the diatom L. mediterraneus, from an isolate obtained from a frustule fully covered by Solenicola. For the first time, the morphology of live specimens of L. mediterraneus and the molecular phylogeny is reported. This provides key information to understand the enigmatic nature of the Solenicola–Leptocylindrus consortium. We additionally report a new sequence of an unidentified species of the genus Dactyliosolen.

MATERIAL AND METHODS

Consortia of Solenicola–Leptocylindrus were gathered from samples collected daily from surface waters with a phytoplankton net (20-μm mesh size) off Ubatuba, São Paulo State, Brazil (23°31′27.80″S, 45°04′59.48″W), from January to December 2014. Aliquots of the net samples were left to settle in a composite settling chamber, examined with an inverted microscope (Nikon TS100, Nikon, Tokyo, Japan) and photographed with a digital camera (Cyber-shot DSC-W300, Sony, Tokyo, Japan) mounted on the microscope eyepiece. After being photographed, the consortium of Solenicola–Leptocylindrus was micropipetted individually with a fine capillary into a clean chamber and washed several times into a series of drops of 0.2-μm-filtered seawater to remove other organisms. The consortium was finally placed in a 12-well tissue culture plate with 0.2-μm-filtered and sterilised seawater supplemented with f/2 medium with silicates (Guillard & Ryther 1962) and incubated at 23°C, with 100 μmol photons m⁻² s⁻¹ from cool-white tubes; the photoperiod was 12:12 light:dark. The free-living diatoms were reisolated and placed into a six-well tissue culture plate and further in 50-ml polystyrene tissue culture flasks and cultured in the same conditions. In addition, one cell of Dactyliosolen sp. was found in September 2014. The cell was isolated and cultured as described above.

Additional light microscopic observations of the culture of L. mediterraneus were carried out at ×100 magnification using an Olympus BX51 epifluorescence microscope equipped with an Olympus DP72 camera (Olympus, Tokyo, Japan). Cells fixed with glutaraldehyde (5% final concentration) were stained with DAPI (4',6-diamino-2-phenylindole, Sigma, St. Louis, Missouri USA). Live cells were observed at ×63 magnification with an inverted confocal microscope Leica TCS SP8 AOBS microscope (Leica, Wetzlar, Germany).

To preserve the cells of L. mediterraneus and Dactyliosolen sp., glutaraldehyde (50% solution) was added to the culture to make a final concentration of 5%. Cells were filtered onto a 0.8-μm pore size Whatman Nuclepore membrane filter (Fisher, Pittsburgh, Pennsylvania USA), washed with distilled water, fixed with osmium tetroxide, dehydrated with a graded series of ethanol (30%, 50%, 70%, 90%, 95%, 99%, 100%) and critical-point dried with CO2. Filters were mounted on stubs, sputter coated with gold and viewed using a Phillips XL30 (FEI, Hillsboro, Oregon USA) scanning electron microscope.

For L. mediterraneus and Dactyliosolen sp., exponentially growing cultures were harvested by centrifugation, and the pellet was placed in a 1.5-ml Eppendorf tube filled with absolute ethanol. The sample was kept at room temperature and in darkness until the molecular analysis could be performed. Prior to polymerase chain reaction (PCR), the sample tube was centrifuged, and ethanol was evaporated by placing the tube overnight in a desiccator at room temperature; 100 μl of DNA lysis buffer (0.1 M EDTA pH 8.0, 1% SDS, 200 μg ml⁻¹ proteinase K) were used to resuspend the cell pellets, and the resuspension was transferred into a 1.5-ml tube. Genomic DNA was extracted from 250 μl using a standard phenol/chloroform protocol adapted from Sambrook et al. (1989). At the end of the extraction process, DNA was eluted in 30 μl of purified water. Fragments of the small-subunit (SSU) rDNA were amplified using the universal primers EukA and EukB (Medlin et al. 1988). PCR amplification was performed in a 25-μl reaction volume containing 0.5 units of Platinum Taq DNA polymerase (Thermo Fisher, Waltham, Massachusetts USA), 1.25× PCR buffer (supplied with the polymerase),
MgCl₂ at 2.5 mM, dNTPs at 0.25 mM and the forward and reverse primers at 0.2 μM. The PCR conditions were initial denaturation (94°C/4 min); 45 cycles of denaturation (94°C/45 s), annealing (50°C/30 s), and extension (72°C/1 min); final extension (72°C/7 min). PCR products were visualised on a 1% agarose gel stained with GelRed (Biotium, Hayward, California USA), purified enzymatically with FastAP Thermosensitive Alkaline Phosphatase and Exonuclease I (Thermo Fisher) and sequenced bidirectionally with an ABI3730xl sequencer (Macrogen Europe, Amsterdam, Netherlands) using the same primers as used for PCR. Sequence reads were aligned and assembled using the software ChromasPro 1.75 (Technelysium, Brisbane, Australia). The newly generated sequences were deposited in DDBJ/EMBL/GenBank under accession numbers KU577433–KU577435.

Matrices comprising SSU rDNA sequences, respectively, were assembled from most similar sequences identified using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Furthermore, available sequences of *Leptocylindrus* spp. and other centric diatoms were included (Ashworth *et al.* 2013; Nanjappa *et al.* 2013; Medlin 2016), and a sequence from the stramenopile *Bolidomonas pacifica* was used as outgroup. The computer program MEGA v6.05 (Tamura *et al.* 2013) was employed to align contigs using the CLUSTALW algorithm, to select the best-fit model of nucleotide substitution and to construct phylogenetic trees using the maximum likelihood (ML), neighbour-joining (NJ) and maximum parsimony methods. The model selection step, using a ML approach and the Akaike information criterion, indicated that the T92+G+I model was most appropriate. We therefore assumed this model for the ML tree search. In addition, a Bayesian inference of phylogeny was performed with MrBayes v3.2 (Huelsenbeck & Ronquist 2001), also assuming the T92+G+I model of nucleotide substitution, along with the following options: Yule tree prior, strict molecular clock, 10 million generations of Markov chain Monte Carlo, state sampling every 200,000 generations and the first 25% of the states discarded as burn-in.

**RESULTS**

**Morphology of *L. mediterraneus***

Our observations of the *Solenicola–Leptocylindrus* consortia off the coast of Ubatuba, Brazil, were rare and in all the cases corresponded to frustules fully covered by *Solenicola*. The diatom as a free-living organism was never detected. Our observations and previous studies based on light and epifluorescence did not reveal the presence of plastids in the *Solenicola–Leptocylindrus* consortium. We assumed that the diatom was dead. Our aim was to examine *Solenicola* under culture conditions, and we fed this heterotrophic protist with natural pico- and nanoplankton assemblages.

Filtered local seawater was enriched with f/2 medium without silicates, and some drops of seawater sample were added in order to grow natural pico- and small nanoplanクトon populations. These cultures were also used to attempt to culture heterotrophic planktonic organisms (flagellates, ciliates and dinoflagellates). Each consortium of *Solenicola–Leptocylindrus* was individually placed in this medium with natural pico-nanoplankton cells and kept in an incubator at 23°C under light or sometimes covered with an aluminium foil in order to reduce the growth of the potential prey.

Microscopic observations during the manipulation revealed that *Solenicola* detached from the diatom frustule. After 24 h, the culture plate was examined at low magnification (×10 or ×20) for the presence of the motile consortium of *Solenicola–Leptocylindrus*, *Solenicola* was not observed, and an apparently empty diatom frustule remained at the bottom of the culture plate. The culture was then discarded without any further observations because the objective had not been accomplished.

A consortium of *Solenicola–Leptocylindrus* observed on 25 February 2014 (Figs 1, 2) was treated with a different protocol. This consortium was considered to be a photosynthetic organism and placed in 0.2-μm-filtered and sterilised seawater supplemented with f/2 medium and incubated at 23°C with a photoperiod of 12:12 light:dark. We expected a single or broken frustule of *L. mediterraneus* free of *Solenicola*; however, a group of colonies of a diatom that coincided with the morphology of *L. mediterraneus* was observed. At higher magnification, the frustules were nearly empty with small amounts of plastid-bearing protoplasm. The cells were isolated into new clonal cultures under the same culture conditions. The growth of this diatom was slower when compared to other diatoms under similar conditions. A second consortium of *Solenicola–Leptocylindrus* was isolated from plankton samples in April 2014 and isolated for culture with no success. No further consortia were found in the plankton samples.

*Leptocylindrus mediterraneus* showed different forms in batch culture. During the first week, after the inoculation of a few cells, *L. mediterraneus* showed tightly formed, straight chains that reached up to eight cells. Despite the optimal growth conditions, the inner frustule remained nearly empty, with reduced protoplasm in the middle of the frustule coinciding with the double-layered structure (Figs 3–6). The protoplasm was displaced to one of the valve faces after each division (Figs 5, 19). Then the double-layered structure appeared in that valve face, and after the extension of the frustule on that side, the double-layered structure and the protoplasm returned to appear in the middle of the frustule (Figs 16–18). The protoplasm was connected to each valve face with two thin filaments (Fig. 6). In some cases, the proximal parts of the filaments were pigmented (Fig. 4). A small nucleus was visible by DAPI staining (Figs 7–10). Each cell showed 5–10 ellipsoid plastids (Figs 11, 12). There was no evidence of the convex walls inside the frustule that has been reported when *L. mediterraneus* was colonised by *Solenicola*. After 2 wk, single- or two-celled colonies of *L. mediterraneus* were dominant, and curved frustules began to appear, sometimes forming sigmoidal colonies. The protoplasm possessed a single nonpigmented filament towards the valve face, and the cell showed a large vacuole (Figs 16–19). In the senescent phase, some of the curved cells showed more intense pigmentation that covered almost the entire frustule (Fig. 13). Round protoplasmic masses, tentatively identified as auxospores, were observed (Figs 13–15).
Figs 1–21. Light micrographs of *Leptocylindrus mediterraneus* (Figs 1–19) and *Dactyliosolen* sp. (Figs 20, 21) from Ubatuba, Brazil. Scale bars = 10 μm.

Figs 1, 2. Consortium of *Solenicola-Leptocylindrus* isolated for the strain culture.

Figs 3, 4. Culture of *L. mediterraneus* in exponential growth phase. The arrow points the pigmented proximal part of the filaments.

Figs 5, 6. Cells in stationary phase. The arrow points two filaments from the protoplasm to the valve faces.

Figs 7–10. Cells stained with DAPI. The blue area corresponds to the nucleus.

Fig. 11. Epifluorescence image of the plastids.

Fig. 12. Plastids release from the protoplasm.

The culture of *L. mediterraneus* was examined by scanning electron microscopy (Figs 22–27). The ends of the girdle bands were wedge shaped and formed a straight line (Figs 22–24). The width of the girdle bands was different between cells of the same culture (Fig. 22) and in the same cell (Fig. 23). As a general trend, there were four or five half bands in 10 μm. However, this number can be variable in the same cell (Figs 23, 24). The cells showed the double-layered structure in the middle of the frustule surface (Figs 22, 23) or displaced towards one side, depending of the growth stage (Fig. 24). This double-layered structure was a unique feature of this species. In senescent cultures, the frustules were more elongated, more or less curved, and the double-layered structure covered a larger proportion of the frustule (Fig. 25). The valve face showed a short labiate process displaced from the centre, eccentric but not marginal (Figs 26, 27).

During plankton observations in September 2014, a cell of an unidentified species of the genus *Dactyliosolen* was observed (Fig. 20). As in the culture of *L. mediterraneus*, this diatom showed the protoplast concentrated to the middle of the cell (Fig. 20). *Dactyliosolen* sp. was isolated and cultured in order to increase the representation of the genus *Dactyliosolen* in the SSU rDNA molecular phylogeny. As in the case of *L. mediterraneus*, even under optimal growth conditions, the protoplast was reduced and concentrated to the middle (Fig. 21). The cells were straight, 20–50 μm in diameter and 60–120 μm in the pervalvar axis. The valve faces were flat (Figs 20, 21); whereas, in *L. mediterraneus* the valve faces were convex (Figs 17–19). Using scanning electron microscopy, the ends of the girdle half bands were wedge shaped and formed a straight line (Figs 28, 29). The cells showed four to five girdle bands in 10 μm (Figs 28, 29). The valve face showed a short marginal labiate process (Fig. 30). The double-layered structure was not observed. The morphology of this species is close to *D. blavynus* (H. Péragallo) Hasle.

**Molecular phylogeny**

We obtained the SSU rDNA sequences (1685 base pairs) of *L. mediterraneus* and *Dactyliosolen* sp. A BLAST search was conducted on the new sequences to find related sequences in GenBank database. The new sequence of *L. mediterraneus* (KU577433) was closely related to *Dactyliosolen blavynus* (KC309491, 99% sequence identity) and the new sequence of *Dactyliosolen* sp. (KU577434–KU577435, 99% sequence identity). The new sequence of *L. mediterraneus* was distant related to the type of the genus *Leptocylindrus*, *L. danicus* Cleve (KC814808, 84%). In the molecular phylogeny, *L. mediterraneus* and *Dactyliosolen* spp. formed a strongly supported clade (Fig. 31). Two sequences of environmental clones (SGYO1439, SGOY1107) from the Gulf Stream surface waters formed a sister clade with *L. mediterraneus*/*Dactyliosolen* sp. These sequences formed a sister clade with *Chaetoceros peruvianus* Brightwell/C. rostratus Launder and C. muelleri Lemmermann/C. calcitrans (Paulsen) Takano. All these species formed a highly supported clade together with the clades of *Acanthoceras* sp., *Urosetonia eriensis* (H.L. Smith) Round & R.M. Crawford, *Hemiaulus* spp., *Eucampia* spp. and *Cerataulina* spp. The sequences of *Dactyliosolen* spp. branched with other radial centric diatoms and were distantly related to the clade of *L. mediterraneus/Dactyliosolen* sp. (Fig. 31). Assuming that *D. blavynus* and *Dactyliosolen* spp. are truly representatives of the genus *Dactyliosolen*, we proposed the reinstatement of *L. mediterraneus* under the genus *Dactyliosolen* as *D. mediterraneus* (H. Péragallo) H. Péragallo.

**DISCUSSION**

**Identity of *D. mediterraneus***

The molecular phylogeny revealed that *L. mediterraneus* should no longer belong to the genus *Leptocylindrus*. Until now, the genus *Dactyliosolen* is represented in the GenBank database only by the sequence of *D. blavynus* (Ashworth et al. 2013; Medlin 2016). The molecular phylogeny revealed that *L. mediterraneus* should no longer belong to the genus *Leptocylindrus*. The sequence of the type species, *D. antarcticus* Castracane, is not available. However, the resemblance of *D. antarcticus*, *D. blavynus* and *D. mediterraneus* supports their placement within the same genus.

The species *D. antarcticus* and *D. tenuijunctus* (Manguin) Hasle showed the girdle band ends in an oblique line; whereas, *D. mediterraneus* and *D. blavynus* showed the band ends in a (nearly) straight line (Hasle & Syvertsen 1997). Von Stosch (1986) illustrated the life cycle of *D. blavynus*. He reported that *D. blavynus* is one of the two centric diatoms known to me . . . in which only the region of the thecal junction, where the girdles overlap, seems to be “inhabited” by the protoplast, though sometimes “pseudopodia”, with or without plastids, extend to neighbouring regions of the cincture. Hasle & Syvertsen (1997) reported that *‘Dactyliosolen blavynus* differs from the other species of the genus by being the only one known to form (endogenous) resting spores by having the protoplasm concentrated to the middle of the cell (von Stosch 1986).

The protoplasm and the filaments, named ‘pseudopodia’ by Stosch (1986), are a common feature for *D. mediterraneus*, *D. blavynus* and *Dactyliosolen* sp. (Figs 4, 21). Hasle (1976) reported that valves of *L. mediterraneus* mounted in a medium of a high refractive index are recognised by their coarse, or double-layered, structure. Ashworth et al. (2013) provided the sequence and light and scanning electron microscopic pictures of *D. blavynus* (available at http://www.protistcentral.org/ Taxa/get/taxa_id/585789). Their pictures show that *D. blavynus* lacks the double-layered structure that characterises *D. mediterraneus* and possesses a marginal labiate process. In contrast, the labiate process of *D. mediterraneus* is eccentrically located but not marginal (Figs 26, 27). The valve face of

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Figs 16–19. Cells observed with confocal microscopy. Red areas correspond to chlorophyll fluorescence. The arrowhead points the large vacuole. The protoplasm possesses a single filament towards each valve face. Note the slightly convex valve faces.

Fig. 20. Cell of *Dactyliosolen* sp. isolated for the strain culture.

Fig. 21. Cultured cells of *Dactyliosolen* sp. Note the flat valve faces.
Figs 22–30. Scanning electron microscopy of *Leptocylindrus mediterraneus* (Figs 22–27) and *Dactyliosolen* sp. (Figs 28–30) from Ubatuba, Brazil. Scale bars = 10 μm, except Figs 26, 27 and inset of Fig. 30 = 1 μm.

Figs 22, 23. The arrowheads point the double-layered structure in the middle of the frustule.

Fig. 24. Double-layered structure close to the valve face. Note the different width of the girdle bands between different cells (Fig. 22) and in the same cell (Figs 23, 24).

Fig. 25. The double-layered structure covers most of the cell.

Figs 26, 27. The arrowhead points the labiate process in the valve face of *L. mediterraneus*.

Fig. 28. Two-celled chain of *Dactyliosolen* sp.

Fig. 29. Detail of the girdle bands.

Fig. 30. The arrowhead points the labiate process in the valve face. The inset shows the labiate process.
*D. blavyanus* is flat as well in *Dactyliosolen* sp. (Figs 20, 21) while convex with slightly rounded edges in *D. mediterraneus* (Figs 17–19).

Under routine phytoplankton microscopic analysis, *D. mediterraneus* devoid of *Solenicola* can be misidentified with other species of *Dactyliosolen*. The number of girdle bands in 10 μm is similar in *D. mediterraneus*, *D. blavyanus* (four to five bands), *Dactyliosolen* sp. (four to five), lower in *D. antarcticus* (two to three) and higher in *D. tenuijunctus* (five to nine) (Table 1). *Dactyliosolen blavyanus* is more commonly found in warm waters (Hernández-Becerril et al. 2010; Ashworth et al. 2013). *Dactyliosolen antarcticus* and *D. tenuijunctus* are common in Antarctic waters, and *D. mediterraneus* shows a cosmopolitan distribution (Hasle...
The nature of the symbiotic consortium

Solenicola setigera is an independent organism phylogenetically unrelated to the diatoms (Gómez et al. 2011; Cavalier-Smith & Scole 2013). In this study, we contributed new data on the diatom to elucidate the nature of this enigmatic symbiotic consortium. The heterotrophic Solenicola needs a substrate to develop its colony. Many centric diatoms have the same size and shape of D. mediterraneus. However, Solenicola develops only on D. mediterraneus. Solenicola often forms highly dense colonies with a mucilaginous layer on the diatom frustule (Taylor 1982; Buck & Bentham 1998; Gómez et al. 2011). For any other diatom species, the colonization by Solenicola will be a barrier for gases and nutrient exchange through the frustule. Under these conditions, any other diatom will be unable to carry out photosynthesis and will finally die. If the diatom dies, the diatom colony decomposes, the silica of the frustule will dissolve and Solenicola will need to look for another host. Dactyliosolen mediterraneus possesses reduced protoplasm and apparently lacks chlorophyll when colonised by Solenicola. However, D. mediterraneus is alive and able to maintain the frustule that is the substrate for the colony of Solenicola. There are no free-living cells of D. mediterraneus in the surrounding waters. Consequently, Solenicola does not need to look for another host if D. mediterraneus is alive.

The diatom D. mediterraneus did not show chlorophyll when colonised by Solenicola (Buck & Bentham 1998; Gómez et al. 2011). However, this study provides evidences that D. mediterraneus remains alive in the frustules fully colonised by Solenicola. Dactyliosolen mediterraneus possesses a double-layered structure that covers the reduced protoplasm. The cells of the colony of Solenicola are preferentially placed on the double-layered structure. Dactyliosolen mediterraneus is not found as a free-living organism, except in our culture under laboratory conditions. When compared to other diatoms, the slow growth of D. mediterraneus could be attributed to its low quantity of protoplasm (and low number of plastids). The protoplasm and number of plastids do not extend inside the frustule even under optimal growth conditions. Consequently, D. mediterraneus with reduced protoplasm is able to provide a wide substrate for Solenicola. The morphology of D. mediterraneus is different when it acts as host of Solenicola or as a free-living organism. In consortium with Solenicola, D. mediterraneus does not produce chlorophyll, and it possesses convex bands inside the frustule enclosing the protoplasm (Buck & Bentham 1998, fig. 3; Gómez 2007b). That structure is unknown in other diatoms. It is uncertain whether D. mediterraneus maintains its metabolism from resources provided by Solenicola. Solenicola setigera needs D. mediterraneus and vice versa because they are not found living independently in natural conditions. The relationship is successful because they are widely distributed throughout the world’s oceans. Epibiosis was defined as a symbiosis in which one organism lives on the outer surface of another (Lincoln et al. 1982). A simple epibiosis does not explain the success of Solenicola–Dactyliosolen. The nature of the association is a phoresy commensalism because the flagellum of Solenicola renders motility to the consortium. Phoresis is defined as the provision of transport, shelter or other support by one organism (the carrier or host) to another organism without metabolic exploitation or exchange (Kinne 1980). We have no evidence that D. mediterraneus uses its photosynthetic capacity when behaving as host of Solenicola. It is uncertain whether D. mediterraneus may benefit from exudates provided by Solenicola. With the current evidence, we discard a parasitic relationship, and we suggest that the consortium Solenicola–Dactyliosolen could be an example of a unique and widespread mutualistic symbiosis.

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REFERENCES


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