

Article

Symbioses of Ciliates (Ciliophora) and Diatoms (Bacillariophyceae): Taxonomy and Host–Symbiont Interactions

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Abstract: The nature of the plankton symbioses between ciliates and diatoms has been investigated from the tropical South Atlantic Ocean, and Mediterranean and Caribbean Seas. The obligate symbioses of the diatoms *Chaetoceros dadayi* or *C. tetrastichon* with the tintinnid *Eutintinnus* spp., and *Chaetoceros coarctatus* with the peritrich ciliate *Vorticella oceanica* are the most widespread, and the consortium of *Chaetoceros densus* and *Vorticella* sp. have been rediscovered. Facultative symbioses between *Eutintinnus lususundae* and *Chaetoceros peruvianus*, *Hemiaulus* spp., and *Thalassionema* sp. are less frequent, often containing three or four partners because *Hemiaulus* can also harbor the diazotrophic cyanobacteria *Richelia intracellularis*. Another three-partner consortium is the peritrich ciliate *Zoothamnium pelagicum*, ectobiont bacteria, and the diatom *Licmophora* sp. The predominantly oligotrophic conditions of tropical seas do not favor the survival of large diatoms, but large species of *Coscinodiscus* and *Palmerina* in facultative symbiosis with *Pseudovorticella coscinodisci* have a competitive advantage over other diatoms (i.e., reduction of sinking speed and diffusive boundary layer). Symbioses allow sessile peritrich ciliates to extend their distribution in the pelagic environment, permit boreal-polar related diatoms such as *C. coarctatus* or *Fragilariopsis doliolus* to inhabit tropical seas, and help large diatoms to extend their survival under unfavorable conditions.

Keywords: commensalism; epibiotic consortia; tintinnid; peritrich ciliate; phoresy; epibiosis

1. Introduction

Ciliates are a highly diverse group of heterotrophic protists [1], and crucial members of the marine microbial loop that predominates in the oligotrophic ocean [2]. Standard protocols of preservation (Lugol's solution, formaldehyde) can distort naked ciliate cells, making their identification in environmental samples difficult. Tintinnid ciliates have vase-shaped hyaline proteinaceous shells called lorica that survive preservation and facilitate species identification. For that reason, there is more information at the species level of tintinnids (loricate ciliates) as compared with the oligotrichs (naked ciliates) [3–5], although the identification exclusively based on the lorica features is sometimes problematic due to the polymorphic character of some species [6–8].

Diatoms are an essential component of phytoplankton that contribute around 40% of marine net primary productivity [9]. Diatoms have a characteristic silica cell wall composed of two valves known as the frustule [10]. They reach high abundances in eutrophic environments such as well-mixed coastal or upwelling regions and, in oligotrophic waters, diatoms can utilize ecological adaptations such as the symbiotic interactions with diazotrophic cyanobacteria [11,12]. Diatoms are potential prey for ciliates [13], but there are also examples of symbiotic relationships between diatoms and ciliates. The most widespread consortia are the diatoms *Chaetoceros dadayi* and *C. tetrastichon* attached to the lorica of species of the tintinnid ciliate genus *Eutintinnus* [14–18], and the consortium of *Chaetoceros coarctatus* and the peritrich ciliate *Vorticella oceanica* [19–22]. Less common examples are the consortia

of the diatom *Fragilariopsis doliolus* and several tintinnid species [23–25], and the consortium of the peritrich ciliate *Pseudovorticella coscinodisci* attached to several large pelagic diatoms [22,26,27]. The research has mainly emphasized morphological and taxonomic aspects of the partners of the consortia, often from fixed material, and little is known about the epibiont/basibiont relationship and the advantages of the symbiotic life. These planktonic symbioses occur preferentially in tropical seas where access to laboratories is limited and, consequently, there are few studies based on live individuals. In addition, while there are numerous publications on the motility of free-living peritrich ciliates attached to inanimate substrates in wastewater treatment plants or shorelines [28–30], nothing is known regarding the motile behavior of symbiotic ciliates such as *V. oceanica* that appear to control the displacement of their diatom host.

This study provides new data on the distribution of these consortia, new examples that include three or four partners (bacteria, ciliate, and diatom), and the first micrographs and video recordings of some consortia. The motile behavior of the ciliate-diatom consortia is investigated by high-speed video recording. Cultures of the symbiotic partners were also attempted in order to test their capability to live as independent organisms, and to track the morphological changes between the symbiotic and free-living stages. This study provides new insights on the symbiotic interactions between diatoms and ciliates.

2. Materials and Methods

Plankton samples were collected with plankton nets from four coastal sites in the Mediterranean and Caribbean Seas and the South Atlantic Ocean (Table 1). The live plankton concentrate was scanned in settling chambers with inverted microscopes. Detailed methods of collection and observations are described in Gómez et al. [22,31]. References were used for the identification of diatoms [10], cyanobacteria [12], naked ciliates [21,22,32], and tintinnid ciliates based on the lorica features [3,4]. In order to test the survival of the diatoms as free-living organisms, the consortia were picked 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 cells were placed in 12-well tissue culture plate with 0.2 µm filtered and sterilized seawater supplemented with f/2 medium with silicates and incubated at 23 °C, with 100 µmol photons·m⁻² s⁻¹. The cultures were monitored using an inverted microscope. Turbulence was added with micro-bubble aeration in cases where culture growth was not immediately apparent.

Table 1. Place, date, and method of collection of the ciliate and diatom consortia.

Place	Coordinates	Date	Method
Marseilles	43°16'48" N–5°20'57" E	October 2007–September 2008	concentrator (20, 40, or 60 µm)
Banyuls-sur-Mer	42°28'50" N–3°08'09" E	October 2008–August 2009	concentrator (20, 40, or 60 µm)
Villefranche-sur-Mer	43°41'10" N–7°19'00" E	September 2009–February 2010	53 µm plankton net
Valencia	39°27'38.1" N–0°19'21.3" E	May 2011–February 2013	20 µm plankton net
Fosforoscente Bay	17°58'30" N–67°01'10" W	February–March 2012	20 µm plankton net
Magüeyes Island	17°58'7.8" N–67°3'41.3" W	February–March 2012	20 µm plankton net
São Sebastião Channel	23°5'4.0" S–45°24'28.8" W	March–December 2013	20 µm plankton net
Ubatuba	23°32'20.1" S–45°5'58.9" W	January 2014–January 2016	20 µm plankton net

The zooid stalk contraction of the peritrich ciliate *V. oceanica* was too rapid to be observed by the time resolution of ordinary video recordings (30 frames·s⁻¹ (fps), 33 milliseconds (ms) between subsequent frames), therefore, the motility was recorded with a high-speed camera (Photon FASTCAM SA2 model 86K-C3, San Diego, CA, USA). The high-speed camera was connected to the lateral port of an inverted microscope (IX73, Olympus, Tokyo, Japan). Initial tests showed that the contraction of the stalk of *V. oceanica* lasted for 2 milliseconds. A recording rate of 5000 fps (0.2 ms between subsequent frames) provided about 10 frames during the contraction that were enough to characterize the movement. To visualize the flow field, aliquots of the non-motile unicellular microalga, *Chlorella* sp., which is approximately ~4 µm in diameter, were added to the media. The flow field was also tested by adding drops of a red-colored food dye. The *V. oceanica* and *C. coarctatus* consortium was continuously moving, therefore, observations at high magnification and recording a zooid travelling parallel to the focal plane were difficult. Because *V. oceanica* is gregarious, forming a pseudo-colony of 100–150 individuals, it was also difficult to determine the feeding current of a single zooid without interference by its neighbors. The motility of the consortium of *P. coscinodisci* and *Coscinodiscus cylindricus* (= *C. wailesii*) was more limited. Some valve faces only showed one or two zooids, and it was easier to record the feeding currents of a single zooid.

3. Results

3.1. Diatoms and Tintinnid Ciliates

3.1.1. Consortia of *Chaetoceros* and *Eutintinnus*

The most widespread consortia were of the diatoms *Chaetoceros dadayi* and *C. tetrastichon* attached to the lorica of *Eutintinnus apertus* and *E. pinguis*, respectively. These consortia were found in all the sampling sites in the western Mediterranean and Caribbean Seas, and the South Atlantic Ocean (Table 2). The species of the genus *Eutintinnus* are characterized by a cylindro-conical lorica, open widely at both ends. One aperture was oral, with the mouth and cilia, and the other aperture was aboral, near the pedicel of the cell. The lorica of *E. apertus* was a slightly tapering short cylinder (~90 µm long, and ~30 µm oral diameter, ~15 µm aboral diameter) with a distinctive abrupt narrowing near the aboral end (Figure 1a,b). The lorica of *E. pinguis* was larger (110–150 µm long, ~40 µm oral diameter, and ~25 µm aboral diameter) and with a less marked aboral constriction (Figure 1e). The diatoms of the genus *Chaetoceros* are characterized by horn-like valve extensions known as setae. The species *C. dadayi* and *C. tetrastichon* are members of the subgenus *Phaeoceros* comprising generally robust species having thick setae, and chloroplasts in both the cell and the setae. The chain of *Chaetoceros* was adhered near the oral aperture where the lorica surface was straight, avoiding the more curved surface near the aboral end (Figure 1a,b,e). Two or more independent chains in the same lorica were never observed (except recently fragmented chains). During the ciliate division, the anterior half of the ciliate formed one daughter cell (the proter), and the posterior half formed the other (the opisthe). The opisthe kept the old lorica, while the proter formed a new lorica. A four-celled diatom chain tended to fragment into two, two-celled chains. One of the two-celled chains remained in the old lorica and the other two-celled chain was attached to the proter, until the completion of the new lorica (Figure 1c). The occasional findings of detached individuals of *C. dadayi* and *C. tetrastichon* are attributed the mechanical damage during the net sampling (Figure 1d).

Table 2. Records of the ciliate-diatom symbioses in this study. The geographical coordinates of the places of collection are reported in the Table 1. -, non-observed; +, occasional records; and ++, common records.

Ciliate and Diatom Consortium	NW Mediterranean	Caribbean Sea	Tropical South Atlantic
<i>Eutintinnus apertus</i> / <i>Chaetoceros dadayi</i>	++	++	++
<i>Eutintinnus pinguis</i> / <i>Chaetoceros tetrastichon</i>	++	++	++
<i>Eutintinnus lususundae</i> / <i>Chaetoceros peruvianus</i>	+	+	+
<i>Eutintinnus lususundae</i> / <i>Hemiaulus</i> spp./ <i>Richelia intracelularis</i>	+	+	+
<i>Eutintinnus lususundae</i> / <i>Thalassionema</i> sp.	-	+	+
<i>Salpingella</i> spp./ <i>Fragilariopsis doliolus</i>	-	+	++
<i>Zoothamnium pelagicum</i> / <i>Licmophora</i> sp./ectobacteria	-	-	+
<i>Zoothamnium pelagicum</i> /ectobacteria	++	++	+
<i>Vorticella oceanica</i> / <i>Chaetoceros coarctatus</i>	-	++	++
<i>Vorticella</i> sp./ <i>Chaetoceros densus</i>	+	+	-
<i>Pseudovorticella coscinodisci</i> / <i>Coscinodiscus</i> spp.	-	+	++

The chains of *C. dadayi* and *C. tetrastichon* were straight, without or with very narrow apertures (the spaces between the cells) and restricted to 2–4 cells (Figure 1a–e), although chains of three were most commonly observed. *C. dadayi* and *C. tetrastichon* differed in the setae orientation. *C. dadayi* showed 1–3 setae directed towards the oral aperture of the host lorica, hereafter oral-oriented (usually a three-celled chain with two oral-directed setae) (Figure 1a–d). *Chaetoceros tetrastichon* showed all the setae directed towards the aboral aperture of the host lorica, hereafter aboral-oriented (Figure 1e). All the setae of *C. dadayi* were in the same plane (Figure 1a–d), while the setae of *C. tetrastichon* were arranged in different planes (Figure 1e). In *C. dadayi*, one half of the setae were shorter and rudimentary, mainly embracing the host lorica, and the other half of the setae were long and oral or aboral-directed (Figure 1d). For typical consortia of three diatom cells, there are six long setae. Two and four long setae are oral and aboral oriented, respectively. The two oral-oriented long setae emerged with an angle of about 60 degrees, and then abruptly changed the orientation being parallel to the diatom axis. The four aboral-oriented long setae were progressively curved. The frustule closer to the aboral aperture showed long posterior terminal setae that laid nearly in parallel to the chain axis (Figure 1d).

The setae configuration of *C. tetrastichon* was less divergent. The setae were almost equal in size (Figure 1e), except in recently divided cells where the setae of the new valve were shorter than in the old one. Setae were curved outward, except those of the frustule closer to the aboral aperture that were turned nearly parallel to the chain axis as for *C. dadayi* (Figure 1e). The setae never protruded beyond the lorica, avoiding interference with the ciliary activity.

The observations of the consortia locomotion of *C. tetrastichon* were more difficult than the consortia with *C. dadayi*. The long setae arranged in different planes of *C. tetrastichon* frequently entangled with other particles in the plankton concentrate. In contrast, the movement of the consortia of *C. dadayi* was more graceful with alternating displacements in both directions. An obstacle (or potential predator) in the trajectory of the ciliate first contacted the thick setae of the diatom. The consortia of *C. tetrastichon* swam preferentially in a forward direction (i.e., the oral aperture in the front) (Video S1 as supporting material at <https://youtu.be/lgxbKjyUu0>, time 0:22–0:30). The attempts to culture *C. dadayi* and *C. tetrastichon* were unsuccessful. The ciliate abandoned the lorica, and the diatom chain settled on the bottom and died. Microbubbles of atmospheric air were added as the source of turbulence without success.

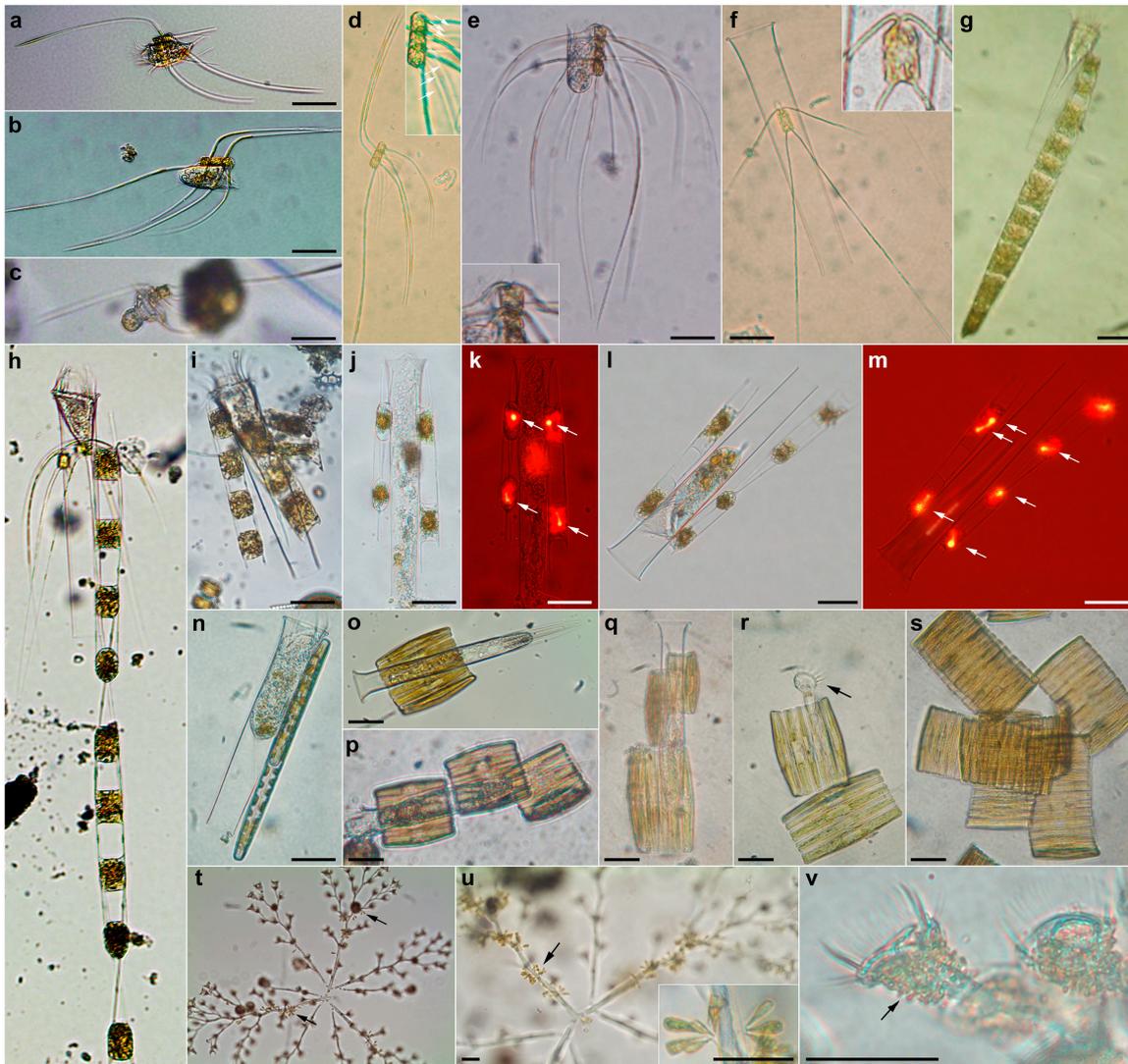


Figure 1. Bright field (a–j,l,n–v) and epifluorescence (k,m) micrographs of consortia between diatoms and ciliates. (a–c) *Chaetoceros dadayi*/*Eutintinnus apertus*; (c) The tintinnid is devoid of the lorica; (d) Detached chain of *C. dadayi*. The arrows in the inset point the short setae; (e) *Chaetoceros tetrastichon*/*Eutintinnus pinguis*. The inset focuses on the terminal setae near the oral aperture; (f) *Chaetoceros peruvianus*/*Eutintinnus lususundae*. The inset focuses on the diatom valves; (g) *Hemiaulus membranaceus*/*Eutintinnus lususundae*; (h) *Chaetoceros peruvianus*/*Hemiaulus hauckii*/*E. lususundae*; (i) *Hemiaulus membranaceus*/*H. hauckii*-*E. lususundae*; (j–m) *Hemiaulus hauckii*/*E. lususundae*; (k,m) The arrows point the cyanobacterium *Rickettsia intracellulare*; (n) *Thalassionema* sp./*E. lususundae*; (o–r) *Fragilariopsis doliolus*/*Salpingella* spp.; (r) The tintinnid is devoid of the lorica; (s) Culture of *F. doliolus*; (t–v) *Licmophora* sp./*Zoothamnium pelagicum*; (u) The inset shows the diatom *Licmophora* sp.; (v) Ectobiont bacteria on the zooids. Scale bar = 50 μ m.

Consortia of the tintinnid *Eutintinnus lususundae* and the diatom *Chaetoceros peruvianus* were occasionally found in the samples from the Mediterranean Sea (Figure 1f,h,j–m). The lorica was almost cylindrical (160–300 µm long), with oral and aboral diameters of ~50 µm and ~35 µm, respectively. The lorica showed a slightly flaring oral aperture that was missing in the aboral end (Figure 1f–n). *C. peruvianus* is another member of the subgenus *Phaeoceros*. The diatom was solitary (two-celled colonies were only observed after recent division), and adhered to the lorica half near the oral aperture. The epivalve was oriented towards the oral aperture, and never protruded beyond the oral aperture (Figure 1f). The outline of the epivalve setae was almost straight or convex. More than one individual of *C. peruvianus* could attach in the different sides of a lorica at more or less the same height (Figure 1f,h).

Consortia of *E. lususundae* and the diatoms *Hemiaulus membranaceus* (Figure 1g) and *H. hauckii* (Figure 1h,j–m) were found in the Mediterranean Sea and the South Atlantic Ocean, and more sporadically formed a three-partner consortia with the co-occurrence of *H. hauckii* and *C. peruvianus* (Figure 1h), or *H. hauckii* and *H. membranaceus* (Figure 1i). The chains of *Hemiaulus* were arranged along the longitudinal axis of the lorica (Figure 1g–m). The diatom chain did not protrude beyond the oral aperture, but it could extend beyond the aboral aperture, in some cases, by a large margin. Only the diatom frustule closer to the oral aperture was attached to the lorica. During swimming, the distal end of a long diatom chain could diverge from the longitudinal axis of the lorica (Figure 1h and Video S1 as supporting material at <https://youtu.be/lgxbKjyUu0>, time 0:54–1:51). *Hemiaulus hauckii* are characterized by long and slender horn-like elevations between the cells that were less prominent in *H. membranaceus*. The chloroplasts and cell contents of *H. hauckii* and *H. membranaceus* were hiding the trichomes of the diazotrophic cyanobacteria *Richelia intracellularis* as revealed by epifluorescence microscopy (Figure 1j–m). This constituted a three-partner consortia, or even four partners in the case of *C. peruvianus* and one species of *Hemiaulus* (Figure 1h), or two species of *Hemiaulus* (Figure 1i). The diatoms *C. peruvianus*, *H. hauckii*, and *H. membranaceus* were commonly found in the surrounding plankton as free-living forms, and there were no apparent differences in the morphology between the free-living and symbiotic forms.

Consortia of an elongated solitary diatom attached to *E. lususundae* were very rarely recorded in the tropical South Atlantic Ocean (Figure 1n). In contrast to *Chaetoceros* or *Hemiaulus*, this diatom lacked setae, horns, or other extension of the valve, although the entire frustule was needle shaped. It corresponded to a solitary form of *Thalassionema*, an araphid (without raphe) diatom found as a free-living form in the surrounding plankton. As usual, the diatom did not protrude beyond the oral aperture of the lorica (Figure 1n).

3.1.2. Consortia of *Fragilariopsis doliolus* and *Salpingella* spp.

The consortia of the diatom *F. doliolus* and the tintinnid *Salpingella* spp. were common in the tropical waters off Brazil (Figure 1o–r), while it was never observed from the Mediterranean samples (Table 2). The lorica of *Salpingella* showed a pointed and closed aboral end, and usually a more marked oral flare. The genus *Salpingella* contains numerous species with nearly identical morphologies which makes species assignation difficult. The most common observations correspond to *S. acuminata*. The frustules of *F. doliolus* were united into bands by the valve surfaces. The perivalval axis (raphe) of the diatom encircled the lorica as the sections of a barrel (Figure 1o–q). Tintinnid cells devoid of the lorica also transported the diatom chains, although the typical alignment (encircled along the longitudinal axis of the lorica) could be lost (Figure 1r). As usual, the diatom did not protrude beyond the oral aperture. Under culture conditions, the diatom chains were almost flat or very slightly curved, losing the barrel-shaped configuration of the epizoic stage (Figure 1s).

3.2. Diatoms and Peritrich Ciliates

3.2.1. Consortium of *Zoothamnium pelagicum* and *Licmophora* sp.

In the previous consortia, the diatom was adhered to the lorica -a relatively hard substrate. In this type of consortia, the diatom can be interpreted as the epibiont, and the ciliate as the basibiont. In the case of the oligotrich ciliates (which are missing rigid surfaces), it is generally interpreted as the diatom as the basibiont, and the ciliate as the epibiont. The peritrich ciliates have a zooid with a stalk attached to the host or substrate using an adhesive disk. Nearly all the peritrich ciliates are sedentary, living preferentially in benthic habitats. They occur in the water column as epibionts on copepods, diatoms, and other organisms. An exception is the consortia of the peritrich ciliate *Zoothamnium pelagicum* that is the only free-floating species in the open ocean among the peritrich ciliates, and in this consortium the diatom is the epibiont. A mature assemblage of *Z. pelagicum* is a pseudo-colony composed of several colonies joined at the bases of their stalks (Figure 1t–v). The branches of the colonies are highly contractile with fast contractions. *Licmophora* is an araphid diatom genus. The species are typically wedge shaped with a basal pole attached to the substrate. In this consortium, *Licmophora* sp. was the epibiont, adhered to the branches of *Z. pelagicum* through the basal pole, avoiding the problems of the elasticity of the substrate (Figure 1t–u). Cells of *Licmophora* sp. were found in up to 30% of the examined pseudo-colonies of *Z. pelagicum* in the South Atlantic Ocean, although the observations were difficult due to the small size of the diatom and the contractions and jumps of the ciliate. There were no observations of the consortia with *Licmophora* sp. in the samples from the Mediterranean Sea. The diatom was isolated and cultured. It grew slowly, and the frustule laid on the bottom of the culture chamber. There were no apparent morphological differences between the frustules of the epizoic and free-living stages. In the Mediterranean and Caribbean Seas, and less commonly in the South Atlantic Ocean, the zooids of *Z. pelagicum* were sometimes heavily covered by ectobiont bacteria (Figure 1v; Table 2). This is another example of three partner consortia with bacteria, diatom, and ciliate.

3.2.2. *Chaetoceros densus*-*Vorticella* sp.

Careful observations revealed that the records from the NW Mediterranean Sea (Figure 2a–j), and partially the records from the Caribbean Sea (Figure 2k) did not correspond to the typical morphology of *Vorticella oceanica*/*Chaetoceros coarctatus* (Figure 2l). The diatom *C. coarctatus* is characterized by distinct horn-like terminal setae, especially the posterior pair, with the intercalary setae emerging almost perpendicular to the chain axis, and then directed posteriorly (Figure 2l). In contrast, this symbiotic diatom did not show the distinct terminal setae, and the intercalary setae bent slightly towards the chain ending (Figure 2a–c,k). This diatom fit with the characteristics of *Chaetoceros densus*, although this species has also been illustrated with the setae directed perpendicularly. The valve face of the frustule was a truncate pyramid that suggested an affinity with the little-known species *Chaetoceros octagonus* (Figure 2a).

The zooids of *V. oceanica* were conical (30–35 µm long and 25–30 µm oral diameter) with a stalk of 100–190 µm in length (Figures 2l and 3). In contrast, the epibiont of *C. densus* showed hemispherical zooids, 20–25 µm long, and with an oral diameter of 40–45 µm. The length of the stalk was highly variable (50–200 µm long) (Figure 2a–k). The stalk of the epibiont of *C. densus* did not fully coil, and the section adjacent to the adhesive disk was always uncoiled (Figure 2g–j). This also contrasted with the stalk of *V. oceanica* that fully contracted, forming five active coils (Figure 3). This evidence suggested that the vorticellid epibiont of *C. densus* is not *V. oceanica*.

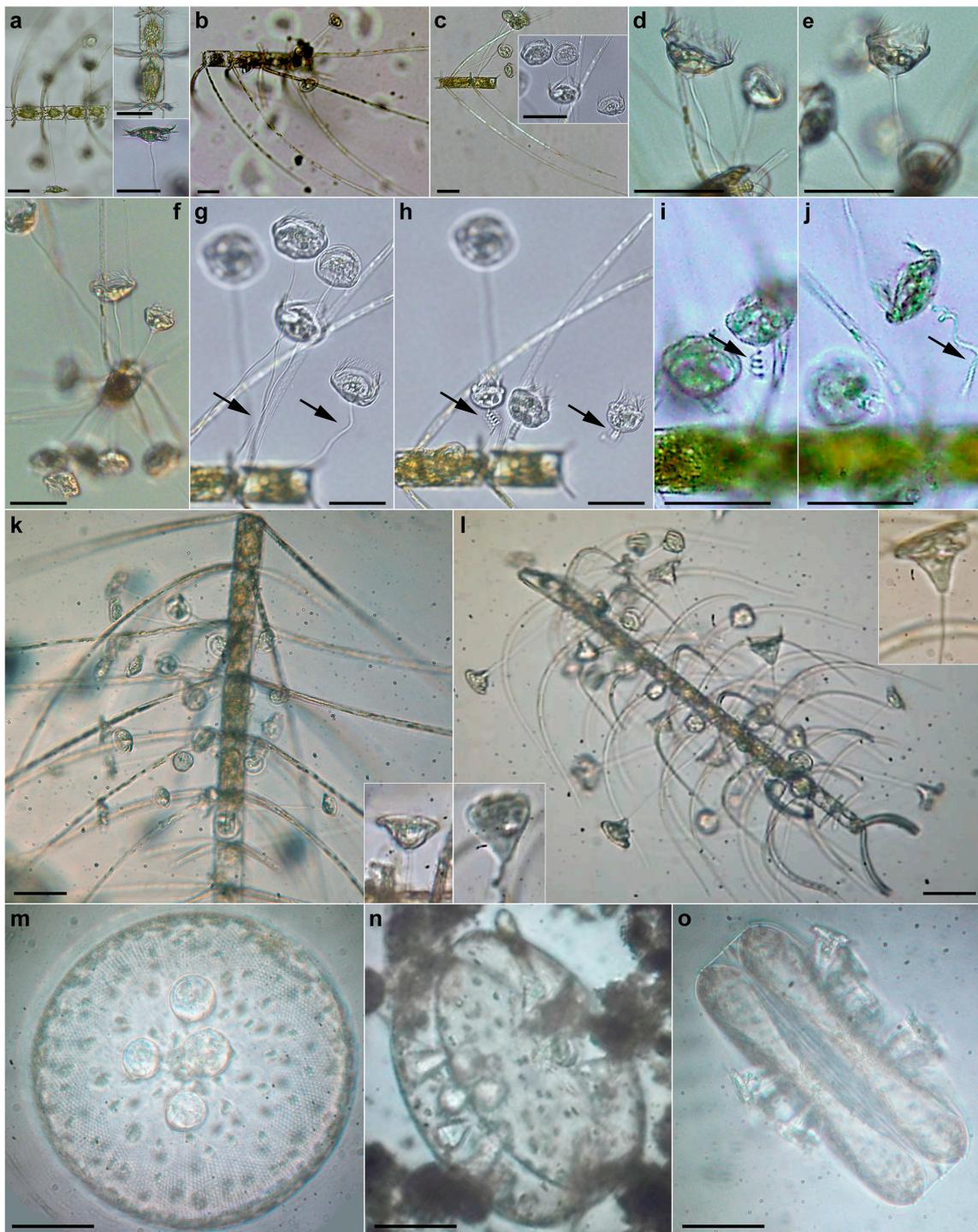


Figure 2. Micrographs of consortia between peritrich ciliates and diatoms. (a–j) *Vorticella* sp./*Chaetoceros densus* from the NW Mediterranean Sea; (a) The insets show the zooid and the frustule contour; (g–j) Frames before and after the stalk contraction; (k) *Vorticella* sp./*Chaetoceros densus* from the Caribbean Sea; (l) *Vorticella oceanica*/*Chaetoceros coarctatus* from the Caribbean Sea; (k–l) The insets show the zooids; (m–o) *Pseudovorticella coscinodisci*/*Coscinodiscus gigas* from the Caribbean Sea. Scale bar = 50 μ m.

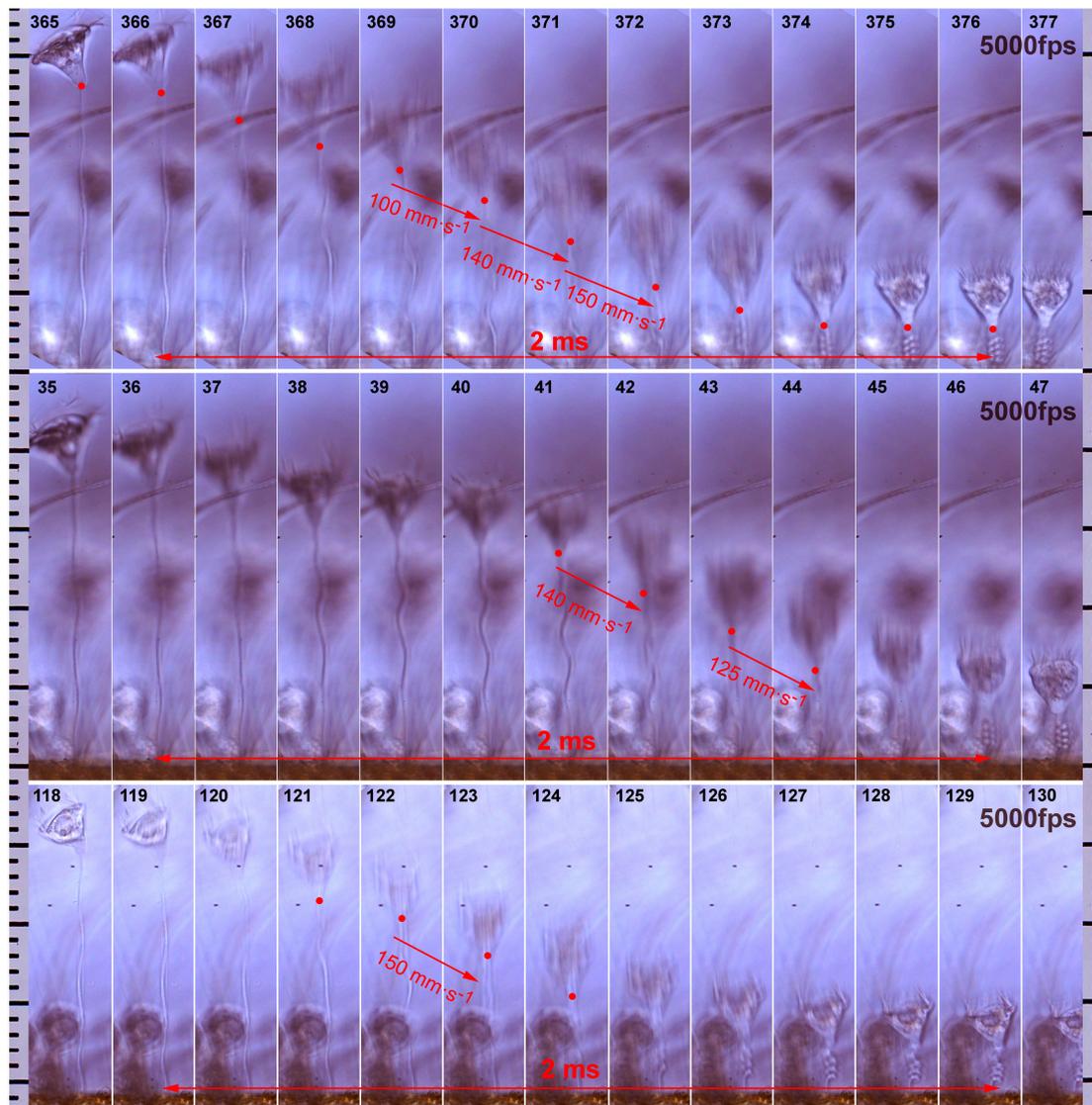


Figure 3. Stalk contraction of *Vorticella oceanica* on *Chaetoceros coarctatus* recorded at 5000 frames·s⁻¹ (0.2 milliseconds (ms) between subsequent frames). The positions of reference points are marked by a red circle. Frame number in the upper left corner. The lateral scale represents 10 μm between two bars.

3.2.3. *Chaetoceros coarctatus*-*Vorticella oceanica*

The consortia of *V. oceanica* and *C. coarctatus* were common in the sampling sites in the South Atlantic Ocean, and also found in the Caribbean Sea, while unrecorded from the NW Mediterranean Sea (Table 2). The morphology of the partners was described in Gómez et al. [19]. This study focused on the lesser known aspects of the consortium, the motility and advantages of the symbiotic life. A diatom chain of *C. coarctatus* usually reached 1–1.2 mm in length and was composed of 25–30 cells. Each diatom cell usually contained 3–4 individuals of *V. oceanica*. In the upright position with an expanded stalk, the zooid protruded beyond the setae (Figure 2l). The zooids responded with a fast stalk contraction to external stimulus (threat) or spontaneously, without any apparent perturbation (Figure 3 and Video S1 as supporting material at <https://youtu.be/lgxbKjyUu0>, time 4:00–10:50).

When the diatom chain was damaged and decomposed into shorter chains, the consortium was unable to swim in a straight trajectory. These short chains seemed to induce irregular helicoidal trajectories by the zooids. Although a single zooid was able to maintain a diatom cell in suspension

with its four setae, the consortium typically possessed about three to four zooids per diatom cell and, consequently, the buoyancy of the diatom chain was guaranteed. More zooids increased the feeding currents and the forward propulsion necessary to exploit new parcels of food resources. Undamaged consortia moved forward (with the distinct posterior terminal setae in the rear) in a straight or slightly curved trajectory. There were three to four zooids with stalks oriented forward, whereas most of the other zooids oriented the stalk perpendicular to the chain axis. When the frontal zooids detected an obstacle, the consortium changed the direction. Consortia lacking the three to four frontal zooids also moved straight forward, but a backwards movement was not observed. Although most of the stalks were perpendicular to the chain axis, each zooid was able to change its angle with respect to the stalk (nicking or polar feeding angle, and azimuthal angle) and, consequently, the angle of propulsion by the ciliary beating propelled the consortium forward (Video S1 as supporting material at <https://youtu.be/lgxbKjJyUu0>, time 6:50–6:58).

The stalk contraction of *V. oceanica* was analyzed by high-speed video sequences. Spontaneous contractions were monitored at a speed of 5000 frames·s⁻¹ (0.2 milliseconds (ms) between subsequent frames). During the contraction, *V. oceanica* stopped ciliary beating and the zooid acquired a more globular shape (Figure 3). The initially straight stalk coiled into a helix, starting from the region near the zooid and moving down towards the base of the stalk (Figure 3). During the contraction, the zooid moved without any observable rotation until the end of the contraction. After the stalk became fully coiled, the zooid started rotating in a clockwise direction. A stalk of 0.18 mm required approximately 1.8–2 ms to reduce its length by about 80% and to form a spring (Figure 3). The number of active coils (including the ends) was five. The median velocity of the zooid during a contraction was 70 mm·s⁻¹. The zooid reached a speed peak of 150 mm·s⁻¹, and in some cases were recorded 165 mm·s⁻¹. Subsequently the stalk re-extended in a few seconds and returned to its original length accompanied by counterclockwise rotation of the zooid (Video S1 as supporting material at <https://youtu.be/lgxbKjJyUu0>, time 9:00–9:42).

The ciliary movement occurred in a wave-like pattern, a metachronal wave that moved at 1.7–2.5 mm·s⁻¹ (Figure S1 in Supplementary Materials). In addition to the forward movement of the consortium, the ciliary beating generated a flow field that brought a continuous stream of food particles to the oral cavity of the zooids. Stalk contraction can also contribute to water flow. Ultimately, feeding currents are the sum of the cooperative ciliary beating of each zooid. Thus, the local flow environment of an individual organism is influenced by active zooids nearby and, consequently, the feeding current created by a single zooid could not be easily determined. Overall, the ciliary beat moved the surrounding seawater up to a distance of at least one mm from the zooid. Using the microalga *Chlorella* sp. for the visualization of the flow field and estimation of the speed, the microalga reached 0.66 mm·s⁻¹ when it approached the zooid (Figure 4). Water and rejected particles were expelled towards the rear of the diatom chain (Figure 5 and Video S1 as supporting material at <https://youtu.be/lgxbKjJyUu0>, time 9:43–10:50).

3.2.4. *Pseudovorticella coscinodisci* and Diatoms

Consortia of *P. coscinodisci* were not observed in the Mediterranean Sea, but in the Caribbean Sea, *P. coscinodisci* was found as an epibiont on large drum-shaped diatoms such as *Coscinodiscus gigas* (Figure 2m–o and Table 2). In the tropical South Atlantic Ocean, the consortium of *P. coscinodisci* and large pelagic diatoms were observed (Video S1 as supporting material at <https://youtu.be/lgxbKjJyUu0>, time 10:51–11:10). The most common host for the clusters of the ciliate was *Coscinodiscus cylindricus*, and more occasionally *C. gigas* and *Palmerina hardmaniana*. Single, or a few, ciliate cells were observed on other large pelagic diatoms (*Chaetoceros*, *Eupyxidicula*), but never formed clusters capable of rendering the consortia motile. These symbiotic relationships were not obligate, and these host diatoms were more commonly found as free-living forms. The percentage of diatoms colonized by the ciliate was higher in post-bloom conditions. Symbiotic cells of *C. cylindricus* and *P. hardmaniana* were isolated and they grew in cultures keeping the typical morphology as free-living species.

The clusters of *P. coscinodisci* rendered the diatom motile. Contrary to the case of *V. oceanica*, the zooid did not need to protrude beyond the host valve extensions (i.e., setae). *Coscinodiscus cylindricus* provided a huge substrate area, but little protection such as spines or horns. The stalk of *P. coscinodisci* was short and after the contraction, the stalk bent, but it did not coil to form a spring. The zooids in the center of the valve showed a perpendicular stalk and the oral cavity parallel to the valve face. In the periphery of the valve face, the stalk or zooid leaned toward the periphery (Video S1 as supporting material at <https://youtu.be/lgxbKjJyUu0>, time 11:10–13:45).

The field flow generated by the ciliary movement was tracked using a drop of red dye placed about 1 mm in front of the valve face. The red colored water registered a flow field with a conical shape of an aperture of 80° and the zooid in the vertex. Subsequently, a toroidal vortex appeared near the periphery of the valve (Figure 6a). Addition of *Chlorella* sp. allowed estimation of the speed of the flow field at a maximum velocity of $0.42 \text{ mm}\cdot\text{s}^{-1}$ at 40–50 μm distance ahead of an individual zooid. A particle present between 50° and 130° with respect to the vertical would conduct to the oral cavity of the zooid. If the particle was rejected, it would enter in the feeding current of the neighbor zooids. If the particle was lower than 50° or higher than 130°, it would be repelled laterally before reaching the zooid (Figure 6b–c and Video S1 in Supporting Information <https://youtu.be/lgxbKjJyUu0>, time 13:45–14:38). This pattern was altered when the zooid changed the nicking or polar feeding angle, and the stalk inclination.

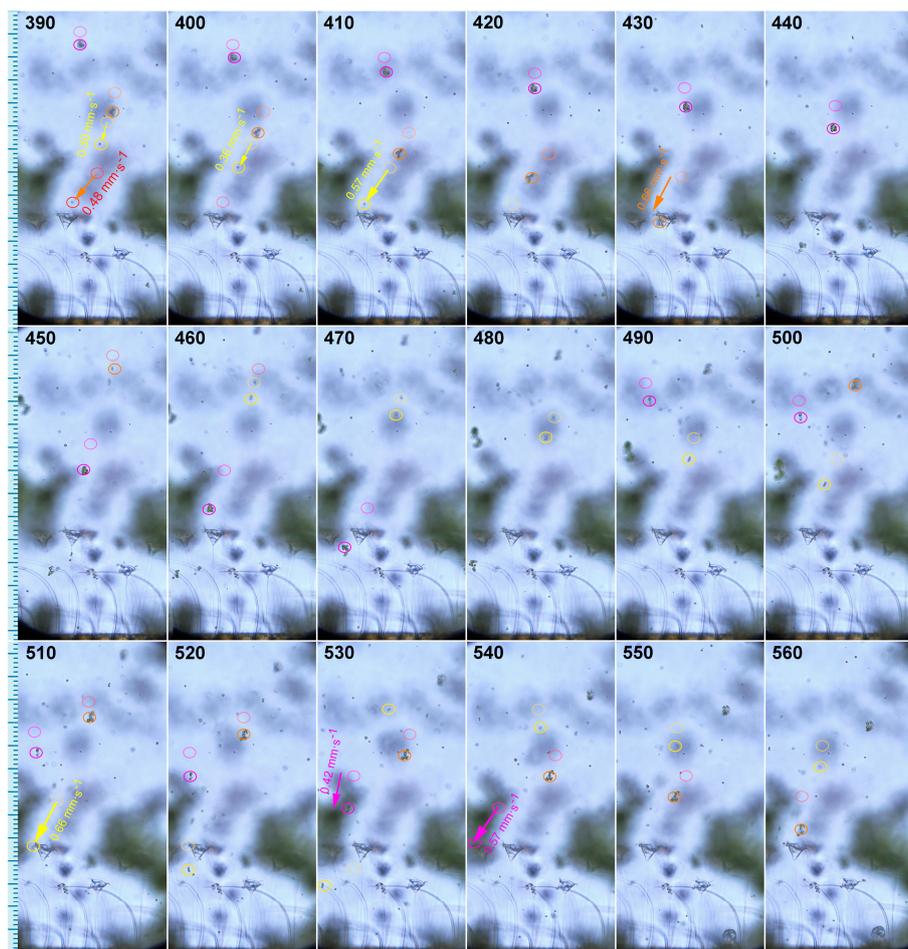


Figure 4. Feeding currents of *Vorticella oceanica* on *Chaetoceros coarctatus* recorded at 60 frames·s⁻¹. The microalga *Chlorella* sp. was used as a tracer of the flow field. Colored circles corresponded to the position of a particle each 10 frames (0.16 s). The colored empty circles represent the previous position of the particle in order to calculate the trajectory and speed fluid path lines. The velocity field is depicted by arrows. The lateral scale represents 10 μm between two bars.

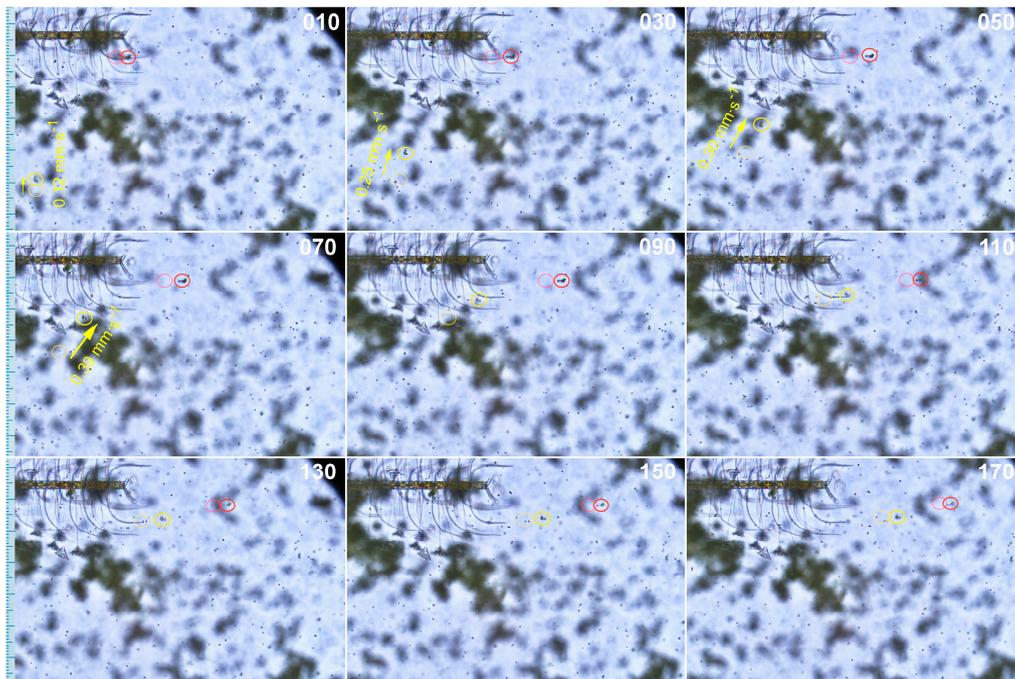


Figure 5. Feeding currents of *Vorticella oceanica* on *Chaetoceros coarctatus* recorded at 60 frames·s⁻¹. The microalga *Chlorella* sp. was used as a tracer of the flow field. Colored circles corresponded to the position of a particle each 10 frames (0.16 s). The colored empty circles represent the previous position of the particle in order to calculate the trajectory and speed fluid path lines. The velocity field is depicted by arrows. The lateral scale represents 10 μm between two bars.

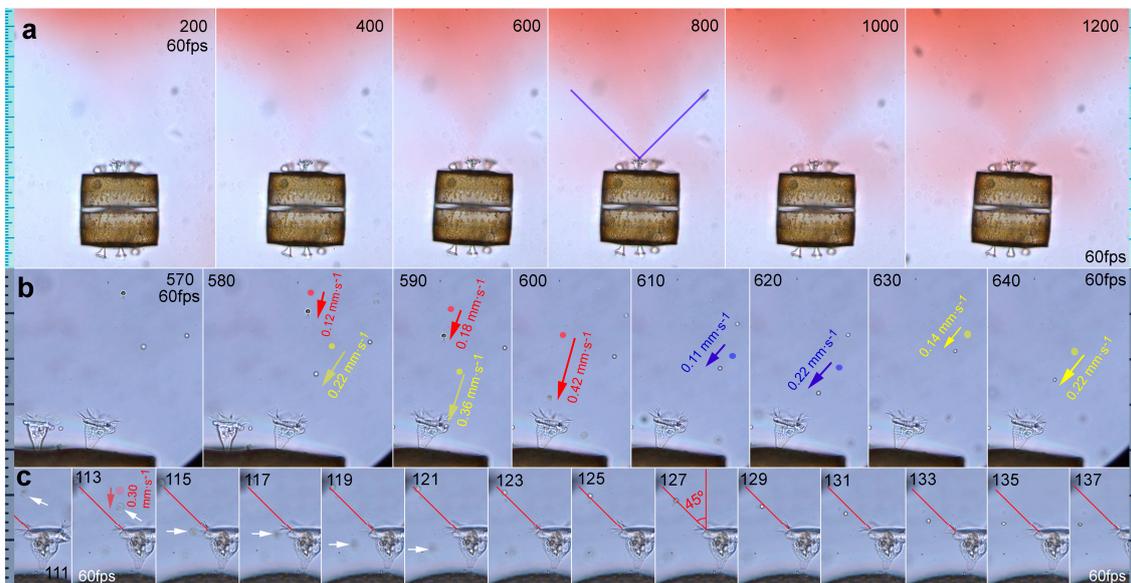


Figure 6. Feeding currents of *Pseudovorticella coscinodisci* on *Coscinodiscus cylindricus* recorded at 60 frames·s⁻¹. (a) Micrographs at intervals of 3.3 s. A red dye was added to visualize the flow field. Note the axis-symmetric conical flow with an opening angle of 80° and the toroidal vortex; (b) The microalga *Chlorella* sp. is used as a tracer of the flow field. Colored circles corresponded to the position of a microalga cell 10 frames before (0.16 s). The velocity field is depicted by arrows; (c) Feeding currents of a single zooid. Micrograph at intervals of 33 milliseconds. Particles aspirated with an angle of more than 40° with respect to the vertical did not reach the cilia. The lateral scale represents 10 μm between two bars.

4. Discussion

4.1. Taxonomy and Biogeography of the Peritrich Ciliate and Diatom Consortia

The morphology of the diatom frustules is well preserved in fixed plankton samples, but the ciliate soft bodies are distorted or lost, and the identification of the diatom hosts is easier than the naked ciliates. Furthermore, in live samples, net sampling can split the partners giving an underestimation of the abundance of the symbiosis, and a biased view of obligate partners as free-living species. In the case of *Chaetoceros coarctatus* and *Vorticella oceanica*, the diatom was described in 1864 from Hong Kong harbor (subsequent records were also illustrated devoid of epibionts), and the ciliate was described in 1906 from the Sargasso Sea [33,34]. *P. coscinodisci*, known since Faria and Cunha in 1917 [26], was described in 2018 [22]. Net sampling can damage the chains resulting in loss of the distinctive terminal setae of *C. coarctatus*. Although the intercalary setae are also different, observations of *C. densus/Vorticella* sp. (Figure 2a–k) could be mistaken for *C. coarctatus/V. oceanica* (Figure 2l). The taxon *Chaetoceros borealis* var. *densus* was described from the North Atlantic Ocean, and later ranked at the species level from the tropical Indo-Pacific Ocean [35,36]. *C. densus* has a cosmopolitan distribution, more commonly recorded in temperate waters [10]. The diatom is illustrated with the setae directed almost perpendicular to the diatom axis, and also with the setae bent towards the chain ending with a convex outside line, as observed in this study (Figure 2a–c, k [37]). Setae bent towards the chain ending are more favorable for zooids with stalks that can protrude beyond the setae (Figure 2a–k). It is possible that the symbiotic *C. densus* with setae bent towards the chain ending, could constitute an independent species. A closely related species is *C. octagonus* with valve face that is a truncate pyramid more pronounced than the symbiotic *C. densus* (Figure 2a). *C. octagonus* was described as a free-living species and it is only known from the original description in tropical waters [38].

The consortium of *C. coarctatus/V. oceanica* is well documented in the warmer Eastern Mediterranean Sea [39], and it has been categorized as a species introduced through the Canal of Suez [40]. More doubtful are the records of *C. coarctatus* in cold areas such as the NW Mediterranean Sea [41], as this study did not record the organism during a four-year survey at four sites in the coastal NW Mediterranean Sea. In the Northern Adriatic Sea, Schröder [42] described without illustration a consortium identified as *C. densus* and *V. oceanica*. Schröder did not provide details on the morphology of the diatom, but described the zooid as flattened spherical, 26–30 µm wide, and 18–24 µm long, and the stalk was 10 to 12 times longer than the diameter of the zooid. A stalk of more than 200 µm long could be an exaggeration for *V. oceanica*. The shape of the zooid is closer to the epibiont of *C. densus* because *V. oceanica* is typically conical (Figures 2l and 3), but the size of the zooid is closer to *V. oceanica*. The species *C. coarctatus* has been only recorded from the southern basin of the Adriatic Sea, while *C. densus* is known from all the Adriatic basins [43]. From the Gulf of Lions, Pavillard [17] reported, without illustration, the consortia of *C. densus* and a vorticellid ciliate, with no details on the morphology of the ciliate. Pavillard [17] knew the morphology of the consortia of *C. coarctatus* and *V. oceanica* as illustrated by Okamura [19], and he considered that the symbiotic diatom corresponded to *C. densus*. Pavillard [17] reported that all the setae were strongly deviated in the same direction. This evidence suggests that Pavillard had observed the consortium *C. densus* and *Vorticella* sp. as illustrated here in the same region (Figure 2a–j).

The consortia of *Chaetoceros densus* and *Vorticella* sp. and *C. coarctatus* and *V. oceanica* co-occurred along the coasts of Puerto Rico (Figure 2k–l), close to the Sargasso Sea, the type locality of *V. oceanica*. This begs the question of whether Zacharias [34] described *V. oceanica* as the epibiont of *C. coarctatus* or *C. densus*. Schröder [44] attributed the host identity of *V. oceanica* in Zacharias to *C. densus*. Zacharias described *V. oceanica* as a bell-shaped zooid (28 µm long and 22 µm oral diameter), which agreed with the shape and size of the epibiont of *C. coarctatus*. Zacharias also reported a stalk length of 80 µm, which was shorter than expected, but could correspond to a partially coiled stalk in the fixed samples. Zacharias [34] described the basibiont as a species of *Chaetoceros*, without details on the morphology. From Sargasso Sea plankton samples, the only diatoms that Zacharias [34] listed were *Chaetoceros*

borealis and two species of *Rhizosolenia*. *C. borealis* was first described from the cold waters of the Gulf of Maine, and later Cleve [35,36] proposed *C. densus* for *C. borealis* var. *densus* observations in warmer regions. It was unexpected to find chains of a large and heavily silicified chain-forming species of *Chaetoceros* in the surface waters of the Sargasso Sea. This suggested that the diatom was probably associated with epibionts, but the ciliate cells were lost due to the sample treatment (except for the two specimens used for the description of *V. oceanica*). It is possible that Zacharias [34] described *V. oceanica* as the epibiont of *C. densus*. However, more evidence is needed, and it is more plausible to maintain the current status with *V. oceanica* as the epibiont of *C. coarctatus*.

In the Caspian Sea, Ostenfeld [45] described without illustration the consortium of the tintinnid *Amphorella borealis* var. *caspiica* adhered to several species of *Chaetoceros*. From the Gulf of Siam, Ostenfeld [46] illustrated the consortia of the large diatom *Palmerina hardmaniana* with semicircular slits that harbored epibionts identified as *Amphorella borealis*. *Amphorella* is currently accepted as the tintinnid *Amphorides*, and Ostenfeld [47] transferred *A. borealis* into *Cothurnia*, a genus of loricated peritrich ciliates of the family Vaginicolidae whose species have sometimes been mistaken for those of the chrysophyte *Dinobryon*. Several species of the genus *Vaginicola* have been reported as symbionts on diatoms [20]. From Northern Australia, Hallegraeff and Jeffrey [27] reported micrographs of *P. hardmaniana* with the distinctive semicircular slits and epibionts identified as *Amphorella borealis*. From the same region, Stosch [48] proposed *P. ostensfeldii* for cells previously identified as *P. hardmaniana* with the semicircular slit that harbored the ciliate. In the South Atlantic Ocean, consortia between *P. hardmaniana* and the peritrich ciliate *P. coscinodisci* have been observed [22]. The diatom did not show semicircular slits in the symbiotic or free-living stages. The consortium of *P. ostensfeldii* and *Cothurnia borealis* or a species of *Vaginicola* remains endemic for the Indonesian region. It is interesting that the diatom valve is characterized by a semicircular slit harboring the peritrich ciliate that can be interpreted as an evolutionary adaptation to the symbiotic life.

4.2. Taxonomy and Biogeography of the Tintinnid and Diatom Consortia

Observations on the tintinnid and diatom consortia have been known since Ehrenberg (1834, fide [3]). In 1883, Fol [14] illustrated the consortium of a species of *Chaetoceros* (later described as *C. dadayi*), and an unidentified tintinnid which showed a lorica with a pointed and closed aboral end. Kofoid and Campbell [4] attributed this tintinnid to *Rhabdonella elegans*. All further authors have reported the tintinnid host with an open aboral aperture and the distinctive aboral narrowing (Figure S2 in Supplementary Materials). Daday [15] illustrated *Tintinnus inquilinus* as a free-living species and attached to *Chaetoceros*. From the open subtropical Atlantic, Cleve [35] described *C. tetrastichon* as a free-living diatom. Famintzin [16] illustrated the consortia of *Tintinnus inquilinus* and chains of *Chaetoceros* with two different morphologies that were considered morphotypes of a single diatom species (Figure S2 in Supplementary Materials). Schröder [49] identified the diatom attached to a lorica of an unidentified tintinnid as *C. tetrastichon*. Pavillard [50] noted the differences between the symbiotic diatoms illustrated by Daday [15] (also by Fol [14]) and *C. tetrastichon*, and he proposed *C. dadayi* for a diatom with oral-directed setae. Schröder [44] considered *C. dadayi* as a synonym of *C. tetrastichon*. Detailed studies of the valve morphologies support the consideration of *C. tetrastichon* and *C. dadayi* as independent species [51].

The symbiotic tintinnid remained identified as *Tintinnus inquilinus* until Kofoid and Campbell [3,4] proposed *Eutintinnus apertus* as an independent species. Some authors continued using the name *T. inquilinus* for the association with *Chaetoceros* spp. [39,52]. *Tintinnus inquilinus* is the first described tintinnid, and the original description of the basionym *Trichoda inquilinus* O.F. Müller 1776 included the species *Tintinnidium fluviatile* Stein 1863. *Trichoda inquilinus* was later reported as *Tintinnus inquilinus* Schrank 1803, *Vaginicola inquilina* Lamarck 1816, *Tintinnidium inquilinum* Fauré-Fremiet 1908, and *Figotintinnus inquilinus* (O.F. Müller) Hada 1976. Kofoid and Campbell [3] proposed *Tintinnus apertus* citing *T. inquilinus* sensu Claparède and Lachmann as an earlier record. Kofoid and Campbell [3] established the Bergen fjord, Norway, as the type locality of *E. apertus*. *Tintinnidium inquilinum* is

known from the cold waters of the North Atlantic as a sessile species that attaches to inert substrates by the aboral end [53,54]. It is difficult to accept that the symbiotic species *E. apertus*, which lives in warm seas, is the free-living species that Claparède and Lachmann described from the cold waters of Norway. The cold and warm water tintinnids are likely independent species.

The species *E. apertus* is commonly reported as the host of *C. dadayi* and *C. tetrastichon*. Pavillard [50] illustrated the lorica with attached *C. tetrastichon* as larger and with a less abrupt aboral narrowing than the lorica with attached *C. dadayi*. Kofoid and Campbell [3] described the following two species closely related to *E. apertus*: *Eutintinnus pacificus* was smaller in size, and *E. pinguis* was larger and with a less abrupt aboral narrowing than *E. apertus*. Balech [55] commented that *C. dadayi* and *C. tetrastichon* were attached to *E. apertus* and *E. pinguis*, respectively, and Taylor [20] also identified the tintinnid with attached *C. tetrastichon* as *E. pinguis*. Gómez [18] reported that the lorica of *E. apertus* in symbiosis with *C. dadayi* was shorter and with a more abrupt aboral constriction than in the symbiotic *E. pinguis*. However, an intraspecific variability of the lorica depending on the consortia with *C. dadayi* or *C. tetrastichon* cannot be excluded from consideration. The lorica of the original description of *E. pinguis* showed a less marked aboral narrowing as compared with the morphology of the host of *C. tetrastichon* (Figure 1e). The shape of the lorica of the symbiont of *C. tetrastichon* is closer to *E. pacificus*, but the lorica of that species was described with a smaller length (~70 µm long). Kofoid and Campbell [3,4] did not comment on the association of *E. pinguis* and *E. pacificus* with diatoms, while they observed *Chaetoceros* spp. attached to *E. apertus* and *E. lususundae*. The intraspecific morphological variability of *Eutintinnus* remains unexplored due to the difficulties in obtaining cultures of these oceanic species. Molecular data of *E. apertus* attached to *C. dadayi* are available [56], but it is unresolved if the host of *C. tetrastichon* is a morphotype of *E. apertus* or an independent species. In the molecular phylogeny, *E. apertus* clustered with *E. tenuis* and an unidentified species with agglutinated lorica (Figure S3 in Supplementary Materials).

Several authors have reported without illustrations the consortia of *Eutintinnus lususundae* and *Chaetoceros* [4,57,58], and *Hemiaulus hauckii* and *H. membranaceus* [58,59]. Kofoid and Campbell [4] commented for *Eutintinnus tenuis* “One lorica ... had a cuirass of four elongated frustules of an unidentified diatom of the genus *Isthmia*, resembling *I. inervis*”. The correct identification of the diatom is questionable as the frustule of *Isthmia inervis* is thick in girdle view, being difficult to harbor four frustules on a lorica. *Isthmia* is a predominately neritic genus, whereas Kofoid’s samples were oceanic. From the Mediterranean Sea, Pavillard [17] provided line drawings of the consortia of *E. lususundae* with *C. peruvianus* and *H. hauckii*. Rampi [60] also reported the diatom *Planktoniella sol* attached to *E. lususundae*. Marshall [58] reported the consortia of *Chaetoceros* and *E. fraknoi* and *E. lususundae*. The synonymy of *E. fraknoi* and *E. lususundae* has been a matter of debate [3,4]. The lorica of *E. fraknoi* is larger than in *E. lususundae*, and it showed an aboral flare that is missing in *E. lususundae*. According to the available molecular data, *E. fraknoi* and *E. lususundae* are independent species (Figure S3 in Supplementary Materials). Both species were described from the Mediterranean Sea, but molecular data of *E. lususundae* were only available from the Pacific Ocean [8,61,62]. Kazama et al. [61] reported DNA sequences of *E. lususundae* that clustered with two species with agglutinated loricae (*Leprotintinnus pellucidus* and *Tintinnopsis dadayi*). Xu et al. [62] provided more sequences of *E. lususundae* from specimens with hyaline or agglutinated loricae. Strüder-Kypke and Lynn [63] also provided the sequence of a species identified as *Eutintinnus* sp. with agglutinated lorica that clustered with *E. apertus*. The diagnosis of the family Eutintinnidae has been recently amended and remains restricted to species with hyaline loricae [56,64]. In a phylogenetic tree including all the currently available sequences of *Eutintinnus*, cells with hyaline and agglutinated loricae cluster within the clade of *E. lususundae*/*E. apertus* (Figure S3 in Supplementary Materials). This suggests that the clade of *E. lususundae*/*E. tenuis*/*E. apertus* contains cells with agglutinated loricae that have been mistaken for species of the genus *Leprotintinnus*, although the placement of *T. dadayi* within the group of *Eutintinnus* is highly questionable. Durán [65] and Marshall [58] reported particles adhered to the lorica of *E. lususundae* and *E. fraknoi*, respectively. All these features suggest a sticky

nature of the lorica of *E. lususundae*, but the consortia are not accidental because there is a clear selection for certain diatom taxa, and the adhesion occurs at defined positions (Figure 1f–n).

Chaetoceros peruvianus and *Hemiaulus* spp., as well as solitary cells of *Thalassionema* sp. are preferentially adhered to the lorica of *E. lususundae* (Figure 1f–n [17,66]). *Thalassionema* sp. is also adhered to *Salpingella* [25,52], although the tintinnid is more commonly found with *F. doliolus*. Balech [23] reported *F. doliolus* attached to *Eutintinnus* in tropical waters. From the Mexican Pacific, Gárate-Lizárraga and Muñetón-Gómez [67] reported the consortium of *F. doliolus* with a ciliate identified as *Vorticella*, but their micrographs corresponded to a tintinnid with an incomplete lorica, probably *Salpingella*. In addition to *Eutintinnus* and *Salpingella*, Vincent et al. [25] illustrated *F. doliolus* surrounding the lorica of *Amphorellopsis*, *Ascampbelliella*, *Dictyocysta*, and *Protorhabdonella* in tropical and temperate oceans. Froneman et al. [24] reported the consortium of *F. doliolus* and *Salpingella* in the sub-Antarctic Ocean. In Antarctic waters, living cells of *Fragilariopsis curta*, *F. cylindrus*, *F. pseudonana*, and *F. rhombica* were attached to loricae of the tintinnids *Laackmanniella naviculaefera* and *Codonellopsis gausi* [68].

The diatoms of the order Fragilariales (=Thalassionematales), Licmophorales and Protoraphiales (=Cyclophorales) are phylogenetically closely related and contain most of the known genera of araphid epizoic diatoms (*Licmophora*, *Protoraphis*, *Pseudofalcula*, *Pseudohimantidium*, and *Synedra*) and also the free-floating genus *Thalassionema* [31]. The etymology of the name *Fragilariopsis* suggests an affinity with the araphid order Fragilariales. However, *Fragilariopsis* is a raphid diatom closely related to *Pseudo-nitzschia*. The raphe of the raphid diatoms is responsible for the ability to adhere and glide over a substratum. This function is atrophied in *F. doliolus*, and therefore it does not glide in the cultures (Figure 1s), but it may still facilitate the orientation of the diatom chains on the lorica (Figure 1o–r).

4.3. Morphological Adaptions and Ecological Advantages

The term symbiosis derives from the Greek *sym*, meaning together and *bios*, meaning life and the etymology refers to describe distinct organisms living together [69]. The main types of symbioses are mutualism, commensalism, and parasitism. Mutualism involves interactions that benefit both organisms and commensalism is beneficial to one or more organisms but has little or no impact on the other partner. However, most of authors restricted the use of symbiosis for the mutualism [70]. Another interpretation considers the evolutionary aspect of relationships and refers to symbioses as long-term interactions that lead to novel capabilities of the partners [71]. The ciliate-diatom symbioses can be considered to be mutualism, as some aspects of the association can be beneficial to the partners, ranging from obligate to facultative depending on the case. Wahl [72], p. 101 defined the epibiosis as “the spatial association between a substratum organism (basibiont) and a sessile organism (epibiont) attached to the basibiont’s outer surface without trophically depending on it”. A problematic issue in some ciliate-diatom symbioses can be the determination of who are considered the basibiont and the epibiont. The term sessile refers to an organism that is anchored to a substrate and cannot move about freely. The diatoms in consortium with ciliates are araphid (except *Fragilariopsis*), without motility in the vegetative stage. The tintinnid ciliates cannot be considered to be sessile organisms (epibiont) as they are free swimmers. In the case of the tintinnid-diatom symbioses, the tintinnids and diatoms are the basibiont and epibiont, respectively. In contrast, Schröder [44] considered the tintinnid as the epibiont, and *Chaetoceros* as the basibiont (as plankton). The vorticellid ciliates can swim in some short life stages, but they are mainly sessile organisms living attached to a surface. In this case, the naked ciliates and diatoms are the epibiont and basibiont, respectively. This can be accepted in the case of the tiny vorticellid ciliates attached to large diatoms, but this is not evident in the case of the tiny cells of the diatom *Licmophora* sp. attached to the stalks of the vorticellid ciliate *Zoothamnium pelagicum*. When there are important differences in size between the partners, the epibiont and the basibiont can be considered to be the small and large partners, respectively. Consequently, it is difficult to establish a general definition of the terms epibiont and basibiont for the ciliate-diatom symbioses.

4.3.1. Symbiotic Diatoms

The interactions between two or more symbiotic organisms change the individual characteristics of each partner. The changes in buoyancy and motility result in access to resources (light, nutrients, and food), an increase in hydrodynamic drag or filtering rates for the ciliate, and a decrease in the diffusive boundary layer for the photosynthetic diatom, and anti-grazing strategies (mechanical protection or camouflage). The combination of these factors determines the success of the symbioses, and it is difficult to isolate the advantages of one factor without the interaction of the others. Laboratory experiments are difficult due to the lack of these symbiotic ciliates in culture [13] and even some of the diatoms, therefore, hypotheses on the ecology of the consortia are speculative. Attempts to culture *Chaetoceros dadayi*, *C. tetrastichon*, and *C. coarctatus* have been unsuccessful, whereas other epizoic diatoms have been cultured with the same method [31]. The chains of *F. doliolus* modified their curvature between the free-living and symbiotic forms (Figure 1o–s). *C. dadayi*, *C. tetrastichon*, and *C. coarctatus* are only known as symbiotic forms, but their setae configuration has diverged from other congeners, suggesting an adaptation to the symbiotic life. A relatively heavily silicified diatom chain could not survive in the surface waters of the tropical seas where the low turbulence and poor nutrient conditions are particularly harsh for free-living species of *Chaetoceros*. The locomotion of the ciliate provides the buoyancy to the symbiotic diatoms and allows the horizontal displacement which is not possible for the free-living counterparts.

The symbiotic *C. coarctatus* is also expected to benefit the flow field (for nutrients and gases) created by the feeding current, and the exudates of the ciliate. The setae configuration of *C. coarctatus* is a cage with bars that protect the vulnerable ciliate from predators, as noted in earlier studies [42]. Copepods are the most common predators, and they grab at the rear of the consortia and consume the ciliate. This seems to be the weak point of the consortia, and the diatom has developed a distinct, horn-like terminal posterior as a defensive strategy (Video S1 as supporting material at <https://youtu.be/lgxbKjJyUu0>, time 6:08–9:00).

Chaetoceros dadayi and *C. tetrastichon* have different construction of the setae. The setae of *C. tetrastichon* represent a higher protection because the lateral and posterior (aboral) flanks of the ciliate are covered. This consortium swims preferentially forward. In contrast, the setae of *C. dadayi* has a protection in the front and rear, and the consortia moves with alternating displacements in both directions (Video S1 as supporting material at <https://youtu.be/lgxbKjJyUu0>, time 0:22–0:54). Hernández-Becerril [51] reported that the valves of *C. tetrastichon* were less heavily silicified than the other member of the subgenus *Phaeoceros*. A reduction of the silicification has been also observed in other epizoic species as compared with benthic counterparts [31].

The consortium *C. dadayi* and *E. apertus* is the most evolved consortia where the diatom has important morphological modifications of the setae and it is unable to have an independent life. In contrast, *C. peruvianus* and *E. lususundae* seems to be an earlier evolutionary stage of the tintinnid and diatom consortia because the diatom is found with similar morphology as an independent species. Species of the genus *Fragilariopsis* are predominantly distributed in cold waters, especially near the Antarctica [68]. The consortia of *F. doliolus* and tintinnids are an exception because they are found in the tropical ocean, as well as in the cold waters such as the sub-Antarctic Ocean [24]. The closer relatives of *C. coarctatus* are polar or cold waters (*C. convolutus*, *C. concavicornis*, *C. danicus*, and *C. borealis*), as well as *C. densus* and *C. peruvianus* with a more widespread distribution [10]. It is possible to speculate that the symbioses have facilitated the spread of native cold-water diatom species such as *F. doliolus* or *C. coarctatus* into the temperate and tropical ocean.

4.3.2. Symbiotic Peritrich Ciliates

An obligate symbiosis is not evident for the tintinnid species. In contrast, peritrich ciliates need a substrate to proliferate in the pelagic ecosystem. The stalk and the adhesive disk are adaptations for a sessile life, and not for swimming. Several studies have revealed that the ciliates attached to substrates have higher filtration rates than their free-swimming counterparts [54,73]. Consequently,

peritrich ciliates that are able to find a substrate in the water column have an advantage. Peritrich ciliates (i.e., *Epistylis*, *Zoothamnium*) are commonly reported as epibionts of copepods [74]. This occurs preferentially in eutrophic coastal waters, being a rare feature in the open ocean. The life of a copepod is finite, and the epibionts must colonize other free-living copepods. Like other crustaceans, copepods periodically shed the exoskeleton, and this empty exoskeleton or exuvia is no longer a suitable motile substrate. In contrast, diatoms are “immortal” and the growth of the chain and further fragmentation provide new substrates that facilitate the expansion of the ciliate.

The length of the stalk of the vorticellid ciliates seems to be an adaptation to the type of host. The stalk of *Vorticella oceanica* has the necessary length to protrude beyond the setae and the ciliary movement is able to create a feeding current without interference of obstacles ahead of the zooid (Figure 3–5). In addition to the alteration of the flow field, the stalk contraction can serve against predation or mechanical injury, as noted in earlier studies (Figure 3 [42]). When the stalk contracts, the zooid is protected behind the entanglement of the diatom setae. In the case of *P. coscinodisci*, a long stalk is not necessary as the diatom host lacked valve extensions that could act as grazing deterrent and interfere with the ciliate feeding currents (Figure 6). The studies on the motility of *Vorticella* have been based on individuals attached to inanimate substrates. The ciliary movement of the zooids of *V. oceanica* is coordinated to propel the consortium straight forward and this facilitates exploring a new parcel of nutrients and food resource (bacteria). Water expelled through the rear of the diatom avoids a rejected particle returning to the feeding currents of the pseudocolony (Video S1 as supporting material at <https://youtu.be/lgxbKjyUu0>, time 10:43–10:50).

Vorticellid ciliates are considered to be the fastest solitary organism relative to their size on Earth (see a review in [28]). *V. oceanica* seems to beat all the records. The peak speed of *V. oceanica* that attained $165 \text{ mm}\cdot\text{s}^{-1}$ (Figure 3) duplicated the value of $88 \text{ mm}\cdot\text{s}^{-1}$ of the most investigated congeneric species, the freshwater species *V. convallaria*. We can find a higher speed of the stalk contraction in other peritrich genera of ciliates, but they are colonial species and, consequently, the speed is due to the summation of numerous individuals joined by a common stalk. During the contraction, the zooid of *V. oceanica* attained $5400 \text{ body length}\cdot\text{s}^{-1}$ ($\sim 30 \mu\text{m}$ in length), being the fastest solitary organism relative to their body length in the Earth. The cheetah, the fastest terrestrial animal, reaches $120 \text{ km}\cdot\text{h}^{-1}$ and moves at $\sim 24 \text{ body length}\cdot\text{s}^{-1}$ [75]. A car ($\sim 4 \text{ m}$ long) moving at this rate of $5400 \text{ lengths}\cdot\text{s}^{-1}$ would be traveling at a speed of $70,000 \text{ km}\cdot\text{h}^{-1}$. The speed of the zooid of *V. oceanica* of $165 \text{ mm}\cdot\text{s}^{-1}$ ($=0.6 \text{ km}\cdot\text{h}^{-1}$) is a low at the human size scale, but it should be taken into account that the zooid ($\sim 30 \mu\text{m}$ in length) moves in a viscous medium (seawater). Flow is laminar when the Reynolds number (Re) is low (i.e., the fluid moves smoothly around the cells and can be considered to be moving in layers between which there is no significant mixing) and flow is turbulent when Re is high [76]. It can be expressed as $\text{Re} = \rho u L \mu^{-1}$ where ρ = density of seawater ($1023.5 \text{ kg}\cdot\text{m}^{-3}$), u = velocity, L = characteristic length, and μ = dynamic viscosity of seawater at $25 \text{ }^\circ\text{C}$ ($0.95 \times 10^{-3} \text{ kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$). The Reynolds numbers are low, i.e., ~ 7 for a zooid that moves at $165 \text{ mm}\cdot\text{s}^{-1}$ during the stalk contraction, and <0.1 for the microalga used as tracer ($\sim 4 \mu\text{m}$ diameter) that was aspirated at $20 \text{ mm}\cdot\text{s}^{-1}$.

The basic feeding mechanism of an individual sessile peritrich ciliate in a quiescent flow field generates an axis-symmetric flow through cilia beat. This can result in fluid trapped in a toroidal vortex and a lower renew with surrounding waters [29] and could constitute a problem for vorticellids attached to immobile substrates (i.e., surfaces of wastewater treatment plants [30]). The close coexistence of sessile peritrich ciliates with diatoms is mutually beneficial from the point of view of fluid dynamics and convective transport of potential prey. *Vorticella oceanica* and *Pseudovorticella coscinodisci* rendered their hosts motile, avoiding the fluid entrapment issue. The gregarious behavior also has the advantage that the presence of other active zooids break-up the regular pattern. The multiple feeding currents due to different orientation of the zooids (nicking angle) and the inclination of the stalk, as well as the spontaneous stalk contraction, introduce variability to the flow field around the consortium [29]. The stalk is short in *P. coscinodisci*, and this can constitute an advantage because a vortex of trapped matter is not formed below the zooid (Figure 6). In a review, Ryu et al. [28] reported feeding currents

within the order of $0.1\text{--}1\text{ mm s}^{-1}$ for *Vorticella* spp. This study recorded for *P. coscinodisci* a maximum speed of $0.66\text{ mm}\cdot\text{s}^{-1}$ of the particles in the feeding current (Figure 6).

The consortium of *V. oceanica* and *C. coarctatus* is an extraordinary ecological and biophysical model that allows the colonization of environments where they cannot live as independent organisms. The epibiosis of *P. concinodisci* on *Coscinodiscus* and *Palmerina* is another successful evolutionary experiment. The buoyancy and a flux of gases and nutrients induced by the epibiont can favor the survival of the symbiotic diatom in post-bloom conditions of decay in turbulence and nutrient levels. The epibiosis increases the survival of diatom cells that can act as a seeding population for the free-living forms when favorable environmental conditions return.

4.3.3. Symbiotic Tintinnid Ciliates

The adhesion of the diatom modified the characteristics of the lorica. The adhered diatom is a kind of postiche allowing a larger, coarser, or spine-protected lorica. The first issue to consider is the function of the lorica, and then the advantages of a modified lorica. The most intuitive view of the lorica is an anti-grazing defense. Agglutinated lorica with particles attached (diatoms frustules, spicules of sponges, coccoliths, and mineral grains) are found preferentially in coastal tintinnids [5]. These particles reinforce the lorica but decrease the buoyancy [77], and higher sinking rates can facilitate the escape from predators in the mixed coastal waters [78]. Mineral grains are scarce in the stratified open ocean, and therefore instead of the inert particles found in coastal waters [62], *E. lususundae* attaches to selected diatoms in the open ocean (Figure 1f–n). The main preferences are diatoms of the subgenus *Phaeoceros*, with coarse and spiny setae. Attachment of diatoms increases the effective size of the tintinnid, and it is expected to decrease its vulnerability to small size predators, but perhaps increase risk from slightly larger predators. The coastal species *Tintinnidium inquilinum* attaches its lorica to solid surfaces, including suspended particles, and it is less susceptible to predation by copepods than is a morphologically similar tintinnid that is entirely free swimming [54]. Changes to the lorica can have other impacts for the ciliate. On the basis of published illustrations of *Eutintinnus apertus* with attached *Chaetoceros dadayi*, Jonsson et al. [54] calculated that the drag resulting from the exposed surface, could increase the flow through the ciliary filter by a factor of three. The lorica of this consortium also showed a distinctive aboral constriction where the diatom did not attach (Figure 1a,b). It is uncertain if the aboral narrowing of the lorica could have any advantage as compared with other species of *Eutintinnus* with a straight lorica. Furthermore, the morphology of the lorica of species of *Salpingella* is close to *Eutintinnus*, but there are no observations of *Chaetoceros* adhered to *Salpingella*. The lorica of most of the oceanic tintinnid is hyaline, and the transparency could help avoid visual predators. Transparency is apparently not a priority in the consortia of *Fragiolariosis doliolus* because the diatom chain encircles the lorica as a green bracelet (Figure 1o–r).

The interaction between diatoms and ciliates is primarily one of prey and predator, but these consortia constitute unexplored ecological and biophysical models. There is still much to be learned regarding the benefits and detriments of the interactions, the balance between obligate and opportunistic interactions, as well as the flexibility and specificity between partners. Although a great deal has been learned from observations and descriptions, cultures of these consortia are necessary to manipulate and understand the interactions.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2673-1924/1/3/10/s1>, Figure S1: Time-lapse sequence of the cilia movement of *Vorticella oceanica* recorded at $5000\text{ frames}\cdot\text{s}^{-1}$ (fps), Figure S2: Earlier illustrations of diatom and tintinnid consortia, Figure S3: Phylogenetic tree based on SSU rDNA sequences, showing the position of the tintinnids of the family Eutintinnidae.

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