Diatom Epibionts on Amphipod Crustaceans: A Possible Vector for Co-introductions?

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Abstract: Epibiotic associations can result in co-introductions of non-indigenous species, which may affect ecosystems in several ways. In fouling communities of three estuaries in southern Brazil, a number of amphipods was found to harbour a dense coverage of epibionts. Three different species, the two globally widespread caprellids *Caprella equilibra* and *Paracaprella pusilla*, as well as the ischyrocerid *Jassa valida*, had been colonised by diatoms. Further scanning electron microscope analyses assigned these diatoms to 14 different species that had previously been reported from benthic habitats. This is one of the scarce records of diatoms attached to amphipods. The occurrence of the diatom *Amphora helenensis* represents the first report for Brazilian waters as well as the second record for the whole SW Atlantic Ocean. As some diatoms were associated with common fouling amphipods, a possible regional spread aided by these crustaceans seems likely. Possible effects of this amphipod-diatom association on the animals and their implications for the underlying ecosystems of this remain to be elucidated.

Keywords: Amphipoda; Bacillariophyceae; basibiont; biofouling; NIS

1. Introduction

Human-based transport mechanisms of non-indigenous species (NIS) are often investigated, but processes of co-introduction through associations like parasitism or epibiosis are still understudied [1,2]. These associations allow for simultaneous multiple introductions that can consequently affect ecosystems on several levels, and may further facilitate secondary invasions (i.e., invasion meltdown hypothesis) [3]. For example, the canopy-forming marine macroalga *Sargassum muticum* (Yendo) Fensholt, 1955, was probably introduced to North American and European shores with the Pacific oyster *Magallana gigas* (Thunberg, 1793) (formerly known as *Crassostrea gigas*), which was probably introduced to North American and European shores with the Pacific oyster *Magallana gigas* (Thunberg, 1793) (formerly known as *Crassostrea gigas*), which was intentionally introduced for aquaculture purposes. Moreover, oyster trade is the presumed vector for the spread of different NIS around the world, including different macroalgae, ascidians, and even amphipods [4,5].

Marine ecosystems face an increased risk of the spread of NIS because even geographically distant locations are virtually connected through the oceans with intensive human shipping activities. Shipping facilitates the dispersal of species through hull fouling, ballast water, and other vessel components, e.g., [6,7]. Besides, ports meet most of the requirements for the successful introduction and establishment of NIS. Ports are subjected to continuous pressure of propagules in usually eutrophic environments and are modified by artificial structures where native species can lose their potential competitive advantage [8,9]. Hard substrates in ports are usually characterised by fouling communities consisting of...
Amphipod crustaceans can dominate marine and estuarine fouling communities in terms of abundance [12,13]. Amphipod fouling assemblages are mainly characterised by either tubicolous or free-living suspension feeders, but different combinations of functional traits are also common including grazers, carnivores, and epibionts associated to other organisms [14]. Common tube constructors such as ischyrocerids (e.g., Jassa, Erricthonius), different corophiids (e.g., Monocorophium, Corophium, Apocorophium), aorids (e.g., Grandidierella, Microdeutopus), and photids (e.g., Photis, Gammaropsis) can dominate these communities along with crawling/clinging taxa such as caprellids (e.g., Caprella, Paracaprella), stenothoids (e.g., Stenothoe), and podocerids (e.g., Podocerus). Moreover, at least 55 species of Amphipoda are recognised as introduced or invasive in at least one marine region around the world, of which more than half are directly associated with biofouling [15]. Many species are recognised as neocosmopolitan (i.e., human-mediated cosmopolitan species; see [16]) as well as widespread in invaded regions [17–20]. Some amphipods are also known to bear different epibionts that, in some cases, would benefit from their basibiont movement without harming the host [21–24]. Accordingly, amphipods might be effective vectors for some organisms that are directly associated with them.

In a field experiment designed to study fouling communities related to ports, artificial plates of polythene and rope (nylon rope wrapped around a squared iron frame) were deployed, at an average depth of 1.5 m, in three estuaries of southern Brazil: the Cananéia-Iguape estuarine complex, the Paranaguá estuarine complex, and the Guaratuba Bay. All investigations were carried out between June 2017 and June 2018. After three months, the plates were collected and separately preserved in 96% ethanol. At the site of the experimental set-up, additional samples of the fouling were scraped from buoys and preserved in ethanol for further comparison. All amphipod individuals were sorted and identified to species level. During this step, some amphipods displayed conspicuous levels of epibiosis and were separated for further examination with a scanning electron microscope (SEM). These specimens were mounted on aluminium stubs with a small piece of adhesive carbon tape, completely air-dried at room temperature, and kept in an air desiccator jar. The stubs were then sputter-coated with gold in a Baltec metallizer and observed in a JEOL JSM 6360-LV electron microscope at an acceleration voltage of 15 Kv and 7 mm working distance (Figure 1A). As no further frustule cleaning with chemicals could be applied, many diatom cells from different samples (i.e., amphipod specimens) were photographed, measured, and preliminary separated into morphotypes to ease identification. The definite identification was then determined by carefully comparing their frustule morphologies and dimensions.
2. Results & Discussion

A total of 14 different diatom species belonging to seven families was identified (Table 1). Most of the taxa recorded fell into frustule size ranges reported in the literature, except for two species, which presented smaller valves than previously reported. *Cocconeis dirupta* Gregory, 1857 valves were shorter (length 16–12 µm, Table 1) than elsewhere (e.g., length 24–17 µm in [25]). The same deviation was found in *Halamphora coffeaeformis* (C. Agardh) Mereschkowsky, 1903, in which valves were 25–11 µm long, but usually ranging from 30 µm to 53 µm, even when our material is compared to another variety of this species, *A. coffeaeformis* var. *aponina* (Kützing) Archibald & Schoeman, 1984 (length 39–15 µm in [26]). The families Catenulaceae and Naviculaceae were the most species-rich with three species each. The diatoms found are typically benthic species, i.e., attached to substrates employing a variety of mucilage secretions. The most common structures were mucilaginous peduncles in *Tabularia* spp. and *Licmophora* spp. (Figures 1I and 2B), along with mucilage pads as in *Amphora helenensis* M. H. Giffen, 1973, *H. coffeaeformis*, and *Cocconeis* spp. (Figure 1E, G and Figure 2I). Other species, such as *Navicula* spp., seemed to
attach to amphipod individuals using mucilaginous secretions of the raphe (Figure 2L,O). Although no quantitative analysis was performed, some diatom taxa were dominant such as *Tabularia affinis* (Kützing) Snoeij, 1992, *Licmophora* spp., *H. coffaeiformis*, and *A. helenensis*, comprising about 50% of the total diatom composition in the majority of analysed amphipod individuals. While some diatoms are known to be specialised epizoic on planktonic copepods [27], only a few studies have dealt with the growth of diatoms on benthic invertebrates. Usually diatoms of the genera *Navicula*, *Amphora*, and *Cocconeis* are known to occur on several species of cnidarians [28], sponges [29], and gastropods [30,31]. Despite some species being known to display specificity to some taxa, all the diatoms found here have been reported elsewhere from other substrates such as macroalgae and rocks [32,33], indicating that these species are rather opportunistic to grow on invertebrates. The fouling communities of the artificial plates inhabited by the amphipods were characterised by dense assemblages of hydrozoans (e.g., *Clytia* spp., *Ectopleura crocea* (Agassiz, 1862), *Obelia* spp., and *Eudendrium carneum* Clarke, 1882) and barnacles (e.g., *Megabalanus coccopoma* Darwin, 1854, *Amphibalanus amphitrite* (Darwin, 1854)), which are both recognised basibionts of either diatoms or amphipods, e.g., [34–38], which would make them the most probable vector of diatoms for the amphipods. Concomitantly, species inhabiting soft bottoms have been reported to host relatively few epibionts in comparison to hard-substrate species [39].

Table 1. Morphometric data of the 14 epibiotic diatom species recorded on the amphipods, sampled in estuaries of South Brazil.

| Species                                      | Length/Breath (µm) | Striae Pattern | N° Striae in 10 µm | Other Features |
|----------------------------------------------|--------------------|----------------|--------------------|----------------|
| *Amphora helenensis* Giffen, 1973            | 22–16/2.5–2.2      | radiate        | 19–22              | apices rounded |
| *Amphora ovalis* var. *pediculus* (Kützing) Van Heurck, 1885 | 16–6/2.2–2.0     | parallel to radiate | 24–28              | apices rounded |
| *Halamphora coffaeiformis* (C. A. Agardh) Levikov, 2009 | 25–11             | radiate        | 20–22              | apices subcapitate |
| *Cocconeis dirapia* Gregory, 1857           | 16–12/9–6         | parallel       | 16                 | striae with 2 rows of poroids |
| *Cocconeis* sp. 1                            | 14–12/5           | slightly radiate | 28                 | striae with 1 row of poroids |
| *Licmophora* cf. *abbreviata* C. Agardh, 1831 | 75–40/18–16      | parallel       | 11–12              | 12–14 slits/multiscissura |
| *Licmophora ehrenbergii* (Kützing) Grunow, 1867 | 75–40/18–16    | parallel       | 12–14              | 14–16 slits/multiscissura |
| *Melosira moniliformis* (O. Müller) Agardh, 1824 | 22–20 (diameter) | radial         |                     | central umbilicus |
| *Navicula platyventris* Meister, 1934 (1935) | 14                | radiate       | 7–5                | 5–7 areolae/striae |
| *Navicula* sp. 1                              | 22–20/5–4        | parallel       | 18                 | 8–9 areolae/stria |
| *Navicula* sp. 2                              | 12–10/2.5        | parallel       | 16–18              | 3–4 areolae/stria |
| *Rhoicosphenia marina* (Kützing) M.Schmidt, 1889 | 20–13/8–6        | radiate       | 18–20              | raphe incipient in one valve |
| *Tabularia affinis* (Kützing) Snoeij, 1992   | 52–32/4.0–3.5    | parallel       | 22                 | biseriate striae |
| *Tabularia fasciculata* (C. Agardh) D.M. Williams & Round, 1986 | 80–40/5.5–4.5   | parallel       | 15–18              | uniseriate striae |
Figure 2. Diatoms found on fouling amphipods in south Brazilian estuaries. (A) overall view of two frustules of *Licmophora abbreviata* (scale: 10 µm); (B) four cells attached by short mucilage stalks of *Licmophora ehrenbergii* (scale: 20 µm); (C,D) details of frustules showing the ocellulimbus at the apices of *Tabularia affinis* (scale: 2 µm) and (E) internal view of valve apex with rimoportula (scale: 1 µm); (F) lateral view of a frustule of *Amphora pediculus* (scale: 2 µm); (G) lateral view of a frustule of *Amphora helenensis* (scale: 5 µm); (H) *Rhoicosphenia marina* (scale: 2 µm); (I) Frustule attached by mucilage extruded through the raphe of *Cocconeis dirupta* (scale: 2 µm); (J) Frustule attached by mucilage extruded through the raphe of *Cocconeis* sp. 1 (scale: 1 µm); (K) *Amphora*, *Halamphora*, and *Tabularia* (scale: 10 µm) on a gill of *Paracaprella pusilla*; (L) Frustule in valve view, with a central raphe system of *Navicula platycentris* (scale: 2 µm); (M) *Melosira moniliformis* (scale: 5 µm); (O) *Navicula* sp. 1 (scale: 2 µm) with (P,Q) two details of apical and central regions (scale: 1 µm); (R) *Navicula* sp. 2 (scale: 2 µm); (S) Frustule in girdle view of *Halamphora coffeiformis* (scale: 5 µm) covered by a seta of *P. pusilla*.

Of the 15 analysed amphipod specimens, twelve individuals bore epibiotic diatoms (Table 2). The amphipod specimens belonged to only three species: the two globally distributed caprellids *Caprella equilibra* Say, 1818, and *Paracaprella pusilla* Mayer, 1890, and the ischyrocerid *Jassa valida* (Dana, 1853). On a single specimen of *Ericthonius brasiliensis* (Dana 1853), as well as on other specimens of *J. valida* and *P. pusilla*, non-identified apostomid ciliates were found, but no diatoms. The highest diversity of epibiont was found on a male specimen of *P. pusilla* with seven different diatom species on its cuticula (Table 2). Three diatom species were each found only once on caprellids: *Melosira moniliformis* C. Agardh, 1824, *Rhoicosphenia marina* (Kützing) M. Schmidt, 1889, and *Tabularia fasciculata* (C. Agardh) D. M. Williams & Round, 1986. Although males generally seemed to display higher
epibiont diversity compared to females, only two female individuals were available for analysis. Possible sex-specific differences in epibiotic colonisation may relate to differences in the moultng cycle. However, due to the low replicate number, any assumptions must be regarded with caution. The diatoms were distributed over the whole body of the animals, but the appendages seemed to exhibit a higher coverage with epibions than the rest of the body. This appears to be counterintuitive as antennas and pereopods are easily and frequently cleaned by the animals, suggesting that the amphipods might be unaffected by the diatoms as reported for epizoic ciliates on the amphipod *Bathyporeia* spp. [40]. This is in contrast to other crustaceans where epibionts have been shown to cause stress to the crustacean host individuals [41]. In addition, the possibility that the amphipods may even benefit from epibiotic diatoms as an additional food source cannot be ruled out as they are known to be omnivores and diatoms were frequently found in amphipod digestive tracts [42].

Table 2. Overview of amphipod and diatom species recorded for each analysed sample. Substrates: PP—polyethene plate; B—buoy; RP—rope plate.

| Estuary                      | Substrate | Lat  | Long  | Amphipod Species | Sex          | Amphora helenensis | Amphora pediculata | Halympha phaerophoroides | Cocconeis sp.1 | Cocconeis dirigua | Licmophora cf. abbreviata | Licmophora ehrenbergii | Melosira moniliformis | Navicula platynis | Navicula sp.1 | Navicula sp.2 | Rhoicosphenia marina | Tabularia affinis | Thalassiosira ascissa |
|------------------------------|-----------|------|-------|------------------|-------------|---------------------|---------------------|------------------------|----------------|----------------|------------------------|--------------------|---------------------|----------------|-------------|-------------|---------------------|----------------|------------------|
| Cananéia-Iguape estuarine complex | PP        | 25.021 | 47.918 | *Paracaprella pusilla* | Male | x      | x      |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |
|                              |           | 25.027 | 47.914 |                | Juvenile | x      |                  |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |
| B                            |           | 25.467 | 48.663 | *Paracaprella pusilla* | Male | x      | x      |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |
| PP                           |           | 25.473 | 48.649 | *Caprella equilibra* | Female | x      |      |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |
| Paraguá estuarine complex    | B         | 25.497 | 48.491 | *Caprella equilibra* | Male | x      | x      |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |
| RP                           |           | 25.549 | 48.389 | *Jassa valida* | Juvenile | x      | x      |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |
|                              |           | 25.549 | 48.388 | *Paracaprella pusilla* | Male | x      |      |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |
| Guaratuba Bay                | PP        | 25.875 | 48.589 | *Caprella equilibra* | Female | x      | x      |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |
|                              |           |         |        | Juvenile | x      | x      |      |                       |                |                |                        |                    |                     |                 |             |             |                      |                 |                  |

Nevertheless, potential interactions of diatoms with their amphipod hosts (e.g., commensalism) are completely unknown. Although it could be possible that high diatom densities on fragile body parts such as the gills or sensory appendages (Figure 1C,H) may lead to handicaps (e.g., interferences in gas exchange, reproduction, or sensorial activity) [43,44], the amphipods and the diatoms seemed to display a basibiont-epibiont association.

The biraphid diatom *Amphora helenensis* found in this work represents the first on record for Brazilian coasts. This species has been recorded repeatedly around the world outside of its type locality (South Africa). Recent records include the coastal waters of Australia and New Zealand [45], Europe (The Netherlands [46]), China (Yellow Sea [33]), and Mexico (Pacific Ocean [47]). We found *A. helenensis* in the Guaratuba Bay and in
the Paranaguá estuarine complex. The latter site is well-studied and has been subject to extensive taxonomic diatom studies since 1960 (see [48]). This suggests that *A. helenensis* was introduced recently to South Brazil. However, it cannot be ruled out that this species had been overlooked or misidentified with other closely related species at other locations in the SW Atlantic, where it was recorded only once in Argentina [49]. Therefore, *A. helenensis* can be classified as cryptogenic.

The presence of diatoms on amphipods might not be a newly recorded association, although it was never further investigated and only briefly mentioned. Previous records on species of the genera *Leucothoe* and *Caprella* are documented, either in the descriptions of amphipods or unintentionally photographed (e.g., [50], see Figures 5 and 6 in [51]). As benthic amphipods can be associated with other sessile fauna (like in this case) or macroalgae [52], they are susceptible for colonisation by motile diatom species (e.g., *Navicula* spp.) or even sedentary species (e.g., *Tabularia* spp., *Licmophora* spp.) if these are abundant on the surface of the shared host. If the new basibiont is abundant (especially as part of a fouling community), this may result in a rapid spread of the diatom. In fact, regional boating is known to aid the spread of NIS among marinas and ports by allocating the whole communities on the ship hulls [53,54]. Diatoms may thus not only benefit from direct human-mediated transport, but also from the active movement of basibiont amphipods to new natural or artificial substrates as well as from nutrients provided by the host.

3. Conclusions

Our findings represent the first documented analysis of amphipod-associated epibiotic diatoms. In the current case, common benthic diatom species were found on the cuticle of three widespread amphipods, suggesting a rather opportunistic epibiosis due to the epifouling lifestyle of these crustaceans. Especially in fouling communities, amphipods can be potent vectors for epibiotic organisms such as diatoms and ciliates (e.g., [22]) aiding the spread of newly introduced epibionts to neighbouring habitats.

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