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# Report of Epibiont Diatom *P. pacificum* on the Cyclopoid Copepod *D. affinis* from the Southwestern Shelf Waters of India (Eastern Arabian Sea)

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**Abstract**
*Pseudohimantidium pacificum*, an epizoic diatom has been reported from different parts of the global oceans, however, has not been reported from the shelf waters of India. This symbiotic association of *P. pacificum* with a specific copepod species (*Ditrichocorycaeus affinis*) was repeatedly noticed from the shelf waters of the Arabian Sea during the winter monsoon from three locations in the South East Arabian Sea. Our study suggests towards host specificity of *P. pacificum* and additionally reveals their preference towards male hosts which is consistent with the earlier reports from other regions. Most importantly, this report extends the range of geographic distribution of the epibiont and this is the third report on this epibiont from the Indian Ocean region. It also suggests that epibiosis in marine zooplankton is much frequent phenomenon that has the potential to play an important role in the marine zooplankton population dynamics.

**Keywords** Epibiont · *P. pacificum* · Zooplankton · Copepod · Epibiosis · Arabian Sea

## Introduction

Marine microorganisms such as bacteria, ciliate, diatoms, etc. show a strong affinity towards attachment. Among other microorganisms, the association of some diatoms on the exoskeletons of chitinous zooplankton is rather common in the marine environment (Ohtsuka et al. 2004). Zooplankton provide suitable sites to the diatoms for attachment, growth, and colonization (Walkusz and Rolbiecki 2007). These epibionts benefit through enhanced photosynthesis due to vertical migration of hosts during the daytime, relief from particle-feeding predators, as well as utilization of nutrients released from the host's body and/or from captured prey (Hiromi et al. 1985). In turn, they increase the buoyancy of hosts, thus, lessening the required energy for swimming (Klevenhusen et al. 1933). Additionally, zooplankton could be benefitted via fresh oxygen supply during intensive bloom (Chiavelli et al. 1993). Diatoms are typically host-specific while associating with zooplankton such as copepods (Hiromi et al. 1985; Prasad et al. 1989; Totti et al. 2010). *P. pacificum* is one of the most abundant epibionts (Simonsen 1974; Navarro 1982; Skovgaard and Saiz 2006) which is principally found in Corycaeidae family (Russell and Norris 1971; Hiromi et al. 1985). Giesbrecht (1892) first reported the association between *Corycaeus elongatus* and *P. pacificum*, collected from the Adriatic Sea. Attachment sites of *P. pacificum* vary between male and female copepods. A higher abundance of the diatoms is generally observed on the second antennae, genital and anal segments of males, and the carapace as well as thoracic segments of females (Russell and Norris 1971).

Although quite a few studies had been carried out about the attachment of diatoms on zooplankton from temperate waters, hardly any information is available from tropical and subtropical waters. Reports on the association between *P. pacificum* on *Corycaeus* sp. had mostly been recorded from the United States (Motoda 1963; Russell and Norris 1971; Gibson 1978, 1979; Gárate-Lizárraga and Muñetón-Gómez 2009; Tiffany 2011; Fernandes and Calixto-Feres 2012; Sunesen et al. 2015; Lizarraga and Esqueda-Escarcega 2018), Indonesia (Früchtl 1924), Korea (Lee et al. 1993),

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Spain (Skovgaard and Saiz 2006). Whereas, only a handful of reports are available from India to date (Sahu et al. 2015; Padmakumar et al. 2015). Besides, those studies mostly encompassed offshore of the Bay of Bengal and Northern Arabian Sea, respectively. As yet, no information is available from the shelf waters of the Arabian Sea. The information will help us to generate baseline information about the association between *P. pacificum* and *D. affinis*. from the west coast of India.

## Material and Methods

Zooplankton samples were collected onboard *RV Sindhu Sadhana* (SSD-068), during December 2019 from three locations (*i.e.*St 1- 11.002°N, 75.1159°E, St 2- 14°N, 73.33°E, and St 3- 16.0028°N, 72.6179°E) along the south-western shelf water of India (eastern Arabian Sea) (Fig. 1). Zooplankton samples were collected with the help of a multi-plankton net (Multinet, Hydro-bios, Kiel, Germany; mouth area of 0.25 m2 and mesh size of 200 µm) operated through the mechanical winch covering the upper 200 m water depth by vertical hauls of plankton net and immediately after collection, preserved with 10% formaldehyde in a clean plastic bottle till further analysis. Microscopic analysis was carried out with the help of a stereo zoom microscope (make-Nikon; Model-SMZ-18) as well as an inverted microscope (Make-Nikon; Model-Eclipse T*i*2). Photomicrographs, measurements of diatom (n = 50), and copepod were obtained by using a compound microscope (Make-Nikon; Model-Eclipse C*i*). The copepod (*D. affinis* McMurrich 1916) was identified using the identification keys by Kang et al. (1990) and Radhika Nandan (2020). Whilst, the terminology and systematic position of diatom (*P. pacificum* Hustedt and Krasske 1941) followed Krasske (1941), Simonsen (1970), Gibson (1978, 1979), Hiromi et al. (1985), Rivera et al. (1986), Round et al. (1990), Lee et al. (1993), Garate-Lizárraga and Muñeton-Gomez (2009).

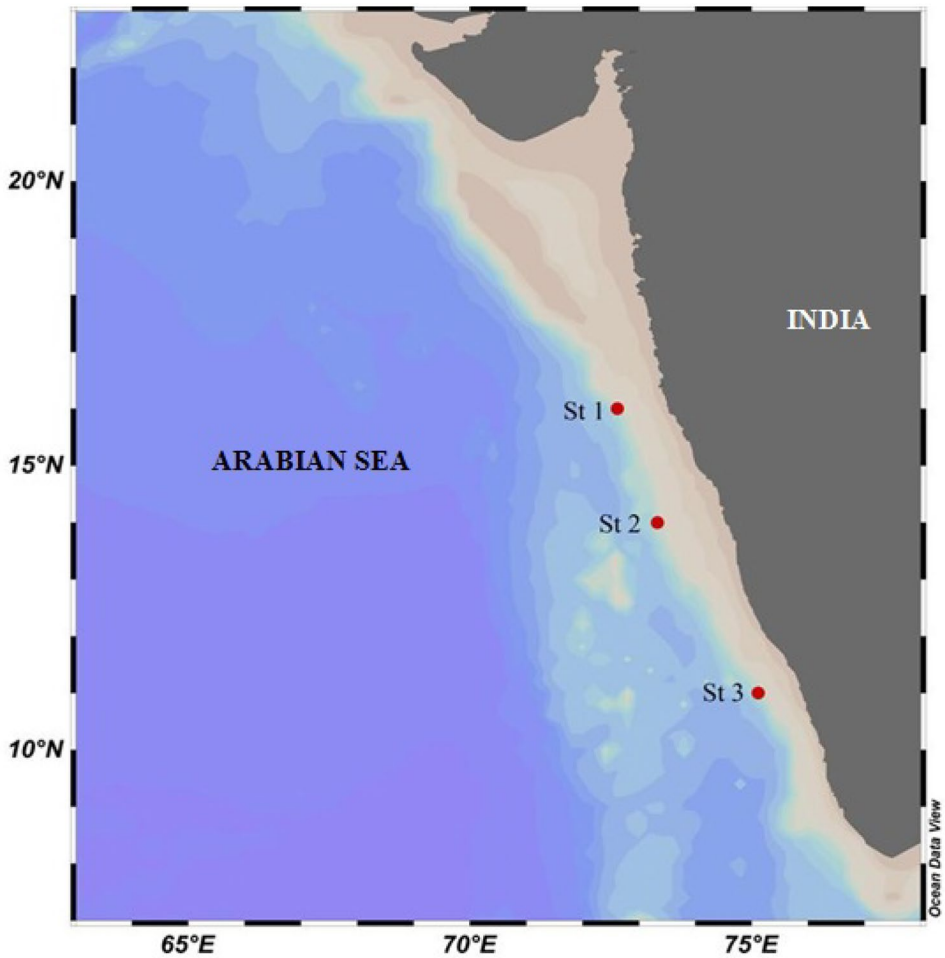


**Fig. 1** Map of sampling locations

## Results and Discussion

The stalked epizoic diatoms which were found attached to the surface of the copepod (*Ditrichocorycaeus affinis*) were morphologically identical with *Pseudohimantidium pacificum*, hence, identified as the same.

## Taxonomic Classification and a Brief Description of the Diatom

Hustedt and Krasske (1941) first described the diatom species *P. pacificum* from the Chilean waters, on the eastern coast of the South-West Pacific.

Classification of the diatom:
**Empire**: Eukaryota Chatton, 1925.
**Kingdom**: Chromista Cavalier-Smith, 1981.
**Phylum**: Bacillariophyta Karsten, 1928.
**Subphylum**: Bacillariophytina Medlin and Kaczmarska, 2004.
**Class**: Bacillariophyceae Haeckel, 1878.
**Subclass**: Fragilariophycidae Round, 1990.
**Order**: Protoraphidales Round, 1990.
**Family**: Protoraphidaceae Simonsen, 1970.
**Genus**: *Pseudohimantidium* Hustedt and Krasske, 1941.
**Species**: *P. pacificum* Hustedt and Krasske, 1941.


**Fig. 2** A: Light micrographs of the *P. pacificum* cells on the exoskeleton of *D. affinis*. The arrows point the diatom cells (Scale bar: 100 µm). B: Magnified view of the diatom colony (Scale bar: 50 µm)

**Table 1** Measurements of *P. pacificum* based on available literature and the present study

| Apical length (µm) | Transapical length (µm) | Reference |
|---|---|---|
| 44-78 | 9-11 | Husted and Krasske, 1941 |
| 31-35 | 7.5 | Voigt 1958 |
| 85-106 | 8-10 | Voigt 1958 |
| 31-102,85-113 | 8-12,8-11 | Simonsen 1970 |
| 28-70 | 9-15 | Russell and Norris 1971 |
| 40-112 | 8-12 | Belyaeva 1973 |
| 70-108 | 8-13 | Belyaeva 1973 |
| 32-53 | 7-15 | Gibson 1978 |
| 38-49 | 9-13 | Navarro 1982 |
| 38-105 (70-90) | 9.8-19 (12-14) | Rivera et al. 1986 |
| 34-76 | 9-14 | Lee et al. 1993 |
| 39-46 | 9-13 | Fernandes and Calixto-Feres 2012 |
| 38-44 | 10-14 | Sahu et al. 2015 |
| 42-47 | 8.5-10.5 | Sunesen et al. 2015 |
| 32-51 | 7-14 | Padmakumar et al. 2015 |
| 28-70 | 10-17 | Lizarraga and Esqueda-Escarcega 2018 |
| 30-35 | 9-12 | **Present study** |

*Hormophora rogallii* (Jurilj 1957), *Hormophora zavodnikia* (Jurilj 1957), *P. pacificum* var. *minor* (Voigt 1958), *P. adriaticum* (Voigt 1958), and *Sameioneis carinaes* (Russell and Norris 1971) are considered to be synonymous with *P. pacificum* (Simonsen 1974).

## Diatom Cell Morphology

*P. pacificum* can be easily identified regarding the valve shape, presence of axial area, as well as occurrence of a row of labiate processes on both ends of the valve (Rivera et al. 1986; Sunesen et al. 2015). Earlier, Simonsen (1970), Hasle (1973), and Gibson (1979) had precisely described the general morphology of the valves of *P. pacificum*. The frustules were curved in girdle view (Fig. 2). Several numbers of the diatom were found to be attached with the copepod through mucilage stalks. Usually, the diatom secretes watery and sticky mucilage (mucopolysaccharides) which becomes the stalk and attaches with the copepod (Russell and Norris 1971). In our present study, most stalks were branched (Fig. 2), having more than one frustules attached with it, while, few were branchless. The stalks emerged from the frustules through a series of fissures present at the apices (Gibson 1979).

Measuring the apical and the transapical length of the diatom is another simple way to verify their identification. The length of the apical axis varied from 30–35 µm and these values are in good agreement with the earlier reported values (Voigt 1958; Simonsen 1970; Russell and Norris 1971; Gibson 1978; Lee et al. 1993; Padmakumar et al. 2015). However, according to Voigt (1958), Belyaeva (1973), and Rivera (1986), the apical axis length could reach > 100 µm. The transapical axis of the diatom ranged from 9–12 µm. The transapical axis length was also in agreement with the studies reported by Husted and Krasske (1941), Navarro (1982), Lee et al. (1993), and Fernandes and Calixto-Feres (2012). Table 1 represents a summary based on the previously published report on the cell length of *P. pacificum*.

## Host Specificity

The interaction between epibiotic diatoms with marine copepods is usually host-specific. Some epibiotic diatoms are facultative epibionts (Gaiser and Bachmann 1994), while, others cannot grow without host copepods (Russell and Norris 1971). *P. pacificum* is an obligate epibiont that cannot survive without a host (Padmakumar et al. 2015). According to the previous findings, the presence of *P. pacificum* can mostly be observed on the copepods belonging to order Cyclopoida (*Corycaeus* sp., *Farronula gracilis*), while, a few reports suggested their association with Harpacticoida (*Euterpina acutiformis*) from a wide range of marine areas (Gibson 1978, 1979; Prasad et al. 1989). *P. pacificum* was only found attached to the surface of the Cyclopoida (*Ditrichocoryca eusaffinis*) irrespective of the presence of other zooplankton orders from the study locations. Our findings are in agreement with the previous studies carried out from the waters of the Brazilian coastline (Fernandes and Calixto-Feres 2012), Bay of Bengal (Sahu et al. 2015), and Gulf of California (Lizarraga and Esqueda-Escarcega 2018).

**Table 2** Worldwide records of different copepod species, colonized by the diatom *P. pacificum*

| Host species | Location | Author(s) |
|---|---|---|
| *Agetus limbatus* (Brady, 1883) (=*Corycaeus limbatus*) | Adriatic sea | Giesbrecht 1892 |
| *Corycaeus crassiusculus* (Dana, 1849) (=*Corycaeus elongatus*) | Gulf of Naples | Giesbrecht 1892 |
| *Corycaeus obtusus* ( Dana, 1849)<br>*Ditrichocorycaeus brehmi* (Steuer, 1910) (=*Corycaeus brehmi*)<br>*Farranula rostrata* (Claus, 1863) (=*Corycaeus rostratus*) | Adriatic sea | Steuer 1910 |
| *Farranula gibbula* (Giesbrecht, 1891) | Aru Islands, Indonesia | Früchtl 1924 |
| *Corycaeus spiciosus* (Dana, 1849)<br>*Agetus flaccus* (Giesbrecht, 1891) (=Corycaeus flaccus)<br>*Urocorycaeus lautus* (Dana, 1849) (=Corycaeus lautus) | South Atlantic Ocean | Klevenhusen et al. 1933 |
| *Corycaeus crassiusculus* (Dana, 1849) | Hawaiian water | Motoda 1963 |
| *Ditrichocorycaeus affinis* (McMurrich,1916) | Northern part of Puget Sound, Washington | Russell and Norris 1971 |
| *Corycaeus spiciosus* (Dana, 1849)<br>*Farranula gracilis* (Dana, 1849) (=Corycaeus gracilis)<br>*Corycaeus* (*Ditrichocorycaeus*) *subulatus* (Herrick, 1887) (=*Corycaeus subulatus*)<br>*Onychocorycaeus giesbrechti* (Dahl 1894) (=*Corycaeus giesbrechti*) | Western North Atlantic Ocean | Gibson 1978 |
| *Corycaeus speciosus* (Dana, 1849)<br>*Euterpina acutifrons* (Dana, 1847)<br>*Corycaeus* (*Ditrichocorycaeus*) *subulatus* (Herrick, 1887) | Ft. Pierce Inlet, Florida | Gibson 1978, 1979 |
| *Corycaeus* sp. | Southern Pacific Ocean | Rivera et al. 1986 |
| *Ditrichocorycaeus affinis* (McMurrich, 1916) | Korean coastal waters | Lee et al. 1993 |
| *Corycaeus* sp. | Barcelona, Spain | Skovgaard and Saiz 2006 |
| *Farranula gibbula* (Giesbrecht, 1891) | The Bay of La Paz, Gulf of California | Gárate-Lizárraga and Muñetón-Gómez 2009 |
| *Euterpina acutifrons* (Dana, 1847) | Mission Bay, California, | Tiffany 2011 |
| *Euterpina acutifrons* (Dana, 1847)<br>*Ditrichocorycaeus amazonicus* (Dahl F., 1894) (=*Corycaeus amazonicus*) | Brazilian coastline | Fernandes and Calixto-Feres 2012 |
| *Euterpina acutifrons* (Dana, 1847) | coastal waters of Argentina | Sunesen et al. 2015 |
| *Euterpina acutifrons* (Dana, 1847)<br>*Corycaeus* sp. (Dana, 1849) | Bay of Bengal, India | Sahu et al. 2015 |
| *Ditrichocorycaeus affinis* (McMurrich, 1916) | North Eastern Arabian Sea, India | Padmakumar et al. 2015 |
| *Ditrichocorycaeus anglicus* (Lubbock, 1857) | Bahía de La Paz, Gulf of California | Lizarraga and Esqueda-Escarcega 2018 |
| *Oithona nana* (Giesbrecht, 1893) | Sao Paulo State, Brazil | Gomez et al. 2018 |
| *Onychocorycaeus giesbrechti* (Dahl F., 1894) (=*Corycaeus giesbrechti*)<br>*Ditrichocorycaeus affinis* (McMurrich,1916) | Coastal waters of Arabian Sea, India | **Present study** |

## Preference Between the Male and Female Hosts

Association of *P. pacificum* was predominantly found on the male *D. affinis*, confirmed by the presence of spermatophore except for fewer cases with the female host. Of note, the diatoms were mostly attached to the dorsal surface of the exoskeleton while few could be found on the antennae. Significantly higher abundances of the diatoms on the male copepods were probably due to the male carapace being more hydrophobic, with a rougher exterior that provides a suitable surface for attachment. Also, toxic compounds associated with the female, carrying eggs, could act as a limiting factor for epibiosis (Totti et al. 2010).

## Distribution

The association between the diatom and the copepod has been reported throughout the world (Table 2), however, only two reports are available from Indian waters to date (Sahu et al. 2015; Padmakumar et al. 2015). In accordance with the previous studies, our study confirms extended biogeographic

distribution of *P. pacificum*, attached with *D. affinis*, from the shelf waters of the Arabian Sea.

## Conclusion

While our study included analysis of samples from both shelf and offshore waters, this epibiosis was only observed in the shelf waters. This is the third report on this epibiont from the Indian Ocean region. The apical and transapical length of the diatom is consistent with the available literature. We suggest that *P. pacificum* is primarily a host-specific epibiont. Interestingly, the male copepods were favoured over the female for the attachment. However, before commenting further on the preferences between male over the female, it is of much importance to accumulate detailed information about the ecology on both of the species. In contrast to previous conjectures, epibiosis could be more frequent, especially from the shelf waters of India. As the cruise was conducted during the winter monsoon season (December), thus, further study is required to understand the effect of different seasons on the epibiosis. Nevertheless, the present study provided much-needed information to generate baseline information about the epibiosis between *P. pacificum* and *D. affinis*. from Indian waters.

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## Declarations

**Conflict of Interests** The authors declare no conflict of interest, financially or otherwise.



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