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Vampyrophrya pelagica (Chatton and Lwoff 1930), an apostome ciliate infectious on calanoid copepod from the Northeastern Arabian Sea

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Abstract

Background: Zooplankton provides a dynamic habitat to various kinds of epibionts in the marine ecosystem and also forms a source of food and nutrients to various parasites and parasitoids. The study was conducted to identify the association of ciliates with calanoid copepod of Northeastern Arabian Sea. Zooplankton samples collected were analysed under stereomicroscope for any kind of ciliate attachments. The infective stage and major characteristics of the ciliates were discussed. The present observation is the first report of apostome ciliate *Vampyrophrya pelagica* on calanoid copepod *Bestiolina similis* from the open ocean waters off Tarapur, Northeastern Arabian Sea, during the early spring intermonsoon season and also a new host record for the ciliate.

Results: Analysis of ~ 2530 copepod revealed the presence of 12 copepod specimens infested with apostome ciliate *Vampyrophrya pelagica*. All the host copepod, identified as *Bestiolina similis*, were females. Approximately 50–60 ciliates occupied the host endocuticular region. *V. pelagica* was represented by fully grown trophont stage characterized by the presence of a large food vacuole surrounded by a thin layer of cytoplasm with numerous lipid bodies.

Conclusion: The study establishes a new host record, calanoid copepod *B. similis* for *V. pelagica*. The report is the first of its kind from the Indian waters and extends the biogeographic boundary of the apostome ciliate.

Keywords: *Bestiolina similis*, Copepod, Northeastern Arabian Sea, Parasitic ciliate, *Vampyrophrya pelagica*

Background

Marine zooplankton provides a diverse and dynamic substratum to various kinds of epibionts in the vast range of water column and also forms a source of food and nutrients to various parasites and parasitoids. Zooplankton, the heterotrophic link between the phytoplankton and organisms of higher trophic level, are primarily associated with many different kinds of ecto and endoparasites/symbionts. Pelagic copepods, most numerous among

the zooplankton, form a major substratum for almost all organisms of lower trophic level such as bacteria, ciliates, diatoms, dinoflagellates, platyhelminthes, nematodes and other crustaceans in the aquatic ecosystem. Some of these organisms infect copepod as intermediate or final hosts, while others become parasitoids and consume them. The exploration of symbionts among the marine zooplankton has been very inadequately understood, so that symbiosis is considered to be of minor importance in the ecological interactions structuring the pelagic ecosystem (Ohtsuka et al., 2007). Only recently, detailed investigations on various symbiotic associations have revealed the complex and significant influence of the association and the associates on population dynamics of the host

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zooplankton (Cachon & Cachon, 1987; Capriulo et al., 1991; Coats & Heisler, 1989; Ohtsuka et al., 2004, 2007; Skovgaard & Saiz, 2006).

Protozoans predominantly, members of Phylum Ciliophora, and subclasses Apostomatia, Suctorina, Hymenostomata, Peritricha and Spirotricha (Corliss, 1979) form very common epibiont on the exoskeleton of zooplankton crustaceans, especially copepods. Among these, subclass Suctorina and Peritricha are the most widely reported epibionts of marine copepods. Apostomes, followed by Suctorina and Peritricha, are ciliates that are symbiotic on both planktonic and benthic crustaceans, mainly copepods, as well as on ostracods, gammarids, isopods, caprellids, euphausiids and decapods (Bradbury, Capriulo & Small, 1986; Capriulo et al., 1991; Chatton & Lwoff, 1935; Gómez-Gutiérrez et al., 2003; Kudo, 1966; Lindley, 1978) and are rarely found on cnidarians (Kudo, 1966; Bradbury 1974b), ctenophores (Kudo, 1966; Skovgaard, 2014), echinoderms (Bradbury 1974b), annelids (Kudo, 1966), chaetognaths (Skovgaard, 2014) and even on other apostomes (Kudo, 1966). Considering the high prevalence of apostome on copepod and its direct influence on their predators, the histophagous feeding of apostome ciliates is relevant towards both the detritus and grazing food chain. The rapid consumption of the host tissues by the ciliate after the injury or predation reduces the nutrient absorption by the predators and hence affect the quality and quantity of detritus matter produced by the host, thus playing an important role in biogeochemical cycling in the aquatic ecosystem (Ohtsuka et al., 2004). Apostome ciliates increase the mortality rate of copepods, consequently reducing the prey availability to carnivorous zooplankton and other higher trophic level organisms in the ecosystem. Apostomes are common along the coasts of the USA, France and Japan, but only a little attention has been given towards their presence in other areas. A major contribution towards the life cycle, morphology and taxonomy of apostome ciliates was made by Chatton and Lwoff (1935). Several studies are still being carried out to understand the apostome host specificity, ecology and their impact on the marine ecosystem in the field. Typical apostome genera include *Vampyrophrya*, *Gymnodiniodes* and *Hyalophysa* (Lynn, 2008), of which *Vampyrophrya* has been widely discovered as symbionts on copepod hosts around the world.

Vampyrophrya pelagica, a common copepod parasite, plays an important role in brackish to coastal pelagic waters of world oceans due to its prevalence and harmful effects on copepods and other invertebrate predators of copepods such as chaetognath, ctenophores and medusa. They have relatively low host specificity and are highly prevalent during warm water seasons. Being a histotrophic apostome, *V. pelagica* possess a large cytostome

for engulfing partially digested host tissues (Chatton & Lwoff, 1935; Grimes & Bradbury, 1992; Ohtsuka et al., 2004). Significant contributions to the study of the life cycle, host specificity and seasonal and distributional occurrences of *V. pelagica* were given by Grimes and Bradbury (1992) from North Carolina, Atlantic Ocean, Ohtsuka et al. (2004) from the Seto Inland Sea, Pacific Ocean and potentially high diversity of apostomes on copepod in the Chinese waters (Guo et al., 2012). Ohtsuka et al. (2004) described the morphological observations, attachments sites, prevalence and intensity, seasonal occurrence and host and stage specificity in the apostome ciliate, *V. pelagica* in the Seto Inland Sea, Japan.

The present study is the first report of apostome ciliate *Vampyrophrya pelagica* on calanoid copepod *Bestiolina similis* from the open ocean waters off Tarapur, Northeastern Arabian Sea, during the early spring intermonsoon season.

Methods

Copepods infected with apostome ciliates were observed in the surface mesozooplankton samples collected from the open ocean waters off Tarapur (Northeastern Arabian Sea- NEAS, Fig. 1), onboard *FORV Sagar Sampada* during the early spring intermonsoon season (March 2013). The sampling was done at 21 locations (0, 10, 20, 30, 40, 50, 75, 100 and 120 m depth) along the transects, viz. off Okha, off Veraval, off Tarapur, off Mumbai and off Ratnagiri. Vertical profiling of physical and chemical parameters such as temperature, salinity, dissolved oxygen and density was obtained using

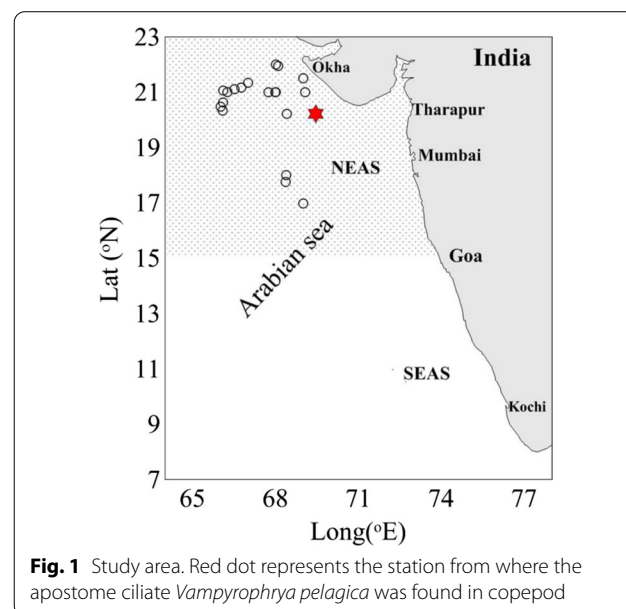


Fig. 1 Study area. Red dot represents the station from where the apostome ciliate *Vampyrophrya pelagica* was found in copepod

Conductivity-Temperature-Depth profiler (CTD, Sea-Bird Electronics model 911 series, Sea-Bird Inc.). Chlorophyll *a* measurements were taken spectrophotometrically (Parsons et al., 1984) using a UV-Visible spectrophotometer. Phytoplankton samples were collected by filtering ~50 L of surface water through 20µ bolting silk. The concentrate was then preserved in 1–3% neutralized formaldehyde solution. Quantitative and qualitative estimation of phytoplankton was done using Sedgewick rafter counting cell following standard identification keys (Tomas, 1997). Surface mesozooplankton samples were collected by the oblique hauling of bongo net (200µ) from the twenty-one stations along the NEAS. Zooplankton samples collected were analysed under a stereomicroscope for copepod infested with ciliates. Individuals were picked up with a Pasteur pipette and preserved in 5% neutralized formaldehyde solution for further analysis and identification. Ciliates were measured and photographed for light microscopy (LM) using *Nikon Eclipse* light microscope attached with *Nikon DN 100* series digital camera and identified following standard keys (Chatton & Lwoff, 1935; Grimes & Bradbury, 1992; Ohtsuka et al., 2004). The copepods were examined under a stereoscopic microscope (*Leica MZ16*) and identified following Al-Yamani et al. (2011).

Results

The mesozooplankton assemblage along the open ocean waters off Tarapur, Northeastern Arabian Sea, was dominated by more than 80% of copepods followed, in dominance, by other planktonic groups such as siphonophores, salps, chaetognaths, mysids, tunicates, fish eggs and decapods during the early spring intermonsoon season of March 2013. Out of the 21 stations sampled, only one station (Lat. 20° 37.73' N; Long. 68° 24.21' E) off Tarapur observed the presence of ciliate infested copepod.

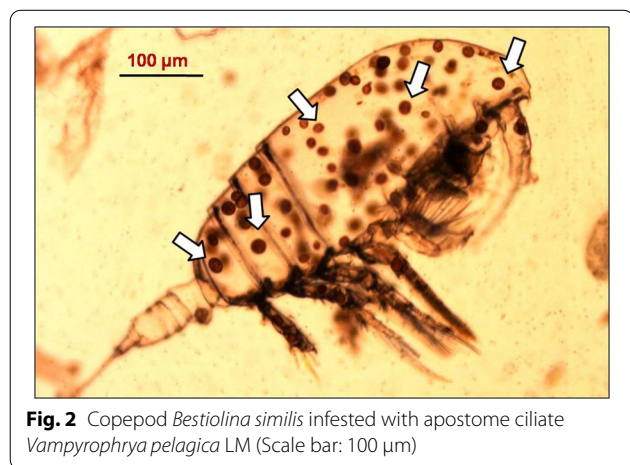


Fig. 2 Copepod *Bestiolina similis* infested with apostome ciliate *Vampyrophrya pelagica* LM (Scale bar: 100 µm)

The copepod population of the particular station was dominated by *Bestiolina similis*, followed by other species such as *Acartia* spp., *Paracalanus aculeatus* and *Eucalanus* spp. Analysis of ~2530 copepod specimens along the respective station showed 12 copepod specimens infested with apostome ciliate *Vampyrophrya pelagica*. The host calanoid copepod was identified as *Bestiolina similis*, with all the host organisms being female. Figure 2 shows the copepod host infested with ciliate. *V. pelagica*, a histotrophic apostome, was represented by the fully grown trophont stage. It is characterized by the presence of a large food vacuole surrounded by a thin layer of cytoplasm with numerous lipid bodies (Fig. 3). The apostome ciliate occupied the endocuticle region of the hosts and fed on the host's tissue. Approximately 50 to 60 apostome ciliates were present in each host copepod.

The physicochemical parameter along the station of occurrence of *V. pelagica* in the Northeastern Arabian Sea during the early spring intermonsoon is shown in Table 1. Temperature, salinity and dissolved oxygen along the station were observed to be 26.2 °C, 36.47 psu and 4.4 ml L⁻¹, respectively. The chlorophyll *a*

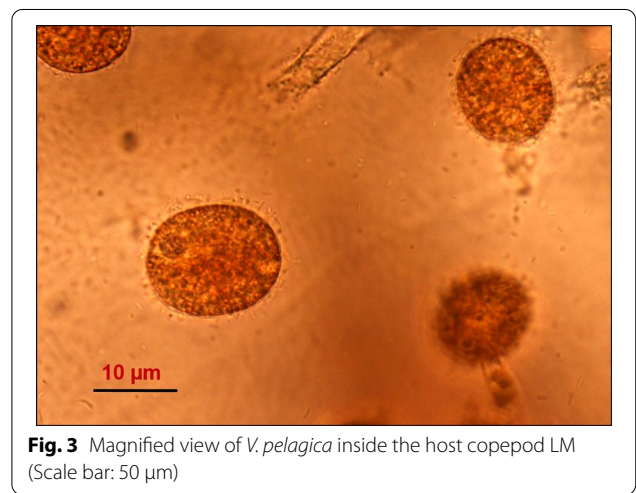


Fig. 3 Magnified view of *V. pelagica* inside the host copepod LM (Scale bar: 50 µm)

Table 1 Physicochemical parameters of the area where the apostome ciliate, *Vampyrophrya pelagica*, was observed in copepod *Bestiolina similis*

Parameter	
Sea surface temperature (°C)	26.2
Salinity (psu)	36.47
Dissolved oxygen (ml L ⁻¹)	4.4
Nitrate (µg L ⁻¹)	0.71
Phosphate (µg L ⁻¹)	0.49
Silicate (µg L ⁻¹)	0.01
Chlorophyll <i>a</i> (µg L ⁻¹)	0.78

concentration was measured to be $0.78 \mu\text{g L}^{-1}$. The station was observed to be non-productive with common diatom species, including *Coscinodiscus* sp., *Thalassiosira* sp., *Skeletonema* sp., *Chaetoceros* sp., *Thalassionema* sp., *Nitzschia* sp., *Odontella* sp. and dinoflagellate *Noctiluca scintillans*.

Discussion

Vampyrophrya pelagica Chatton & Lwoff, 1930

Kingdom	Ciliophora
Phylum	Chromista
Subphylum	Intramacronucleata
Infraphylum	Ventrata
Class	Oligohymenophorea
Subclass	Apostomatia
Order	Apostomatida
Family	Foettingeriidae
Genus	<i>Vampyrophrya</i>
Species	<i>V. pelagica</i>

Vampyrophrya pelagica, first described by Chatton and Lwoff (1930), is a well-investigated histotrophic apostome in its morphology, cytology and ecology. According to Grimes and Bradbury (1992), Ohtsuka et al. (2004), Ohtsuka et al. (2011) and Ohtsuka et al. (2015), there are four functionally different stages in the life cycle of *V. pelagica*. It includes phoront (resting stage), trophont (feeding stage), tomont (division stage) and tomite (infective stage). The phoront are oval, dormant, encysted stage on the body surface of the host. They are mainly attached to the ventral side and appendages of prosome region of copepod by means of a short stalk of $\sim 5 \mu\text{m}$ length. The site of attachment is highly influenced by the feeding and swimming current created by the host copepod. Phoront stage is characterized by specialized intracellular structure, numerous lamellae, which were identified as the precursor of the food vacuole membrane of the trophont. These phoront excyst in two ways: either by a physical injury to the host (single-host cycle) or the predation of apostome infected copepod by invertebrate predators such as chaetognath, medusa, ctenophore (two-host

cycle) or rarely due to unsuccessful moulting (Grimes & Bradbury, 1992; Ohtsuka et al., 2004). In any condition, the trigger for phoront hatching, a critical point in the life cycle, is the oozing body fluid of the copepod. Trophont, excysted from the phoront, is the feeding stage. It is characterized by the presence of an expanded food vacuole that occupies most of the cytoplasm, pushing the macronucleus and other organelles to the edges. Numerous lipid bodies are also sparsely distributed in the cytoplasm. Trophont enters through the wound into the host body and consumes the entire host tissue with the help of its large cytostome. The cell volume increases 30 times the initial trophont size. The fully grown trophont then settles in the cuticle of the totally consumed/dead copepod and metamorphose into encysted tomont. The tomont undergo palintomy, and up to 2 to 32 tomites are released from a tomont. These tomites form the infective stage and search for new copepod host. Once on the new host, they again settle and metamorphose to form encysted phoront.

The host specificity of *V. pelagica* is considerably low, but they prefer calanoid to cyclopoid copepods. Table 2 gives the details of the previously reported copepod host of *V. pelagica*. Various reports suggest that calanoid and poecilostomatoid copepods are the most preferred (Boxshall & Halsey, 2004) along with cyclopoid and harpacticoid copepods (Ohtsuka et al., 2004) irrespective of their size, sex and behaviour. The major copepod host includes *Parvocalanus parvus*, *Calanus sinicus*, *Euterpina acutiformes*, *Corycaeus affinis* from the Seto Inland Sea, Japan (Ohtsuka et al., 2004), *Centropagus hamatus*, *C. typicus*, *Acartia tonsa*, *A. longiremis*, *Corycaeus* sp., *Eurytemora* sp., *Labidocera aestiva*, *Eucalanus* sp., *Paracalanus* sp., *Oncaea minuta* from North Carolina, Atlantic ocean (Grimes & Bradbury, 1992). Despite having a large host range belonging to calanoid, cyclopoid and harpacticoid copepods, there are exceptions where certain copepods included within the same genus such as the cyclopoid *Oithona* and harpacticoid *Microsetella norvegica*, are rejected by the apostome ciliate (Ohtsuka et al., 2004, 2015). The tomites are observed to crawl around these un-preferred copepods to seek attachment sites but finally swim away from the water (Ohtsuka et al., 2015).

Table 2 Summary of previous information on *Vampyrophrya pelagica*

Author	Host copepod	Location
Hockin (1984)	Harpacticoid copepod	Ythan estuary, Aberdeenshire, Scotland
Grimes and Bradbury (1992)	<i>Centropagus hamatus</i> , <i>C. typicus</i> , <i>Acartia tonsa</i> , <i>A. longiremis</i> , <i>Corycaeus</i> sp., <i>Eurytemora</i> sp., <i>Eucalanus</i> sp., <i>Labidocera aestiva</i> , <i>Paracalanus</i> sp., <i>Oncaea minuta</i>	North Carolina, Atlantic Ocean
Ohtsuka et al., (2004)	<i>Parvocalanus parvus</i> , <i>Calanus sinicus</i> , <i>Euterpina acutiformes</i> , <i>Corycaeus affinis</i>	Seto Inland Sea, Japan, North Pacific Ocean
Present study	<i>Bestiolina similis</i>	North eastern Arabian Sea

The present study establishes a new host, calanoid copepod *Bestiolina similis* for *V. pelagica* from the Northeastern Arabian Sea, which inflicts the host specificity of the apostome ciliate towards the calanoid copepods. Also, the high dominance of the females of *B. similis* along off Tarapur might be a reason for all the host copepods being similar. This report, thus, extends the biogeographic distribution of *V. pelagica* to the Northeastern Arabian Sea apart from North Atlantic and Pacific Ocean (Grimes & Bradbury, 1992; Ohtsuka et al., 2004). The naupliar and copepodite stages are also observed to be infected with the phoront (Ohtsuka et al., 2004); however, the phoront are cast-off after the moulting of the juvenile copepods.

V. pelagica parasitizes the planktonic copepods and are considered to be pathogenic towards their host. The species infecting the marine copepods kills the injured copepod by partially or entirely consuming their tissues (histophagous feeding) and greatly influences the population dynamics of copepods and their invertebrate predators like chaetognath, medusa and ctenophores. Physically wounded copepod trigger the excystation of the phoront, leading to consumption by the trophont. Since *V. pelagica* lives at the host's expense and eventually kills the host, they are considered more as a parasitoid rather than as a parasite (Ohtsuka et al., 2015). The apostome-infected copepod, when ingested by their invertebrate predators, are resistant to the predators digestive enzymes. The predators, thus, fail to digest and absorb nourishment as the ingested copepod are consumed prior by the trophont rather than by the predators (Grimes & Bradbury, 1992; Ohtsuka et al., 2004, 2015). Thus, an increase in the *V. pelagica* infestation can decrease the copepod abundance and other invertebrate predators (Ohtsuka et al., 2004), thereby influencing the population dynamics of zooplankton and higher trophic level organisms in the water column.

Conclusions

Apostome ciliates have been mainly investigated from the Pacific Ocean, Atlantic Ocean and Chinese waters. In contrast, only fewer studies have been carried out in other areas. The present study, thereby, summarizes the presence and description of apostome ciliate, *Vampyrophrya pelagica*, collected from the open ocean waters off Tarapur, Northeastern Arabian Sea on calanoid copepod *Bestiolina similis*, which is a new basibiont record. So far, there is no published report of this parasitic association in the Indian waters, and therefore, this is the first report of *V. pelagica* in the Northeastern Arabian Sea. This report also extends the biogeographic boundary of the ciliate to NEAS. However, further studies are still required to understand the life cycle, negative impacts

on the host and the role of these associations in the vast marine ecosystem.

Abbreviations

USA: United States of America; NEAS: Northeastern Arabian Sea; FORV: Fishery oceanographic research vessel; CTD: Conductivity–temperature–depth; UV: Ultraviolet; LM: Light microscopy; μ : Microns; °C: Celsius; psu: Practical salinity unit; ml L⁻¹: Millilitres per litre; μ g L⁻¹: Micrograms per litre; sp.: Species; μ m: Micrometre.

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Author contributions

AP wrote the manuscript with input from LCT. LCT and KBP conducted the field study. LCT, AP and SVF contributed to the taxonomic identifications. KBP supervised this study and provided research materials. All authors read and approved the manuscript.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

No animal/human testing was performed during this study.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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