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Invasion of biofouling mussel *Mytilopsis* Conrad, 1857 (Bivalvia: Dreissenacea) in the Cochin backwaters, southwest coast of India

The black-striped mussel, *Mytilopsis sallei* is an invasive biofouling bivalve native to the tropical and subtropical waters of the western Atlantic, which extends from Colombia to the Gulf of Mexico[1–4]. It is generally believed that their distribution towards the West Africa coast and beyond has been through attachment to the hull of ships since the 16th century. Later they were introduced to the Eastern Pacific via the Panama Canal, Fiji, Japan, Taiwan, Hong Kong, China, Philippines, Thailand, Singapore, Malaysia and India through ballast waters and also by attachment to the hull of ships. This species is reported to have invaded Indian waters through Visakhapatnam harbour during 1960s (ref. 5), and further reported from Mumbai harbour during 1975 (refs 6, 7).

Here we report on the occurrence of *Mytilopsis* sp. from the southwest coast of India (near Cochin harbour: 9°50′43.9″N, 76°17′17.2″E). During the study, the basic water quality parameters were measured using a standard multiparameter instrument (model: Eutech PC 450). Water column temperature was 27°C with 5 ppt salinity and 5.8 mg/l dissolved oxygen. Population density was estimated by quadrate method. The estimated population density of *Mytilopsis* sp. in Ezhupunna region of Vembanad Lake, Kerala, was 748 ind.m^{-2} (Figure 1). Specimens were identified based on key characters and other morphological features[8]. The right valve of the bivalve was slightly larger and overlapped the left valve. The shell colour was light yellowish-brown, while the juvenile mussels showed light and dark bands veering alternately to the right and left. The highly variable shell morphology of this species makes morphological identification difficult[9]. Therefore, a tissue sample from the specimen was subjected to molecular examination for species-level confirmation. A live individual was put in hot water for a few minutes and then preserved in 100% ethyl alcohol[10]. Prior to DNA extraction, the alcohol-preserved sample was hydrated at 26°C in 1 ml sterile distilled water for 10–12 h. Genomic DNA from the macerated tissue was extracted using the Qiagen DNeasy Blood and Tissue Kit (Germany, Catalog No: 69504) by following the spin column procedure. The polymerase chain reaction (PCR) mixture contained 25 µl Master Mix (Takara Clontech EmeraldAmp® GT PCR Master Mix), 1 µl reverse primer, 1 µl forward primer, 8 µl template DNA and 15 µl nuclease free water. The universal end primers 18S F (5′-CTGGTTGATCCTGCCAGT-3′) and 18S R (5′-TAATGATCCTTCCGCAGGTTCACCT-3′) were used for amplifying 18S ribosomal RNA gene. Amplification was carried out in a thermal cycler (Agilent Technologies, model no. Sure cycler 8800). The protocol for amplification started with denaturation at 95°C for 1 min, annealing at 55°C for 2 min and extension at 72°C for 3 min; 30 cycles were performed. Amplified products were run on agarose gel (1.2%) electrophoresis. An intense band was developed, which was purified and sent for sequencing (SciGenom Labs Pvt Ltd, Ernakulam, Kerala). The acquired sequences were assembled by BioEdit 7.0.9 (ref. 11) and later aligned by ClustalX (ref. 12). A 757 base-pair length of ribosomal RNA gene sequence was developed in respect of *M. sallei* and submitted to the NCBI database with accession number KY013490. Two other sequences of *M. sallei* were obtained from NCBI for constructing a phylogenetic tree (maximum likelihood). The sequences of *M. sallei* specimens were arranged in a single clade with a high bootstrap value (100%). The selected out-group *Congeria kusceri* exhibited a divergent array (Figure 2). The sequences of *M. sallei* exhibited an intraspecific sequence divergence of 0%, which confirmed that the sequence obtained from the current geographic position (KY013490) clearly matched with those from Lam Tsuen River, Hong Kong, China (JX099476, JX099477)[4]. This result leads to the confirmation of bioinvasive occurrence of *M. sallei* in the Cochin backwaters. Five specimens,

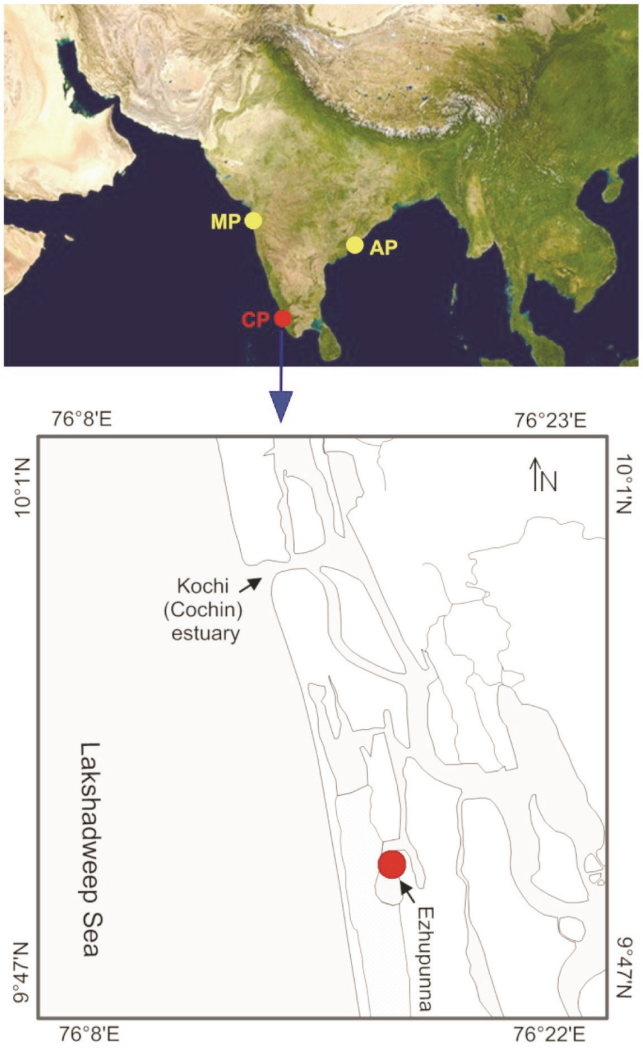

Figure 1. Occurrence of *Mytilopsis* sp. in Indian waters: red dot showing the present study and yellow dots showing previous reports from Indian waters (MP, Mumbai Port; AP, Visakhapatnam Port; CP: Cochin Port).

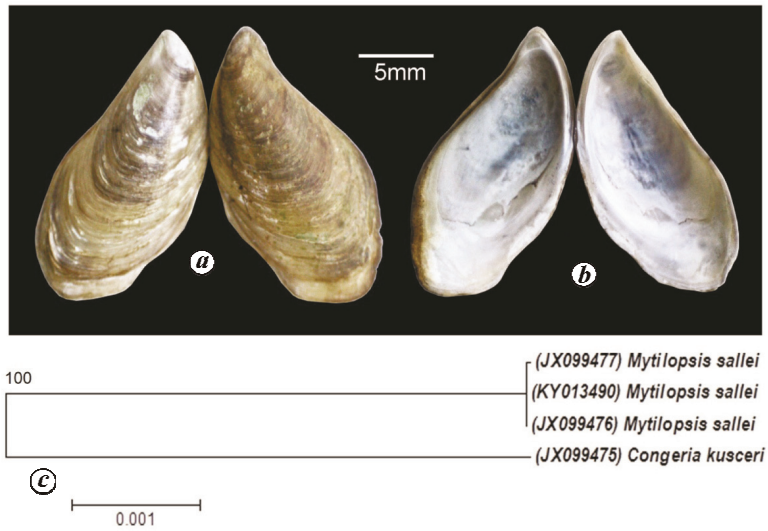

Figure 2. (***a***) Exterior and (***b***) interior valve view of *Mytilopsis* sp. collected from the Cochin backwater; (***c***) Maximum likelihood tree for *Mytilopsis* sp. based on 1000 bootstrap pseudoreplicas.

including one voucher specimen used for the molecular study were deposited in the museum collections of the Department of Marine Biology, Cochin University of Science and Technology (CUSAT), Cochin (MBM/SBN/JCPR/11-15/2018). However, some researchers argue that the earliest studies on *Mytilopsis* in India have been wrongly reported as *M. sallei*, and that subsequent studies used the same name for specimens collected from Japan, Hong Kong and Australia[13–15]. According to Morton, *M. sallei* was carried through the Panama Canal by shipping and onto Fiji and the east coast of India, but a species of *Mytilopsis* (as *Congeria*) was identified from Fiji by Hall[16] before the opening of the Panama Canal in 1914. Some researchers also suggest that *Mytilopsis allyneana* (type locality Fiji) and *Mytilopsis adamsi* (type locality San Jose Island, Panama) are conspecific and the same species of *Mytilopsis* are found in India, Hong Kong, Japan and other localities in Asia. They are also concerned about the chances of error in GenBank data deposition from Asia, which can lead to wrong identification of *M. sallei.* However, there are no GenBank data available from type locality of *M. sallei* (Rio Dulce, Gautemala) to clarify the concern about taxonomic identity of *Mytilopsis* from India, Hong Kong, Japan and other localities in Asia. A comparative molecular study on Asian population and Caribbean *M. sallei* population may resolve this issue.

The massive population of *Mytilopsis* sp. was first noticed on wooden materials partially submerged in the water column having a salinity of 5 ppt. Juvenile mussels were primarily attached to filamentous algae proliferated in the wooden poles. It has also been reported that they are often found in large colonies by forming mat-like structures attached to hard substrate in intertidal range up to +1.0 m above chart datum[13], or the subtidal estuarine environment. However, their distribution is restricted to intertidal depth range. *Mytilopsis* sp. is an epibyssate, filter-feeding bivalve with wide temperature, salinity and oxygen tolerances, leading to very high risk for its invasion in coastal waters. It is also capable of living in the turbid water column of estuarine environment. The survival experiment in the laboratory conditions confirmed its tolerance to wide temperature, salinity and oxygen variations. The adult individuals can survive in a wide

salinity range, i.e. 0–50 ppt (refs 6, 17). The latitudinal distribution pattern of *Mytilopsis* sp. suggest that it can be survive and reproduce in a wide temperature range (10–35°C). Its aggregation behaviour on substratum was also found to be prominent[18]. This fast-growing opportunistic mussel demonstrates high fecundity rate and early maturity; it can attain maturity within one month of larval settlement. Their average life span is about 18 months with a maximum of 22 months[13]. It also shows sex changes, with a proportion of hermaphrodite population[18,19]. It is reported to perforate in the hard substrate and cause serious fouling damage to coastal infrastructure. The present specimen size ranged from 5 to 24 mm in length with a maximum height of 12 mm and width of 9 mm. Morton[13] suggested that individuals attained shell lengths between 6 and 10 mm within one month of settlement, and subsequently required another three months to reach 20 mm during the summer months. Kalyanasundaram[20] reported that many individuals grew up to a size of 30 mm within six months. Their prolific growth results in the exclusion of other species; leads to biodiversity loss and huge economic losses[13,18,21–23]. The species is a massive fouler on the outer hull of ships and boats, pipelines, fish-farming cages and other coastal structures. In Visakhapatnam port[5,9], Hong Kong[13] and Taiwan[24], it was reported to cause severe risk of fouling. They are ecologically similar to related species zebra mussel, *Dreissena polymorpha*. To eradicate *Mytilopsis* sp. from Darwin Harbour, Australian agencies spent approximately US$ 2.2 million (www.dpi.nsw.gov.au). Even though there is high risk of invasion in Asian ports, there have been limited efforts to study the impact, management and control of the species. The timely record of bioinvasion and biofouling activities in the backwaters will be helpful for prevention and early management of *Mytilopsis* species.

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