Rapid Communication

First record of the alien invasive biofouling mussel *Mytella strigata* (Hanley, 1843) (Mollusca: Mytilidae) from Indian waters

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Abstract

The invasive tropical American brackish water mussel *Mytella strigata* is recorded for the first time from the Indian sub-continent and this is the fourth report from the Indo-Pacific. The mussels were found attached in high densities (120 ± 24 ind. 25 cm⁻²) to floating plastic bottles, wooden pilings, walls of fish cages, hulls of boats and bottom sediment in the Cochin backwater, Kerala, India during summer 2019. The only other bivalve species found with this population was *Perna viridis*. The mean length to height ratio for the Cochin population of *M. strigata* was 2.06 ± 0.26 cm with the largest individual having a length of 5.9 cm. The general external colour of the shells in Cochin population was uniformly black when wet but they exhibit dark green over the dorsal and posterior areas with faint paler streaks when dry. In addition some of the individuals rarely form bright green colour and pattern as seen in Singapore populations. The mitochondrial DNA cytochrome *c* oxidase subunit I gene sequences of mussels from this study were consistent with specimens in their native range, from Colombia, and from Singapore where it has recently been reported as invasive. The invasion of this species in Cochin may be through ballast water or fouling on ships hulls from its native range or from Singapore where it has been established recently. Their rapid growth, early maturity and wide salinity tolerance make them a potentially alarming fouling species in all brackish waters of India and neighbouring countries.

Key words: bioinvasion, bivalve, brackish water, Kerala, India

Introduction

Biological invasions are one of the most serious problems confronting native species and marine ecosystems around the world (Boudreaux and Walters 2006; Molnar et al. 2008; Lim et al. 2018). The most successful invasive species are able to survive in a wide range of areas and conditions outside of their home range and are capable of reproducing rapidly, often establishing large populations (Jayachandran et al. 2018b). Indian brackish water ecosystems are home to many endemic species and these invasive species may seriously affect their survival and natural habitats (Arathi et al.
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Figure 1. Locations in Cochin (Kochi) backwater, India where the non-native mussel *Mytella strigata* was observed during summer 2019, as indicated by red circles (Site A: Ezhupunna, Site B: Marine Science boat jetty, Ernakulam). Left bottom corner showing the fouling of *M. strigata* on a boat at site B with its magnified image. Photo by P.R. Jayachandran.

2018; Jayachandran et al. 2018a, 2019; Oliver et al. 2018a). Cochin (Kochi) backwater is susceptible to invasive species due to its proximity to an International Container Transshipment Terminal. The alien invasive bivalve, *Mytilopsis sallei* (Récluz, 1849) is already established in Cochin backwater (Jayachandran et al. 2018b) and this study adds another new non-indigenous invasive species record to Indian waters.

The present study aims to report the dense population of a small fouling mytilid in the Cochin backwater that could not be identified from current Indian literature (Subba Rao 2017). This study also confirmed the identity of this species as *Mytella strigata* (Hanley, 1843) through morphological and molecular techniques. The correct identification and consequent early detection of invasive species is necessary to provide a rapid response to prevent their establishment and mitigate any ecological and economic damage. This paper brings initial attention to this invasive species in India while further studies will investigate its ecology and impact in the Cochin backwater.

Materials and methods

The presence of small black mussels, not previously recognised in the Indian literature (Subba Rao 2017), was noted from two locations in the Cochin backwater, south-west coast of India (Figure 1) between May and June 2019. The mussels were attached to various materials, including plastic bottles, wood, nettings, frames of fish cages, bottom sediment and the hull of boats. They were first detected on plastic and wooden materials at the Ezhupunna region (9°50′43.9″N; 76°17′17.2″E) of Cochin backwater and also at the School of Marine Sciences boat jetty (9°57′51.67″N; 76°16′56.05″E), Ernakulam in March 2019. Their morphology was compared with that of native mussels and known invasive species such as
Xenostrobus spp. and Mytella spp. (Sanpanich and Wells 2019; WoRMS 2019). Samples for DNA extraction were stored immediately in absolute ethanol, and additional samples were preserved in 4% formalin for morphological and anatomical examination. Water temperature was measured using a standard mercury thermometer, and salinity was measured with a standard refractometer (Model MCP0-100). A standard quadrat (5 × 5 cm) was randomly placed over surfaces of bivalve mats and individuals in the quadrats were removed and brought to the laboratory for identification, counting and measurement. A standard Aerospace Vernier Caliper (Thermo) was used for shell measurements. The individuals were acclimatized in the laboratory conditions. Individuals with similar size (~ 2 cm) were kept in 100 litre pre-cleaned glass tanks having 10 litre water with various salinities such as 0, 2, 5, 10, 15, 20, 25, 30, 35 and 40 PSU in duplicates to establish their salinity tolerance. Experiment was conducted for 96 hours with proper aeration and stable room temperature (26 °C).

Molecular analysis.

Genomic DNA was extracted from a sub-sample of tissue collected from the foot of mussels using a DNeasy Blood and Tissue extraction kit (Qiagen) according to the manufacturer’s instructions. The mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) region was amplified using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). The PCR mix consisted of 12.5 μL of EmeraldAmp GT PCR Master Mix (Takara Bio), 0.4 μM primers and 4 ng template DNA in a 25 μL reaction volume. The amplification conditions consisted of an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 52 °C for 30 s and 72 °C for 1 min, with a final extension of 72 °C for 5 min. Sequencing was done with an ABI PRISM Big Dye Terminator v3.1 cycle sequencing kit in an AB 3730 DNA analyzer (Life Technologies). The sequences obtained were viewed using the ABI sequence scanner v2.0 and compiled, aligned and analysed using BioEdit v7.2.5. (Hall 1999). The sequences were uploaded and compared with available sequences in GenBank using the Standard Nucleotide Basic Local Alignment Search Tool (BLAST). A phylogenetic tree was constructed based on the maximum likelihood method using MEGA 10.0.5 with bootstrap values for 1000 replicates (Tamura and Nei 1993; Kumar et al. 2018). Reference sequences were retrieved from GenBank under the accession numbers indicated on the phylogenetic tree. The partial mtDNA COI sequences were submitted to GenBank under the accession numbers: MN165292, MN165293, MN165294, MN165295, MN165296, and MN165297. Voucher specimens were deposited in the Museum of the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology (MBM/SBN/JCPR/15-20/2019).
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**Results**

*Identity and description of shell.*

At first sight it was thought that the Cochin mussels were a species of *Xenostrobus* (Mytilidae) due to the similarity in shape and the black unsculptured shells. However, the presence of small but distinct teeth on the inner anterior margin (Figure 2i) is not seen in *Xenostrobus* or *Limnoperna* but is present in the genus *Mytella*. *Mytella strigata* (Hanley, 1843) = *M. charruana* (d’Orbigny, 1846) has recently been recorded as an...
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alien invasive species in Southeast Asia (Lim et al. 2018) and a comparison with shells from Singapore showed them to be identical. Lim et al. (2018) reported its presence in Johor Strait, Singapore, while Michael et al. (2016) and Vallejo et al. (2017) reported it from Luzon Island, Philippines. In a recent study, their occurrence was also confirmed from the inner Gulf of Thailand (Sanpanich and Wells 2019). Here we report its presence in the Cochin Backwater, Kerala, India, for the first time from the Indian subcontinent and the fourth for the Indo-Pacific. Included within the phylogenetic tree presented by Lim et al. (2018) is one sequence identified as *Mytella brasiliensis* Chemnitz = *M. guyanensis* (Lamarck, 1819) and it suggests that the species level systematics may not be totally clarified but our data is totally consistent with *Mytella* from Singapore. In this paper, we have adopted the nomenclature of Lim et al. (2018), where they present a cogent case for accepting the earliest available name as *M. strigata* (Hanley, 1843) and accepting that the name *M. charruana* d’Orbigny dates from 1846 and not 1842 as suggested in MolluscaBase (2019).

**Shell description.**

Shells reaching 59.2 mm in length with height of 26.3 mm and turbidity of 15 mm, thin, brittle. Umbos low, beaks subterminal. Morphometric ratios were: length:height 2.06 ± 0.26, length:tumidity 3.45 ± 0.73 and height:tumidity 1.68 ± 0.31 (n = 160). Outline wedge shaped, dorsal (ligament) margin long and straight, sloping steeply; posterior flared, rounded; ventral more or less straight, sometimes weakly concave. Anterior region very small, with distinct radial ridges corresponding to 2–4 teeth on the inner margin (Figure 2e, f). Elsewhere sculpture of fine, regularly spaced commarginal ridges. External colouration consistently dark, almost black when wet but is black tinged with dark green over dorsal and posterior areas with faint paler streaks; ventral area paler, greenish-brown; anterior ribbed area often bright green (Figure 2f). Small shells are typically paler (Figure 2c). Interior (Figure 2g) margin smooth except for 2–4 (typically 2 larger) rounded teeth (at) on the anterior margin (Figure 2i). Resilial ridge under ligament (lig), pitted (prr) (Figure 2h). Anterior adductor scar (aa) small on inner edge behind the toothed margin, posterior adductor scar (pa) rounded, continuous with elongate pedal/byssus retractor muscle scar (brm). Internal colouration tinged bluish to purplish. The anatomy of the Indian specimens agrees with that given by Lim et al. (2018). The Indian shells are more uniform in colour and pattern compared with those reported from Singapore. The majority of Indian shells agree with the “black” forms illustrated by Lim et al. (2018) on their figure 2, bottom row and also having rare occurrence of “bright green” forms illustrated on top row of their figure 2 and different shell pattern on their figure 2 (MS3), middle row (Figure 2a–k).
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**Figure 3.** Maximum likelihood tree based on the COI gene showing the phylogenetic relationship between *Mytella strigata* (= *charruana*), *Modiolus brasiliensis* (= *Mytella guyanensis*), *Musculista senhousia*, *Modiolus metcalfei* (= *modulaides*), *Xenostrobus securis*, *Perna viridis*. Bootstrap results from 1000 replicates with > 50% support are shown on the branches. Five *M. strigata* sequences obtained from the Cochin backwater, India in the present study are marked with an asterisk. Sequences of *M. strigata* Hap A (EU917173) and Hap H1 (KP013759) were based on Gillis et al. (2009); Hap F1 (JQ685158) from Alves et al. (2012); F2 (MG736075), F1 (MG736074) and M1 (MG736069) from Lim et al. (2018); *M. brasiliensis* (EU917181) from Gillis et al. (2009); *M. senhousia* from (AB076942) Matsumoto (2003); *X. securis* 01 (KU714794) from Miralles et al. (2016) and *M. metcalfei* (AB076940) from Matsumoto (2003).

**Molecular description of Indian specimen of *M. strigata***

Six specimens were identified by sequencing the mtDNA COI gene. The nucleotide BLAST results showed 99% similarity with the *M. strigata* sequences reported recently by Lim et al. (2018) from Singapore. Out of the six, four individuals CE1 (MN165292), CE2 (MN165293), CE4 (MN165295) and CE5 (MN165296) showed a close match with the mtDNA COI sequence of the *M. strigata* haplotype F2 from Singapore and haplotype A from Colombia; while the other two individuals CE3 (MN165294) and CE6 (MN165297) were identical to the haplotype M1 from Singapore. The evolutionary relationship of the mussels was depicted on the phylogenetic tree (Figure 3) constructed using mtDNA COI sequences based on the maximum likelihood method. The phylogenetic analysis also proved the relationship of mussels with Singapore haplotypes F2 and M1, and the Colombian haplotype A of *M. strigata*.

**Field and laboratory observations.**

The colonies were dominated by *M. strigata* with small numbers of *Perna viridis*. The densities of these clumps reached a maximum of 120 ± 24 ind. 25 cm⁻². Salinity readings at the Ezhupunna and Marine Sciences boat jetty regions were 23 and 25 PSU, respectively. The water temperatures were within
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**Figure 4.** Distribution of *Mytella strigata* based on published sources.

the range of 25–28 °C at both the study sites. *M. strigata* along the tidal line formed biogenic reefs by accumulating fine clay particles and other debris that includes floating plastic pieces. Rat-tailed maggot (immature *Eristalis tenax*) was found in the intertidal biogenic reef of *M. strigata* at the School of Marine Sciences boat jetty, which is an indicator of high organic matter accumulation. In laboratory conditions, they showed a 100 % survival rate between the salinity of 2 to 35 PSU at 26 °C but all individuals failed to survive at salinities of 0 and 40 PSU.

**Discussion**

The American Charru Mussel *Mytella strigata* is an invasive biofouling mussel which is capable of colonising a variety of hard and soft substrates in brackish water environments (Lim et al. 2018). Its native range includes the Pacific and Atlantic coasts of tropical America, from the Gulf of California to Ecuador in the eastern Pacific (Coan and Valentich-Scott 2012); in the Caribbean and Atlantic (Soot-Ryen 1955) from Florida (Boudreaux and Walters 2006) to Argentina (Castellanos 1967). In the Indo-Pacific it is known from Luzon Island, the Philippines (Michael et al. 2016; Vallejo et al. 2017), from Johor Strait, Singapore (Lim et al. 2018) and from the inner Gulf of Thailand (Sanpanich and Wells 2019). The records here are the fourth record from Indo-Pacific and the first for the Indian subcontinent. The introduction of this species to India may be from Thailand or Singapore through shipping routes. Given the extensive brackish water systems present on both east and west coasts of India further records would not be surprising. There is a great chance for further introduction of this species to western region of Arabian Sea through shipping of the Kochi International Container Transshipment Terminal (Figure 4).
Lim et al. (2018) suggest that the method of invasion was by ballast water through the extensive shipping trade in Singapore and a similar mode is suggested here as the Cochin backwater is home to a major international sea port. The spread of *M. strigata* constitutes the second substantive estuarine invasion of intertidal habitats by a non-native marine bivalve species in Cochin backwater, India, after the establishment of the Caribbean dreissenid bivalve *Mytilopsis sallei* in brackish waters during the post-monsoon of 2018 (Jayachandran et al. 2018b). The population size of *M. strigata* exceeds that previously observed for *M. sallei* in Cochin backwater (748 ind. m$^{-2}$). In the Cochin backwater, the density of *M. strigata* was 120 ± 24 ind. 25 cm$^{-2}$ and is similar to that in their native habitats (Kishore 1995) and to that in Singapore (Lim et al. 2018). *Mytella strigata* usually attains reproductive maturity at a length of 1.25 cm (Stenyakina et al. 2010), and here the majority of individuals were already above this suggesting that the population is well established and reproductive. At this time there is not sufficient data to postulate when the first settlement took place but is clear that they were not found in any of our previous monthly field collections appearing as recently as the summer of 2018 (Jayachandran et al. 2018a, b; Oliver et al. 2018a, b).

In the present study the individuals survived salinities between 2 to 35 PSU in laboratory conditions, but failed to survive in lower than 2 PSU. Thus, the salinity tolerance of the species is similar to that in their native range (Wei et al. 2010). The Cochin backwater is subject to very large fluctuations in salinity due to the seasonal monsoon and the survival of *M. strigata* may depend on this ability to tolerate extreme fluctuations. The rapid colonisation of *M. strigata* in natural and artificial habitats of Cochin backwater is alarming, as it may exclude native species present in the Indian backwaters (including Cochin backwaters). The very dense populations of small mussels currently identified as *Brachidontes striatulus* (Hanley, 1853) and *B. undulatus* (Dunker, 1857) can become important fouling organisms and would thus appear to be in direct competition with *Mytella strigata* (Subba Rao 2017). Given the variability of these native mussels there is an urgent need to define them precisely through a proper morphological and molecular study and then to define their ecologies.

India has already adopted Aichi Biodiversity Target 9 as its National Biodiversity Target 4 to identify the invasive alien species and identify their route of introduction, for developing better management plan as a part of National Biodiversity Strategies and Action Plans (NBSAP) formulated by Convention on Biological Diversity (CBD) signatory countries by the year 2020. In this scenario, it is important to discuss conservation and management challenges of alien invasive species introduced in India to develop invasive species management, policy and practice.
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