Environment Conservation Journal 24 (2):48-53, 2023

Journal homepage:https://www.environcj.in/

Environment Conservation Journal

ISSN 0972-3099 (Print) 2278-5124 (Online)

Bioinvasion of *Mytella strigata* (Hanley, 1843) in Ashtamudi Lake, Kerala, India – is pollution aggravating environmental degradation in Ramsar wetland?

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ARTICLE INFO

Received : 26 August 2022
Revised : 10 December 2022
Accepted : 15 December 2022

Available online: 09 March 2023

Key Words:
Ashtamudi Lake
Gene sequencing
Invasive
Mytella strigata
Phylogeny

ABSTRACT

Polluted or degraded aquatic ecosystems accumulate more invasive species than less polluted areas. The alien invasive mussel *Mytella strigata* (=*charruana*) was reported to be affecting wild spats of mussels as a dominant competitor for space on floating substrates which is native to Central and South America later outspread to east coast of central Florida and subsequently reported from Philippines. The tremendous increase of *M. strigata* population in Ashtamudi Lake is raising an alarming situation over the native bivalve species as it compete for space, breeding ground, substrates and food. The broad salinity ranges allows them to invade via ballast exchange of water and the fouled ship hulls. When salinity levels goes beyond the thresholds of the native species, *M. strigata* can sometimes outcompete them. Moreover, the molecular phylogenetic analysis via COI sequences shows a close genetic relationship shared between the native mussel *Perna viridis* and the alien invasive mussel *M. strigata*. The increasing pollution load along with the solid waste disposal in the Lake, is accelerating the spat fall of *M. strigata* over the native species and therefore should be a priority in the bioinvasion control, otherwise can lead to displacement or local extinction of the native species.

Introduction

Ashtamudi Lake (8°59'N 76°36'E) is the second largest and deepest wetland ecosystem in Kerala, well known for its unique biodiversity which has been considered as one among the Ramsar sites in India. The Neendakara Fisheries Harbour of Ashtamudi Lake is the second biggest fish trading centre in the State and it provides landing and berthing facilities to the existing flet of mechanized crafts operating from Kollam region. The establishment of the new industrial projects, have increased the pollution load on the Ashtamudi Lake which is adversely effecting native bivalve populations. Municipal solid waste and sewage from Kollam Municipal Corporation oil spillage, hydrocarbon/ fuel pollution, sand mining, pollution by effluents from various industries situated in the banks of estuary are the major environmental issues facing by the Lake (Sitaram, 2014) and thereby increases the risk of invasive species. *M. strigata* which is native to Central and South America (Boehs *et al.,* 2004), was first observed in Jacksonville, FL, United States in 1986 and was considered eliminated after 1987. However in 2004, few individuals were reported along the east coast of central Florida which was 212 km south of the previous site (Boudreaux & Walters 2006). In 2014, *M. strigata* was reported to be affecting wild spats of mussel and other bivalves in the Philippines which was facing serious environmental threats by land and sea based pollution (Vallejo *et al.,* 2017).

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Doi:https://doi.org/10.36953/ECJ.14662447

The invasive mussel *M. strigata* was known as *M. charruana* (d'Orbigny, 1846) in the previous literature. However, currently *M. charruana* is known to be a synonym of *M. strigata*. The first report of *M. strigata* in Indian waters came from Cochin backwaters, Kerala (Jayachandran *et al.,* 2019) and followed by the reports from Ashtamudi Lake and other Kerala coasts by Biju et al. (2019). However, the genetic basis for the invasion of *M. strigata* from Indian waters is very scare and impeded by the lack of molecular taxonomic studies and phylogenetic utility. Since, bivalves show phenotypic plasticity, it becomes a prerequisite to provide an integrated taxonomic information including a solid molecular evidence for the proper identification of the invasive mussel and for the management of the native bivalves especially the native *Perna viridis* population. Since, Ashtamudi Lake is one among the major contributors of bivalve fishery of India, it is imperative to analyse the genetic relatedness of *M. strigata* with that of the native mussel *P. viridis.* Therefore, this paper documents the COI gene sequencing data of *M. strigata* for the first time from Ashtamudi Lake, Kerala, along with the sequence data of native Asian green mussel *P. viridis* and discusses its genetic relationship through molecular phylogenetic analysis. Furthermore, the environmental indices have been discussed along with the possible threats that may arise in the near future.

Material and Methods

Individuals of *M. strigata* were collected from Ashtamudi Lake (Lat- 8.961508, Long- 76.607392) in March, 2019, which was primarily attached to the hardy shells of Oysters. The spats of *M. strigata* was found to be increasing tremendously and the fouling effects of *M. strigata* were clearly visible as it were attached to the hard substrates like the native bivalve shells, damaged boats and other concrete remains (Figure 1). The collected specimens were brought to the laboratory and the species identification were done according to Medioda *et al.,* 2017 and Vallejo *et al.,* 2017. The water quality parameters such as Surface water temperature, pH, Salinity, Dissolved oxygen, Hardness and TDS were estimated according to (APHA, 1989). Total genomic DNA from the muscle tissue was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel). A partial fragment of mitochondrial COI was amplified via PCR using the primers LCO1490 (GGTCAACAAATCATA AAGATATTGG) and HCO2198 (TAACTTCAGG GTGACCAAAAAATCA) (Folmer *et al.,* 1994). Polymerase chain reactions was carried out in 10µl volume including 1 µl DNA template, 0.2 µl dNTP mix, 2.0 µl 5x phire buffer, 0.25 µl of primer and 0.2 µl (1 U) Taq phire polymerase, 0.15 µl of 5 % DMSO, 1 µl BSA and the PCR cycles were performed in a Mastercycler PCR System (Eppendorf) with a pre-denaturation for 1 min at 94°C, followed by 35 cycles for 20 sec at 94°C, annealing temperature for 20 sec at 45-55°C, extension at 72°C for 1 min followed by a final extension step for 5 min at 72°C. Using ExoSAP-IT (GE Healthcare) the PCR products were purified and the sequencing reaction was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) by the Big Dye Terminator V.3.1 Cycle sequencing kit (Applied Biosystems). For checking the sequence quality, Sequence Scanner Software v1 (Applied Bio systems) was employed and the sequence alignment were carried out using Bioedit (Hall 1999). Analysis for homology of the CO1 gene sequence against nucleotide databases was performed with the help of NCBI- BLAST server. The taxonomy of the sequences were thus confirmed and the sequences were deposited in GenBank under the accession numbers MN603972 (*M. strigata =charruana*) and MW722974 (*P. viridis*). Using MEGA 7 the Maximum Likelihood phylogenetic tree was constructed using Tamura-Nei model (Kumar *et al.,* 2016) with a bootstrap values for 1000 replicates.

Results and Discussion

M. strigata in Ashtamudi Lake was observed with a temperature range of 32 to 36°C, Salinity from 8.337 ppt to 32.066 ppt and dissolved oxygen from 5.236 to 7.653 mg/L (Table 1).

The diagnostic characters such as morphology and other images are given below.

Morphology

Moderately large sized, thick and equivalve shells, with mytiliform and narrow-wedge shaped shell outline, having smooth and shiny shell surface characterised by the concentric or semi-circular

Table 1: Water Quality parameters of the Study Site.

Environmental parameter	Range
Surface water temperature	32 - 36°C
pH	7.6 - 8
Salinity	8.337 ppt - 32.066 ppt
Dissolved Oxygen	5.236 - 7.653 mg/L
Hardness	225 - 310 mg/L
TDS	36.25 - 40.83 mg/L

rings (Fig. 2). Posterior dorsal margin crescent shaped with thick and dark black coloured periostracum of wavy dark pattern. Downturned beaks with curved umbones having narrow hinge area, without teeth in the anterior region bearing pitted resilial edge. Internal colouration white with a broad band of iridescent purple and deep purplish black at the smooth posterior margin. Foot is characterised by deep orange colour along with brown pigmentation at mid-hinged area, bearing byssal threads protruding outside. Pallial line curved towards the adductor scar and the byssal retractor scar seen beneath the adductor muscle which forms a thick straight line going towards the middle portion of the shell, while anterior retractor is greatly reduced in the umbonal area (Fig. 3).

Figure 1: Cluster of *M. strigata* collected from Ashtamudi Lake

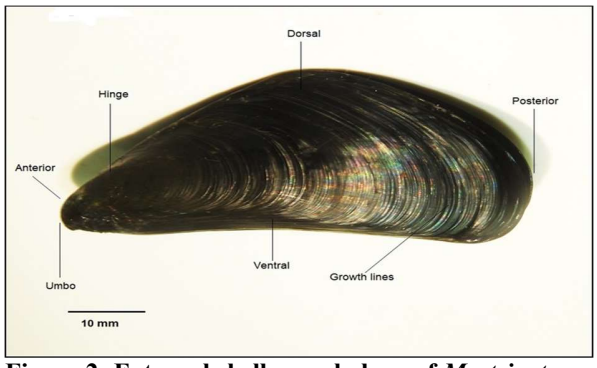

Figure 2: External shell morphology of *M. strigata*

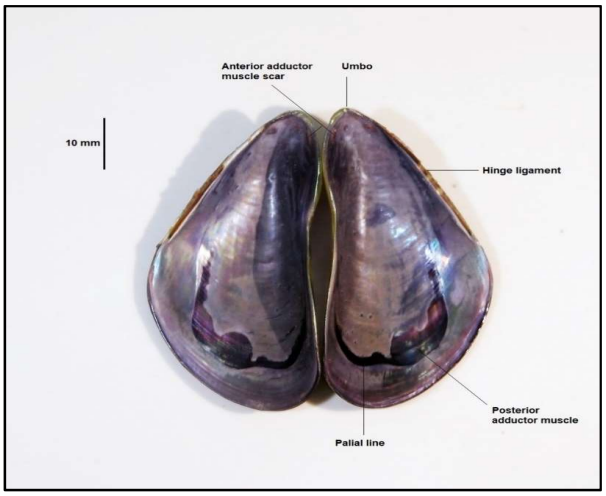

Figure 3: Internal shell morphology of *M. strigata*

Molecular Phylogenetic Analysis

From the chromatogram, FASTA sequences with 533 (*M. strigata*) and 558 (*P. viridis*) base pairs were interpreted and the taxonomy were confirmed through BLAST analysis and deposited in GenBank under accession numbers MN603972 (*Mytella strigata= charruana*) and MW722974 (*Perna viridis*). The COI gene sequence analysis showed the nucleotide frequencies as 24.4 (A), 42.4 (T), 18.6 (G), 14.6 (C) for *M. strigata* and 24.2 (A), 42.5 (T), 20.8 (G), 12.5 (C) for *P. viridis*. Maximum likelihood tree was constructed to infer the evolutionary history of *M. strigata*. Analysis of CO1 gene dataset involved, 2 original sequences and 11 related sequences retrieved from NCBI-GenBank. The molecular sequencing data of *M. strigata* was reported for the first time from Ashtamudi Lake, Kerala. The relationships of *M. strigata* with other Mytilids are illustrated in the Fig. 4. The members of the tree fall under two major well-resolved clades as *Perna*-I and *Mytilus*-II respectively with well supported bootstrap values. It is noteworthy to observe *M. strigata* under the *Perna* clade and not under the *Mytilus* clade. The K2P genetic distance between the *Perna* clade was found to be 0.202 and only a distance of 0.193 was observed between *M. strigata* and *P. viridis*. The observed sequence of *M. strigata* (MN603972) was compared between the Philippine sequence (EU917168) as well the sequence from Kochin (MN165292) backwaters, Kerala, India and the closest distance was observed

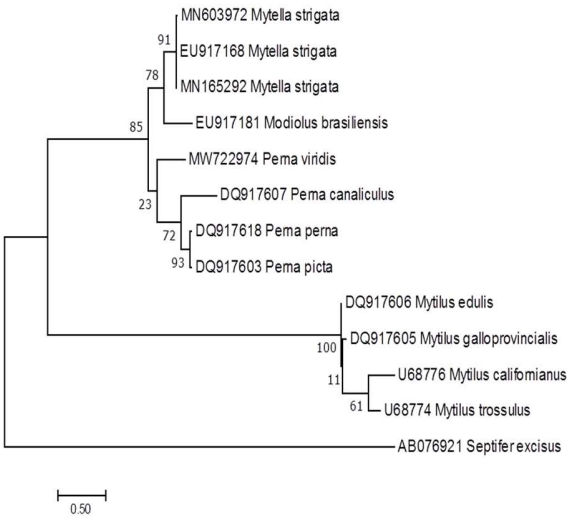

Figure 4: Maximum-likelihood tree (Tamura-Nei Model) using COI sequences of Mytilids

between the Philippine sequence (*M. strigata =charruana*) rather than the Kochin sequence with a bootstrap value of 100. The mean K2P distance between *Mytilus* and *Perna* clade was observed as 0.399.Among the other closely related species, *M. strigata* showed the closest relationship with *Modiolus brasiliensis* (Gray) (*=Mytella guyanensis*). The curve towards the adductor scar created by the pallial line is the unique morphological means of identification of *M. strigata (=charruana)* (Medioda *et al.,* 2017). Also the curved pallial line is unique to *M. strigata* compared to other species like *Perna canaliculus*, *P. viridis*, *P. perna* and *Mytilus edulis* (Quayle and Newkirk 1989). Moreover, distinct brown to black colouration of the shell observed in the samples confirmed the species identification of Rice et al. (2016) and Vallejo et al. (2017) as *M. strigata* (*=charruana*). External features described were the same as those of Gosling (2003) and Spinuzzi et al. (2013) for *M. strigata*. The phylogenetic relationships of *M. strigata* is congruent with the findings of Rice *et al.,* 2016 and Vallejo *et al.,* 2017 and fall under the lineage of *Perna* and the alien invasive mussel *M. strigata* was found to share a close relationship with the native mussel *P. viridis*. The closest relationship between the *M. strigata* with the sequence from Philippines rather than from Kochi indicate the possibility of an independent invasion of *M. strigata* through the Neendakara harbor.

Impact on the ecosystem

The observed salinity ranges of *M. strigata* agrees with the previous records from salinities ranging from 2–40 ppt and even to 55 ppt (Rice *et al.,* 2016). Therefore it can invade and survive in broad saline environments (Yuan *et al.,* 2010; Sanpanich and Wells, 2019). As far as coastal ecosystems are concerned, increase in salinization likely possess a significant effect on the pathways of invasive species. Increased physiological cost has to incur by the salinity-intolerant species to keep-up the osmotic balance when salinity increases, and therefore will gradually grow slower than the tolerant ones. Therefore, increase in salinity may favour invasive species when they are tolerant than the native species. Therefore, the foremost means to bring down the *M. strigata* spread is by means of open-ocean ballast-water exchange (Rahel and Olden, 2008). An emergency action on this invasive species is highly recommended as it has been considered as the potent species to compete with the native species for living space and food resources and can lead to the displacement or the local extinction of the indigenous species (Gurevitch & Padilla, 2004; Spinuzzi *et al.,* 2013). Moreover, the ability of *M. strigata* to accumulate in high densities as 11000 m^2 could also lead to the native species displacement (Pereira *et al.,* 2003). This will worsen when the native species is larger in size like *P. viridis* that may rise up the competitive edge of the species for space and food and has reported to be economically costly (Spinnuzi *et al.,* 2013). This makes *M. strigata* as a dominant competitor and henceforth must be a preference in the bioinvasion control management (Rocha *et al.,* 2010). However, no successful control strategies has been so far reported.The potential of *M. strigata* to outcompete the *P. viridis* is a serious concern that urgently needs to be assessed (Lim *et al.,* 2018; Sanpanich and Wells, 2019) as they occupy similar ecological niche (Vallejo *et al.,* 2017). It is a growing matter of concern that the concentration of the invasive species during the low-flow conditions especially during the hot summer seasons may also rise up the hybridization rate between the non-native and native aquatic species. However, more studies are needed in this concern, to understand its long-term effects. Apart from the consequences on species richness of the invasive species and the corresponding native species

extinction, few invasions may govern multiple influences that lead to gross ecosystem functioning, together with factors like primary production, material flow in between the trophic groups, expansion of organic material decomposition and of benthic-pelagic coupling (Occhipinti-Ambrogi, 2007) and even lead to cascading effects of ecosystem (Ivanov *et al.,* 2000; Shiganova *et al.,* 2001). However, the advantages offered to the invasive species by human-activities in any ecosystem cannot be overlooked. The habitat disturbance by human activities and increasing emission of greenhouse gases, deposition of nitrogen and pollutants thereby global climatic change can effect resource dynamics and species distribution in both aquatic and terrestrial ecosystems and subsequently leads to bioinvasion (Dukes and Mooney, 1999, Occhipinti-Ambrogi, 2007). The rapidly evolving ones in these are the total alkalinity, changing pH and temperature. Furthermore, the reverberation on ecosystem and climate will be carrying a long-term effect, even if human activities slowdown in the near future.

Conclusion

Since the studied sites are the breeding grounds of the native Bivalves such as *Villorita cyprinoides*, *Marcia recens*, *Magallana bilineata*, *P. viridis* etc., *M. strigata* puts a high risk over their vital habitats by rapid increasing and also by competing for space, substrates and food. The broad salinity tolerance in turn allows this species to spread through the ballast water exchange process and fouled ship hulls and sometimes can outcompete the native species when their salinity levels goes beyond the thresholds. Therefore, an incessant monitoring on the ecosystem dynamics of Ashtamudi Lake should be guaranteed allied with environmental factors that promote the steady pace in the establishment of *M. strigata* in the Lake. The change in the alkalinity, salinity, pH and temperature associated with the increasing pollution load has been a growing concern and now the advantages offered by the organic pollution and plastic and other solid waste disposal may favour the spat-fall and growth of the invasive species to flourish and outcompete the native species. Therefore, the invasion of *M. strigata* in Ashtamudi Lake can be assessed as an indicator of environmental degradation of this Ramsar Wetland. Henceforth, an effective solution should be derived through the co-operation of the natives and fisher folk of Ashtamudi Lake to mitigate further spat-fall of *M. strigata* through removal of substrates and to ensure a viable population of *P. viridis* and other indigenous bivalves in the wild.

Acknowledgement

The authors are grateful to Rajiv Gandhi Center for Biotechnology, Thiruvananthapuram, Kerala, India.

Conflict of interest

The authors declare that they have no conflict of interest.

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