Rapid Communication

First record of the invasive biofouling mussel *Mytella strigata* (Hanley, 1843) (Bivalvia: Mytilidae) from clam ponds in Taiwan

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Abstract

In 2019, a survey of hard clam-cultured ponds along the southwestern coast of Taiwan revealed an unknown, almost ubiquitous, brackish water mussel. The mussels were attached to the concrete walls and drainage systems of the clam ponds, hulls of boats, bottom sediment, and riverbanks of the estuary. The largest individual had a shell length of over 6 cm. The general external color of the shells was uniformly dark brown or black, but shells with a dark greenish color were also found. The mitochondrial DNA cytochrome *c* oxidase gene sequences obtained from specimens were consistent with *Mytella strigata* from Singapore, India, and the Philippines. Based on interviews with farmers, the occurrence of this species has become common since 2014 and is increasing in abundance and density, suggesting that its first invasion may have occurred before 2014. The introduction vector of *M. strigata* may be ballast water discharged from ships or bio-fouled ship hulls from its native range or somewhere else in Asia. This report represents the first time this invasive tropical mussel (*M. strigata*) has been recorded in Taiwan and the fifth time it has been reported from the Indo-Pacific region. The rapid growth, high fecundity, and broad salinity tolerance of this species makes it a competitive threat to clams in the ponds as well as other native species.

Key words: Asia, bioinvasion, brackish water, ecological threat

Introduction

Biological invasions are serious problems for marine and brackish water ecosystems globally (Boudreaux and Walters 2006; Molnar et al. 2008; Lim et al. 2018). Successful invasive species often have short life cycles and can survive a wide range of habitats (Jayachandran et al. 2018). Taiwan is located in a tropical/subtropical region and is surrounded by oceans and a strait with a complicated oceanic current system. Thus, it has diverse marine and coastal ecosystems suitable for many species to grow and reproduce
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Figure 1. Locations of hard clam ponds (purple) in Changhua, Yunlin, and Chiayi counties and Tainan city in Taiwan, where the non-native mussel *M. strigata* was observed during summer 2019.

(Liao and Chao 2007). Moreover, with an optimal climate and good water and soil quality for aquaculture, Taiwan has been invaded by many exotic species via the aquaculture trade over the past several decades. Some of these species have escaped from culture ponds and become wild invasive species, such as the apple snail *Pomacea canaliculata* (Lamarck, 1822) and the green-lipped mussel *Perna viridis* (Linnaeus, 1758), which compete with native species and/or hurt crop cultures (Taiwan Invasive Species Information Networks: http://taibif.tw/invasive/).

The hard clam *Meretrix petechialis* (Lamarck, 1818) (Family: Veneridae), which is broadly distributed in Korea, China, and Taiwan (Huang et al. 2020), is an important aquaculture species in Taiwan. The clam ponds are distributed mainly along the southwestern coastal areas of Taiwan (Changhua, Yunlin, Chiayi, and Tainan, Figure 1) and occupy more than 15% (> 8000 ha) of the overall aquaculture area in Taiwan (Fisheries Statistical Yearbook 2020). The ponds are filled with brackish water, and the salinity is controlled either by underground water or water from nearby drainages that are directly connected to coastal seawater. In a 2019 survey on hard clam ponds, farmers complained that there were small, dark-colored mussels in the ponds.
that had been present in high densities since 2014. At the time, the farmers noted that the new mussels seemed to seriously impede the growth and survival of the hard clams. Subsequent visits to other clam farms revealed that these dark-colored mussels existed in most clam ponds. At first, the mussels were thought to be *Perna viridis* due to their resemblance in shell shape; however, on closer examination of the color and size, it was confirmed not to be this species.

This study aimed to investigate the prevalence of these fouling mussels in clam ponds and identify the species genetically. Once the species was determined, the genetic relationships between the Taiwanese population and those occurring elsewhere were also evaluated. The correct identification of invasive species is the first step necessary to mitigate their ecological and economic damage.

### Materials and methods

Hard clam farms are distributed in the counties of Changhua, Yunlin, and Chiayi as well as in Tainan City, Taiwan (Figure 1). The invasive mussels were found attached to various materials, including the concrete walls and drainage systems of clam ponds, hulls of boats, bottom sediment, and on the hard clams themselves (Figure 2). Specimens (*n* = 8) of the invasive mussels were collected from three clam ponds in Yunlin (DS1–DS3) and 5 clam ponds in Chiayi (BD1–BD5) between June and August 2019, kept on ice, and immediately transported to the laboratory. Mantle tissues (20 mg) were stored in absolute ethanol for DNA extraction. The salinity of the pond water was measured with a standard refractometer (ATAGO, Japan, Model MASTER-S/MillM). Water temperature, pH, and dissolved oxygen (DO) were measured using a meter (HORIBA, Japan, Model D-75G). Ammonia was measured using the VISCOLOR ECO Tests (MACH ERET-NAGEL, Germany). The water quality parameters were measured every two months between June 2019 and August 2020.

The mitochondrial cytochrome c oxidase gene (COI) was employed to identify the species of mussels. DNA was extracted using an EasyPure DNA spin Kit (BIOMAN Scientific, Taiwan) according to the manufacturer's instructions and then stored at −20 °C before undergoing polymerase chain reaction (PCR). The universal primers used for PCR amplification of COI were LCO1490 (F): 5’-GGTCAACAATCATATAAGATATTGG-3’ and HCO2198 (R): 5’-TAAACTTCAGGGTGACCAAAAAATCA-3’ (Folmer et al. 1994). The PCR mix consisted of 12.5 μL super mix buffer (AMPLIQON, Danish), 0.5 μL of each primer (2.5 μM), 1 μL crude DNA, and then filled with water to 25 μL. The amplification conditions consisted of an initial denaturation for 2 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. PCR products were resolved by agarose gel electrophoresis (expected size 710 bp) and then
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Figure 2. The fouling of *M. strigata* on a plastic boat (A), with a magnified image (B), to the hard clam (C). External view (D) and internal view (E) of *M. strigata* shells.

Nucleotide sequences were edited using BioEdit v7.0.5 (Hall 1999). The sequences were uploaded and compared with available sequences in GenBank using the Standard Nucleotide Basic Local Alignment Search Tool (BLAST). The partial mtDNA COI sequences were submitted to GenBank under the accession numbers DS1 (MW020362), DS2 (MW020363), DS3 (MW020364), BD1 (MW020357), BD2 (MW020358), BD3 (MW020359), BD4 (MW020360), and BD5 (MW020361). A phylogenetic tree was constructed based on the maximum likelihood method using MEGA X 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 in the phylogenetic tree.
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### Table 1. *Mytella strigata* prevalence and water quality indices in the hard clam aquaculture ponds.

| Items          | Changhua | Yunlin | Chiayi | Tainan |
|----------------|----------|--------|--------|--------|
| Prevalence     | > 80%    | > 80%  | > 70%  | < 50%  |
| Salinity (psu) | 15–27    | 17–27  | 15–35  | 16–42  |
| Temp. (℃)      | 20–33    | 19–34  | 21–34  | 21–33  |
| pH             | 8.6      | 8.4    | 8.3    | 8.4    |
| DO (ppm)       | 5.1      | 4.6    | 4.4    | 3.7    |
| NH₃ (ppm)      | 0.2      | 0.6    | 0.5    | 0.5    |

### Results

**Species identity and field observations of mussels**

The maximum size of the invasive mussels was 6.8 cm, and the mussels were dark bluish to brown in exterior color with a bluish to purplish nacreous interior (Figure 2). The conchological features of the specimens agree well with the American charru mussel *Mytella strigata* (Hanley, 1843) found in Singapore (Lim et al. 2018) and India (Jayachandran et al. 2019). In the case of the specimens from Taiwan, the majority of the shell was more uniform in color and agreed with the “black” forms illustrated by Lim et al. (2018). These observations differed from another invasive mussel, *Perna viridis*, which reaches a maximum size over 8 cm and presents with green shell edges.

Using the descriptions provided above, eight specimens were identified as *Mytella strigata*. In Taiwan, this species forms high-density fouling colonies attached to the concrete walls and drainage systems of clam ponds, hulls of boats, and bottom sediment. Sometimes, *M. strigata* was also found attached to hard clams (Figure 2C). Field investigations showed that these mussels were distributed in most clam ponds in Changhua, Yunlin, and Chiayi counties, and in Tainan city in southwestern Taiwan (Figure 1, Table 1). They also commonly occurred on the concrete riverbanks of estuaries in southwestern Taiwan. Salinity readings in the ponds that had *M. strigata* differed between region, but were between 15 and 42 psu (Table 1). Interestingly, the prevalence (the number of ponds that had the mussel/the number of ponds surveyed) of *M. strigata* was highest in Changhua and Yunlin counties (> 80%), but lowest in Tainan City (< 50%), and inversely proportional to the pond salinity. The water temperatures were within the range of 19–34 °C at all sites. The mean pH, DO, and ammonia concentrations were all similar among these ponds (Table 1).

**Phylogenetic description of the Taiwan specimen of M. strigata**

The nucleotide BLAST results of the suspected *M. strigata* from the Taiwanese ponds were 99–100% similar to *M. strigata* sequences recently reported in Singapore, India, and the Philippines. 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. Of the eight specimens, three individuals (DS3, BD2, and BD5)
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**Discussion**

The American charru mussel *M. strigata* (Hanley, 1843), previously known as *M. charruana* (d’Orbigny, 1846), is an invasive biofouling mussel that is capable of colonizing 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 Central and South America, from Mexico, the
Caribbean, Ecuador, and Argentina (Castellanos 1967; Coan and Valentich-Scott 2012; Soot-Ryen 1955). It has also been recorded as an invasive species in Florida in 1986 (Boudreaux and Walters 2006). In the Indo-Pacific, it has invaded Luzon Island, in the Philippines in 2014 (Michael et al. 2016; Vallejo et al. 2017), Johor Strait, Singapore in 2016 (Lim et al. 2018), the inner Gulf of Thailand in 2017 (Sanpanich and Wells 2019), and brackish water in Cochin, India in 2019 (Jayachandran et al. 2019). This record of *M. strigata* in Taiwan is the fifth record from the Indo-Pacific region and the first for Taiwan. Given the extensive brackish water systems present on both the east and west coasts of Taiwan, further records would not be surprising.

Lim et al. (2018) suggested that the invasion of *M. strigata* in Singapore was mediated by ballast water discharge from ships or biofouling on the hulls of shipping boats. A similar model is suggested here, as there are many international seaports in western Taiwan. *Mytella strigata* can attain reproductive maturity at a shell length of 1.25 cm (Stenyakina et al. 2010); in Taiwan, the majority of fouling colonies were above this size. This suggests that the population is well established and already reproducing in Taiwan. At this time, there is insufficient evidence to postulate when the first settlement took place, but it is clear that they must have been in Taiwan before 2014. Lim et al. (2018) reported that three specimens in the collection of the Natural History Museum in London from the 1840s had a locality label in the Philippines. Therefore, it is possible that *M. strigata* may have been introduced to the Philippines centuries ago and have remained undetected until 2014. However, since there are many instances of mislabeling of 19th-century collections, and there has been extensive malacological work in the Philippines over the last century, it is probable that *M. strigata* was not in the Philippines until very recently. Phylogenetic analyses demonstrated the close genetic relationship between mussels in Taiwan, Singapore, India, and the Philippines (Figure 3). With the recent detection of *M. strigata* in the Philippines (2014), Singapore (2016), Thailand (2017), and India (2019), the introduction of this species into Taiwan may be from its native population in the Central Americas or somewhere in Asia. The first country invaded by *M. strigata* in tropical Asia requires further investigation.

In Tainan City, the clam ponds usually do not have underground water sources, which can result in pond water salinity over 40 psu in the winter dry season due to high evaporation rates. In these ponds, the salinity can only be diluted by rain during the summer season. However, in the Changhua and Yunlin counties, underground water is used to decrease the pond salinity to below 30 psu to promote clam growth throughout the year. Interestingly, the prevalence of this species seemed to be the lowest in ponds with the highest salinity in Tainan City (Table 1). Under laboratory conditions, *M. strigata* can tolerate salinities between 2 and 40 psu (Yuan
et al. 2010; Jayachandran et al. 2019) and temperatures ranging from 6 to 36 °C (Brodsky et al. 2011). Therefore, the high salinities at the Tainan clam ponds in winter may inhibit the colonization of *Mytella strigata*.

The rapid colonization of *M. strigata* in wild and aquaculture habitats in Taiwan is alarming, as it may compete with and exclude native species. After 2016, mass mortalities of hard clams were commonly reported in Taiwan (Huang et al. 2020). Factors such as high temperature, large rainfalls, industrial pollution, disease, inbreeding depression, and eutrophication potentially explain this high mortality rate (Huang et al. 2020). Interestingly, clam farmers said that *M. strigata* became more prevalent after 2014–2015, which also coincided with the high mortality rate of hard clams starting in 2016. *Mytella strigata* is a filter feeder that typically has high fecundity and a rapid growth rate while tolerating a wide range of environmental conditions, all of which make them ideal for survival in clam ponds. Thus, potential competition and possible unknown pathogens brought by *M. strigata* may contribute to the mortality of hard clams. In addition, *M. strigata* has also been recorded in marine ponds in the Philippines and likely competes with milkfish *Chanos chanos*, which are also plankton feeders, as reported by the Manila Bulletin newspaper in 2019 (https://www.pna.gov.ph/articles/1007840).

Although *M. strigata* are sold in Thailand and have been trialed as a potential food supply for rock lobsters (Sanpanich and Wells 2019), this species currently has no economic benefit in Taiwan. Thus, there is an urgent need to define their distribution in Taiwan’s waters in order to develop invasive species management policy and practice.

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