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Research Article

First record of the Charru mussel *Mytella charruana* d'Orbignyi, 1846 (Bivalvia: Mytilidae) from Manila Bay, Luzon, Philippines

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Abstract

This study reports the presence of the Charru mussel *Mytella charruana* d'Orbignyi, 1846 (Bivalvia: Mytilidae) in Manila South Harbor, Manila Bay, Luzon Island, Philippines. In 2014, mussels previously identified as *Mytilus* spp. were reported in Manila Bay. The species was detected as part of an ecological dynamics study of previously-recorded marine non-indigenous mollusc species. DNA barcoding results suggest that the previously identified *Mytilus* are in fact *Mytella charruana* with an average identity match of 94%. The trends in abundance of *Mytella* during the 2014–2015 sampling season are described and the potential of this new species to become invasive and competitive with native *Perna viridis* (Linnaeus, 1758).

Key words: marine non-indigenous species, invasive mussels, estuary, harbors

Introduction

The Port of Manila (Figure 1) is the Philippines biggest port and is the main shipping port of the country (PPA 2010). Manila's international harbors, North and South Harbors have recorded a total of 4,793 foreign ship calls in 2009 representing 73,000 gross tons of shipping and an estimated 13,000 hours of port service calls (PPA 2010). Manila Bay is almost completely surrounded by highly urbanized and rapidly urbanizing communities. It has many environmental issues ranging from land and sea based pollution (Prudente et al. 1994; Prudente et al. 1997; Sta. Maria et al. 2009), sedimentation, harmful algal blooms (Azanza et al. 2004), overexploitation of fishery resources (Munoz 1993), reclamation, land conversion and most recently, biological invasions (Jacinto et al. 2006; Chavanich et al. 2010). Manila Bay is also used for mariculture and fisheries. With all of these factors at play, the risk of biological invasions is high.

Studies in the marine non indigenous species of Manila Bay started in 2011 at the Manila Ocean Park (Ocampo et al. 2014; Ocampo et al. 2015) (Figure 1). The goals of the studies included confirming the presence and invasive potential of *Mytilopsis* as a fouling species, investigate its community ecology especially in interactions with indigenous malaco-fauna, and to detect new non indigenous species.

An introduced mussel species was previously reported by the Philippines Bureau of Fisheries and Aquatic Resources as *Mytilus* spp. due to its similarity of its shape, morphometric measurements and valve color to that of *Mytilus galloprovincialis* (Rinoza

2015). The Bureau noted these as "blue mussels" and that this introduced species is used for mariculture in Lingayen Gulf. However *Mytilus* is a temperate and subtropical species and, given its biological requirements, it was unlikely to reproduce successfully in tropical estuarine conditions such as found in Manila Bay (Bayne 1976; Branch and Nina Steffani 2004; Bownes and McQuaid 2006). Thus the initial diagnosis of *Mytilus* was questionable. A more likely candidate, the Charru mussel *Mytella charruana* d'Orbignyi, 1846, is indigenous to the Tropical Atlantic and Pacific coastlines of Central and South America. In 2014, we first detected and formally report in this study the species from Manila Bay as a fouling organism on the pilings of Manila Ocean Park at the South Harbor, Port of Manila. It was also observed on the recruitment collectors of researchers from the University of the Philippines who were investigating the ecological dynamics of an earlier documented marine non-indigenous species (MNIS), *Mytilopsis sallei* (Récluz, 1849) and *M. adamsi* (Morrison, 1926) (Bivalvia: Dresseinidae) now established in Manila Bay. To confirm the species identity, we did DNA barcoding of the newly detected species to ascertain its identity and we came to the conclusion that this species is *Mytella charruana*. Thus, this paper represents the first record of *Mytella charruana* in Manila Bay.

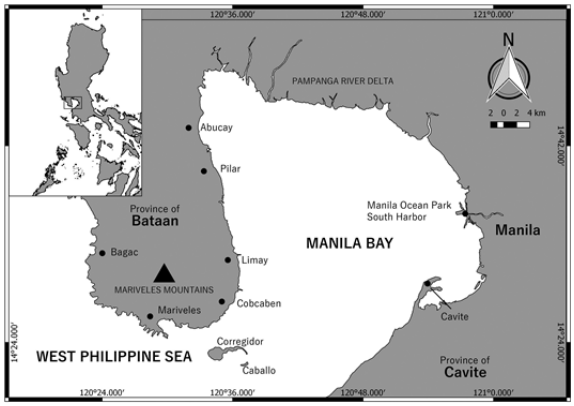

Figure 1. Bay and the location of the Manila Bay-South Harbor study site (filled triangle).

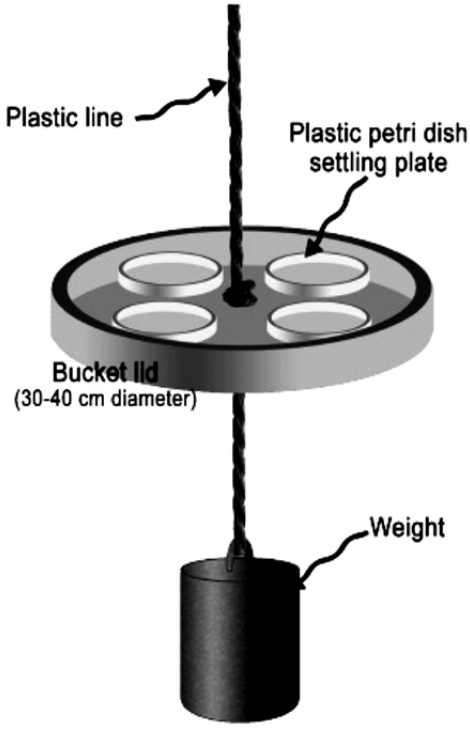

Figure 2. Design based on PICES fouling species recruitment collectors, modified for use in tropical environments and tested for use in harbors in Southeast Asia.

Material and methods

Sampling and preliminary identification

We used fouler collectors designed by the North Pacific Marine Science Organization (PICES) for ports in temperate East Asia including Japan, China and Korea (Figure 2). These collectors were tested for use in ports in South East Asia as part of the International Oceanographic Commission Subcommission for the Western Pacific (IOC-WESTPAC) training programs on rapid assessment of marine non indigenous species. Modified collectors (Figure 2) consisted of a 30 cm diameter bucket lid holding four recruitment plastic petri dishes each with an area of 64 cm^2 each.

Seventeen collectors were deployed in the Port of Manila, South Harbor marina, around the Manila Ocean Park 14º34′43.06″N; 120º58′18.69″E. Following the sampling strategy of Ocampo et al. (2014), the collectors were tied to the jetties at five points in the Manila Ocean Park deck for 16 months from January 2014 to April 2015. All collectors were suspended at 1 m depth from the lowest tide level.

The other 12 collectors were deployed in the marina. The sampling strategy followed that of Ocampo et al. (2014). At each of the four sampling points in the marina, three collectors were deployed. The collectors were soaked in seawater tub at the Manila Ocean Park for one week before deployment. The collectors were retrieved every 60 days and the petri dishes photographed, foulers initially identified, and two of the petri dishes were randomly selected and removed then sent to the laboratory for curation, fixation and

species identification. The petri dishes were replaced with new ones when the collectors were returned. This was done to investigate patterns of recruitment and ecological succession as part of the ecological monitoring. Only foulers on the petri dishes were counted although foulers including *Mytella* which attached on the rest of the lid were identified and noted. The total number of *Mytella* on each petri dish were counted and this is reported as a total for each collector. The total number observed was the sum of *Mytella* from all 17 collectors (an area of 0.435 m^2).

Specimens collected from the plates were identified in situ to the species level whenever possible based on the identification keys for Manila Bay (Ocampo et al. 2014). The attachment habit of the specimens also was noted. *Mytella* was differentiated from *Perna* and *Modiolus* by its dark bluish to brown color and a bluish to purplish nacreous interior, shape of the valve which is more angular (Figure 3). It was differentiated from *Brachidontes* by the lack of radial ribs on the valve. It was also differentiated from the non-indigenous *Mytilopsis* by its larger size, *Mytilopsis* in Manila Bay having a size not exceeding 1 cm in shell length. When further verification was needed, specimens collected were identified at the curatorial laboratory of Manila Ocean Park, Institute of Environmental Science and Meteorology in the University of the Philippines, Diliman and at the University of the Philippines Los Baños. If species diagnosis using morphological keys resulted in uncertain identification, the specimens were sent to the DNA Barcoding laboratory of the University of the Philippines Institute of Biology (UPD-IB).

Environmental characteristics (pH, salinity, temperature, NH_4-N, NO_2-N, NO_3-N) of the collection sites were taken by the curatorial staff of Manila Ocean Park.

Samples were not collected in December 2014 due to the passage of Category 5 Typhoon Hagupit on December 5. Thus there are no counts for January 2015.

DNA barcoding and phylogenetic reconstruction

Live specimens for DNA barcoding were brought to the University of the Philippines Institute of Biology. DNA was extracted from mantle tissue using the NaOH method Bimboim and Doly 1979, as modified by Fontanilla 2010 (Bimboim and Doly 1979; Fontanilla 2010). Amplification of 655 bp segment of COI was performed using the following primers: LCO1490: 5'-ggtcaacaaatcataaagatat tgg-3' HC02198: 5'-taaacttcagggtgaccaaaaaat ca-3' (Vrijenhoek 1994). Polymerase chain reaction (PCR) was done in a final volume of 25 µL having the following mixture: 5 µL 5× PCR buffer with dNTPS, 0.125 µl Taq polymerase (MyTaq), 14.375 µl ultrapure water, 0.75 µl of each primer, 2 µl DMSO and 2 µl of DNA template. The PCR conditions were: 95 °C initial denaturation for 3 min, followed by 30 cycles of 95 °C of denaturation at 30 s, 52 °C annealing at 38s, 72 °C extension for 45s min, and at 72 °C final extension for 3 min. PCR products were confirmed on 1% agarose gel electrophoresis and positive products were sent to 1st Base Asia in Malaysia for bidirectional sequencing using ABI® 3730xl analyzer (AB, USA) with BigDye v3.1 (AB, USA).

Figure 3. *Mytella charruana* from the Manila Bay PICES collectors. Photographs by J. Conejar-Espedido.

Forward and reverse sequences were edited and assembled using Geneious v.8.1.5 (Biomatters). Assembled sequences were then subjected to BLASTn searches at the GenBank National Centre for Biotechnology Information (NCBI). Phylogenetic reconstruction was performed in MEGA v. 6.0 (Tamura et al. 2013) using the Kimura-Two-Parameter (K2P) model (Kimura 1980) of DNA substitution and based on reference sequences found in GenBank.

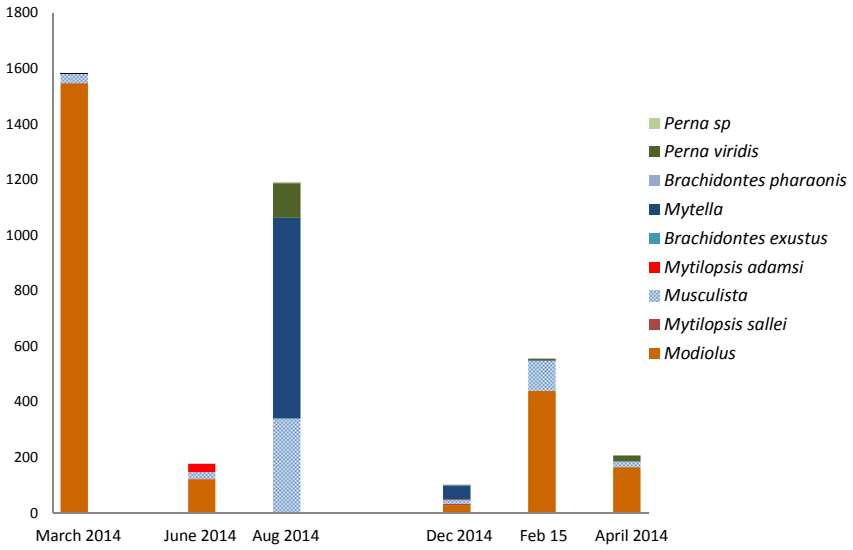

Figure 4. Abundance (total numbers from 17 collectors, an area of 0.432 m^2) of *Mytella charruana* (*Mytella*) during the 2014–2015 sampling season. No samples were collected in December 2014 due to the passage of Typhoon Hagupit.

Table 1. Genetically-determined identities of Manila Bay "blue mussels" previously identified as *Mytilus edulis*.

Specimen		Closest Species	Family	Genbank results Sequence ID	Identity Match (%)	Gap
ME1	1	*Mytella charruana*	Mytilidae	EU917174	99%	0%
ME2	2	*Mytella charruana*	Mytilidae	JQ685158	93%	0%
ME3	3	*Mytella charruana*	Mytilidae	JQ685158	94%	0%
ME5	5	*Mytella charruana*	Mytilidae	JQ685158	93%	0%
ME6	6	*Mytella charruana*	Mytilidae	JQ685158	93%	0%
ME7	7	*Mytella charruana*	Mytilidae	JQ685158	93%	0%
ME8	8	*Mytella charruana*	Mytilidae	JQ685158	93%	0%
ME9	9	*Mytella charruana*	Mytilidae	JQ685158	93%	0%

Results

The mussels were observed to be clumped on the PISCES collectors and were dark brown to blue in color with a bluish nacreous shell valve interior (Figure 3). The average shell length was 2.8 cm. The mussels were first recorded from the PICES collectors in July 2014 and peaked in August 2014. They have been observed continuously since then, although in lower numbers (Figure 4). The range of temperatures recorded at the collection sites were from 25.9 °C recorded during the cool northeast monsoon season of January and 31.3 °C recorded during the May monsoon break and dry season. Salinities ranged from 21.8 in August during the southwest monsoon rainy season to 31.4 in May during the monsoon break. The average levels of standard water quality variables in the collection sites were pH 8.0, NH_4-N at 0.297 mg/l, NO_2-N at 0.439 mg/l and NO_3-N at 1.81 mg/l.

DNA barcoding results for eight sequences with those matched from GenBank suggested that the previously identified *Mytilus* matched *Mytella charruana* with an average match of 94% (Table 1). One of these samples, ME1, had a match of 99%, confirming its identity as *Mytella charruana*. All eight sequences were submitted to GenBank with the following accession numbers: KX499630–KX499637.

The phylogenetic position of the Manila Bay samples is shown in Figure 5 based on 277 nucleotide positions that are common in the reference sequences and the Manila Bay MNIS samples. The results showed that ME1 was closest to *Mytella charruana*, as supported by 99% NJ bootstraps, and belong to the *Perna* clade. The other samples also clustered with the *Perna* clade; although their identity could not be definitively ascertained at this time; this could be due to the limited number of nucleotide positions used for the phylogenetic analysis.

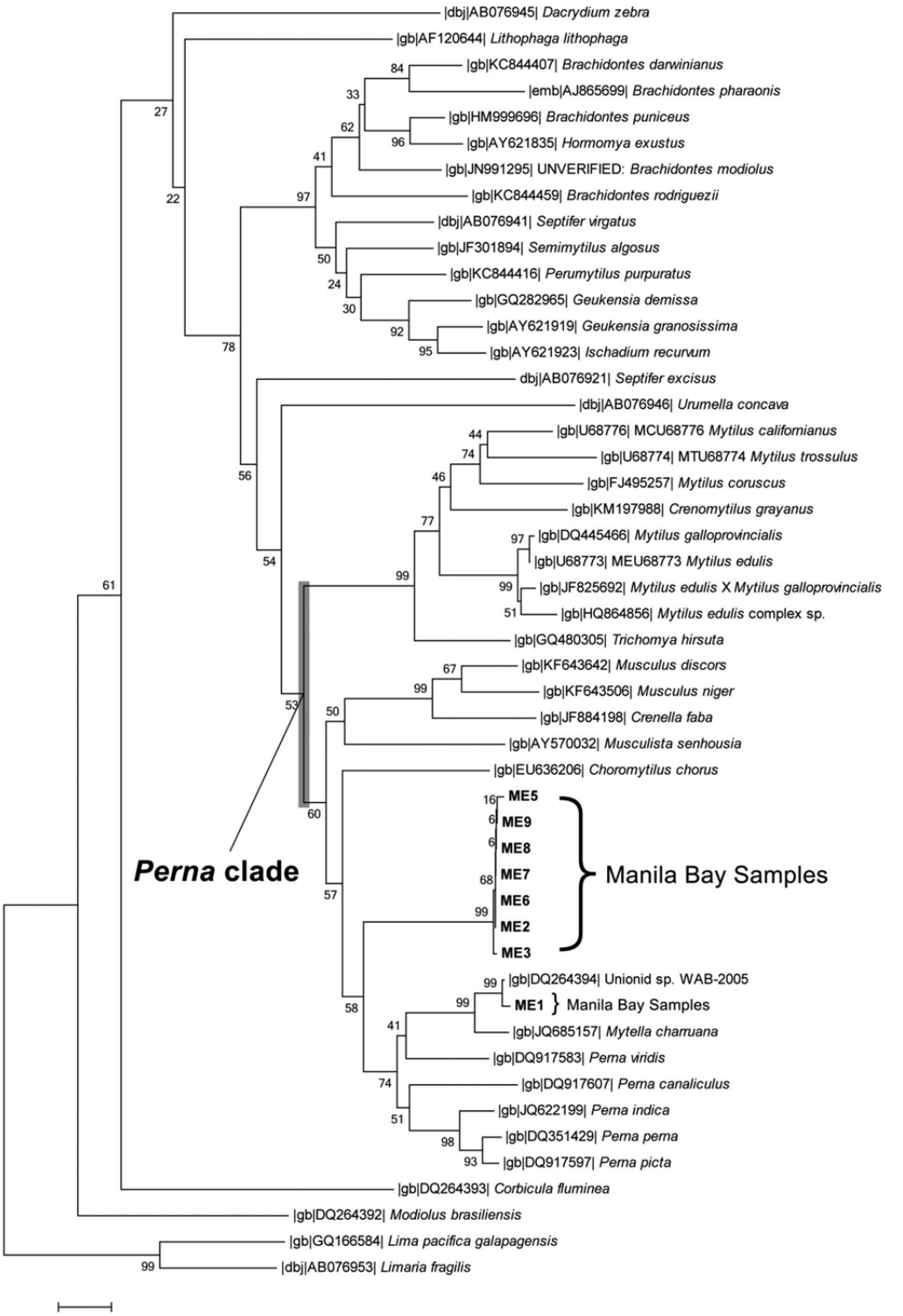

Figure 5. Phylogenetic position of the Manila Bay Charru mussels within the *Perna* clade based on the COI gene. The tree was constructed using the ML method based on 277 nucleotides and the K2P model of DNA substitution. GenBank accession numbers are indicated after each reference species. Numbers on nodes refer to bootstrap support out of 1000 samples. Scale bar represents five nucleotide changes for every 100 nucleotides.

Discussion

Mytella charruana is a documented invasive species outside of its native range. It has been recorded as an invasive in Florida, USA (Boudreaux et al. 2006), and the major vectors for invasion are fouling of ship hulls (Farrapeira et al. 2010) and ballast water exchange (Spinuzzi et al. 2013). The species, while described as primarily marine, is capable of tolerating a wide range of salinities from 2 to 40 (Yuan et al. 2009) and a wide range of temperatures (6–36 °C) (Brodsky et al. 2011).

Given these characteristics, the species may establish and perhaps become invasive in estuarine environments like Manila Bay. The range of temperatures recorded at the collection sites were from 25.9 to 1.3 °C. Salinities ranged from 21.8 to 31.4. Given these favourable environmental condition, it is highly possible that *Mytella* can be established and be reproductive upon maturity in Manila Bay.

Reproduction in the northern hemisphere occurs in the summer months of July to September (Stenyakina et al. 2010). Stenyakina et al. (2010) suggest that food availability may cause sex reversal in *Mytella* individuals wherein starvation results in a male biased sex ratio. When food becomes more available, males change into females thus guaranteeing an extended spawning period. However the larval duration of *Mytella charruana* has not been studied. There is also higher genetic diversity among some invasive populations, which is a result of admixture, defined in population genetics as interbreeding of distinct genetic lineages from the native range when populations are introduced beyond their original range. (Kolbe et al. 2008; Gillis et al. 2009).

The *Mytella* spat were first observed at the start of the southwest monsoon rainy season. They were collected from the PICES collectors in July 2014 at 5–8 mm in length. If the life history and growth parameters of this population are similar to the native *Perna viridis* (Linnaeus, 1758), which has a 3 to 4 week planktonic stage before spatfall, then the first introduction of this species occurred in late April or early May 2014. This is consistent with many tropical mussels which spawns in time with the onset of the southwest monsoon rainy season. Introduction was very likely via ballast water or through fouled ship hulls. Given the adaptability of mytilids to estuarine and coastal conditions and their high reproductive rates in eutrophic conditions, it is highly likely that *Mytella* will be established and be an invasive in Manila Bay. Its abundance on the PICES collectors increased as compared to other non-indigenous species, which have remained at low abundances.

Mytella charruana has also been reported from Lingayen Gulf 250 km north of Manila Bay, where it has been the subject of mariculture, and it has also been reported from the Bolinao Marine Laboratory of the University of the Philippines located in the Lingayen Gulf area. How this non indigenous species will affect the populations of the indigenous *Perna viridis* is not known. But there is some evidence that they occupy the same ecological niche. *Perna viridis* is also maricultured in Lingayen Gulf as well as in Manila Bay.

The results of this study suggest that phylogenetic position of *Mytella charruana* is within the *Perna* clade. While *Mytella* has not previously been included in a phylogenetic assessment of *Perna*, there is plausibility in this hypothesis since it is possible that *Perna* originated in the Atlantic in the Miocene and the modern distribution of *Perna* reflects vicariant speciation related to the closing of the Tethyan Seaway (Wood et al. 2007). The distribution of modern *Mytella* in the eastern Pacific species grouping provides support to its membership in the *Perna* clade (Gillis et al. 2009).

With establishment highly likely, and known invasiveness of the species elsewhere, we recommend more in depth studies on the reproduction, ecology, community dynamics, population genetics and the phylogenetics of *Mytella charruana* in Manila Bay and in the Philippines.

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