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Shell Morphology and Anatomy of the Philippine Charru Mussel *Mytella charruana* (d'Orbigny 1842)

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Abstract

A study on the shell morphology and internal anatomy of charru mussel *Mytella charruana* (d'Orbigny 1842) in the Philippines was conducted. Sampling sites in Bataan, Cavite, and Pangasinan were chosen based on the occurrence and abundance of the mussel. This study is the first to reveal that *M. charruana* can be uniquely identified from other mussels based on the presence of a curved pallial line aside from its black-purplish colour. It has a dark orange foot with brownish pigmentation that is located at the mid-hinged area which can extend to a considerable distance. This and other parts of the internal anatomy did not differ from other mussel species. Charru mussel was found in waters with a wide range of salinity (3.85–25.96 ppt), dissolved oxygen (2.07–7.09 $mg.L^{-1}$), pH (7.51–8.07) and total dissolved solids ($4.45x10^3$–$26.28x10^3$ $mg.L^{-1}$). The ability to inhabit a wide range of physico-chemical conditions indicates potential invasive characteristics.

Keywords: mussel, *Mytella charruana*, Philippines, morphology, anatomy

Introduction

Introduction of non-native species is a serious problem around the world. Invasive species do not have natural competitors to limit their reproduction and they are able to spread from the point of introduction to other areas during their larval stages. The introduction of non-native zebra mussel in the Great Lakes region in the United States and the Asian mussel in Florida altered biodiversity by competing with or outgrowing native species (Griffiths et al. 1991).

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In 2014, a non-native species *Mytella charruana* (d' Orbigny 1842) or charru mussel was reported to be affecting wild spats of mussel and other bivalves in the Philippines (Rice et al. 2016; Vallejo et al. 2017). Introduction of the charru mussel was believed to be through ballast water discharges from vessels. It was first spotted in the northern area of Manila Bay specifically in Cavite which serves as a naval base and docking zone for international vessels. From there, charru mussel was able to disperse rapidly to some neighbouring areas in Bataan, Bulacan and Pangasinan because of its ability to readily colonise a variety of habitats (Rice et al. 2016).

Rocha et al. (2010) stated that charru mussel is a dominant competitor for space on floating substrates and should be a priority in the management for bioinvasion control. It may compete for food and space with *Perna viridis* which are actively cultured in the Philippines. Thus, it is timely to investigate the biology of *M. charruana*, specifically its shell morphology and internal anatomy, to provide baseline information. This study aims to describe and compare the shell morphology and internal anatomy of charru mussel with other mytilid species and to identify its distinct morphological characteristics. Physicochemical characteristics of the sites where charru mussels are located were also described. Information derived from this study will also serve as a field identification guide.

Materials and Methods

Samples of charru mussels were collected from three sites in the Philippines – Abucay, Bataan (N 14° 43.057′; E 120° 33.862′**),** Bacoor, Cavite (N 14° 29.087′; E 120° 56.820′) and Lucao, Pangasinan (N 16° 01.282′; E 120° 18.848′) in November 2016. Sites were chosen based on the abundance of charru mussel reported during a preliminary interview with the mussel farmers. Sampling points in Bataan and Cavite were part of Manila Bay. Manila Bay is a harbour for both local and international ships, a tourist destination, a fishing ground and an aquaculture area for different species such as milkfish, oyster and mussel. However, the bay is severely polluted because of the number of factories and households located nearby that release wastes directly into the bay. Sampling points in Pangasinan were within the Gamayan River where oyster culture is dominant. This river serves as a ferry terminal, docking area for motorised boats and catching basin for Linoc Creek. The site is near Dagupan City, the largest market city of the Province of Pangasinan. Effluents coming from different sources were also released into the river.

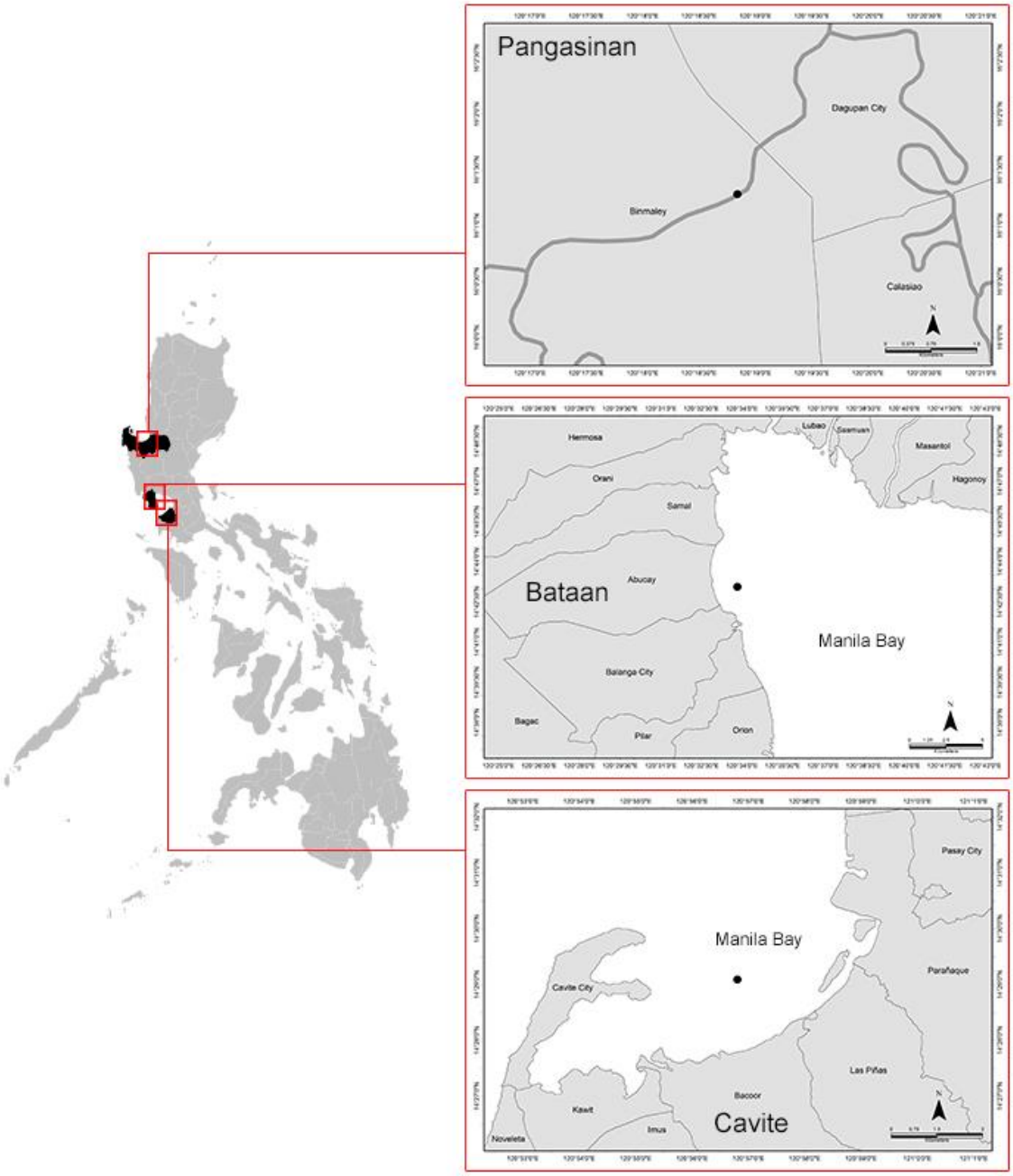

Fig. 1. Locations of sites in Bataan, Cavite, and Pangasinan**,** Philippines where water quality parameters were measured and mussel samples taken. Base maps were taken from PhilGEPS and projected to WGS 1984 UTM 51 N.

In each site, three sampling points were identified and five replicates of temperature, salinity, pH, turbidity, total dissolved solids and dissolved oxygen were measured using YSI multiparameter (Professional Plus, USA) meter. Depth in each sampling point was also measured using HawkEye (H22PX, USA) depth sounder. The sampling time of between 8:00 a.m. and 10:00 a.m. was strictly observed.

Mussels **(**>40mm) were selected and scraped from the bamboo, nets and rubber tire substrates at 2–3 m depth. Samples were washed to remove adhering organisms and other debris. Charru mussels were measured for shell length (SL), the widest part from posterior to anterior portion of the shell, with a Vernier caliper (Caddy and Bazigos 1985) and weighed for total weight (TW) to the nearest 0.01 g on a Shimadzu field-type top loading balance. Pooled samples from different points were photographed with size reference and were sent to Michael Rice for validation. Samples were preserved in 10 % buffered formalin and then brought to the laboratory at the College of Fisheries and Ocean Sciences, University of the Philippines Visayas, for analysis. Charru mussels were dissected using a scalpel. Shell and meat were photographed and general anatomy was identified using the descriptions of Poutiers (1998). Illustration of the anatomy was done and a computerised visual representation was developed. Secondary data and published reports on bivalves were the basis for identification (Narchi and Galvao-Bueno 1997; Gosling 2003; Spinuzzi et al. 2013; and Rice et al. 2016).

Results

Physico-chemical characteristics of the sampling sites

Physico-chemical characteristics of each site are summarised in Table 1. Temperature in the three sites ranged from 29.1 ˚C to 30.1 ˚C and pH from 7.35 to 8.13. Salinity was 3.72 ppt in Pangasinan, 17.24 ppt in Bataan and 25.84 ppt in Cavite. Bataan had an average dissolved oxygen of 6.95 $mg.L^{-1}$, Cavite 3.19 $mg.L^{-1}$ and Pangasinan 3.23 $mg.L^{-1}$. Total dissolved solids (TDS) were 26,276.63 $mg.L^{-1}$ in Cavite and 18,299.20 $mg.L^{-1}$ and 4,447.63 $mg.L^{-1}$ in Bataan and Pangasinan, respectively. Depth ranged from 5.0 m to 9.4 m in Cavite, 3.1 m to 6.7 m in Bataan and 2.3 m to 3.2 m in Pangasinan.

Table 1. Water quality characteristics of Bataan, Cavite and Pangasinan (mean±SE)

Site	Temp (^oC)	Sal (ppt)	DO ($mg.L^{-1}$)	pH	TDS ($mg.L^{-1}$)	Depth (m)
Bataan	29.20±0.06	17.24±0.65	6.95±0.42	8.06±0.03	18.29×10^3 ±0.63	4.78±0.30
Cavite	29.53±0.17	25.84±0.48	3.19±0.12	7.66±0.59	26.27×10^3 ±0.21	7.47±0.33
Pangasinan	29.71±0.16	3.72±0.13	3.23±0.80	7.67±0.13	4.44×10^3 ±0.79	2.79+0.07

Shell morphology of M. charruana

The maximum shell length of charru mussel collected at the three sites was observed to be 56.5 mm (n=1,080). Charru mussel has a smooth and shiny symmetrical shell and has predominantly dark brown to black colour with wavy dark pattern. Sculpture of fine concentric lines or

semicircular rings is visible. It has two similar shaped valves joined by a hinge without teeth at the anterior portion. The interior of the shell is white with a broad band of purple or dark blue and deep purplish black at the posterior margin (Fig. 2A). It has two muscle scars, the large posterior adductor muscle scar and the greatly reduced anterior adductor muscle (Fig. 2B). The byssal and pedal retractor muscle scar is located below the adductor muscle forming a thick straight line moving towards the middle portion of the shell. The pallial line was seen as a curved line towards the adductor scar that was only visible in mussels of size >40mm (n=360).

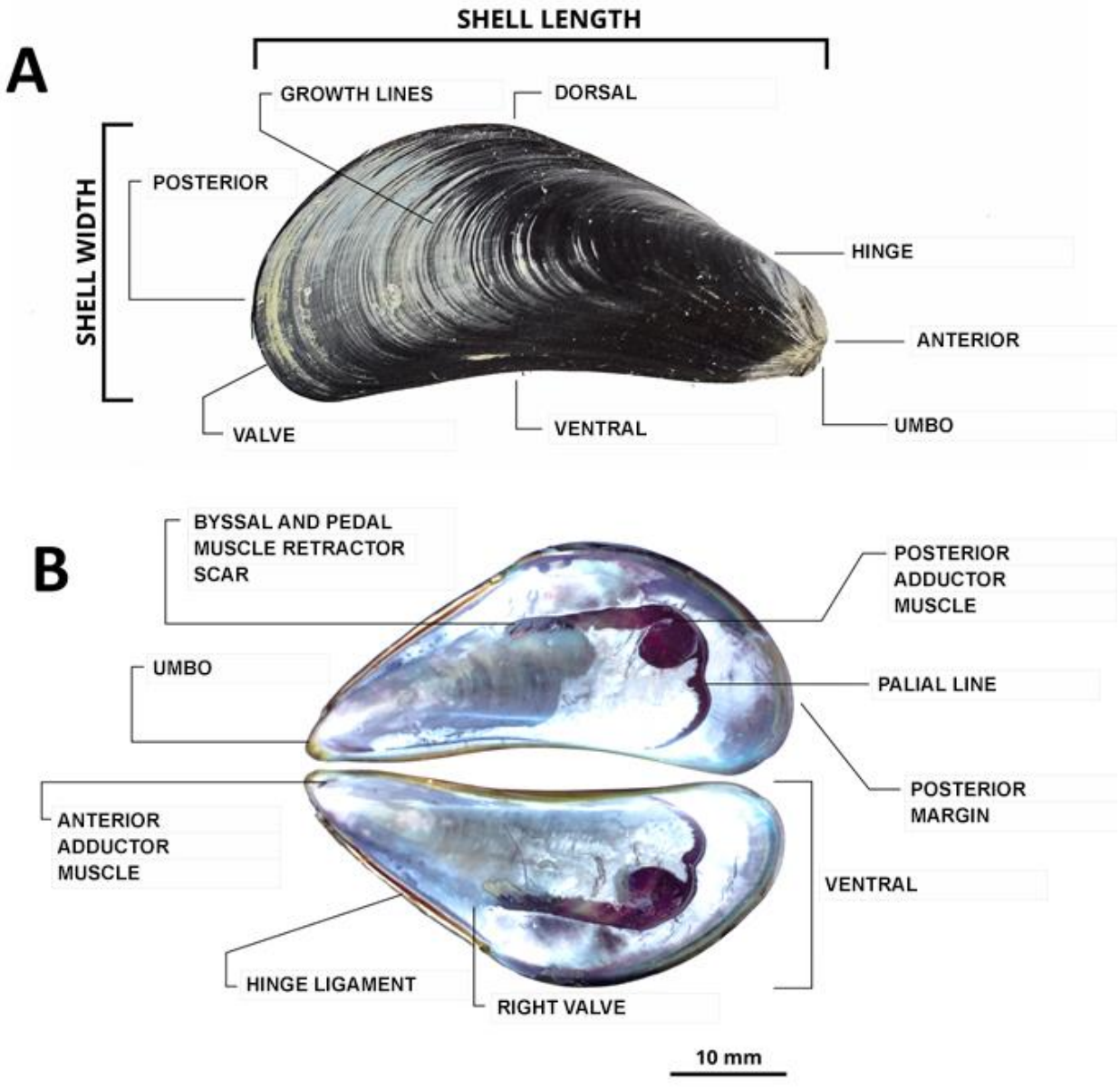

Fig. 2. External (A) and internal (B) shell morphology of charru mussel, *Mytella charruana*.

Internal anatomy of M. charruana

The foot of *M. charruana*, which is dark orange with brownish pigmentation, is located at the mid-hinged area (Fig. 3). The foot can extend to a considerable distance that limits movement of mussel species. A byssus gland that produces a strong thread, which aids in the attachment of sessile organisms is located above the posterior portion where the foot is connected. The byssus thread is visible extending outside the closed external shell of the mussel. The adductor muscles are larger in the posterior area than the anterior. Attached to the adductor muscle are retractor muscles connected to valves in the posterior dorsal area while a smaller anterior retractor is attached to the umbonal area. A fleshy fold of tissue known as the mantle follows the curvature of the valves but is not attached to the shell. Two large curtain-like structures known as lamellibranch gills are also suspended from the ctenidial axis. Gills can be easily identified with their prominent hair-like structure because of the interlocking clumps of cilia. The cilia serve as a gateway for water into the mantle cavity to the exhalant chambers and openings. Orange coloured gonads are embedded in the mantle and in the body. No distinct colour difference was observed in the male and female samples. The mouth is ciliated and used to propel food to the esophagus and towards the stomach. The stomach, black gray in colour, is oval-shaped and is smaller compared to other bivalves. A semi-transparent gelatinous rod at the posterior end of the stomach is also observed. The heart is located at the mid-dorsal region of the body close to the hinge line of the shell. The kidney is located in the pericardial cavity that surrounds the heart, dorsal to the gills and extending from the labial palps to the posterior adductor muscle (Fig. 4).

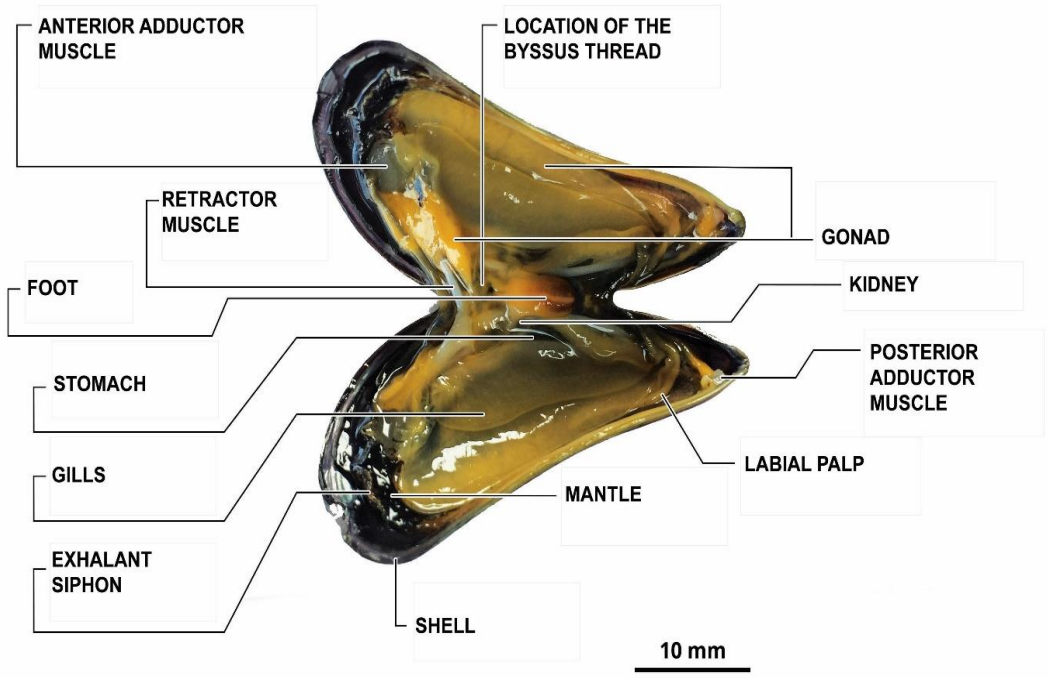

Fig. 3. Internal anatomy of *Mytella charruana*

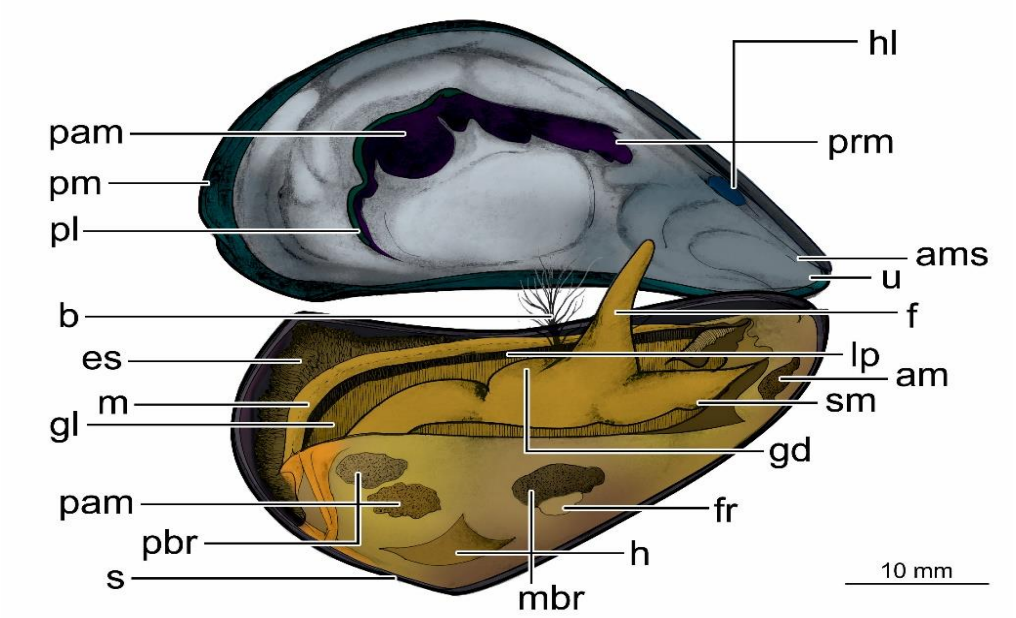

Fig. 4. Graphical representation of the internal anatomy of *M. charruana*. (am: anterior adductor muscle; amr: anterior adductor muscle scar; b: byssus thread; es: exhalant siphon; f: foot; fr: foot retractor muscle; gd: gonad; gl: gill; h: heart; hl: hinge ligament; lp: labial palp; m: mantle; mbr: median byssus retractor muscle; pam: posterior adductor muscle scar; pbr: posterior byssus retractor muscle; pl: palial line; pm: posterior margin; prm: posterior retractor muscle; s: shell; sm: stomach; and u: umbo (Narchi and Bueno 1997).

Discussion

The curved pallial line first found in this study was unique to charru mussel compared to other species like *Perna canaliculus*, *Perna viridis*, *Perna perna* and *Mytilus edulis* (Quayle and Newkirk 1989) (Table 2). This morphological difference observed in the pallial line can be used as a unique feature for identifying charru mussel from other mussel species (Fig. 5).

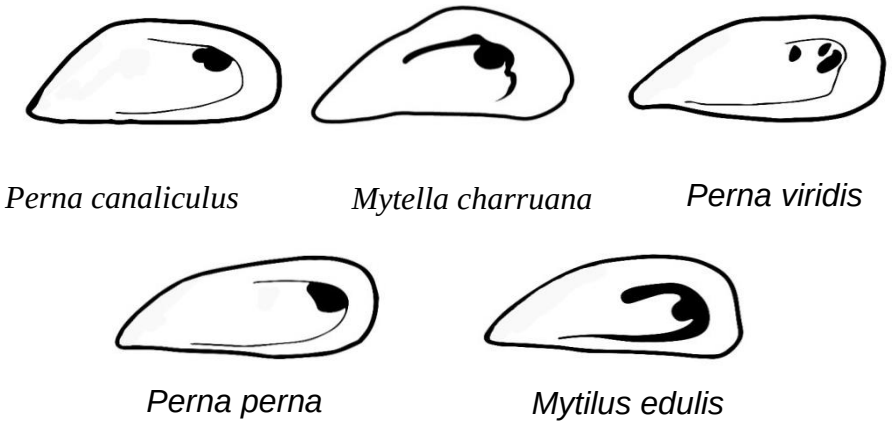

Fig. 5. The pallial line of *Mytella charruana* (arrowed) observed in this study and other mussel species (Drawings modified from Figure 16 of Quayle & Newkirk 1989).

Table 2. Muscle scars in different mussel species

Species	Description of muscle scar	Author
Perna spp.	Has no anterior adductor scar muscle and two adductor muscle scars	Quayle and Newkirk (1989)
Mytilus spp.	Has anterior scar with only one retractor scar	
Modiolus spp.	Has continuous retractors	
Aulacomya spp.	Has no anterior adductor scar present in adults	
Mytella charruana	Has two muscle scars, the large posterior adductor muscle scar and the reduced anterior adductor muscle	Present study; Gosling 2004; Spinuzzi et al. 2016; and Rice et al. 2016
	Pallial line showed a curve line towards the adductor scar	Present study

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. charruana*. External features described were the same as those of Gosling (2003) and Spinuzzi et al. (2013) for charru mussel. However, the morphological differences between species must be accompanied with identification of genetic markers (Bates et al. 2013) to validate the claim. According to the results of Rice et al. (2016), sequences obtained from samples collected in Calmay River, Barangay Tucok, Dagupan City, Pangasinan, Philippines had highest affinity to female-lineage haplotypes from the charru mussel and had 100 % identity with *M. charruana* "Haplotype A" as previously reported by Gillis et al. (2009). Vallejo et al. (2017), using DNA barcoding, obtained a 94 % match to *M. charruana* and not *Mytilus* sp. as previously identified. Samples exhibited similar morphological patterns among sites which may indicate that environmental conditions are not sufficient in obscuring species-specific differences (McDonald et al. 1991), contrary to the report of Seed (1968) which states that differences in shell morphology are due to environmental conditions.

Morphological features of the shell are commonly used in species identification since colour, shape and marking on the shell vary considerably in the different groups of bivalves (Gosling 2003). The colour of mussel shell varies with age and distribution (Mitton 1977) and is controlled by several genes (Newkirk 1980). Bivalves like mussels have two similar valves that are roughly triangular in shape. Mussel species have outer shell colour controlled by several genes (Innes and Haley 1977) and habitat (Mitton 1977) that varies from one species to another. *Mytilus edulis* has a blue-black shell, *Perna perna* red-maroon, and *Perna viridis* bright or blue-green (Siddall 1980). The non-native species that was recently found in the Philippines has dark brown to black shell colour but a closer look at the shell reveals a violet to deep blue colour. It has a shiny and smooth thin triangular shell with evident semicircular rings. Valves are similar with no distinct ribs.

Ligament is found in the internal portion while the umbo is located at the posterior portion of the shell. The hinge has no teeth (edentulous), common to mytilid species (Gosling 2003). The presence of concentric rings that are extensively used to estimate age (Lutz 1980) may provide an accurate estimate of age for charru mussel because of its prominent feature.

Like any other bivalves, *M. charruana* has a nacreous layer that has markings or scars that determine the attachment of the muscles (Quayle and Newkirk 1989). These scars may be used to identify mussel species. The internal anatomy of *M. charruana* is similar to all mytilid species as described by Poutiers (1998), Giribet and Wheeler (2002**)** and Gosling (2003) and no unique feature in the orientation of different internal organs was found.

Conclusion

The curve towards the adductor scar created by the pallial line is the unique morphological means of identification of *M. charruana* that was first identified in this study. No evident differences were observed in the internal anatomy of the charru mussel in comparison to closely related native Philippine mytilids. The ability to inhabit a wide range of physico-chemical parameters indicates potential invasive characteristics. Strategies on how to utilise charru mussel as a potential protein source for human consumption or feed for aquaculture must be taken into consideration to control its ecological impact.

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