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First record of *Tridacna crocea* Lamarck, 1819 (Bivalvia, Cardiidae) from Patnanungan Island, Philippines

Jane Abigail Santiago[1*], Ma Carmen Ablan-Lagman[1]

1 Biology Department and Center for Natural Sciences and Environmental Research, De La Salle University, Manila, Philippines • JAS: jane_santiago@dlsu.edu.ph • MCAL: ma.carmen.lagman@dlsu.edu.ph

* Corresponding author

Abstract

We present a new record and information on the distribution of the IUCN listed *Tridacna crocea* Lamarck, 1819 in the Philippines. The new record in Patnanungan Island extends the known distribution of this species by 80 km east of the nearest previously known occurrence. The collected specimens are found in shallow water at a depth of 3 m, exhibit a relatively small size, and showed the characteristic of completely burrowing its valves in coral substrates. DNA barcoding was also done, and the constructed phylogenetic tree demonstrated that the giant clams created a monophyletic group. *Tridacna crocea* has a wide distribution and is relatively abundant throughout the Philippine reefs. We recommend updating the population status and stock assessment of giant clams in the country for local regulation and conservation management.

Keywords

Biodiversity, boring clam, crocus clam, DNA barcoding, giant clams

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Introduction

Giant clams (Cardiidae: Tridacninae) belong to the list of the Convention on International Trade in Endangered Species (CITES) Appendix II and the International Union for Conservation (IUCN) Red List of Threatened Species. In the Philippines, giant clams are protected under the Philippine Fisheries code, Fisheries Administrative Order (FAO) 208 and Republic Act (RA) 8550, which prohibit the collection of living specimens for raw shells, meat consumption, and any byproducts of giant clams. The Philippine law only permits the collection of giant clams for research purposes where the proponent must abide with the requirements under the Fisheries Administrative Order 233. The FAO Order 233 allows research for giant clams if they are not taken from their habitat and ensure that they remain alive during the study. The rigorous processing of local and national permits for the collection of giant clams can take 1–3 years, particularly for marine protected areas. These prohibitions highlight the importance of giant clam conservation to Philippine marine biodiversity. The status of giant clams in the Philippines was assessed in 1989 (Junio et al. 1989) and included seven extant species which ranged from Endangered to Vulnerable. Five of these species belong to the genus *Tridacna* Bruguière, 1797 and two to the genus *Hippopus* Lamarck, 1799. *Tridacna crocea* Lamarck, 1819 is listed by the IUCN as of Least Concern, but in the Philippines this species is considered Endangered. Thus, discovery of thriving populations of these clams are important. There is no recent update on the conservation and population status of giant clams in the Philippines since 1989.

Polillo archipelago in the eastern Philippines consist of 27 islands with three major islands: Polillo, Patnanungan, and Jomalig. *Tridacna crocea* was reported to occur in Polillo Island more than 30 years ago by Junio et al. (1989) and more recently by Ravago-Gotanco et al. (2007). There are no records of either surveys or collection data from Patnanungan and Jomalig islands which are further off the Philippine Pacific seaboard. Here, we document a new record from Patnanungan Island, Quezon Province. This new record represents a small range extension of 80 km east of the nearest previously known occurrence.

Materials and Methods

Acquisition of permits. Local and national CITES permits were acquired before specimen collection. The Philippines' Bureau of Fisheries and Aquatic Resources - Fisheries Quarantine and Wildlife Regulation Section of the Fisheries Regulatory and Quarantine Division under the Philippine National Government is the issuing office for National-Gratuitous Permits. The Gratuitous Permit No. 0203-20 was issued for this study.

The permit only allowed two giant clams to be collected and held during the collection of mantle tissue. After being sampled, he giant clams were returned to their natural habitat, and only the tissue samples for molecular analyses were allowed to be transferred to other locations. All standard methods according to the permit were followed accordingly.

Collection of *Tridacna crocea*. *Tridacna crocea* were collected using scuba equipment from Patnanungan Island, Quezon Province. Two live specimens were brought back to the wild after identified, measured using vernier calipers, and photographed with Fujifilm X-A2. A small, approximately 10–15 mm sample of mantle tissue was cut from each specimen for DNA barcoding analysis. A phosphate-buffered saline (1X-PBS) solution in a 1.5 ml microcentrifuge tube was used to preserve the sample tissues. All collected tissues are initially stored in an ice chest and later placed in a −20 °C freezer in the Practical Genomics Laboratory of De La Salle University.

The earliest record in the Philippines for *T. crocea* was from 1983 (Wells et al. 1983). Data from compiled literature from 1983 to now were used to ascertain the distribution of *T. crocea* in the Philippines. Data points from the Global Biodiversity Information Facility (GBIF 2020) and records from published literature were the basis of mapping the occurrences of *T. crocea* in the Philippines. SimpleMappr (Shorthouse 2010) was used to produce the map.

Morphological characterization. The shell size, boring nature, valve thickness, byssal gape, and shell margin characteristic were observed. Braley (1992), Naguit (2009), and Hernawan (2012) are the main literature used in describing the morphological characteristics of *T. crocea*.

DNA extraction, PCR, and sequencing. DNA was isolated from the mantle tissue of giant clam voucher specimens. DNA extractions were performed using 10% Chelex (biorad) solution (Walsh et al. 1991). A fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the tridacnid specific primer (forwards: LCO: 5′-GGG TGA TAA TTC GAA CAG AA-3′; reverse: RCO: ′′-TAG TTA AAG CCC CAG CTA AA-3′) (Nuryanto et al. 2007). Polymerase chain reaction (PCR) reactions were performed in a total volume of 25 μL containing approximately: 1 μL DNA template, 17.1 μL ddH2O, 5 μL 5× Vivantis TaqBuffer, 1.5 μL 25MM MgCl2, 0.5 μL 10 MM dNTPs, 0.4 μL of each primers (10 μM). The amplified products were visualized on 1% agarose gel stained with ethidium bromide. PCR purified products were sent to Asiagel-Malaysia for bidirectional sequencing.

Genetic analysis. Nucleotide sequences were aligned using MUSCLE using default parameters. The aligned sequences were 426–480 bp long and free from gaps after trimming using ExPASy server (Gasteiger et al. 2003). All DNA sequences were deposited in GenBank under accession numbers MW582697 and MW581938. The sequences from the collected *T. crocea* were identified using NCBI-blast (Altschul et al. 1990). Additional sequences of *T. maxima*, *T. noae*, *T crocea*, and *T. squamosa* in the subgenus *Chametrachea* Herrmannsen, 1846, *Tridacna gigas* (Linnaeus, 1758) in the subgenus *Tridacna* Bruguière, 1797, *Hippopus Hippopus* (Linnaeus, 1758) were derived from Genbank. The DNA sequence of *Cerastoderma glaucum* (Bruguière, 1789) was used as the. Phylogenetic trees were constructed using Molecular Evolution Genetic Analysis (MEGA) v. 10.0 using the neighbor-joining method. Kimura's 2-parameter distance model was used to construct neighbor-joining trees.

Results

We identified our two collected specimens as *Tridacna crocea* (Fig. 1) using both morphological and COI gene data. These specimens represent the first record of the genus *Tridacna* and of *T. crocea* from Patnanungan Island.

New record. PHILIPPINES • West Philippine Sea, Quezon Province, Polillo group of islands, Patnanungan Island; 14°48′00″N, 122°18′00″E; 3 m depth; 3-XII-2020; JAM Santiago leg; GenBank accession numbers MW582697 and MW581938

Identification. *Tridacna crocea* was recognized due to its habit of burrowing and completely embeds to hard coral heads (Hernawan 2012; Neo et al. 2015). *Tridacna*

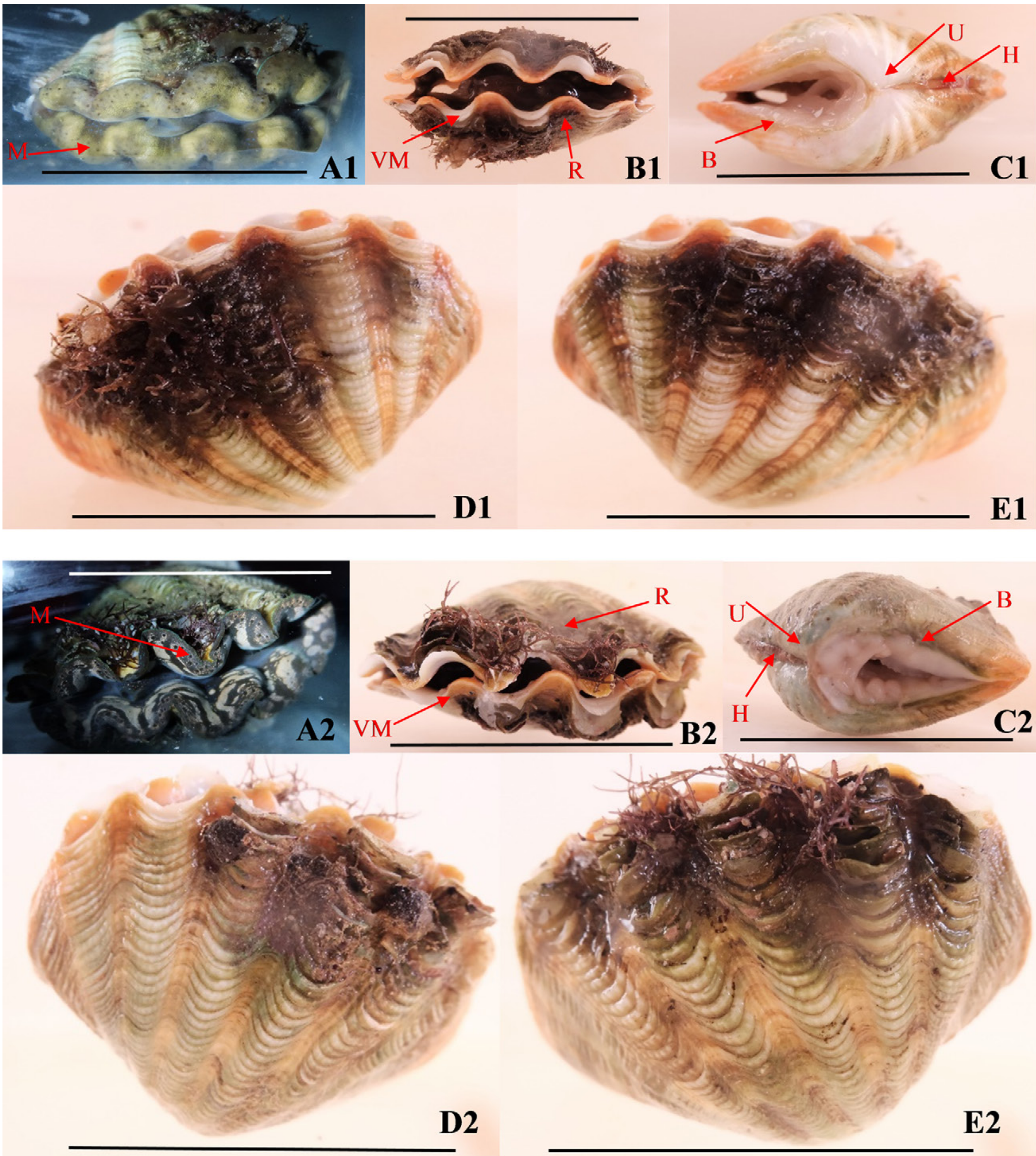

Figure 1. *Tridacna crocea* collected from Patnanungan Island. **A1, A2.** Mantle (M) view. **B1, B2.** Valve margins (VM) and rib (R) interstices view. **C1, C2.** Byssal gape (B), umbo (U) and hinge (H) line view. **D1, D2, E1, E2.** Lateral view. A1–E1 = specimen 1; A2–E2 = specimen 2. Scale bars: 50 mm.

crocea is relatively smaller size compared with other tridacnid species. The length of shells in this study are 62mm and 105 mm, which is within the range found in Palawan (10 mm–147.6 mm; Daño et al. 2020). The mantle was brownish. The other distinguishable characteristics of *T. crocea* are the following: the valve thickness is moderate, with swollen valve margins; there is a large byssal gape and the hinge line is less than half of the shell length; the outline of the shell is triangular-ovate; the outer shell sculpture consists of tattered, low but raised ribs and smooth toward umbos (Braley 1992); and there are scattered hyaline organs on the siphonal mantle (Naguit 2009).

Discussion

Tridacna crocea has only been recorded in the Indo-Pacific Region, where it is widely distributed and abundant (Ravago-Gotanco et al. 2007; Huelsken et al. 2013) that have occurrences from Japan, Australia, Palau (Wells 1997) and Indonesia (Hernawan 2012). However, it is considered extinct in Guam and the Northern Marianas (Wells 1997). In the Philippines, *T. crocea* was recorded from 55 localities since 1983 (Table 1). The Luzon group of islands has the most records of *T. crocea* among the 25 studies that has been conducted in the Philippines (Table 1; Fig. 2). The Palawan region has the most records of *T. crocea* that includes morphological and molecular characterization, distribution pattern, and population genetic structure. The list of occurrences (Table 1) show that *T. crocea* has a wide distribution in Philippines, and this species is relatively more abundant than other tridacnid species on Philippine reefs (Ravago-Gotanco et al. 2007; Dolorosa and Jontilla 2012). The current population density and distribution pattern of *T. crocea* in the Philippines need updating to allow for a proper recommendation to the local resource management and possible mariculture. Currently, all *Tridacna* species are Endangered in the Philippines. For *T. crocea*, this status may be possible to change to Vulnerable or Least Concern status if population density and

Table 1. Updated list of *Tridacna crocea* Lamarck, 1819 occurrences in the Philippines.

Island group	Locality	Reference	Remarks
Luzon	Palawan Regions	Wells et al. 1983	Inventory
		Alcala 1986	Inventory
		Gomez and Alcala 1988	Inventory
		Munro 1989	Inventory
	Cagayancillo, Palawan	Junio et al. 1989	Inventory
	Western Pangasinan	Junio et al. 1989	Inventory
	Polillo Island	Junio et al. 1989	Inventory
		Ravago-Gotanco et al. 2007	Population genetics
	Zambales	Junio et al. 1989	Inventory
	Albay	Junio et al. 1989	Inventory
	Sorsogon	Junio et al. 1989	Inventory
	Calatagan, Batangas	Junio et al. 1989	Inventory
	Lubang Island	Junio et al. 1989	Inventory
	Apo Reef	Junio et al. 1989	Inventory
	Puerto Galera	Junio et al. 1989	Inventory
	El Nido, Palawan	Junio et al. 1989	Inventory
	Tubbataha Reef, Palawan	Estacion et al. 1993	Inventory
		Calumpong and Cadiz 1993	Inventory
		Yamaguchi 1996	Inventory
		Yu et al. 2000	Genetic marker
		Junio-Menez et al. 2003	Genetic marker & structure
		Dolorosa and Schoppe 2005	Inventory
		Dolorosa and Jontila 2012	Inventory
		Dolorosa et al. 2015	Inventory
		Conales et al. 2015	Morphology
	Linapacan Strait in northern Palawan	Junio-Menez et al. 2003	Genetic marker & structure
	the Balabac Strait in southern Palawan	Junio-Menez et al. 2003	Genetic marker & structure
	Ulugan Bay, Palawan	DeBoer and Barber 2010	Genetic marker
		DeBoer et al. 2014	Population genetics & structure
		Santiago et al. 2020	Phylogenetics
	Honday Bay, Palawan	DeBoer et al. 2014	Population genetics & structure
		Santiago et al. 2020	Phylogenetics
	Romblon	DeBoer et al. 2014	Population genetics & structure
	Apulit Island, Taytay, Palawan	Gonzales et al. 2014	Inventory
	Saint Paul Bay, Western Palawan	Gonzales 2015	Inventory
	Kalayaan island group	Junio-Menez et al. 2003	Genetic marker & structure
	Eastern Philippine Seaboard	Ravago-Gotanco et al. 2007	Population genetics
	Cagayan Valley	Ravago-Gotanco et al. 2007	Population genetics
	Isabela	Ravago-Gotanco et al. 2007	Population genetics
	Aurora	Ravago-Gotanco et al. 2007	Population genetics
Luzon	Catanduanes	Ravago-Gotanco et al. 2007	Population genetics
	Masbate	Ravago-Gotanco et al. 2007	Population genetics
	Spratly Islands	Naguit 2009	Genetic markers
	Camarines	(DeBoer et al. 2014	Population genetics & structure
	Batanes Island	Santiago et al. 2020	Phylogenetics
	Rita Island	Daño et al. 2020	Inventory
	Patnanungan Island, Quezon	This study (2021)	Morphology & DNA Barcoding
Visayas	Central and Western Visayas	Alcala 1986	Inventory
	North East Negros	Junio et al. 1989	Inventory
	Sumilon Island Cebu	Calumpong and Cadiz 1993	Inventory
	Balicasag Island, Bohol	Calumpong and Cadiz 1993	Inventory
	Bolisong, Negros Occidental	Calumpong et al. 2002	Inventory
	Balicuatro, Northern Samar	Ravago-Gotanco et al. 2007	Population genetics
	Divinubo, Eastern Samar	Ravago-Gotanco et al. 2007	Population genetics
	Homonhon, Eastern Samar	Ravago-Gotanco et al. 2007	Population genetics
	Southeastern Samar	Naguit 2009	Morphology & genetic markers
		Naguit 2015	Genetic markers
	Pamilacan, Bohol	Naguit 2009	Morphology & genetic markers
	Tañon Strait	Naguit 2009	Morphology & genetic markers
		Naguit 2015	Genetic markers
	Panglao, Bohol	ter Poorten 2009	Inventory
	Carbin, Sagay, Negros Occidental	Naguit 2009	Morphology & genetic markers
		DeBoer et al. 2014	Population genetics & structure
	Guian, Samar	DeBoer et al. 2014	Population genetics & structure
	Bohol Sea	Naguit 2015	Genetic markers
Mindanao	Cagayan, Sulu Sea	Alcala 1986	Inventory
		Calumpong and Cadiz 1993	Inventory
	Camiguin Island	Juinio et al. 1989	Inventory
		Naguit 2009	Morphology & genetic markers
	Dinagat Islands, Northern Mindanao	Ravago-Gotanco et al. 2007	Population genetics
		DeBoer et al. 2014	Population genetics & structure
	Lianga, Surigao del sur	Ravago-Gotanco et al. 2007	Population genetics
	Mati, Davao	Ravago-Gotanco et al. 2007	Population genetics
	Tawi-Tawi	DeBoer et al. 2014	Population genetics & structure

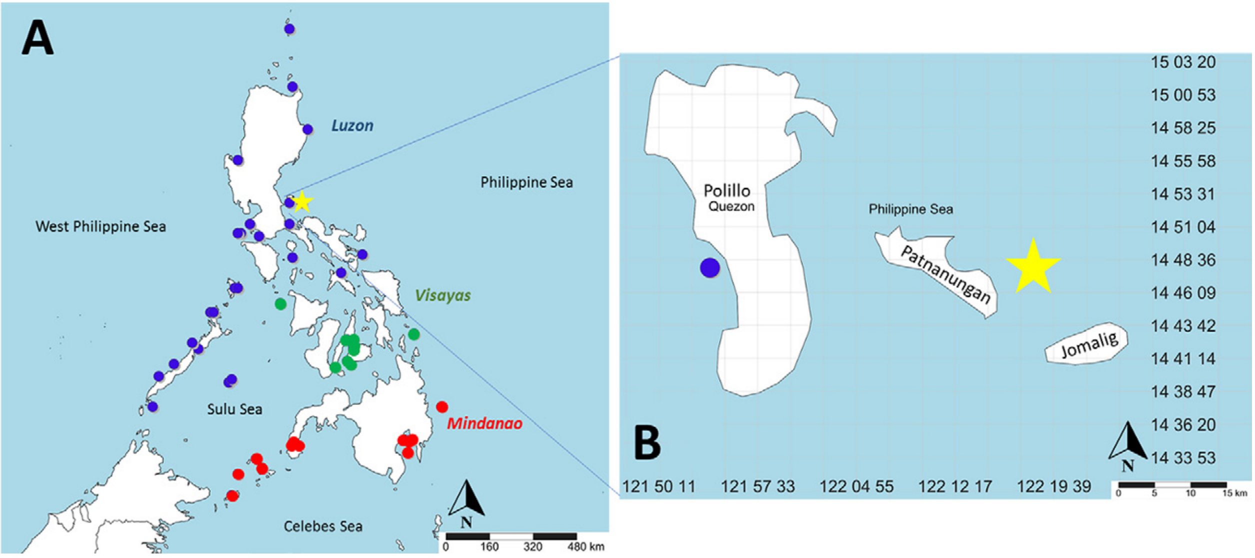

Figure 2. Distribution Map of *Tridacna crocea* in the Philippines. **A.** Occurrences in the Philippines where blue dots represent previous records in Luzon, green dots in Visayas, red dots in Mindanao. **B.** Map showing the 3 major islands of Polillo Group of Islands. Blue dot was the previous record while the yellow star is the new record by this study.

stock assessments are updated. Certainly, we still recommend that *T. crocea* remain protected under the law and by enforcement, but that regulated mariculture is promoted. Relatively smaller species, such as *T. crocea*, *T. maxima* (Röding, 1798), and *T. squamosa* Lamarck, 1819, are better option for mariculture because it only takes 5–7 months to grow to a size suitable for export (Foyle et al. 1997). Most of local people illegally collect wild *T. crocea* for their food consumption and for the aquarium trade (Gomez and Mingoa-Licuanan 2006; Gomez et al. 2006), but this could be lessened if regulated mariculture of this species was established.

Tridacna crocea was recorded from Polillo Island in 1989 and 2007, but there are no more recent records from this area. Since the last records, Polillo Island has become a popular tourist spot where the human population and congestion has increased (Lagbas and Habito 2016). Polillo Island is also a focal point where commercial fishes are marketed. The coastal community consumes giant clam meat during the typhoon and monsoon season when fishermen are prevented from going to the open sea. This happens in other areas of the Philippines, resulting in declining *Tridacna* populations (Gomez and Mingoa-Licuanan 2006; Lebata-Ramos et al. 2010). There was an effort made on Polillo Island to restock giant clams by the Local Government Unit (LGU) with University of the Philippines-Marine Science Institute (Gomez and Mingoa-Licuanan 2006; Gomez et al. 2006). Our new record of a *Tridacna* species in neighboring Patnanungan Island may be helpful to guide the LGU in conservation of *Tridacna* species in Quezon Province. Patnanungan Island, with lesser disturbances due to its smaller human population and lesser amount of tourism, could be an additional restocking site. The new record of *T. crocea* from Patnanungan Island will also help to give awareness and encouragement to *Tridacna* and other marine species in Patnanungan Island.

The genetics of giant clams in the Philippines have been given little attention. A recently rediscovered species, *Tridacna noae* (Röding, 1798), has been proposed to belong to subgenus *Chametrachea* which includes *T. squamosa*, *T. maxima*, *T. crocea*, and *T. noae*. There is ambiguity and confusion with the morphological identification of these species due to their similar size and shell pattern (Nuryanto et al. 2007; Naguit 2009). Although there several phylogenetic trees are available (Nuryanto et al. 2007), there are still uncertain relationships among tridacninae species, specifically in the subgenus *Chametrachea* (Nuryanto et al. 2007). We present a phylogenetic tree that includes all species of the subgenus *Chametrachea* to confirm the identification of the *T. crocea* on Patnanungan Island. Our phylogenetic tree also lends support to the claim that *T. noae* belongs to *Chametrachea*. In our phylogentic tree (Fig. 3), the two specimens collected for this study from Patnanungan Island grouped with other *T. crocea* sequences from GenBank. Giant clams form a monophyletic group in our tree, *T. crocea* is more closely related to *T. squamosa* than *T. noae*, and *T. maxima* is a sister group to the other three giant clams of the subgenus *Chametrachea* (Fig. 3).

A survey throughout the country to determine the population status and genetic diversity of the wild and restocked populations of giant clams is vital to assist the local conservation efforts and at the same time help establish sustainable mariculture which would provide economic and environmental benefits.

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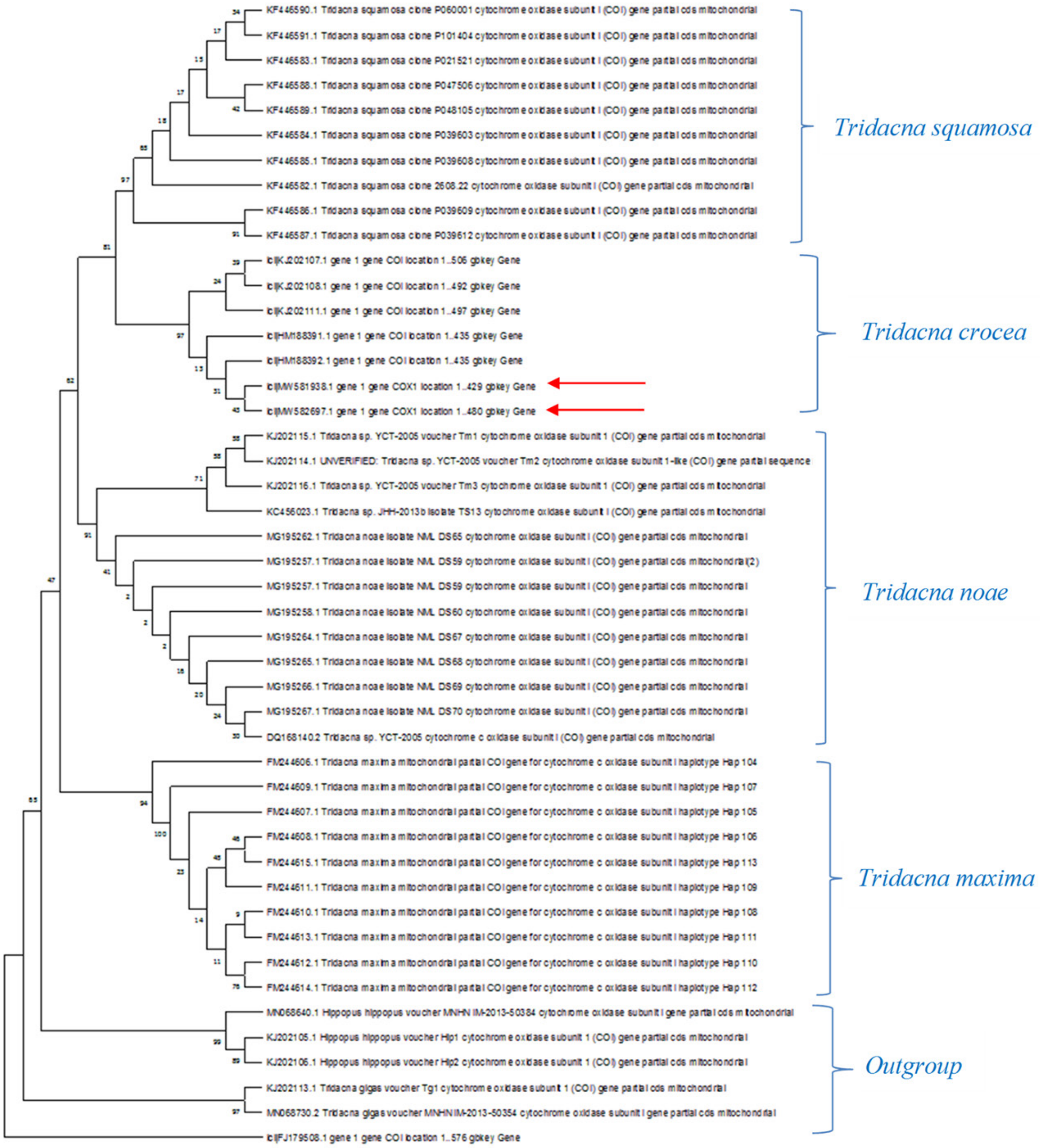

Figure 3. Phylogenetic tree of giant clams based on 400 bp of the mitochondrial DNA COI gene using the neighbor-joining method. Red arrows mark the two new records of *Tridacna crocea* from Patnanungan Island.

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Author's Contribution

Conceptualization: JAS, MCAL. Data curation: JAS. Formal analysis: JAS, MCAL. Funding acquisition: MCAL. Project administration: MCAL. Software: JAS. Supervision: MCAL. Validation: MCAL. Visualization: JAS. Writing – original draft: JAS. Writing – review and editing: MCAL.

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