DLSU RESEARCH CONGRESS 2020
"Building Resilient, Innovative, and Sustainable Societies"
June 17-19, 2020

Updates on the status of *Tridacna crocea* in the Philippines with Notes on Molecular Approach Analysis

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Abstract: The collection sites of *Tridacna crocea* in the Philippines was listed based on the previous studies from 1986 up to the present date. Eighteen (18) studies showed the wide distribution of *T. crocea* within the country. New samples were also added to the collection by the present study from Ulugan Bay and Honda Bay in Puerto Princesa Palawan. First record of *T. crocea* in Batanes Island was also presented by this study using molecular approach identification. The amplified mitochondrial DNA cytochrome c oxidase I gene (COI) sequences of *T. crocea* and the sequences from Genbank of the *Tridacna maxima*, *Tridacna squamosa*, *Tridacna noae* and outgroup were used to construct Neighbour Joining and Maximum Likelihood tree. The information in this study contributes to the growing data on genetics of the giant clams (Cardiidae: Tridacninae). This can serve as a guide to the possible population structure and adaptation patterns of habitat associated marine invertebrate species.

Key Words: *Tridacna crocea*, Giant clam, Phylogenetics, Philippines, Checklist, COI

1. INTRODUCTION

Giant clams (Cardiidae: Tridacninae) are locally known as taklobo in the Philippines. Two extant genera are under Tridacninae subfamily which are *Hippopus* (2 species) and *Tridacna* (9 extant species) (Su et al., 2014). The nine giant clam species in the Philippines are *T. crocea* Lamarck, 1819, *T. derasa* (Röding, 1798), *T. gigas* (Linnaeus, 1758), *T. maxima* (Röding, 1798), *T. noae* (Röding, 1798), *T. squamosa* Lamarck, 1819, *Hippopus hippopus* (Linnaeus, 1758) and *Hippopus porcellanus* (Rosewater, 1982) (Ragaza et al. 2020). The giant clams are listed in the Convention on International Trade in Endangered Species (CITES) appendix II and International Union for Conservation of Nature (IUCN) Red List of Threatened Species. Brood-stock imported from Palau and Solomon Islands of *Tridacna* were made to protect its population (Gomez and Alcala, 1988; Gomez et al., 2006) although most of the restocking efforts in the Philippine mainly focuses with *Tridacna gigas*. Culturing giant clams could be the best way to conserve the population by reseeding depleted reefs (Beckvar 1981) and also help coastal communities for aquaculture industry. A larger amount of capital fund and investment is needed for the nursery facilities for *T. gigas*. It could take 7-year growing period for *T. gigas* to grow and be suitable for meat harvesting which could be a disadvantage for many coastal community villagers (Foyle et al. 1997). Smaller species *T. crocea, T. maxima,* and *T. squamosa* will be a better option for villagers because it only takes 5-7 months to be ready for export (Foyle et al. 1997).

Tridacna crocea is the smallest among the giant clam species. It is the most abundant tridacnid species in the Philippines reefs (Gotangco et al. 2007) which makes it a better candidate for aquaculture-aquarium trade. *T. crocea* has been popular for aquarium enthusiast for its size and colorful bright mantle. *T. crocea* is recommended giant clam for community aquaculture industry. In order to do this, identification of the sites where it can be found in the Philippines is essential for seeding purposes and target stocking areas. This study list the sites of existing *T.crocea* in the Philippines with first record and additional notes on its phylogeny for future reference of biodiversity hotspot studies.

2. METHODOLOGY

2.1 Sample Collection.

Literatures were compiled from 1986 up to date where *Tridacna crocea* was collected and recorded in the Philippines (Table 1).

Small piece of mantle tissue from *T. crocea* were collected through scuba diving or snorkeling from Ulugan Bay and Honda Bay in Puerto Princesa, Palawan and Chanarian, Basco, Batanes in April 2019. The collected specimens were preserved in 1X Phosphate-Buffered Saline (PBS) solution in a 1.5ml microcentrifuge tube. Voucher specimens were initially stored in an ice chest and were deposited to -20°C freezer in De La Salle University-Practical Genomics Laboratory.

2.2 DNA extraction, PCR and sequencing.

DNA extractions were done using 10% chelex (biorad) solution (Walsh et al. 1991). A fragment of mitochondrial cytochrome oxidase subunit-I gene (COI) was amplified using the tridacnid specific primer (forwards: LCO: 5'-GGG TGA TAA TTC GAA CAG AA-3' and reverse: RCO: 5'-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 5X Vivantis TaqBuffer, 1.5 μL 25MM MgCl2, 0.5 μL 10 MM dNTPs, 0.4 μL of each primers (10μM). Amplified PCR products were sent to Asiagel-Malaysia for sequencing. Additional sequences from GenBank were also utilized for comparison: *T. maxima* (Nuryanto and Kochzius 2009); *T. squamosa* (DeBoer et al., 2014); *Tridacna sp.* (Lizano and Santos, 2014); *T. noae* (Neo et al. 2018).

2.3 Genetic analysis.

The sequences from the collected T*ridacna crocea* were identified using NCBI-blast (Altschul et. al., 1990). All sequences with additional sequences from GenBank and *Laevicardium crassum* (Lundin 2019) as the outgroup were utilized for molecular phylogenetic analysis. Nucleotide sequences were aligned using MUSCLE using default parameters. Phylogenetic trees were done using Molecular Evolution Genetic Analysis (MEGA) version 10.0 in two different phylogenetic modes of Neighbor-Joining (NJ) and Maximum-Parsimony (MP). Kimura's 2-parameter distance model was utilized to construct Neighbor- Joining trees (Kimura 1980). Maximum-Parsimony tree was acquired using the Close-Neighbor-Interchange algorithm. NJ and MP trees are distance-based methods that transform the sequence data into pairwise distances, and then use the matrix during tree building. The bootstrap values indicate the robustness of nodes in the result of phylogeny trees inferred in 1000 replicates.

3. RESULTS AND DISCUSSION

From 1986, occurrences of *T. crocea* has been observed within the Philippine reefs. A total of 18 studies has been done with *T. crocea* that includes morphological and molecular characterization, distribution and genetic structure. High densities of *T .crocea* has been repeatedly observed in Tubbataha reefs, Palawan (Conales, 2015). The provinces and regions of Samar, Tubbataha Reefs, Palawan, Sorsogon, Western and Central Visayas, Batangas,

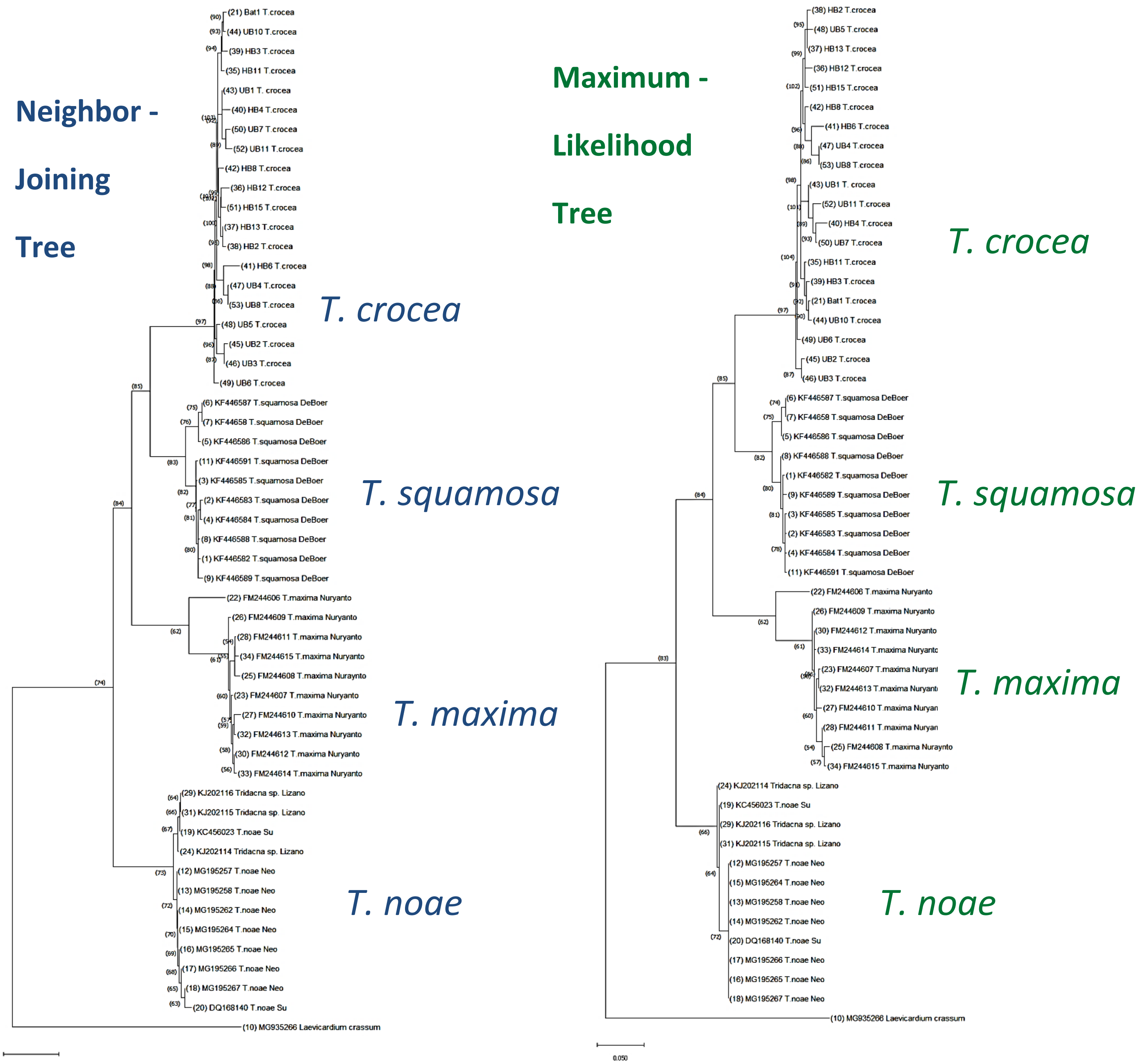

Figure 1. Phylogenetic tree of giant clams based on 400 bp of the mitochondrial DNA COI gene using genetic distances Kimura 2-parameter on NJ approach; bootstrap analysis with 1000 replicates. Similar topology was constructed with Maximum likelihood tree.

Table 1. List of occurrences of *Tridacna crocea* in the Philippines

Place of Collection	Reference	Remarks
Central and Western Visayas; Cagayan, Sulu Sea; Palawan Regions	Alcala 1986	Survey data
Cagayancillo, Palawan;Western Pangasinan; Polillo; Zambales Albay; Sorsogon; Calatagan; Lubang Island; Apo Reef; Puerto Galera; North East Negros; El Nido, Camiguin	Junio et al., 1987	Survey data
Tubbataha Reef, Palawan	Estacion et al., 1993	Survey
Tubbataha Reef, Palawan	Yamaguchi, 1996	Survey
Palawan Pangasinan	Yu et al., 2000	Genetic Marker
Linapacan Strait in northern Palawan, the Balabac Strait in southern Palawan, the Kalayaan island group in the South China Sea, and the Tubbataha shoals in the Sulu Sea	Juinio-Menez et al., 2003	Genetic Markers and Structure
Tubbataha Reef, Palawan	Dolorosa and Schoppe, 2005	Survey
Eastern Philippine Seaboard: Cagayan, Isabela, Aurora,Polillo, Catanduanes, Masbate, Balicuatro, Divinubo, Homonhon, Dinagat, Lianga and Mati	Ravago-Gotanco et al. 2007	Population Genetics
Pamilacan, Bohol; Negros: Tanon Strait, Carbin; Camiguin Island; Southeastern Samar; Spratlys Island	Naguit 2009;	Morphology and Genetic Markers
Ulugan Bay, Palawan	DeBoer and Barber, 2010	Genetic Marker
Tubbataha Reef, Palawan	Dolorosa and Jontila, 2012	Survey
Apulit Island, Taytay, Palawan	Gonzales et al., 2014	Survey
Ulugan and Honda Bay Palawan; Romblon; Dinagat; Carbin, Negros Occidental; Camarines; Quezon; Tawi-Tawi	DeBoer et al. 2014	Population Genetics and Structure
Guiuan, Samar	Lizano & Santos, 2014	Morphology and Genetic Markers
Tañon Strait, Bohol Sea, Visayan Sea, Southeastern Samar	Naguit, 2015	Genetic Markers
Tubbataha Reef, Palawan	Dolorosa et al., 2015	Survey
Saint Paul Bay, Western Palawan	Gonzales, 2015	Survey
Tubbataha Reefs Natural Park, Cagayancillo, Palawan	Conales et al., 2015	Morphology
Ulugan Bay Honda Bay Chanarian, Batanes	This study	Genetic Marker

Mindoro, Quezon Province, Negros Occidental, and Davao are the locations where *T. crocea* has been studied (refer Table 1).

Most of the survey and genetic studies were conducted in Palawan regions. DeBoer et al. (2014) presented the population genetic structure of *T. crocea* in the western side of the Philippines while Ravago-Gotanco et al. (2007) provided for the eastern side. Naguit (2015) has showed both morphological and population genetics analysis for *T. crocea* in Bohol and

Negros region. This information demonstrated that natural populations of *T. crocea* are widely distributed in the Philippine waters wherein it can be found in Luzon, Visayas and Mindanao regions. Since this study has presented the occurrences of *T. crocea* in the Philippines, the next step is to identify the biodiversity hotspot or seeding pools of giant clams within the country and identify the stress resilient populations. Population genetics and structure analysis is a great tool to identify the target populations.

Since genetic data is significant for the next steps, 10 samples each from Ulugan Bay and Honda Bay and one sample from Batanes Island were identified as *Tridacna crocea* through NCBI-Genbank BLASTN search in this study. The first record of *T. crocea* in Batanes Island is showed in this present study. Marine biodiversity of Batanes is still unknown up to date. There are numerous phylogenetic trees that has been presented but there is still uncertainty for the relationships among giant clam species of Tridacninae, in particular among species belongs to subgenus Chametrachea (Nuryanto et al., 2007). The species of *T. squamosa*, *T. maxima* and *T. crocea* are under the Subgenus Chametrachea that can be found in the Philippines. Lizano and Santos (2014) has proposed to include *T. noae* under Chametrachea. The four aforementioned species have almost near size range and usually confusing when morphologically identified. Since this is the first record for Batanes Island, we constructed a phylogentic tree for better identification of the specimen where *T. noae* were added since this is a recent rediscovered species of giant clam. Ecube et al. (2019) has recorded *T. noae* in Palawan with morphological identification. Lizano and Santos (2014) suggested that the specimen they collected in Sibulan, Negros Oriental is *T. noae* DNA sequences were deposited in NCBI-Genbank but with *Tridacna sp.* tag only and not a verified *T. noae*. We included the unverified *T. noae* (Tridacna sp. YCT 2005) from Lizano and Santos (2014) in the constructed phylogenetic tree. *Laevicardium crassum* (Lundin 2019) as the outgroup.

The results (Figure 1) showed that the giant clams created a monophyletic group. *T. crocea* was more closely related to *T. squamosa* than *T. maxima*. *T. noae* become a sister group to the other three giant clams. This data showed that the four species are ecologically similar with each other. The phylogenetic analysis is in line with the results of Borsa et al., (2015). These results will support the claim that *T. noae* is a valid giant clam species and should be added to the subgenus Chametrachea.

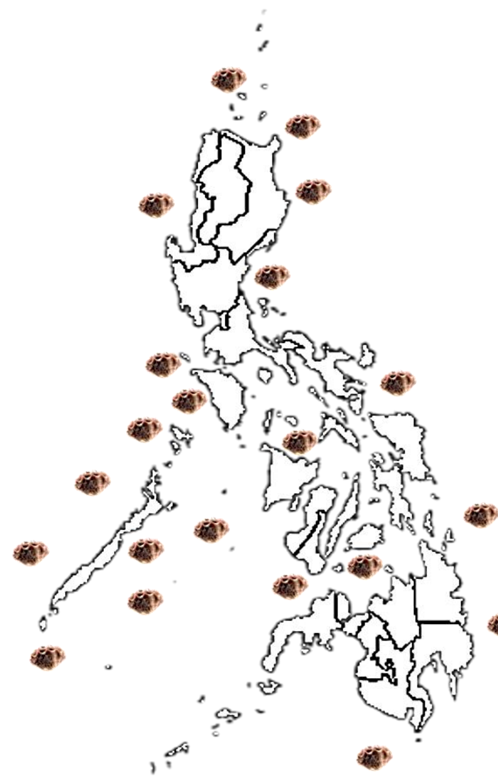

Figure 2. Recorded sampling sites of *Tridacna crocea* in the Philippines. The image represents sampling sites.

4. CONCLUSIONS

Collection sites check-list has shown that *Tridacna crocea* has a wide distribution and can be considered reasonably abundant throughout the Philippine reefs. Molecular approach is a valuable tool in taxonomic research, especially for organisms that are closely related. A population structure and adaptation for the Philippine waters could be the next step since the occurrence sites and DNA sequences are publicly available through Genbank. This will greatly help in conservation efforts of giant clams by possible identification of the resilient populations for aquaculture purposes.

5. ACKNOWLEDGMENTS

This is under the MECO-TECO Project funded by DOST-PCAARRD. The authors would like to extend their gratitude to Western Philippines University research staff: Dante Limbagan, Philip Habal, Jaysee Matillano, and Michael Manlavi for specimen collection in Palawan and Jefferson Elento for Batanes sampling. Special thanks to Janlyn Santos and Crissa Lilagan for their unconditional support.

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