# MOLECULAR TAXONOMY AND SOME ASPECTS OF THE BIOLOGY OF MUDCLAMS IN THE ISLAND OF GUIMARAS, PHILIPPINES

# MOLECULAR TAXONOMY AND SOME ASPECTS OF THE BIOLOGY OF MUDCLAMS IN THE ISLAND OF GUIMARAS, PHILIPPINES

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*Key words*: mudclam phylogeny, 18s rRNA gene, COI gene sequence, reproductive biology

## Abstract

Mudclams inhabit the mangrove areas around the island of Guimaras. The two species have been identified as *Austriella corrugata* and *Anodontia edentula*. The two mudclam species exhibit the morphological characteristics of lucinids and differ only in external shell morphology and ligament position. Comparison of their 18s rRNA gene sequences show they belong to different branches of the family Lucinidae. In addition, *A. edentula* specimens obtained from different sites in the island are quite homogenous with no significant differences in their COI gene sequences. *A. corrugata* is mostly dioecious with a small percentage of hermaphrodite and reaches sexual maturity at shell length of 28 mm for both male and female clams. Likewise *A. edentula* is dioecious with occasional occurrence of hermaphrodite and reaches sexual maturity at shell length of 28 mm for males and 33 mm for females. Brooding is also observed in some adults which harbor juveniles under their gills or mantle cavity.

Mudclams (Veneroida, Lucinidae) are bivalves which inhabit the shallow waters of mangrove areas around the island of Guimaras, Philippines. Two species known locally as *imbaw baye* and *imbaw laki* are usually harvested by local fishermen by digging in the sandy-muddy substrate. The two species are identified as *Anodontia edentula* (Linne, 1758) and *Austriella corrugata* (Deshayes, 1843) respectively. Both species are classified under family Lucinidae and are said to harbor sulfur-oxidizing bacteria[1-2]. *Anodontia edentula* is widely distributed from East Africa to the Phillipines and Australia [3]. Specimens of *Austriella corrugata* have been reported in Zamboan ga, Mindanao, Philippines, the Indo-West Pacific region, New South Wales, Queensland, Northern Territory to south Western Australia [4].

In Guimaras, *A. edentula* is usually found buried in the mud at a depth of 20-30 cm[5]. *A. corrugata*, on the other hand, is said to burrow deeper into the mud (personal communication from clam collectors) at the fringes of mangrove areas [4]. The difficulty in collecting and high demand from seafood restaurants make the mudclams quite expensive at local markets. Frequently sold at local markets is *A. edentula* and occasionally *A. corrugata*. These mudclams are considered as one of the commercially important resources of coastal communities in Guimaras and are potential aquaculture species.

Recent studies have used molecular data to support morphological characteristics and to understand relationship of bivalve families[6-8], relationship of species belonging to the same family[2,9] or discriminate variation within species[10-11] . This study was undertaken to describe the features of the two species of mudclams found in Guimaras and some aspects of its reproductive biology. The present study also attempts to establish a phylogenetic framework for mudclams that will be independent of shell morphology through molecular analysis of 18s rRNA genes within the family and other related bivalves. Preliminary work on the use of molecular marker to distinguish *A. edentula* populations from different sites will also be done.

## Materials and Methods

Samples of mudclams were collected from mangrove areas in Guimaras and transported to the laboratory wrapped in newspaper moistened with seawater. Clams were measured by their length, width, and height. The

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characteristic shell sculpture, ligament structure and position, hinge teeth, size and shape of anterior adductor muscle scar were noted. Clams were dissected, their internal morphology was studied and gonads biopsied to determine sex ratio and size at sexual maturity. Some gonads were processed for histology when biopsy results did not provide clear distinction between male and female mudclams. Anatomical features were compared with known descriptions of *Anodontia edentula* and *Austriella corrugata* to validate the identification of specimens found in Guimaras.

**DNA extraction, amplification and sequencing**

Gill, foot, and gonad tissues were obtained from specimens of *Anodontia edentula* and *Austriella corrugata* that were brought to the Faculty of Fisheries, Kagoshima University. The tissues were ground in liquid nitrogen and genomic DNA was extracted using the nucleic acid purification kit SepaGene (Sanko Junyaku, Tokyo, Japan) following the manufacturer's instruction. Extracted DNA (10 ng) was used for PCR amplification of the 18s rRNA region using the following primers: 18SF 5'-AACCTGGTTGATYCTGCCAG-3'; 18SR 5'-TGATCCTTCYGCAGGTTCACCTAC-3'. PCR amplification of 18s rRNA gene was conducted for *A. edentula* at 35 cycles under the following conditions: 15 sec at 95°C, 30 sec at 55°C, 2 min at 72°C and final extension of 7 min at 72°C. The following conditions were used for *A. corrugata*: 35 cycles with 15 sec at 95°C, followed by 30 sec at 50°C annealing temperature, 2 min at 72°C, and final extension of 7 min at 72°C.

Since *A. edentula* specimens were obtained from three sampling sites in Guimaras (Tando, Panabulon, and Lactawan), differences in the population was studied using cytochrome oxidase subunit I (COI) gene sequence. DNA was extracted from gill, foot, and gonad using a nucleic acid purification kit (DNeasy Plant Mini Kit, Qiagen). PCR amplification was carried out with the following primers: forward primer LCO1490 (5'-TCAACAAATCATAAAGATATTGG-3') and reverse primer HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') under the following conditions: 1 min at 95°C for initial denaturation of template DNA, followed by 30 cycles of 15 sec at 95°C, 30 sec at 46°C, 1 min at 72°C and final extension step of 7 min at 72°C. PCR product was purified with MinElute Gel Extraction Kit (Qiagen).

Purified PCR products were used for automated sequencing with ABI Prism 310 genetic analyzer (Applied Biosystems) using BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems). Mudclam 18s rRNA and COI gene sequences were aligned by the Clustal X multiple sequence alignment program version 1.8. Mudclam 18s rRNA gene sequences were aligned with known sequences of other representative bivalves present in GenBank to determine phylogenetic relationship of the two species and with other lucinids. COI gene sequences of *A. edentula* from the three sampling sites were compared.

**Table 1.** Primers used for sequencing 18s rRNA gene.

| Primer Name | Sequence (5' → 3') |
|---|---|
| 18SF | AACCTGGTTGATYCTGCCAG |
| 18SR | TGATCCTTCYGCAGGTTCACCTAC |
| EuSSUR.3 | CATCACAGACCTGTTATTGCC |
| EuSSUR.5 | CTTCGATCCCCTAACTTTCG |
| EuSSUR.6 | CTACGAGCTTTTTAACTGCAACAA |

## Results

**Description of mudclam species from Guimaras**

As members of family Lucinidae, both species of mudclam have oval moderately inflated shell covered with thick greenish brown periostracum (Fig. 1). On the inner side of the shell, the anterior adductor muscle scar is separated from the pallial line three quarters of its length, hinge teeth are absent and shell ligament is long and broad. The mantle is thick with folded edges and exhibits fusion below the inhalant aperture. Foot is tubular, terminating in a muscular tip. The gills consist of two demibranch covering the visceral mass with a prominent globular gonad.

*A. corrugata* can be recognized by its ovate shell with more regular comarginal lamellae and prominent anterior and posterior sulci (see Fig.1-top). The long, broad ligament is at the rim of the hinge region of the shell and is visible externally when the valves are closed. Sampling of the population of *A. corrugata* showed that sex can

be male, female or hermaphrodite. Males comprised 51% of the collected clams, females 33% and hermaphrodite, 11%. Hermaphrodites usually show mature spermatozoa and developing (vitellogenic) or mature oocytes. The occurrence of mature spermatozoa or vitellogenic oocytes in the gonad was first observed in clams with shell length of 28 mm. All specimens of *A. corrugata* with shell length greater than 45 mm were found to be sexually mature.

The ovate shell of *A. edentula* could be distinguished from *A. corrugata* by their fine growth lines (Fig. 1-bottom). The long, broad ligament is shifted towards the interior and cannot be seen externally when the valves come together. Sexes are separate with males comprising 49 % of the samples and females about 51% (1:1 sex ratio).

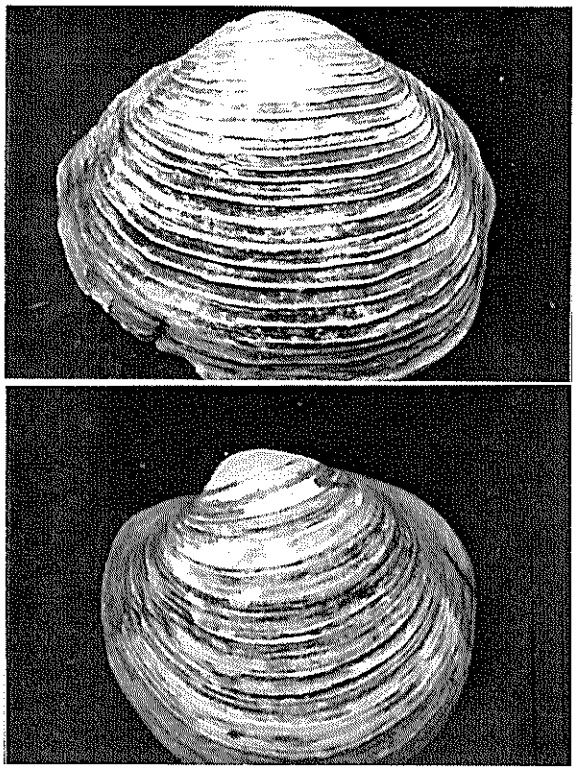

**Fig. 1.** Shown are left valves of *A. corrugata* (Top) and *A. edentula* (Bottom). Note the prominent anterior and posterior sulci and regular growth lines of *A. corrugata*. *A. edentula*, on the other hand, has fine growth lines. Both valves are covered by greenish brown periostracum.

However, three hermaphrodite specimens were found with gonads containing both mature spermatozoa and oocytes. In female *A. edentula*, vitellogenic oocytes were first observed at shell length of 30 mm. However, only 30% of female clams were sexually mature at shell length of less than 35 mm. At shell length of 35-40 mm, about 67% were mature and at shell length >40mm, all female clams examined were sexually mature. In contrast, males were first observed to have mature spermatozoa at shell length of 28 mm. It was also noted that half of the male population were sexually mature at shell length of 30 mm. At shell length ≥ 35 mm, all male clams biopsied were sexually mature. It was noted that about 10% of clams obtained from one study site brood juveniles under their gills or within their mantle cavity. Although the majority of brooding clams had one juvenile, some had more than one juvenile at varying sizes (range: 2.8-13 mm shell length, maximum number of juveniles 13).

**Gene sequences of 18s rRNA and COI genes**

Full length sequences of 18s rRNA gene consisting of 1860 bps for *A. edentula* and 1735 bps for *A. corrugata* were obtained. However regions which were ambiguously aligned were not included in the calculation. As a result, only 836 bps of 18s rRNA gene sequences were aligned and used for phylogenetic tree construction (Fig. 2). 18s rRNA sequences of *A. corrugata* and *A. edentula* show they belong to two different clades under family Lucinidae. *A. edentula* clustered with other *Anodontia* species. *A. corrugata* specimen from Guimaras was in a branch separate from the *Anodontia* group, in a cluster containing an *Austriella* species from Western Australia.

A full sequence of cytochrome oxidase subunit I gene (COI) of *A. edentula* was obtained from Lactawan sampling site while partial sequences were obtained from the two other sites (Tando and Panabulon) (Fig. 3). Comparison of nucleotide sequences showed no difference among specimens obtained at the three sites.

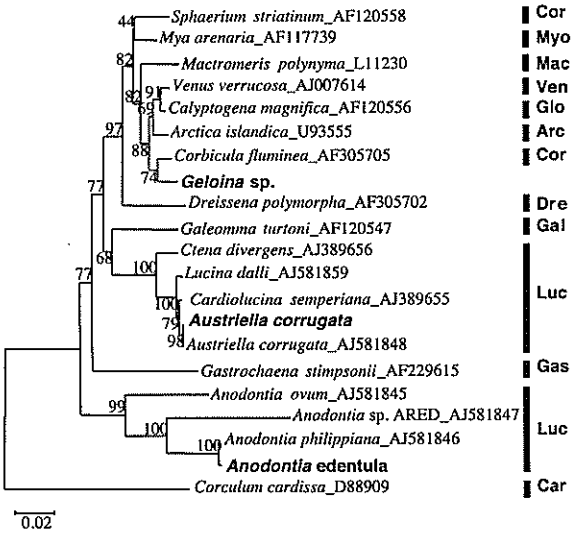


**Fig. 2.** Phylogenetic relationship inferred from 18S ribosomal RNA gene nucleotide sequences. Cor, Corbiculoidea; Myo, Myoidea; Mac, Mactroidea; Ven, Veneroidea; Glo, Glossoidea; Arc, Arcticoidea; Dre, Dreissenoidea; Gal, Galeommatoidea; Luc, Lucinoidea; Gas, Gastrochaenoidea; Car, Cardiidae. Codes after each species indicate GenBank accession numbers. Names in bold letters are specimens obtained in Guimaras.

## Discussion

The molecular data confirm the observed morphological characteristics that mudclams in Guimaras belong to family Lucinidae and consists of two distinct species namely *Anodontia edentula* and *Austriella corrugata*. 18s rRNA gene sequences appear to be able to distinguish interspecific differences from the two recognized species of mudclams. When compared with sequences from other bivalves, this resulted in clustering of the two species into different branches of family Lucinidae. Such clustering has also been observed in tree construction using combined 28s and 18s RNA gene sequences[11].

*Anodontia edentula* clustered with other *Anodontia* species and appeared to be closely related to *A. philippiana* (fromWestern Australia). The high bootstrap value suggests that the two specimens although collected from quite distant geographic sites may be similar or the same species. This however needs to be verified until specimens of *A. philippiana* could be obtained and sequenced. While 18 s rRNA gene sequences were used to distinguish the two mudclam species, COI gene sequences were used to determine differences in *A. edentula* population from three different sites in Guimaras. There were however no distinct variation in nucleotide sequences of COI gene from specimens collected at different localities in Guimaras. The results of COI gene sequence confirm the identity of *A. edentula* from the three sites. It also implies that the population in Guimaras may be homogeneous. This finding may have an implication on recruitment of *A. edentula* around the island.

*Austriella corrugata*, on the other hand, was found to cluster at a different branch of family Lucinidae, the group of lucinids living in shallow waters and deep-sea vents[9]. The 18s rRNA gene sequence of the specimen from Guimaras was found to be very similar with *A. corrugata* specimen from Western Australia. The high bootstrap value between the specimens from Guimaras and Western Australia confirms this relatedness.

Other than their taxonomic features, the two mudclam species were found be to dioecious with a small percentage of hermaphrodites in the population. Incidence of hermaphroditism was slightly higher in *A. corrugata* compared to *A. edentula*. Hermaphrodites have also been observed in other clam species [12-14] which imply that its occurrence may be a common phenomenon among bivalves. Some *A. edentula* specimens collected in mangroves near the mouth of a river, brood juveniles, a habit found mostly in oysters and freshwater clams[15-16]. Both hermaphroditism and brooding in this species may be important adaptations to insure reproductive success in the harsh environment of mangrove areas.

Size at sexually maturity differ between the two species. Both male and female *A. corrugata* reach sexual maturity at 28 mm shell length. In contrast, male *A. edentula* mature earlier at 28 mm shell length than females which first showed vitellogenic oocytes at 33 mm shell length. However, the population of both mudclam species were found to be all mature at shell length greater than 40 mm. As commercially important species, these results suggest that clam collection should be done at shell length of more than 40 mm to insure the existence of a breeding population and avoid depletion of the resource.

```
                        10         20         30         40         50         60
Tando_For               TTTACTTACT TGTAGGGTTT TGATCTGGCT TAGTAGGCAC AGGTCTTAGA GTACTAATTC
Lactawan_For            ---------- -GTAGGGTTT TGATCTGGCT TAGTAGGCAC AGGTCTTAGA GTACTAATTC
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- ---------- ---------- ---------- ----------
Anodontia_contig        TTTACTTACT TGTAGGGTTT TGATCTGGCT TAGTAGGCAC AGGTCTTAGA GTACTAATTC

                        70         80         90         100        110        120
Tando_For               GTCTAGAACT TGGACGACCT GGCGAAAATC TAATAGACAG CCAAACATAC AACGTAGTCG
Lactawan_For            GTCTAGAACT TGGACGACCT GGCGAAAATC TAATAGACAG CCAAACATAC AACGTAGTCG
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- ---------- ---------- ---------- ----------
Anodontia_contig        GTCTAGAACT TGGACGACCT GGCGAAAATC TAATAGACAG CCAAACATAC AACGTAGTCG

                        130        140        150        160        170        180
Tando_For               TTACGATTCA CGGATTCGTA ATGATTTTCT TCCTTGTTAT ACCTATGCTA ATTGGAGGAT
Lactawan_For            TTACGATTCA CGGATTCGTA ATGATTTTCT TCCTTGTTAT ACCTATGCTA ATTGGNNGAT
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- ---------- ---------- ---------- ----------
Anodontia_contig        TTACGATTCA CGGATTCGTA ATGATTTTCT TCCTTGTTAT ACCTATGCTA ATTGGAGGAT

                        190        200        210        220        230        240
Tando_For               TCGGTAATTG ACTAGTTCCT CTAATACTAG CAGCACCTGA CATGGCCTTC CCTCGTCTAA
Lactawan_For            TCGGTAATTG ACTAGTTCCT CTAATACTAG CAGCACCTGA CATGGCCTTC CCTCGTCTAA
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- ---------- ---------- ---------- ----------
Anodontia_contig        TCGGTAATTG ACTAGTTCCT CTAATACTAG CAGCACCTGA CATGGCCTTC CCTCGTCTAA

                        250        260        270        280        290        300
Tando_For               ACAACCTAAG ATTCTGACTA CTCCCAGGAT CAATGGCAAT GATGATGATT TCTATGATAA
Lactawan_For            ACAACCTAAG ATTCTGACTA CTCCCAGGAT CAATGGCAAT GATGATGATT TCTATGATAA
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- ---------- ---------- ---------- ----------
Anodontia_contig        ACAACCTAAG ATTCTGACTA CTCCCAGGAT CAATGGCAAT GATGATGATT TCTATGATAA

                        310        320        330        340        350        360
Tando_For               CAACACAAGG TCCCGGAACT GGGTGAACTC TTTATCCTCC ACTAAGAGGA GTAATGCAAC
Lactawan_For            CAACACAAGG TCCCGGAACT GGGTGAACTC TTTATCCTCC ACTAAGAGGA GTAATGCAAC
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- ---------- ---------- ---------- ----------
Anodontia_contig        CAACACAAGG TCCCGGAACT GGGTGAACTC TTTATCCTCC ACTAAGAGGA GTAATGCAAC

                        370        380        390        400        410        420
Tando_For               ACTGAGATCG AGGGGTCGAC ATGGCTATTT TCTCACTTCA CCTAGCAGGC GTATCGTCCG
Lactawan_For            ACTGAGATCG AGGGGTCGAC ATGGCTATTT TCTCACTTCA CCTAGCAGGC GTATCGTCCG
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- ---------- ---------- ---------- ----------
Anodontia_contig        ACTGAGATCG AGGGGTCGAC ATGGCTATTT TCTCACTTCA CCTAGCAGGC GTATCGTCCG

                        430        440        450        460        470        480
Tando_For               TACTTGGTGC CGTTAATTTC TTCTCCACAA TTTGAAATAT GCGTCCAGAC GGTATCACTC
Lactawan_For            TACTTGGTGC CGTTAATTTC TTCTCCACAA TTTGAAATAT GCGTCCAGAC GGTATCACTC
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- ---------- ---------- ---------- ----------
Anodontia_contig        TACTTGGTGC CGTTAATTTC TTCTCCACAA TTTGAAATAT GCGTCCAGAC GGTATCACTC

                        490        500        510        520        530        540
Tando_For               TAGGTCGAGT ACCCCTTTTC CCATGATCAA TCCTCGTTAC CGCAGTACTT CTAATCCTAG
Lactawan_For            TAGGTCGAGT ACCCCTTTTC CCATGATCAA TCCTCGTTAC CGCAGTACTT CTAATCCTAG
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           ---------- ---------- -CATGATCAA TCCTCGTTAC CGCAGTACTT CTAATCCTAG
Anodontia_contig        TAGGTCGAGT ACCCCTTTTC CCATGATCAA TCCTCGTTAC CGCAGTACTT CTAATCCTAG

                        550        560        570        580        590        600
Tando_For               CAGTACCAGT GCTAGCCGGA GCCCTAACCA TGCTACTATT GGATCGACAT TTTAACACCT
Lactawan_For            CAGTACCAGT GCTAGCCGGA GCCCTAACCA TGCTACTATT GGATCGACAT TTTAACACCT
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           CAGTACCAGT GCTAGCCGGA GCCCTAACCA TGCTACTATT GGATCGACAT TTTAACACCT
Anodontia_contig        CAGTACCAGT GCTAGCCGGA GCCCTAACCA TGCTACTATT GGATCGACAT TTTAACACCT

                        610        620        630        640        650        660
Tando_For               CATTCTTCGA TCCTGCCGGT GGCGGCGACC CCGTTCTATT TGAACATCTA TTCTGATTCT
Lactawan_For            CATTCTTCGA TCCTGCCGGT GGCGGCGACC CCGTTCTATT TGAACATCTA TTCTGATTCT
Lactawan_Rev            ---------- ---------- ---------- ---------- ---------- ----------
Panabulon_Rev           CATTCTTCGA TCCTGCCGGT GGCGGCGACC CCGTTCTATT TGAACATCTA TTCTGATTCT
Anodontia_contig        CATTCTTCGA TCCTGCCGGT GGCGGCGACC CCGTTCTATT TGAACATCTA TTCTGATTCT
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**Fig. 3.** Comparison of Cytochrome oxidase subunit I gene (COI)nucleotide sequences of *Anodontia edentula* from three sites: Tando, Lactawan, and Panabulon.

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