

DNA Barcoding of Marine Bivalve *Meretrix* (Karibuyo) in Northern Philippines

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Abstract

The *Meretrix Karibuyo* marine bivalve mollusc is an important economic mollusc species found primarily in the coastal regions of Northern Cagayan, Philippines. This study specifically aimed to identify specific species of the bivalve *Meretrix Karibuyo* collected using morphological identification, identify bivalve species collected using mitochondrial cytochrome c oxidase subunit (COI) gene, compare DNA sequences to sequences available in GenBank using the BOLD search and compare identified bivalve species identified using morphological and molecular methods. The study is descriptive and includes methods for sample collection and preservation, DNA extraction, PCR amplification, gel electrophoresis, gene sequencing, and software and program analysis of DNA samples. The collection of samples was carried out in Northern Cagayan, specifically in the towns of Aparri, Buguey, and Sta. Teresita. Identification of the species was based on morphology. NA sequences from bivalve samples were compared to available sequences in GenBank and BOLD Systems. A Neighbor-Joining (NJ) tree was built using 23 *Meretrix* sp. sequences from GenBank as further confirmation of specimen identity, using the program MEGA X. The selected outgroup is a *Petricolalapicida* COI sequence from Genbank. *P. lapicida* which belongs to the same family (Veneridae) as *Meretrix* sp. The resulting 658 base pair (bp) sequence was matched using the identification tool of the Barcode of Life Database (BOLD). This study illustrated how the combination of morphological and DNA taxonomic analysis can help in the monitoring of commercially available marine *Meretrix* specie *Karibuyo*, which were all identified as *Meretrix lyrata*.

Keywords: DNA barcoding, bivalves, karibuyo, COI gene

Introduction. The Philippines, as an archipelagic region, is rich in aquatic resources. In particular, it hosts about 10% (22,000) of mollusk species (Cabrera 1987)-about 10% of the worldwide resources of conservative global mollusk species (200,000 species) (Rosenberg 2014). Molluscs accounted for 28 percent of inland fisheries production in the Philippines and squid was the ninth largest contributor to 123 marine fisheries by volume and commercial value in 2015 (PSA 2016). In the environment, molluscs play essential roles, contribute to the Philippine economy and influence public health.

As the daily demand rises, the accumulation of aquatic items significantly increases worldwide. This is due to the growing diversity in the local market of aquatic species available. Molluscs are important to society and the economy, but there is limited detailed knowledge of the Karibuyo bivalve species in northern Cagayan. *Meretrix* bivalve, also known locally as karibuyo, is a marine bivalve mollusk, an edible saltwater clam of the Veneridae family, found mainly in the Northern Cagayan coastal region.

There has been a reduction in biodiversity over the years and a distinct rise in the number of endangered species observed in marine molluscs caused by climate change, degradation of the coastal ecosystem and anthropogenic activities. Hence for economic and conservation purposes, the need for proper and precise identification of species. DNA

barcoding is a method used to verify species in popular economic aquatic products in order to obtain accurate information for buyers, efficiently track trade in the aquatic market and encourage conservation of species.

As a technique that will make species identification quicker and more available, DNA barcoding has been proposed. As the common identifier of all living species, a brief stretch of DNA was suggested (Blaxter 2003; Hebert et al 2003a, 2003b; Kress et al. 2005; Saunders 2005; Calmin et al. 2007). The probability of species being differentiated by DNA barcodes depends on the variation of the sequence within and between the taxa. Bivalves constitute a wide and diverse group, but within this class of mollusks, little is known about molecular variation. About half of the existing mollusk species are estimated to have been described so far (Brusca&Brusca 1990). In addition, in ecological surveys, the richness of mollusk species is grossly underestimated (Bouchet et al. 2002).

DNA barcoding requires the production of PCR amplicons to sequence them from specific regions and these sequence data are used to classify or "barcode" the organism to differentiate it from other organisms (Lebonah et al., 2014). (Lebonah et al., 2014). Taxonomic knowledge about unidentified species is increasingly used to acquire it. DNA barcoding includes sequencing from taxonomically unknown specimens a short fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, "DNA barcodes," and allowing comparisons with a DN A barcode library of known taxonomy.

The purpose of this study was to provide DNA barcoding data for economically important bivalve *Karibuyoin* Northern Cagayan, it specifically aimed to identify specific species of the bivalve *Meretrix Karibuyoc* collected using morphological identification, identify bivalve species collected using mitochondrial cytochrome c oxidase subunit (COI) gene, compare DNA sequences to sequences available in GenBank using the BOLD search and compare identified bivalve species identified using morphological and molecular methods

Methods. The study is descriptive and includes methods for sample collection and preservation, DNA extraction, PCR amplification, gel electrophoresis, gene sequencing, and software and program analysis of DNA samples. The collection of samples was carried out in Northern Cagayan, specifically in the towns of Aparri, Buguey, and Sta. Teresita. Identification of the species was based on morphology. The samples were preserved and then deposited in ethyl alcohol at 95 percent. The study was conducted for species identity confirmation at the DNA Barcoding Laboratory of the Institute of Biology, University of the Philippines College of Science, Diliman, Quezon City.



Figure 1. Five jars containing five individual bivalves were brought to the laboratory for species identity confirmation

In this analysis, DNA Barcoding was performed using PCR amplification of the Cytochrome Oxidase subunit 1 gene in five bivalve samples grouped according to their morphological character (B1, B2, B3, B4, and B5), following the Herbert et al. (2003) process. Sanger sequencing has been carried out by South Korea's Macrogen Inc. The resultant 658 base pair (bp) sequence was matched using the Barcode of Life Database (BOLD) recognition method. A tree for Neighbor-Joining (NJ) was constructed using 23 *Meretrix* sp. GenBank sequences as further proof of specimen identification, using the MEGA X program (Kumar et al., 2018).

Result and Discussion. DNA sequences from bivalve samples were compared to available sequences in GenBank and BOLD Systems. The resulting 658 base pair (bp) sequence was matched using the identification tool of the Barcode of Life Database (BOLD).

Table 1 shows BOLD search results, indicating complete or almost complete sequence identity with other *Meretrix* sp. sequences in the database.

Species ID	Sample ID	Percent Match
<i>Meretrix lyrata</i>	KF009624.1	99.68%
<i>Meretrix lyrata</i>	KF009624.1	98.77%
<i>Maretrixlyrata</i>	KF009624.1	99.55%
<i>Meretrix lyrata</i>	KF009624.1	99.20%
<i>Meretrix lyrata</i>	KF009624.1	99.24%

Table 1. BOLD search results for the five bivalve specimens. Marker used is a 658-bp region of the COI gene.

A Neighbor-Joining (NJ) tree was built using 23 *Meretrix* sp. Sequences from GenBank as further confirmation of specimen identity, using the program MEGA X (Kumar et al., 2018). The selected outgroup is a *Petricolalapidica* COI sequence from Genbank. *P. lapidica* belongs to the same family (Veneridae) as *Meretrix* sp.

Figure 2 shows the NJ tree indicating that the sample closely matches of *M. lyrata*. It was determined that the samples belong to the species *Meretrix lyrata* using DNA Barcoding following the methods of Herbert et al. (2003).

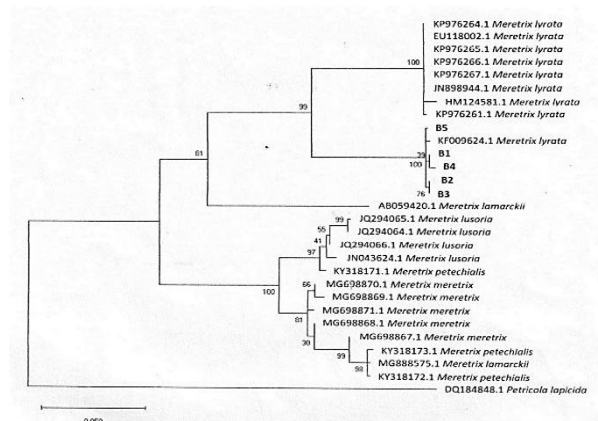


Figure 2. Neighbor Joining Tree Showing that the samples (B1, B2, B3, B4, and B5) cluster with *Meretrix lyrata* sequences from GenBank

Conclusion. The use of DNA sequences in taxonomy is of great importance. DNA Barcoding is potentially useful if accurate results are provided adequately and comprehensively by databases. Integrative approaches, however are needed; methods where molecular data can be complemented by information based on morphological characters assembled in past years. DNA barcoding is an invaluable tool for distinguishing species of mollusks. It can be used for species identification and food safety. It also makes it possible to discover the bivalves of putative species that can be found in the markets of Northern Cagayan. This study demonstrates how the combination of morphological and DNA taxonomic analysis will help to track the commercially available species of Karibuyo marine bivalve *Meretrix*, all of which have been identified as *Meretrix lyrata*.

Recommendations. Further studies in the field should consider adding more sampling sites to widen the scope of the study.

Conservation management of *Meretrix lyrata* shall be conducted by knowledgeable researchers to maintain or even increase the availability of the specie.

Biochemical analysis of *Meretrix lyrata* shall be studied, which is very essential for product development purposes.

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