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Research Article

Molecular identification of *Ergalatax contracta* from Yeongdo island, Korea

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ABSTRACT

The current study reports the cytochrome oxidase subunit 1 sequence of Gastropoda *Ergalatax contracta* from Busan, Korea. Four specimens were collected and extracted to obtain genomic DNA for the sequencing analysis. This is the first *E. contracta* sequence from Korea submitted to the NCBI for an accession number.

Key words: Molecular identification, DNA barcoding, cytochrome oxidase subunit 1, COI, Gastropoda, *Ergalatax contracta*

INTRODUCTION

Gastropoda is the taxa with the largest population among the Mollusca phylums, and it is one of the few animals that show ubiquitous distribution over a wide range of habitats such as marine, freshwater, and territorial (Loker, 2010). There are over 80,000 reported species of Gastropoda, accounting for more than 80% of all mollusks (Bieler, 1992).

Ergalatax contracta is a Gastropoda belonging to the Muricidae family of the Neogatropoda order. The shell is slightly fusiform, constricted, and canalized at the base, and the canal is slightly distorted. The nasal layer prominently protrudes transversely, with longitudinally layered ribs, which are angular, and the lip tubercle is on the inside. The shell is variegated with rusty brown, columella, and white inside the aperture (Reeve *et al*., 1845).

Traditionally, species are classified based on morphological differences (Taylor and Sohl, 1962). Since a classification is structured on empirical criteria, the subjectivity of the classifier can be reflected and may lead to of misidentification. The recent employment of DNA sequence analysis in the field of classification provides more accessible and objective results for identification purposes. With the advancement of DNA sequencing technology, DNA analysis can be easily performed at a lower cost, and accordingly, the classification of species has become simpler through DNA sequencing. This identification is based on comparing the DNA sequencing data with previously registered entities in the database. For identification purposes, DNA sequencing information with precise classification is necessary. Such precise classification can provide more accurate information for understanding the ecosystem and is expected to confirm genetic diversity. However, the current DNA sequencing data for Gastropoda are not enough for identification purposes in most regions. Herein, we report cytochrome oxidase subunit 1 (COI) sequencing data of *E. contracta* collected offshore of Yeongdo island, Busan, south-east Korea.

MATERIALS AND METHODS

Collection of sample

A total of four samples were collected from two locations. Three samples were collected from the coast near the Korea Maritime and Ocean University, Busan, Korea (35°04'36.1"N, 129°05'16.2"E) on 22 June 2020. The other sample was collected from Jung-ri, Busan, Korea (35°04'07.0"N, 129°03'54.5"E). All samples were frozen at −80 °C in a 50 mL falcon tube with seawater until DNA extraction.

DNA extraction

Genomic DNA was extracted using a Biofact® Genomic DNA prep kit (Biofact, Daejeon, Korea) from the edible part using the by manufacturer's animal tissue protocol. The quality of the extracted DNA was checked spectrophotometrically using NanoDrop spectrophotometer. The extracted genomic DNA was stored into a 2 mL tube at −20 °C.

PCR amplification

From the extracted genomic DNA, the COI gene was amplified using the forward and reverse primers LCO1490 (5'-TGTAAAACGACGGCCAGTGGTCAACAA ATCATA AAGATATTGG-3') and HCO2198 (5'- CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAA AAAATCA-3') (Folmer *et al*. 1994). Each PCR

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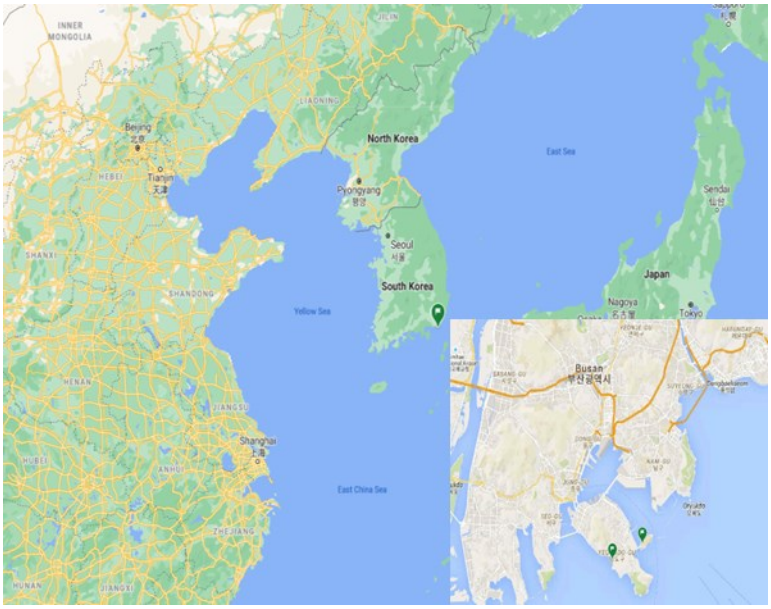

Figure 1. Map of Yeongdo island, Busan, Korea showing the collection sites (indicated by Google).

reaction included a 2 µL DNA template, except sample A (3 µL DNA template used). It contained two 12.5 µL Lamp Taq PCR Master mixes (Biofact, Daejeon, South Korea) and a 1 µL primer mix (each 10 pmol/µL), made up to 25 µL with distilled water. The thermocycling regime consisted of one cycle of 1min at 95 °C, 35 cycles of 20 s at 95 °C, 40 s at 50 °C, 1 min at 72 °C, and finally 5 min at 72 °C. Then, 2% agarose gels were used to screen for amplification success and sent out for DNA sequencing (Biofact, Daejeon, South Korea). The sequence was subjected to a BLAST search on NCBI to identify the most similar sequences in the database.

RESULTS AND DISCUSSIONS

Four samples were collected from the sampling sites (Figure 1 and 2). Each sample was extracted to obtain genomic DNA for the sequence analysis. The cytochrome oxidase subunit 1 (COI) gene fragment of the samples was amplified, and the COI sequences were obtained.

Based on the BLAST search, all COI sequences showed the most similar sequences with the *Ergalatax contracta* mitochondrial partial COI gene with 99% similarity (Table 1). The *E. contracta* has only four COI gene sequence in the NCBI database. All of the four sample's sequence were compared with the accession number FR853882 and showed a similarity of over 99.12%. Most of the previously reported sequences were from the coast of Hainan, China, which is located at the southern end of China. A sequence of the sample B was submitted to the NCBI as the accession no. MW411799.1.

Phylogenies were built in MEGA7 (Kumar *et al*., 2016), using the maximum likelihood (ML) and neighbor-joining (NJ) algorithms. Based on the BLAST search, a set of seven COI sequences of the genus *Ergalaxta* were selected and aligned along with the four samples A–D (Figure 3).

Table 1. BLAST results of samples compared with the NCBI accession no FR853882.

Collected samples	E-value	Similarity (%)	Species Name
Sample A	0.0	99.27 (676/681)	*Ergalatax contracta*
Sample B	0.0	99.12 (675/681)	*E. contracta*
Sample C	0.0	99.12 (674/680)	*E. contracta*
Sample D	0.0	99.26 (672/677)	*E. contracta*

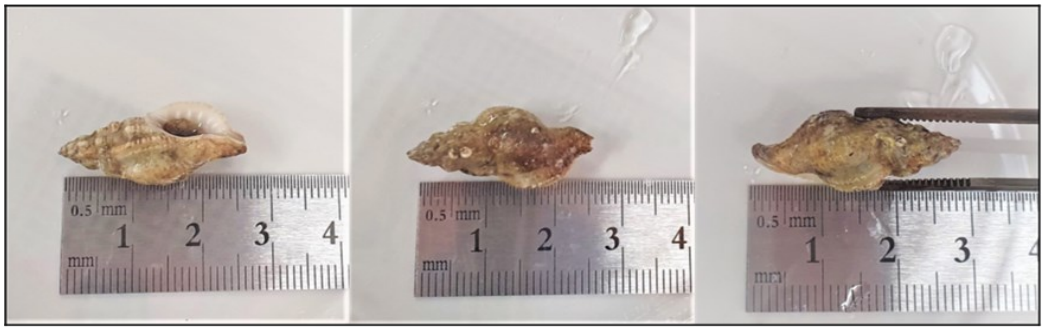

Figure 2. The images of sample-B, representing each sample collected.

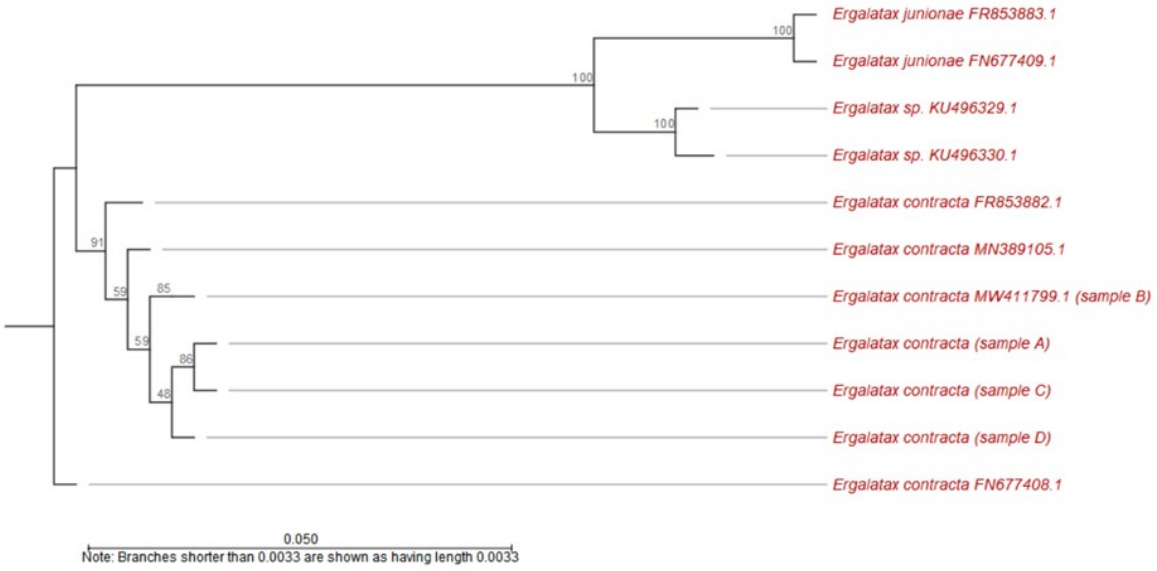

Figure 3. Maximum likelihood phylogenetic tree of *Ergalatax* sp. showing the position of samples A–D.

One of the famous movements for the conservation of mollusks was encouraged by the Freshwater Mollusk Conservation Society in North America (Bradley *et al*., 2016). The society suggested not only the academic background but also a national strategy for the movement. Another recent study indicates the importance of the genetic structure and geographical variation of Gastropoda *Neotricula aperta*, which is the major host of the parasitic blood-fluke *Schistosoma mekongi* (Attwood *et al*., 2019).

However, basic molecular studies on Gastropoda in many areas are not sufficient. As shown above, molecular indicators for *E. contracta* have not been reported much worldwide (Barco *et al*., 2010; Claremont & Reid & Williams, 2011; Claremont *et al*., 2013; Ip *et al*., 2019). Archives of sequencing information about *E. contracta* are limited and need expansion for applications. Based on the current study, it would be helpful to explore not only species, but also eco-system diversity.

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