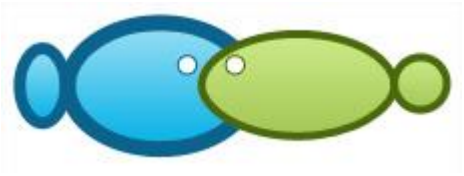

Genetic identification of *Clithon oualaniense* (Gastropoda: Neritidae) from Madura, Indonesia

Anisya E. Juniar, Reni Ambarwati, Dwi A. Rahayu

Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Indonesia. Corresponding author: R. Ambarwati, reniambarwati@unesa.ac.id

Abstract. *Clithon oualaniense* is a member of the Neritidae (Mollusca: Gastropoda), which has many shell variations, hence it often causes erroneous morphological identifications. The DNA barcode provides a molecular taxonomy solution by using short DNA sequences to identify species. This study aimed to characterize the DNA barcode of *C. oualaniense* and to analyze the phylogenetic relationship of *C. oualaniense* from Madura, Indonesia. Samples were taken from Labuhan Beach Bangkalan Madura using a purposive sampling method by taking three dominant variations and two non-dominant variations, then preserved in absolute ethanol. The molecular characterization was performed using Cytochrome Oxidase subunit I (COI) markers, through: DNA isolation, DNA amplification (using COI barcode primers), electrophoresis and genetic analysis (using a bioinformatics software). The genetic identification was carried out using research sample data and comparison gene data from GenBank NCBI. The COI DNA barcoding resulted in a DNA sequence length of 667 bp consisting of 10 types of transition mutations and 657 of conserved nucleotide bases. The phylogenetic tree reconstruction of the *C. oualaniense* research samples from GenBank, through the Neighbor-Joining and Maximum Likelihood methods, formed one cluster with a bootstrap value of 100, with a similarity value between 98.26-100%. The analysis using COI barcode DNA markers has successfully identified the species *C. oualaniense* from Madura, Indonesia.
Key Words: DNA barcoding, cytochrome oxidase subunit I (COI), neritid snail, phylogenetic.

Introduction. Neritids live in marine habitats (littoral zone), brackish waters and freshwater. Members of the Neritidae are found along the coast, in the mid to surface intertidal zone. Generally, neritids are herbivorous and usually live in colonies. Neritidae is one of the gastropods families that has a polymorphic shell type, with a variety of patterns and colors among the species (Tan & Clements 2008; Mujiono 2011).

The genus *Clithon*, included in the Neritidae family, is a group of small to medium sized snails that live in freshwater, estuary and marine habitats (Tan & Clements 2008). Some of the *Clithons* are living scattered in the Indo Pacific region, one of which is in Indonesia (Gruneberg 1976). The result of Mujiono (2011) revealed that there are three species of the genus *Clithon* that can be found around the Java Island, namely *Clithon flavovirens*, *Clithon fuliginosus* and *Clithon oualaniense.*

C. oualaniense is a member of the genus *Clithon* which has a shell as big as a pea, measuring 8-9 mm high and 7-8 mm wide, with no spines but a shiny shell surface with many color variations (Mujiono 2011). Ecologically, *C. oualaniense* plays a role of detritus eater (decomposers) (Choirunnisa & Ambarwati 2018). The morphology and morphometrics of the shell motifs of this species have been studied extensively. Based on the collection of the Museum Zoologicum Bogoriense, Indonesia has at least ten types of *Clithon*, one of which is *C. oualaniense*. According to Gruneberg (1976), who examined the polymorphism of *C. oualaniense* in several Indo-Pacific countries covering India, Sri Lanka, Hong Kong Malaysia and Singapore, there can be found 12 shell motifs: axial, black and white spiral, axial with numerous little tongues, spiral tongues, ladder, purple spiral, tiger, black color, little tongue with dilution, giant tongue, narrow spiral and yellow spiral (Gruneberg 1976). Gardner et al (1995) investigated the relation between the frequency of shell motifs

and microhabitat variations in Queensland, Australia.

Mujiono (2016) found that *C. oualaniense* from Java Island has ten motif variations. The research of Choirunnisa & Ambarwati (2018) also showed that there are ten types of shell motifs in Bangkalan, Madura, Indonesia, namely: axial, axial with numerous little tongues, spiral tongues, ladder, purple spiral, tiger, black, dilution little tongues, giant tongues and narrow spiral. Gruneberg (1978) divided *C. oualaniense* into two groups of patterns: "Western *Clithon*" and "Eastern *Clithon*", according to the 10 above-mentioned motif variations. Axial variation, Axial with numerous little tongues, Spiral tongues, Ladder and Purple spiral are included in the Western *Clithon* (WC) group, while tiger variation, dilution little tongues, giant tongues, black and white spiral and black are included in the Eastern *Clithon* (EC) group.

Gruneberg (1978) explained the hypothesis of grouping motifs based on the environmental factors: at a salinity below 33‰, the Eastern Clithon group motifs are more dominant than the Western *Clithon* motifs. However, Choirunnisa & Ambarwati (2018) recently found that the Western *Clithon* group was dominant among the shell pattern variations of *C. oualaniense* in Bangkalan Madura. Since the results of the relevant studies are contradictory, they should be confirmed by the genetic characters of *C. oualaniense*.

Several researches on *C. oualaniense* related to the connection of shell motifs frequency with microhabitat variations (Gardner et al 1995), morphology, distribution, kinship (Mujiono 2011) and morphometrics, shell pattern variations and habitat profiles (Choirunnisa & Ambarwati 2018) have been conducted. *C. oualaniense* shell pattern is unique and diverse, therefore the morphological identification often causes misclassification, hence molecular identification is needed. However, in Indonesia, research on *C. oualaniense* through genetic identification has not been carried out yet. Genetic identification of a species can be done using genetic markers. This method uses a short DNA sequence for species identification. The use of DNA as a characteristic of this species has several advantages: it is more thermo stable and sensitive than proteins, it is not influenced by the environment and the growth factors and almost all tissues can be used as a source of genetic material (Teletchea et al 2005). Besides, the genetic identification has the advantages of being accurate. Using molecular genetics to determine a species begins to develop and can be used to corroborate the morphological analysis. The standard target for DNA barcodes in animals is the mitochondrial marker cytochrome oxidase subunit I (COI), commonly known as COI markers. COI markers have a high level of diversity, hence that they are able to identify species with a broad taxonomic level (Madduppa et al 2014). Thus, the genetic identification of *C. oualaniense* and its phylogenetic analysis can be carried out by using the cytochrome oxidase subunit I (COI) gene. Neapolitan (2009) stated that phylogenetic studies could be carried out through molecular analysis.

Based on the described context, this study was conducted to identify the characteristics of the *C. oualaniense* COI DNA barcode that has been found in Bangkalan, Madura, Indonesia and to analyze the phylogenetic relationship of the *C. oualaniense* specimens found in Bangkalan, Madura.

Material and Method

Sampling of *C. oualaniense*. Research sampling locations of *C. oualaniense* refer to the research of Choirunnisa & Ambarwati (2018). Labuhan Beach (6°53'22"S112° 59'51°E) is coast of the northern coast of Madura Island (Figure 1). Molecular analysis was carried out in the Laboratory of Molecular Biology, Maulana Malik Ibrahim State Islamic University, Malang.

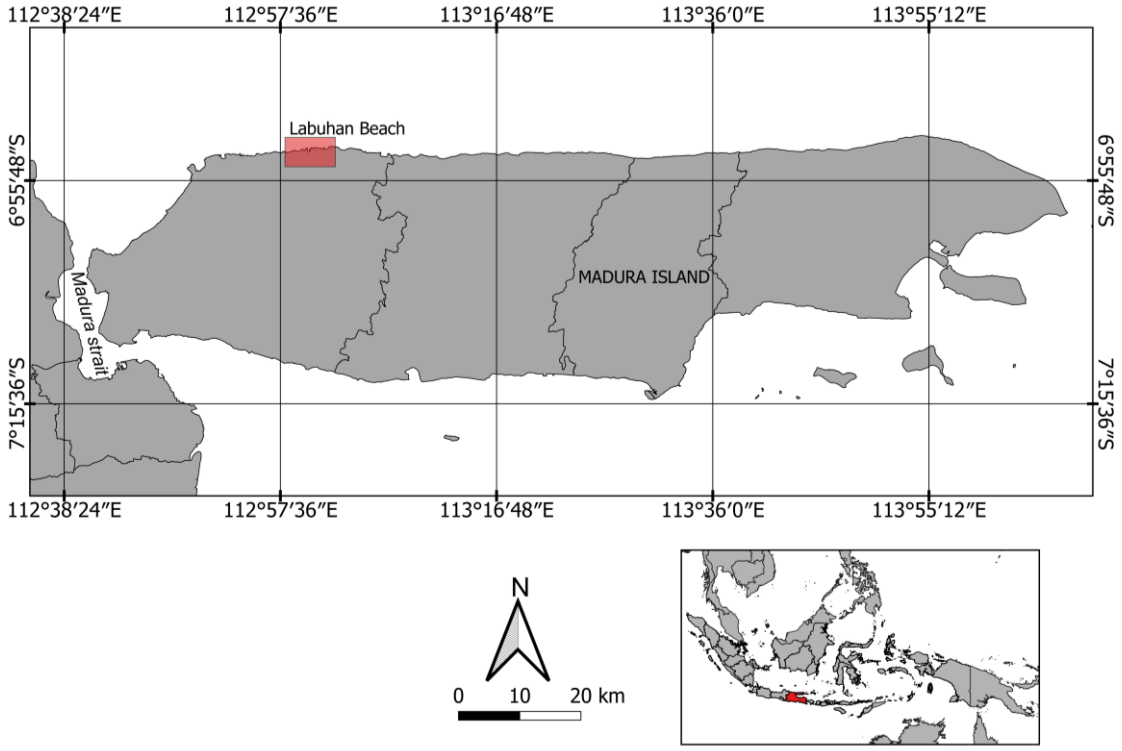

Figure 1. Research location (marked with a red box).

Sampling of *C. oualaniense* was conducted through the purposive sampling method. The dominant variants (collected on the Labuhan Beach, Bangkalan Regency, Madura) were: Axial with numerous little tongues, Black and Spiral tongues, while the non-dominant variants were: Dilution little tongues and Narrow spiral, which were (Choirunnisa & Ambarwati 2018). Thus, the total number of *C. oualaniense* samples collected from Labuhan beach was five individuals, one individual of each variant. The *C. oualaniense* samples were put in a 40 mL collection bottle containing absolute ethanol and each collection bottle was labeled with the identity of the sample.

DNA isolation. The isolation of total DNA (whole genome) from stomach tissue samples was carried out using the DNA Isolation Kit (Roche), with several modifications. The DNA isolation procedure for *C. oualaniense* comprised several stages: (1) chopping 0.05 g of stomach tissue; (2) adding 100 µL of Tissue Lysis Buffer in a petri dish; chopping until smooth and even, then putting in a tube and adding with 200 µL of Tissue Lysis Buffer and 40 µL of Proteinase K; immediately vortexing and incubating at 55°C; (3) adding the sample suspension with 230 µL binding buffer, then immediately vortexing and incubating at 70°C for 10 minutes on a waterbath; adding 210 µL of ethanol absolute 96% to the tube, and immediately vortexing; adding 500 µL of inhibitor removal buffer centrifuging at 9,200 rpm for 1 minute; discarding the flow through (the high filter tube is reconnected with the new collection tube); (4) washing DNA from various contaminants; (5) releasing the DNA bound in the column by adding 100 µL of elution buffer (which has been incubated at 70°C); (6) adding 100 µL elution buffer (which was incubated at 70°C), then centrifuging at 9,200 rpm for 1 minute.

Amplification. Once isolated, the DNA was amplified with a Thermocycler using primers with the COI gene target, namely LCO: (5 'GGT CAA CAA ATC ATA AAG ATA TTG G 3') and HCO: (5 'TAA ACT TCA GGG TGA CCA AAA AAT CA 3') (Folmer et al 1994). The hotstart PCR method uses a Kapa master mix and two Taq master mixes. The PCR process was carried out in 35 cycles. Each cycle consisted of a double thread attachment process (pre-denaturation) at 95°C for 3 minutes, a denaturation at 94°C for 45 seconds,

an annealing at 45°C for 45 seconds and an extension at 72°C for 2 minutes. Then it was proceed further, with final elongation at 72°C for 10 minutes.

Electrophoresis and sequencing. Prior to electrophoresis, the medium was prepared, namely 1% agarose (0.5 g agarose and 50 mL TAE Buffer) mixed with 4 µL Ethidium Bromide (EtBr) as a dye. The next step was to mix 3 µL of the PCR samples with 1 µL of loading dye, the mixture was then put into an agarose well. The electrophoresis was performed using an electrophoresis machine with a voltage of 220 V and a current of 400 mA, for 25 minutes. The length of the DNA base strands can be measured using a 4 µL low mass ladder that is inserted into the agarose of the above-mentioned well. The results of electrophoresis appeared in the form of bands that could be seen using a UV transilluminator. Further, the PCR product from the *C. oualaniense* sample was then sent to PT Genetika Science Indonesia for DNA sequencing.

Molecular analysis. The results of the *C. oualaniense* DNA sequencing were then continued by carrying out the main analysis, namely the chromatogram, by using Finch TV and translating proteins online via the Expasy web (https://web.expasy.org/translatee). Then, data from the *C. oualaniense* research samples sequencing with the COI gene were checked through the BLAST, also known as (Basic Local Alignment Search Tool) (https://blast.ncbi.nlm.nih.gov.cgii). Then the analysis was carried out using bioinformatics software: the alignment stages of the *C. oualaniense* DNA sequence were performed using the Clustal X program and then checked manually using the Bioedit program. The alignment results were then continued with the online identification through the BOLD System (www.barcodinglife.org).

Phylogenetic tree construction. The next step is to create a phylogenetic tree through the MEGA 6 program with the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods using the Kimura-2 parameter (K2P) model. Then the formed phylogenetic trees were analyzed bootstrapping 1,000 times and the similarity value was checked again through GenBank to be compared with close relatives of *C. oualaniense*. The purpose of making phylogenetic trees is to see the grouping of different variations in 1 species of *C. oualaniense.* The test sample is determined based on the grouping of phylogenetic tree clusters that are formed. The calculation of similarity values was performed as follows: Similarity percentage = (1-Genetic Distance) x 100%. The substitution of transitions and the transversion of nucleotide bases was calculated by the Kimura 2 parameter (K2P) model.

Results

Identification via BOLD system. In the nucleotide base of *C. oualaniense* there have been identified as much as 667 bp nucleotides. The analysis and translation checking of the five samples revealed that there was no stop codon in the nucleotides. Furthermore, the *C. oualaniense* nucleotide base was analyzed online through the BOLD System (Table 1). The results of the analysis showed similarities among the five samples of *C. oualaniense*. The matching scores (with the BOLD System data for *C. oualaniense*) were: 98.42-100% for the A sample (the Axial with numerous little tongues shell motif variant), 98.58-99.53% for the B sample (the Black shell motif variant), 98.58% for the C sample (Spiral tongues shell motif variant) of 98.26-99.84% for the D2 sample (Dillution little tongues with dilution shell motif variant) of and 98.42-100% for the E (Narrow spiral shell motif variant) (Table 1).

Table 1

The three highest compatibility values from identification through the BOLD system with a representation of the similarity value

Sample name	*Identification BOLD*	*Similarity (%)*
Clithon oualaniense A (variant Axial with numerous little tongues)	*Clithon oualaniense*	100
	Clithon oualaniense	100
	Clithon oualaniense	98.42
Clithon oualaniense B (variant Black)	*Clithon oualaniense*	99.53
	Clithon oualaniense	99.53
	Clithon oualaniense	98.58
Clithon oualaniense C (variant Spiral tongues)	*Clithon oualaniense*	98.58
	Clithon oualaniense	98.58
	Clithon oualaniense	98.58
Clithon oualaniense D (variant Dillution little tongues)	*Clithon oualaniense*	99.84
	Clithon oualaniense	99.84
	Clithon oualaniense	98.26
Clithon oualaniense E (variant Narrow spiral)	*Clithon oualaniense*	100
	Clithon oualaniense	100
	Clithon oualaniense	98.42

Composition of the nucleotide base. Among the five *C. oualaniense* research samples, the COI gene barcode sequences showed an average variation of the G+C nucleotide base composition of 37.37%, while the A+T nucleotide base composition was 62.26 on average (Table 2). Based on these average results, the composition of the nucleotide base G+C is lower than the composition of the nucleotide base A+T. The composition of the G+C nucleotide base ranges from 37.50 to 38.33% (Table 2).

Table 2

Composition of the nucleotide base of *Clithon oualaniense* at Labuhan Beach Bangkalan Beach, Madura

Species	*A (%)*	*C (%)*	*G (%)*	*T (%)*	*G+C (%)*	*A+T (%)*
Clithon oualaniense A	23.00	16.33	21.17	39.50	37.50	62.50
Clithon oualaniense B	22.83	16.33	21.33	39.50	37.67	62.33
Clithon oualaniense C	22.50	16.67	21.67	39.17	38.33	61.67
Clithon oualaniense D	23.00	16.50	21.17	39.33	37.67	62.33
Clithon oualaniense E	23.00	16.33	21.17	39.50	37.50	62.50
Average	22.87	16.43	21.30	39.40	37.73	62.26

A-Adenine; C-Cytosine; G-Guanine; T-Thymine.

Variation of the nucleotide bases. Based on the alignment stages of all samples (research samples with GenBank data), there were 667 bp consisting of 10 variations of nucleotide bases and 657 bp of conserved nucleotide bases (Table 3).

The unique nucleotide bases were found in nucleotide bases number 78, 162, 213, 345, 351, 405, 411, 462, 622 and 661. For example, in nucleotide bases number 622 and 661, *C. oualaniense* E had nucleotide base Thymine (T), while others *C. oualaniense* group had Cythosin (C).

Table 3

Clithon oualaniense nucleotide base variations based on the COI gene

Specimen	*Variation nucleotide base*									
	78	*162*	*213*	*345*	*351*	*405*	*411*	*462*	*622*	*661*
Clithon oualaniense D	T	C	T	C	C	A	T	T	C	C
Clithon oualaniense E	.	.	.	.	.	.	.	.	T	T
Clithon oualaniense A	.	.	.	.	.	.	.	.	.	.
Clithon oualaniense B	C	T	C	T	.	G	.	.	.	.
Clithon oualaniense C	.	.	.	.	T	.	C	C	.	.

. are conserved nucleotide bases.

The unique nucleotide bases were found in nucleotide bases number 78, 162, 213, 345, 351, 405, 411, 462, 622 and 661. For example, in nucleotide bases number 622 and 661, *C. oualaniense* E had nucleotide base thymine (T), while other *C. oualaniense* group had cythosin (C).

The DNA sequence of C. oualaniense. The DNA sequences are succession of nucleotide bases generating alleles within DNA molecules, especially the COI gene which is used as a mitochondrial marker in the phylogenetic studies (Kim et al 2017). The genetic identification using *C. oualaniense* DNA sequences was obtained from research samples collected at Labuhan Beach Bangkalan Madura and from *C. oualaniense* samples stored in GenBank (Table 4).

Table 4

Sample code with relatives from GenBank and BOLD system

Sample name	*Sample location*	*Acc number gene bank*	*Acc number bold system*
Clithon oualaniense A	Labuhan Beach, Bangkalan, Madura	Research sample	Research sample
Clithon oualaniense B	Labuhan Beach, Bangkalan, Madura	Research sample	Research sample
Clithon oualaniense C	Labuhan Beach, Bangkalan, Madura	Research sample	Research sample
Clithon oualaniense D	Labuhan Beach, Bangkalan, Madura	Research sample	Research sample
Clithon oualaniense E	Pantai Labuhan, Bangkalan, Madura	Research sample	Research sample
Clithon oualaniense	Cina	MN389016.1	
Clithon oualaniense	Cina	MN389017.1	
Clithon oualaniense	Cina	MN389018.1	
Clithon spinosus	USA	AY820387.1	
Clithon spinosus	USA	AY820386.1	
Clithon spinosus	USA	AY820382.1	
Clithon spinosus	USA	AY820381.1	
Clithon spinosus	USA	AY820380.1	
Clithon corona	USA	EU732362.1	
Nerita helicinoides	USA	EU732251.1	
Nerita helicinoides	USA	EU732252.1	

Genetic distance. The genetic distance between *C. oualanienese* and the close relatives in Neritidae family was counted using the p-distance model. The average value of genetic distance among *C. oualaniense* variants from Labuhan Bangkalan Beach, Madura was 0.81%. The average genetic distance between the research sample and the in-group was 0.89% while, the average genetic distance between the research sample and the out-group was 21.8% (Table 5).

Table 5

Genetic distance between groups (OTU) of gastropods of the Neritidae based on the COI gene barcode sequence using the p-distance calculation model (percentage)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Clithon spinosus AY820380.1															
Clithon spinosus AY820382.1	0														
Clithon spinosus AY820387.1	0	0													
Clithon spinosus AY820381.1	0	0	0												
Clithon spinosus AY820386.1	0	0	0	0											
Clithon corona EU732362.1	0.1	0.1	0.1	0.1	0.1										
Clithon oualaniense D	0.1	0.1	0.1	0.1	0.1	0.2									
Clithon oualaniense E	0.1	0.1	0.1	0.1	0.1	0.2	0								
Clithon oualaniense A	0.1	0.1	0.1	0.1	0.1	0.2	0	0							
Clithon oualaniense MN389016.1	0.1	0.1	0.1	0.1	0.1	0.2	0	0	0						
Clithon oualaniense MN389017.1	0.1	0.1	0.1	0.1	0.1	0.2	0	0	0	0					
Clithon oualaniense B	0.1	0.1	0.1	0.1	0.1	0.2	0	0	0	0	0				
Clithon oualaniense C	0.1	0.1	0.1	0.1	0.1	0.2	0	0	0	0	0	0			
Clithon oualaniense MN389018.1	0.2	0.2	0.2	0.2	0.2	0.2	0	0	0	0	0	0	0		
Nerita helicinoides EU732251.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	
Nerita helicinoides EU732252.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0

Phylogenetic. The phylogenetic topology of *C. oualaniense* (Figure 2) was obtained from the comparison of five sample sequences of *C. oualaniense* from Labuhan Bangkalan Beach Madura with several GenBank DNA sequences of *C. oualaniense*, *C. spinosus* and *C. corona*, as in groups and *Nerita helicinoides* as the *out* group. The results of the phylogenetic topology of *C. oualaniense* formed two clusters, the first cluster consisted of the species *C. oualaniense* originating from Labuhan Bangkalan Madura Beach, *C. oualaniense* from China, *C. spinosus* from the USA and *C. corona* from the USA while the second cluster consisted of species of *C. helicinoides* originating from USA. Based on the Figure 2, it can be observed that *C. oualaniense* C2 was in the same cluster as *C. oualaniense* from China, with a bootstrap value of 100. The sample *C. oualaniense* B forms a cluster with *C. oualaniense* C (Figure 3).

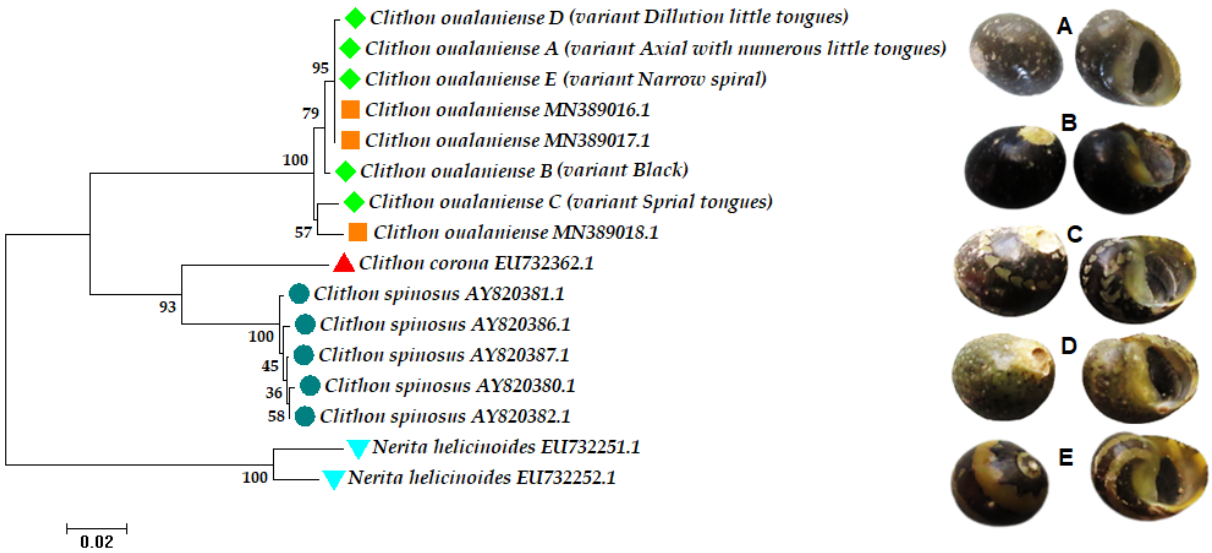

Figure 2. Phylogenetic topology of *Clithon oualaniense* from Labuhan Beach, Bangkalan, Madura, with reference to the COI gene from GenBank (Neighbor Joining with bootstrap 1,000 times).

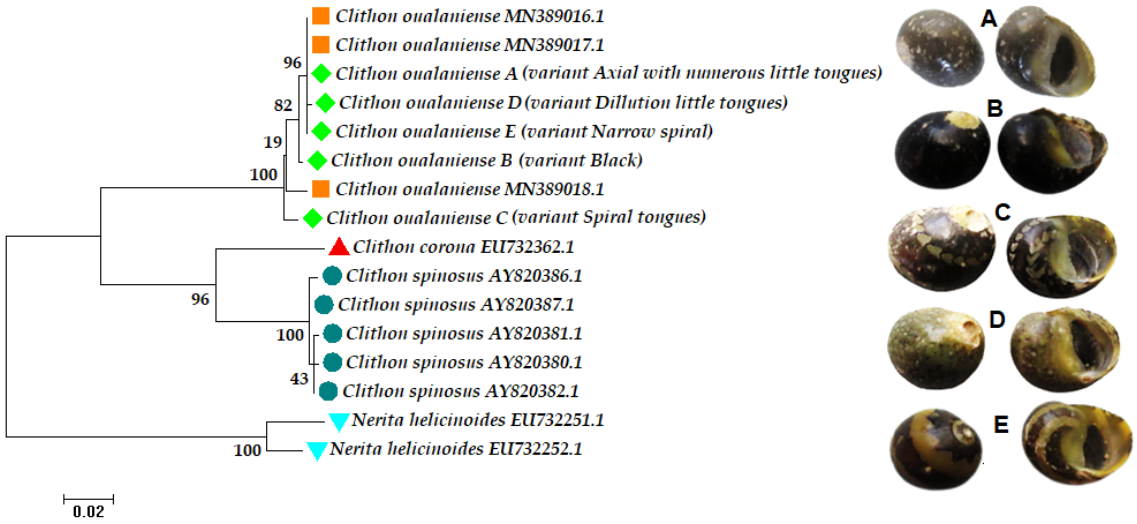

Figure 3. Pylogenetic topology of *Clithon oualaniense* from Labuhan Beach, Bangkalan, Madura, with reference to the COI gene from GenBank (Maximum Likelihood with bootstrap 1,000 times).

Discussion. Genetic identification of *C. oualaniense* based on the nucleotide base *Cytochrome Oxidase I* (COI) gene markers through the BOLD System obtained a high similarity value between the five *C. oualaniense* samples taken from Labuhan Beach, Bangkalan, Madura with similar percentage results of 98.26-100% (Table 1). It confirmed that the species was *C. oualaniense*. This finding confirms that COI gene can be used to identify mollusks up to species level. Besides, COI gene also can be used to identify other invertebrates, for instance, brachiopod *Lingula anatina* (Ambarwati et al 2021). The high similarity between *C. oualaniense* and in-group were due to the high homology of the COI barcode sequence with the BOLD System database. Cryptic species or the similarity of a species based on morphology generally occurs in invertebrates, hence conventional and molecular identification approaches are important as an accuracy of species identification (Vrijenhoek 2010). This research on genetic identification of *C. oualaniense* adds to data on marine biota, especially on the Neritidae family of gastropods at Labuhan Beach, Madura, to the DNA barcoding library collection (BOLD System), directly by following the requirements according to the GenBank database, including (1) having a nucleotide sequence of 500 bp, derived from the COI gene barcoding site, (2) amplification refers to universal primer sequences designed by the Consortium for the Barcode of Life (CBOL), (3) the file track record is accessible and open, and (4) the species naming refers to the approved documents as stated by Rahayu et al (2019).

The nucleotide bases composition of the DNA sequences of the *C. oualaniense* samples from the Labuhan coast, Bangkalan, Madura presented differences between the five samples, due to mutations of mitochondrial nucleotide bases. In the composition of the nucleotide bases in the samples of *C. oualaniense* at Labuhan Beach, Bangkalan, Madura, the percentage value of the nucleotide base G+C was lower than the composition of the nucleotide base A+T (Table 2). Mutation events generally still occur and accumulate in the next generations. Thus, the form of mutations that occur within a species can be considered an evidence for the species evolution (Dailami et al 2018).

Mutations between Guanine (G) and Adenine (A) and Cytosine (C) and Thymine (T) bases are called transitional because they occur between the purines adenine and guanine or between the pyrimidines cytosine and thymine. Transition mutation types are mutations that occur with a higher frequency than transversion mutations, which are generally non-synonymous (Saleky et al 2020). Changes in the nucleotide base of a species can be caused by the species' adaptation patterns to environmental changes (Prehadi et al 2015).

This genetic distance showed the relationship of each sample variant with the species GenBank data. The difference in the mean values of the genetic distance can be caused by the presence of intragroup genetic diversity. The value of the genetic distance

is said to be very low if, after being converted in percentage, it is of less than 2%, showing the same species. If the genetic distance value is greater than 2%, it indicates the existence of a different species, from other group members (Wong 2008). High genetic distance can indicate family and genus level identification (Ran et al 2020). According to Chiu et al (2013), the genetic distance diversity can be influenced by environmental factors. Differences in geographic location and environmental conditions of a species can also lead to changes in phylogenetic morphology and population (Twindiko et al 2013). Due to their wide genetic variations, Mollusca species are generally not identified only based on the intraspecies and interspecies genetic distance (Mikkelsen et al 2007).

Phylogenetics is a taxonomic method of species classification based on the evolutionary history analysis, carried out through both morphological and DNA sequence analysis of a species. The phylogenetic tree of *C. oualaniense* from Labuhan Bangkalan Madura Beach, built with the Neighbor Joining (NJ) and Maximum Likelihood (ML) methods (Figure 2 and Figure 3), shows that the five research samples *C. oualaniense* form together one cluster and are included in the group with a range of bootstrap values between 57-100 for the NJ and between 19-100 for the ML. *C. oualaniense* variant C (*Spiral tongues*) and the *C. oualaniense* originating from China form one clade, with a bootstrap value of 100. Molecularly, a high bootstrap value indicates the validity of the phylogenetic tree branching, and the closeness of the interspecies phenotypes (Wirdateti et al 2016). The higher the bootstrap value, the higher the stability and similarity of the sample. Phylogenetic studies show that the geographic location of a species' presence also affects the bootstrap value of phylogenetic trees (Kim et al 2017).

Conclusions. Genetic identification of *C. oualaniense* resulted in a COI gene sequence data of 667 bp and had a similarity value between 98.26-100% with the data sequence from GenBank. The phylogenetic tree shows that the *C. oualaniense* sequence from Madura, Indonesia is in the same clade with the Chinese *C. oualaniense* from GenBank, with a bootstrap value of 100. Analysis using COI barcode DNA markers has been successful in identifying the *C. oualaniense* species from Madura, Indonesia.

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Conflict of interest. The authors declare no conflict of interest.

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Authors:
Anisya Eka Juniar, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jln. Ketintang, Gayungan, 60231 Surabaya, Indonesia, e-mail: anisyanesya@gmail.com
Reni Ambarwati, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jln. Ketintang, Gayungan, 60231 Surabaya, Indonesia, e-mail: reniambarwati@unesa.ac.id
Dwi Anggorowati Rahayu, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jln. Ketintang, Gayungan, 60231 Surabaya, Indonesia, e-mail: dwirahayu@unesa.ac.id

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