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Revealing species identity of edible razor clam *Cultellus* sp. (Bivalvia: Pharidae) from Madura Island, Indonesia

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Abstract. *Ambarwati R, Rahayu DA. 2024. Revealing species identity of edible razor clam* Cultellus *sp. (Bivalvia: Pharidae) from Madura Island, Indonesia. Biodiversitas 25: 1505-1513.* Razor clams have an essential role as commercial commodities. One of the poorly-known edible razor clams from Madura, Indonesia, has not been identified as the species. This research aimed to identify the edible razor clam (Bivalvia: Pharidae) species from Madura Island, Indonesia. The samples of razor clams (*Cultellus* sp.) were collected from Bangkalan, Madura Island, Indonesia. Identification was conducted based on morphological and molecular characterization. The foot tissues of three individuals were processed for DNA isolation, electrophoresis, amplification, and sequencing to obtain the sequences of COI gene. The morphological characters of razor clam shells were analyzed descriptively and quantitatively. Data were collected from the COI gene sequences of razor clam *Cultellus* sp. and descriptively analyzed using bioinformatics software. The morphological identification revealed razor clams (*Cultellus* sp) from Bangkalan, Madura, Indonesia, *Cultellus subellipticus*. The nucleotide base composition sequences of *C. subellipticus* A, B & C consisted of Thiamine (T), Cytosine (C), Adenine (A), and Guanine (G) with a mean of 18.25, 12.92, 23.42, and 46.39%. Maximum Likelihood (ML) and Minimum Evolution (ME) phylogenetic analysis was also conducted using the Kimura 2 Parameter model to establish one major clade on *C. subellipticus* from the gene bank and a different group with *Cultellus attenuatus* and one out-group significantly different from the *Ensis macha*. This finding was strengthened by molecular identification results, which indicated the similarity with *C. subellipticus* from GenBank, with very high BOLD System data between 98.10-98.86%.

Keywords: Coastal ecosystem, COI gene, *Cultellus subellipticus*, DNA barcoding, marine organisms

INTRODUCTION

Madura is an island that has many beaches with different substrates; the northern shores are usually rocky and sandy (Ambarwati and Faizah 2017, Wijaya et al. 2023a), meanwhile the southern shores dominate with muddy sand substrate and have mangrove vegetation (Islamy and Hasan 2020; Ramadhani et al. 2022). Differences in coastal substrates cause a high diversity of macro-benthic organisms. One of the macro-benthic fauna that is found in Madura coastal waters comes from bivalves. Several publications reported the existence of razor clams belonging to Solenidae (*Solen* spp.) as a significant commodity in the Madura region (Wahyuni et al. 2017; Ambarwati and Irawan 2020)

Apart from *Solen* spp., on Madura Island, other razor clams can be consumed (edible) and traded. Apriliana and Ambarwati (2018) reported an edible Pharidae family member, locally known as '*eres'* (*Cultellus* sp.). The presence of this razor clam in Madura is relatively abundant. The habitat of '*eres'* is beaches with muddy substrates and mangrove vegetation.

Cultellus sp. reported by Apriliana and Ambarwati (2018), needs to be studied further; hence, the species can be identified. Globally, there are five species of *Cultellus*, namely *Cultellus maximus*, *C. attenuatus*, *C. hanleyi* (Huber 2010; MolluscaBase-Eds 2023), *C. subellipticus*, and *C. vitreus* (MolluscaBase-Eds 2023). In comparison, *Cultellus maximus* and *C. attenuatus* are well known and studied, for instance, the reproductive and growth of *C. maximus* (Lai et al. 2022) and the genome of *C. attenuatus* (Li et al. 2022). Thus, further research must be conducted to identify *Cultellus* sp. from Madura. Molecular characterization can be used to complement morphological data for species identification. DNA barcoding is one of the tools used to strengthen identification based on morphological data (Moritz and Cicero 2004; Hebert and Gregory 2005; Ferri et al. 2009; Packer et al. 2009).

Moreover, DNA Barcoding has been applied for the identification of Mollusca successfully (Jaksch et al. 2016; Sun et al. 2016; Juniar et al. 2021; Sari et al. 2021) and various other animal taxa, for example, using Cytochrome Oxidase Subunit I (COI) sequences applied in Pharidae bivalve (Márquez et al. 2020). In addition, DNA barcoding has successfully identified several variations of *Clithon oualaniense* (Gastropoda) from Madura (Juniar et al. 2021) and *Donax incarnatus* from Madura (Wijaya et al. 2023b). Sequencing of the Cytochrome Oxidase I (COI) gene is a valuable tool in molecular identification due to its widespread use in species identification research across various organisms (Valen et al. 2023a). COI sequences have been extensively utilized to identify animal species, including Mollusca (Palanisamy et al. 2020). Furthermore, the COI gene, located in mitochondrial DNA (mtDNA), offers several advantages for molecular identification purposes. One of the key advantages of using COI

sequences for molecular identification is its high accuracy level (Valen et al. 2023b). Studies have shown that COI sequences can accurately identify multiple species within the same genus, even across different geographical locations (Insani et al. 2022). This accuracy is crucial in distinguishing between closely related species where morphological characteristics may not be sufficient for accurate identification. Additionally, COI sequences offer a rapid and efficient method for species identification (Buckley et al. 2011; Kelly et al. 2007; Modica et al. 2016) to evaluate phylogenetic relationships (Blacket et al. 2016). DNA barcoding using COI sequences allows for quick and reliable differentiation between species, supporting and sometimes surpassing traditional morphological identification methods (Hasan et al. 2021). This rapid identification is particularly useful in large-scale biodiversity assessments and conservation efforts. Furthermore, the COI gene in mtDNA is known for its stability, with minimal deletions and insertions in its sequences. This stability ensures that the genetic information obtained from COI sequences remains consistent and reliable for species identification. Reserving natural characteristics in COI sequences enhances their utility in molecular identification studies.

Based on this background, this study aims to identify *Cultellus* sp. from Madura based on morphological and molecular characterization using Cytochrome Oxidase Subunit I.

MATERIALS AND METHODS

Study area

Samples of *Cultellus* sp. were collected from Bangkalan Beach, Madura Island, Indonesia 7°01'42.9"S 112°43'58.9"E (Figure 1). The beach has muddy substrate and mangrove vegetation. Samples were collected from the lower intertidal zone during the lowest tide. Samples were preserved in alcohol 70% for morphological and morphometric analysis, while three individuals was preserved in absolute alcohol for molecular analysis.

Procedures

Morphological identification

Morphological identification refers to Huber (2010). Morphological characters, including the shell's exterior and interior, were observed using a magnifier lamp. Exterior characters include shell shape, shell outline, shell color, periostracum color, shell sculpture, umbo type, and shell ligaments. Interior characteristics include the type of hinge teeth, adductor muscle attachment scars, pallial grooves, pallial line, and shell interior color. A total of 77 specimens were measured using calipers. Shell measurements include shell length, width, and height (Figure 2).

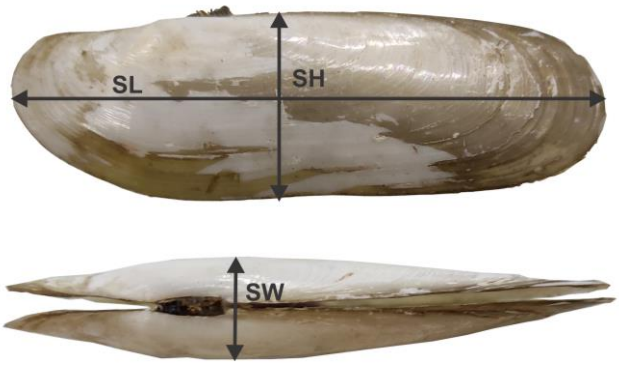

Figure 2. Shell measurements; SL: shell length, SH: shell height, SW: shell width

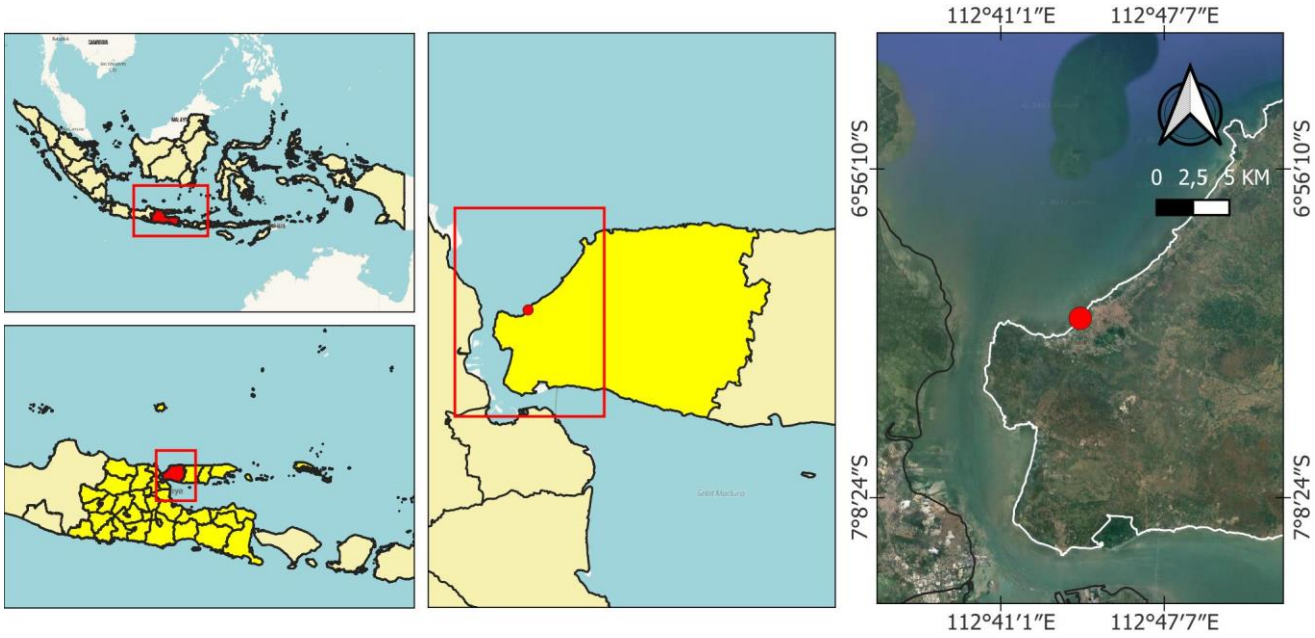

Figure 1. The sampling site of *Cultellus* sp. in Bangkalan, Madura Island, Indonesia

DNA extraction

The isolation of total DNA (whole genome) samples was carried out using the DNA Isolation Kit (Roche). A 20 mg foot tissue of razor clam sample was crushed until smooth and put into 1.5 tubes. The Buffer GT1 200 µL pipette was put into a 1.5 tube with a vortex. Next, 200 µL of GT2 buffer and 20 µL of Proteinase K were added to the mixture and mixed using a vortex. The mixture was incubated for 10 minutes at 56°C, during which the incubation tube was turned back and forth every 5 minutes. Next, 200 µL of absolute ethanol was added and mixed shortly with a vortex. The sample was put into a Spin Column and centrifuged for 1 minute at 13,000 rpm. The flow-through was discarded, and 500 µL of buffer W1 was added to the Spin Column and then centrifuged for 1 minute at 13,000 rpm. Next, the flow-through was discarded, and 700 µL of W2 buffer (added with ethanol) was added and then centrifuged for 1 minute at 13,000 rpm. Flow-through was thrown back and centrifuged again for 2 minutes at 13,000 rpm. DNA in the Spin Column in a new 1.5 mL tube. 50-100 µL Elution Buffer was added and incubated at room temperature for 1 minute; next, centrifuge for 1 minute at 13,000 rpm. The DNA Spin Column is removed, and the DNA is purified for the next step. DNA is stored at -20°C for a few days, -70°C for long-term storage.

Amplification

The isolation results were then amplified using a Biorad PCR machine in 30 µL of solution consisting of 15 µL consisting of PCR Master Mix Nexpro, 3 µL DNA Template samples (100 ng/µL), 6 µL Water, 3 µL primer (10 pmol each forward primer and reverse). The primers used were LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). The hot start PCR method was employed, using a Kapa master and two Taq master mixes. Amplification was carried out with the following of 35 cycles with temperature settings: pre-denaturation at 94°C for 1 minute, then continued with 40 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 45°C for 45 seconds, and extension at 72°C for 1 minute 30 seconds. Next, the post elongation process was conducted at 72°C for 10 minutes. The resulting PCR was electrophoresed on 1% agarose; PCR products were also purified using a Qiagen purification kit according to the manufacturer's instructions and subsequently sequenced at First Base, Malaysia.

Data analysis

Morphological data analysis

The morphological characters of razor clam shells were analyzed descriptively and quantitatively.

Molecular data analysis

Data were collected in the form of the COI gene sequences of razor clam Cultellus sp. were descriptively analyzed using the FinchTV program, resulting in good nucleotide base sequences. The resulting chromatograms were then copied using the DNA Baser Assembler program. Each sequence was initially translated into an amino acid to check and remove pseudogene (Song et al. 2008; Buhay 2009). Nucleotide sequencing was then continued by carrying out the chromatogram analysis using Finch TV software and translating into amino acid sequence through the ExPASy website (Duvaud et al. 2021). Subsequently, the nucleotide bases were compared to the NCBI database using BLAST (https://blast.ncbi.nlm.nih.gov/) (Boratyn et al. 2013), which yielded information regarding the nucleotide identity and similarity between the query and the hit. The nucleotide identity was then translated into an amino acid sequence using Expasy (https://web.expasy.org/translate/), resulting in the *Cultellus* sp. COI gene protein sequence. The amino acid sequences were then analyzed with the BOLD System (https://www.boldsystems.org/) to check for homology, using GenBank to compare with related species (Ratnasingham and Hebert 2007). Next, the sequence results were aligned with ClustalX (Larkin et al. 2007) to create multiple alignments between the COI gene samples and the closely related *Cultellus* sp. database collected from Bangkalan, Madura Island, Indonesia. Finally, the alignment sequences were analyzed with BioEdit (Hall 1999), producing DNA sequences in fasta format. A phylogenetic tree was then obtained using MEGA 6.0 (Kumar et al. 2018).

RESULTS AND DISCUSSION

Morphological characterization

The research results revealed the description of *Cultellus* sp from Bangkalan Beach, Madura: equivalve shell, elongated shape, rounded anterior, posterior edge, straight ventral part, thin, fragile, gaping at both ends. The anterodorsal is short and slanted, while the posterodorsal is long and straight. The shell color is white and gray on the dorsal part and yellow on the edge, smooth shell carving. The periostracum layer is thin, shiny, and yellowish-white; Umbo is low, one-third of the length of the shell. The ligament is brown, one third of the length of the shell, opisthodetic type. The heterodont hinge tooth type has three teeth on one side of the shell and two teeth on the other (Figure 3). The shell length is 36.2-66.35 mm (52.73±6.59 mm). The shell height is 10.6-21 mm (16.69±2.73 mm). The shell width is 5.85-10.85 mm (8.61±1.23 mm). The shell length and height ratio is 3.16:1 (Figure 4).

This description matches the picture of *C. subellipticus* in Huber (2010) and Low and Tan (2017) and the description of *C. subellipticus* in Dharma (2023). Furthermore, Dharma (2023) also reported that *C. subellipticus* is found in Sunda Strait, Java Sea, and Singapore. This razor clam was also reported to be consumed and traded.

Molecular characterization

Electrophoresis results

DNA amplification of the COI gene target was carried out using the conventional PCR method by using universal primers, 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'). The results of DNA amplification of the target COI gene were then electrophoresed using a 1% agarose gel and visualized with a UV transilluminator. The well-amplified COI gene target was characterized by the presence of a thick DNA band and no smear with DNA visualization results obtained at ±620 bp (Figure 4). The absence of stop codons in these sequences indicates that amplification effectively captured functional mitochondrial COI sequences. Consequently, nuclear DNA sequences originating from mitochondrial DNA (NUMTs) were not included in the sequencing, given that vertebrate NUMTs typically have a length of less than 600 base pairs (Wong et al. 2009).

The results of DNA amplification of the COI gene target after the sequencing process showed that 620 bp of nucleotide bases were obtained from the three *C. subellipticus* research samples. The sequencing results were processed using Expasy through translation into protein until no stop codons were found in the middle of the nucleotide bases. The use of COI gene for species identification has been widely recognized for its accuracy due to the elimination of pseudogenes during the protein translation process. Studies have shown the effectiveness of COI sequences in differentiating species across various taxa. For instance, research on various biodiversity in generation of new COI sequences for a significant percentage of species (Venera-Pontón et al. 2020). The COI gene stands out as a highly accurate tool for species identification across different taxa due to its reliable sequences resulting from the translational process that eliminates pseudogenes. The extensive use and success of COI sequences in various studies underscore its importance in DNA barcoding and taxonomic research (Williams and Knowlton 2021). The results of this analysis show similarities between the three *C. subellipticus* research samples, with *C. subellipticus* from GenBank (Table 1), namely with very high BOLD System data between 98.10-98.86% (Table 2).

Figure 3. The morphology of *Cultellus subellipticus* from Bangkalan, Madura Island, Indonesia

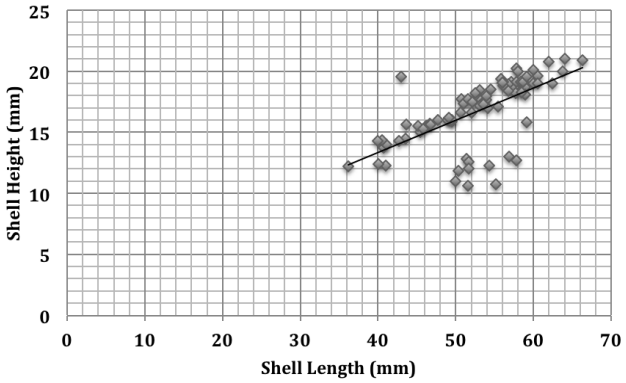

Figure 4 The morphometric of *Cultellus subellipticus* from Bangkalan, Madura Island, Indonesia

Table 1. Sequences from NCBI GenBank were used as reference species in this study

Species	GenBank	Locality
Cultellus attenuatus isolate 211012-2	OQ434723.1	China
Cultellus attenuatus isolate XDC01	JN859998.1	China
Cultellus subellipticus isolate 211119-9	OQ434727.1	China
Cultellus subellipticus isolate 211119-10	OQ434728.1	China
Ensis macha voucher CNP_INV-NA10	MN454380.1	Argentina
Cultellus subellipticus A (*this study*)	PP593776	Madura Island, Indonesia
Cultellus subellipticus B (*this study*)	PP595806	Madura Island, Indonesia
Cultellus subellipticus C (*this study*)	PP595805	Madura Island, Indonesia

Table 2. The three highest match values from identification via the BOLD System with similarity value representation

Samples	3 Highest BOLD Identification	Similarities (%)	Status
Cultellus subellipticus A	*Cultellus subellipticus*	98.86	Published
	Cultellus subellipticus	98.53	Published
	Cultellus subellipticus	98.37	Published
Cultellus subellipticus B	*Cultellus subellipticus*	98.86	Published
	Cultellus subellipticus	98.21	Published
	Cultellus subellipticus	98.10	Published
Cultellus subellipticus C	*Cultellus subellipticus*	98.86	Published
	Cultellus subellipticus	98.47	Published
	Cultellus subellipticus	98.43	Published

Composition of nucleotide bases

The *Cultellus subellipticus* COI gene barcode sequence data among the three research samples showed that there was an average G+C nucleotide base composition of 35.35% while the average A+T nucleotide base composition was 58.3% (Table 3). The partial sequence of the COI gene of *Cultellus subellipticus* showed that the values of the nucleotide base composition of G+C and A+T were between 35.35 and 58.3%, as shown in Table 3. The value of the nucleotide base composition and content of the A+T result was higher than G+C, consistent with the characteristics of the mitochondrial base composition. The analysis of the partial sequence of the COI gene showed that AT content (58.3%) was higher than GC content (35.35%).

Variation of nucleotide bases

The number of nucleotide bases undergoing transition substitutions is five, and those undergoing transversion substitutions are five. Transversion nucleotide base substitutions such as nucleotide base number 52 indicate a change in base T (Thymine) to base A (Adenine). Transition nucleotide base substitutions such as nucleotide base number 253 indicate a change in base T (Thymine) to base C (Cytosine). From these results, seven automorphic nucleotide base patterns were found as markers of characterizing nucleotide bases, such as base numbers 52, 217, 253, 280, 304, 365, and 412, which other species do not share (Table 5).

Genetic distances

Genetic distances (Table 6) between samples and their close relatives were calculated using a 2-parameter kimura model. The average genetic distance between *C. subellipticus* is 0.2% compared to *C. subellipticus* from GenBank, which is 1.1%. This shows that intragroup genetic diversity is very low or less than 2%. A genetic distance value of more than 2% indicates that there is a species that is different from other group members, whereas a genetic distance value of less than 3% indicates that the group or cluster originates from the same species or one species (Wong et al. 2009).

Phylogenetic tree

The results of the reconstruction of the phylogenetic tree of the *C. subellipticus* Madura research samples (A, B, C) with ingroup species using the Neighbor Joining Tree (NJT) method show the existence of two clusters. Sub-cluster 1 consists of *C. subellipticus* research samples, and Sub-cluster 2 consists of *C. subellipticus* originating from China. Cluster 2 is the *C. attenuatus* group, separated from *C. subellipticus*. Out-group groups are separate clusters. Figure 5 shows that the *C. subellipticus* research sample forms a bootstrap value of 100%. These findings strengthened the results' reliability and underscored the COI gene's suitability as a marker for distinguishing between *C. subellipticus* and *Cultellus attenuantus* from GenBank.

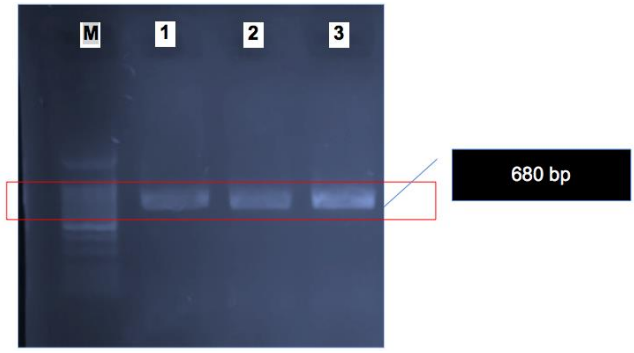

Figure 5. Electrophoregram of COI gene amplification results of *Cultellus subellipticus* specimens in 1% agarose gel (Note: 16: DNA Ladder 1 kb; 17: M: Marker, 1. *Cultellus subellipticus* A, *Cultellus subellipticus* B, and *Cultellus subellipticus* C

Table 3. Composition of nucleotide bases

Samples	A (%)	C (%)	G (%)	T (%)	A+T (%)	G+C (%)
Cultellus subellipticus A	18.87	12.42	24.68	44.03	62.9	37.1
Cultellus subellipticus B	18.18	12.97	21.16	47.69	66.49	34.13
Cultellus subellipticus C	17.71	13.39	21.43	47.47	55.18	34.82
Average	18.25	12.92	22.42	46.39	58.3	35.35

Note: A: Adenine; C: Cytosine; G: Guanine; T: Thymine

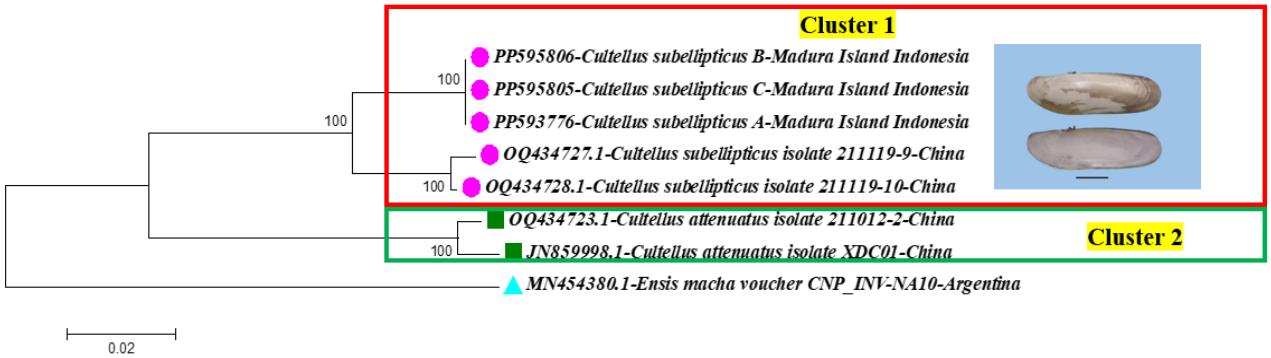

Figure 6. Phylogenetic tree of *Cultellus subellipticus* from Bangkalan, Madura, based on COI gene sequences using Neighbor Joining Tree

The results of the reconstruction of the phylogenetic tree of the *C. subellipticus* Madura research samples (A, B, C) with ingroup species using the Neighbor-Joining Tree (NJT) method show the existence of two clusters (Figure 6). Sub-cluster I consists of *C. subellipticus* research samples, and Sub-cluster 2 consists of *C. subellipticus* originating from China. Cluster 2 is the *C. attenuatus* group, separated from *C. subellipticus*. Out-group groups are separate clusters. Figure 5 shows that the *C. subellipticus* research sample has a bootstrap value of 100%.

Referring to the bootstrap value of each OTU ranging from 99 to 100, it can be said that the grouping has a high level of similarity (Figure 7). The NJ and ML methods show a constant relationship between *C. subellipticus* and its relatives, which differ only in the bootstrap value (Madduppa et al. 2017). Hesterberg et al. (2023) states that the bootstrap percentage of 1000 repetitions with a value above 80% on the branch shows very good results because this value strongly supports that the samples in one branch are correct or in the same species.

The phylogenetic tree was reconstructed by analyzing the partial sequence of the COI gene in a dataset consisting of 8 sequences; the software MEGA 6 version 10.2.6 was employed for this purpose. The primary objective was to ascertain the arrangement of distinct species and two methods, Minimum Evolution (ME) and Maximum-Likelihood (ML). The process began by configuring a bootstrap consensus tree derived from 1000 replicates, representing the evolutionary history of the taxa (Felsenstein 1985). Additionally, evolutionary distances were computed using the Kimura 2-parameter (K2P) method, measured in terms of base substitution numbers per site (Kimura 1980; Nishimaki and Sato 2019). The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm at search level 2 (Nei and Kumar 2000), and the neighbor-joining algorithm was used to generate the initial tree (Saitou and Nei 1987; Mailund et al. 2006).

Further analysis was carried out to strengthen the grouping of *C. subellipticus* with its close relatives through the ABGD web (Puillandre et al. 2012). ABDG separates species based on maximum intraspecific distance ranges. The image shows that the initial partition of the OTU *C. subellipticus* compared to its relatives is constant at a value of 7. This follows the results of the Haplotype networking grouping, which divides the species into 2 haplogroups with 5 haplotypes.

The utilization of ABGD analyses, with a prior maximal distance set at 0.025, further reinforced the separation of the *C. subellipticus* and *C. attenuatus* into distinct partitions (Figure 8). These supplementary analyses align with the differentiation observed between these species. Consequently, the combination of genetic distance, phylogenetic analysis, and ABGD analyses collectively confirmed the successful identification of *C. subellipticus* from Madura as *C. subellipticus*. Based on the comprehensive evidence derived from DNA barcoding with morphological characteristics, it can be concluded that the targeted utilization of these tools offered an efficient and reliable means of identifying *C. subellipticus* at the species level.

Table 5. Variation of nucleotide bases

Species	Variation of nucleotide bases									
	52	**217**	**253**	**256**	**280**	**304**	**365**	**412**	**451**	**453**
Cultellus subellipticus isolate 211119-9	T	T	T	A	T	C	G	C	G	C
Cultellus subellipticus isolate 211119-10	●	●	●	●	●	●	●	●	●	●
Cultellus subellipticus A	A	A	C	●	C	T	A	A	T	●
Cultellus subellipticus B	A	A	C	T	C	T	A	A	●	●
Cultellus subellipticus C	A	A	C	●	C	T	A	A	●	A
Cultellus attenuatus isolate 211012-2	●	●	G	●	●	●	T	T	●	●
Cultellus attenuatus isolate XDC01	●	●	G	●	●	●	T	T	●	●
Ensis macha voucher CNP_INV-NA10	●	●	●	G	●	●	T	T	T	●

Note: ● conserved nucleotide bases according to ingroup species. Transversion mutations are yellow, and transition mutations are pink

Table 6. Genetic distances

Samples	1	2	3	4	5	6	7	8
Cultellus attenuatus isolate 211012-2								
Cultellus attenuatus isolate XDC01	1.1							
Cultellus subellipticus A	11.9	12.1	0					
Cultellus subellipticus B	11.9	12.1	0.2	0				
Cultellus subellipticus C	11.9	12.1	0.4	0.2	0.4			
Cultellus subellipticus isolate 211119-9	12.3	12.6	4.2	4.2	4.2			
Cultellus subellipticus isolate 211119-10	12.1	12.3	3.7	3.7	3.7	0.6		
Ensis macha voucher CNP_INV-NA	16.5	16.9	16.7	17.9	17.9	18.4	17.9	18.1

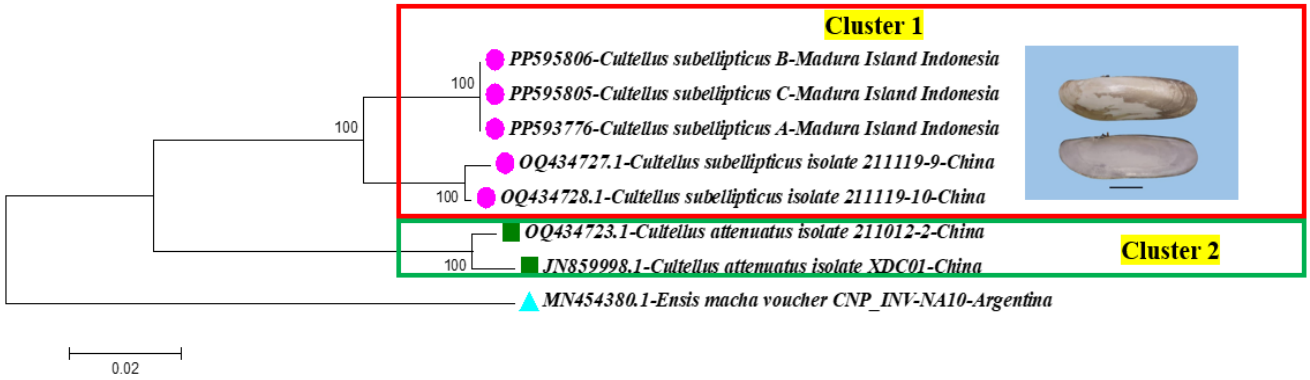

Figure 7. Phylogenetic tree of *Cultellus subellipticus* from Bangkalan, Madura, based on COI gene sequences using Maximum Likelihood

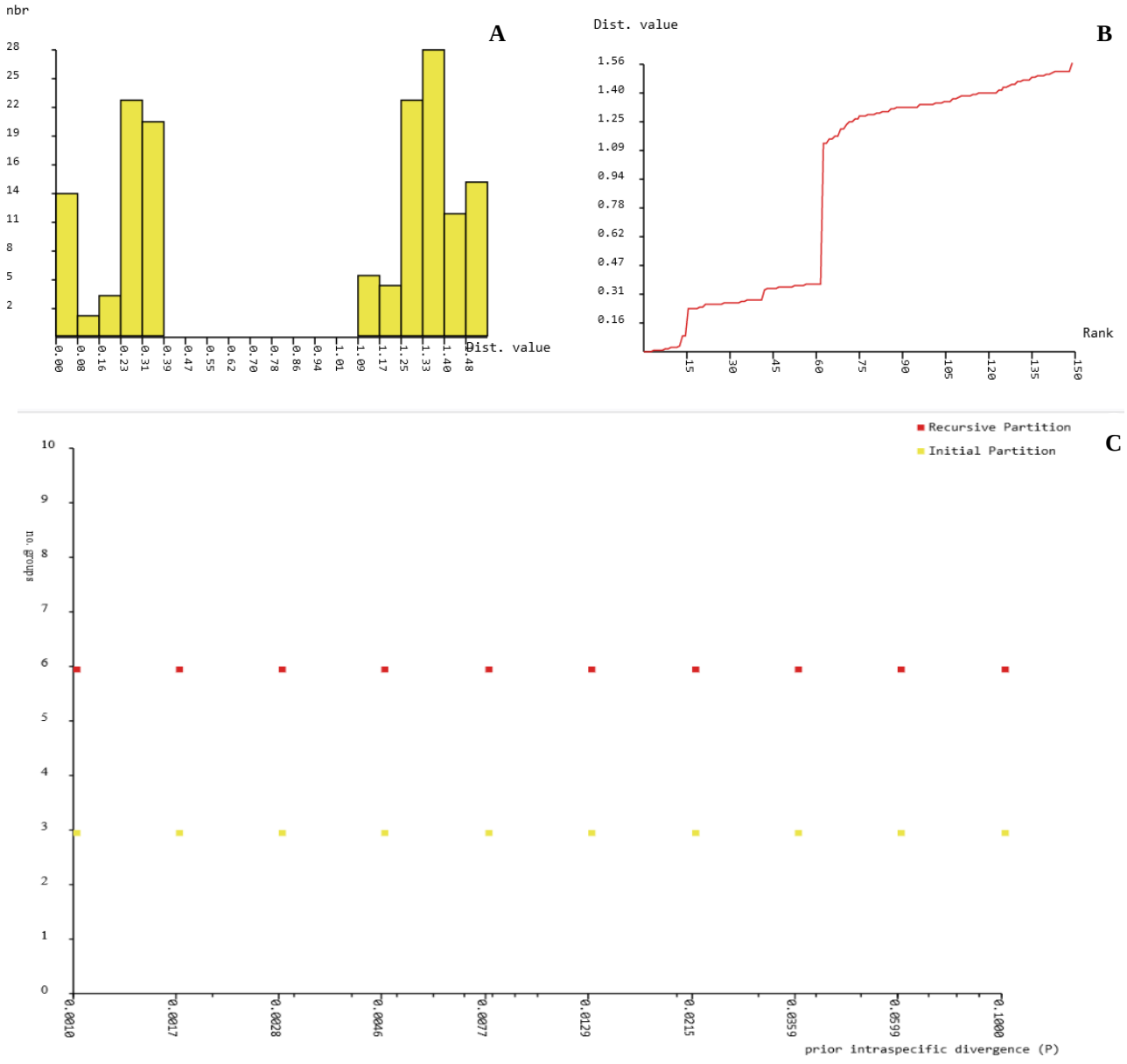

Figure 8. Analysis of Gap Barcodes of *Cultellus subellipticus* species generated by Automatic Barcode Discovery Gap Discovery (Puillandre et al. 2012). Distribution of K2P distances between each pair of specimens for the COI gene; A: distance histogram, B: rank distance, and C: number of PSH obtained for each previous intraspecific divergence

Based on the research results obtained, it can be concluded as follows. Molecular identification of *C. subellipticus* obtained COI gene sequence data of 620 bp. BOLD System identification of three *C. subellipticus* individuals showed similarity values between 98.10-98.86%. Analysis of nucleotide bases consisting of ten variations of nucleotide bases showed that there were 5 transversion nucleotide base substitutions and 5 transition nucleotide base substitutions; in these nucleotide bases variations, seven automorphic nucleotide base patterns were found. A phylogenetic tree using the Neighbor-Joining Tree (NJ) and Maximum Likelihood (ML) methods with a bootstrap of 1000 repetitions shows that the *C. subellipticus* research sample from Madura is in the same cluster as *C. subellipticus* from China with a bootstrap value of between 99-100%.

Both morphological and molecular data support that *Cultellus* sp. from Madura Island, Indonesia, is *C. subellipticus* Dunker 1862. This finding adds to the list of members of the Pharidae family that occur in Indonesia. Previously, Dharma (2005) reported five Pharidae species in Indonesia: *Ensiculus cultellus, Phaxas attenuatus, Pharella javanica, Siliqua winteriana,* and *Siliqua radiata*. Limited access to references for identifying *Cultellus* sp. from Madura causes this clam is poorly known. It is hoped that the results of this research can be widely used to identify *Cultellus subellipticus.* In addition, there are still many other razor clams (Pharidae and Solenidae) from Madura Island that have not been properly identified. The results of this research can encourage research to reveal the species identity of bivalves from the Pharidae and Solenidae families.

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