Original article DOI: 10.19027/jai.23.2.147-156

Characterization of CO1 and 18S rDNA genes from Indonesian native ornamental shrimp *Caridina boehmei* cultured in Jakarta

Karakterisasi gen CO1 dan 18S rDNA dari udang hias asli Indonesia *Caridina boehmei* yang dibudidayakan di Jakarta

Ekky Ilham Romadhona*, Handang Widantara, Novi Megawati, Sutanti Sutanti, Arif Rahmat Ardiansyah, Kiki Mariya Dewi, Annisa Fitri Larassagita, Aditia Farman, Moh Risky, Rahmat Rahmat, Ayub Ayub

Research Center for Fisheries, National Research and Innovation Agency, Cibinong, Bogor
Corresponding author: ekky001@brin.go.id

Received November 28, 2023; Received in revised form December 20, 2023; Accepted January 16, 2024

ABSTRACT

Caridina boehmei is an ornamental shrimp native to Sulawesi that has been successfully cultured and marketed in Indonesia. However, molecular information about this shrimp is not yet available. This study aimed to characterize cytochrome c oxidase subunit I (CO1) and small subunit ribosomal RNA (18S rDNA) genes. Shrimp samples were extracted, and DNA was amplified using CO1 and 18S rDNA primers. The sequencing results were then analyzed by the neighbor-joining method. The 654 bp partial coding sequence (cds) of the CO1 and 380 bp partial cds of 18S rDNA have been successfully amplified from genomic DNA. The CO1 sequencing produced fragments consisting of 25.84% adenine, 20.95% cytosine, 19.11% guanine, and 34.10% thymine. The 18S rDNA sequencing produced fragments consisting of 24.47% adenine, 26.05% cytosine, 28.16% guanine, and 21.32% thymine. Phylogenetic analysis based on the CO1 gene revealed that *C. boehmei* was grouped with *C. variabilis* (MK190014), *C. brachydactyla* (MK190011), and *Caridina* sp. (MK190012). Phylogenetic analysis based on the 18S rDNA gene revealed that *C. boehmei* was identical to *Caridina* sp. Suphan Buri-1 MF622000, *C. serratirostris* KP725708, *C. multidentata* JF346236, *C. formosae* GQ131924, and *Caridina* sp. Suphan Buri-2 MF622001. The lowest-highest pairwise distance based on CO1 and 18S rDNA was consecutively 0.0017-0.2247 (0.17-22.47%) and 0.0000-0.1218 (0-12.18%).

Keywords: 18S rDNA, *Caridina boehmei*, CO1, DNA barcoding, ornamental shrimp

ABSTRAK

Caridina boehmei merupakan udang hias asli Sulawesi yang telah berhasil dibudidayakan dan dipasarkan di Indonesia. Kendati demikian, informasi molekuler mengenai udang ini belum tersedia. Tujuan penelitian ini yaitu melakukan karakterisasi menggunakan gen sitokrom c oksidase subunit I (CO1) dan RNA ribosomal subunit kecil (18S rDNA). Sampel udang diekstraksi kemudian DNA diamplifikasi dengan menggunakan primer CO1 dan 18S rDNA. Hasil sekuensing kemudian dianalisis dengan metode neighbor-joining. Sekuen koding (*coding sequence*, *cds*) parsial 654 bp dari CO1 dan cds parsial 380 bp dari gen 18S rDNA) telah berhasil diamplifikasi dari DNA genom. Sekuensing CO1 menghasilkan fragmen yang terdiri dari 25,84% adenin, 20,95% sitosin, 19,11% guanin, dan 34,10% timin, sedangkan 18S rDNA menghasilkan fragmen yang terdiri dari 24,47% adenin, 26,05% sitosin, 28,16% guanin, dan 21,32% timin. Analisis filogenetik berdasarkan gen CO1 menunjukkan bahwa *C. boehmei* sekelompok dengan *C. variabilis* (MK190014), *C. brachydactyla* (MK190011), dan *Caridina* sp. (MK190012). Analisis filogenetik berdasarkan gen 18S rDNA menunjukkan bahwa *C. boehmei* identik dengan *Caridina* sp. Suphan Buri-1 MF622000, *C. serratirostris* KP725708, *C. multidentata* JF346236, *C. formosae* GQ131924, and *Caridina* sp. Suphan Buri-2 MF622001. Jarak berpasangan terendah hingga tertinggi berdasarkan CO1 dan 18S rDNA berturut-turut adalah 0,0017-0,2247 (0,17-22,47%) dan 0,0000-0,1218 (0-12,18%).

Kata kunci: 18S rDNA, *Caridina boehmei*, CO1, DNA barcode, udang hias

INTRODUCTION

Indonesia is a tropical country with high diversity, one of which is shrimp from the Atydae family. This family of shrimp is widely distributed in waters such as rivers, lakes, and ponds. Atydae has nearly 500 species and around 300 described species, most are included in the genus Caridina (De Grave *et al*., 2015). As of 2016, 62 species of Atydae shrimp have been found in Indonesia, 52 of which are located in Sulawesi and surrounding islands (Annawaty *et al*., 2016).

Caridina boehmei is a small ornamental shrimp found in the Tomori area of Central Sulawesi and it was first described by Klotz and Rintelen (2013). Presently, this shrimp has been cultured outside its native habitat and marketed in Indonesia under the name mambo bee. Despite being cultured and marketed in Indonesia, molecular characterization of this shrimp has not been done. DNA barcoding is a method that uses standardized genetic markers to identify species.

This method has seen a significant increase in usage over the past decade (DeSalle & Goldstein, 2019). The cytochrome oxidase subunit 1 (CO1) gene can effectively differentiate between species with low intraspecific and high intergeneric genetic distances (Ayesha *et al*., 2019). Imtiaz *et al.* (2017) highlight its potential in biodiversity conservation, forensic tools, species identification, food safety, and various practical applications. There are a lot of barcode gen used in this method. In animal DNA barcoding, mitochondrial genes particularly the CO1 region, have been extensively used as a DNA barcode (Rach *et al*., 2017; Tang *et al*., 2023).

These studies have generally found the CO1 region to be effective for discriminating closely related species. Genes on chromosomes such as the 18S region can also be used as DNA barcodes because it has a slow evolutionary rate, easy for universal primer design, and easy to align (Machida & Knowlton, 2015). Previous research shows that 18S rDNA successfully identified marine zooplankton, nematode, and mollusk through metabarcoding (Carta & Li, 2018; Semmouri *et al*., 2021; De Jonge *et al*., 2021). In this study, we used both CO1 and 18S rDNA genes to represent DNA sequences from mitochondria and chromosomes. By obtaining these sequences, the phylogeny of *C. boehmei* can be constructed and it can serve as information for future DNA barcoding studies.

MATERIAL AND METHODS

Sample collection and preparation

The shrimp samples were obtained from an ornamental fish farmer in Jakarta, Indonesia. The species confirmation was done by observing five representative shrimp in the Laboratory for the Development of Industrial Technology for Agricultural and Biomedic (LAPTIAB), National Research and Innovation Agency (BRIN). The coloration and morphological characters of cephalothorax, abdominal somites, scaphocerite, pereiopod, and pleopods of observed shrimp were then compared with the features of *C. boehmei* that had been described by Klotz and Rintelen (2013). For the molecular examination, we used one 1 cm long shrimp that died during rearing as a representative of the population. The shrimp was put into tubes and stored in a -20°C freezer for DNA extraction.

DNA extraction, PCR, and sequencing

In this study, we used the phenol-chloroform DNA extraction described by Sambrook *et al*. (1989). About 10 mg of shrimp tissue was placed in a 1.5 mL tube and then 700 µL lysis solution and 10 µL proteinase K (1 mg/mL) were added to the tube. The mixture was then incubated at 37°C overnight. An amount of 700 µL of phenol: chloroform: isoamyl alcohol (P:C:I) 25:24:1 mixture then added to the tube. This mixture is rotated on a rotator for 10 minutes and then centrifuged 7,000 rpm for 10 minutes at 4°C. Total supernatant is collected and placed on a new 1.5 mL tube.

The addition of P:C:I and supernatant collection are repeated three times. After this process is finished, an equal volume of chloroform: isoamyl alcohol (C:I) 24:1 mixture is added to the supernatant. This mixture is homogenated on a rotator for 10 minutes and then centrifuged 7,000 rpm for 10 minutes at 4°C. The supernatant is collected and the addition of the C:I mixture is repeated three times. Sodium acetate and alcohol 100% are added with volume 1/10× and 2× final supernatant, respectively.

The mixture is incubated for 10 minutes at -20°C and then centrifuged 13,000 rpm for 10 minutes at 4°C. The supernatant was then discarded. One mL of alcohol 70% then added to the tube and then centrifuged 13,000 rpm for 10 minutes at 4°C. The supernatant is discarded and the DNA pellet is dried on a vacuum dryer. Forty

µL of TE buffer and 2.5 µL RNase were added to the DNA pellet.

The mixture is incubated at 37°C for 1 hour. The DNA is then stored at -20°C. PCR was performed using Bioline 5× master mix. The two primers were used to amplify CO1 and 18S. For CO1, the primer was FF2d: 5'-TTC TCC ACC AAC CAC AAR GAY ATY GG-3' and FR1d: 5'-CAC CTC AGG GTG TCC GAA RAA YCA RAA-3' (Ivanova *et al*., 2007). The PCR protocol for CO1 amplification employed a predenaturation at 94°C two minutes, followed by 35 cycles consisting of denaturation at 94°C 30 s, annealing at 52°C 40 s, extension at 72°C one minutes; and a 10 minutes final extension at 72°C.

The expected PCR product for CO1 is 654 bp. For amplifying 18S rDNA we used primer TAReuk454FWD1: 5'-CCA GCA SCY GCG GTA ATT CC-3' and TAReukREV3: 5'-ACT TTC GTT CTT GAT YRA TGA-3' that targeted V4 region (Stoeck *et al*., 2010). The 18S rDNA PCR was carried out by predenaturation at 94°C five minutes, 35 cycles consisting of denaturation at 94°C one minutes, annealing at 53°C one minutes, extension at 72°C one minutes; and final extension at 72°C five minutes. The expected PCR product for 18S rDNA is 380 bp. DNA extraction and PCR were done in LAPTIAB BRIN, South Tangerang. Apical Scientific Sequencing in Malaysia performed bidirectional sequencing using the Sanger method.

Data analysis

The results from sequencing were first edited using Mega-X to remove low-quality nucleotides at the beginning and ending parts of the sequences. Low nucleotide-free sequences were then aligned with the database in GenBank using Basic Local Alignment Search Tolls (BLAST) on the page https://blast.ncbi.nlm.nih.gov/Blast.cgi. To show the similarities, the obtained sequences were compared with the other 14 sequences from the family Atydae. Phylogeny tree construction was carried out using Mega-X and we used another 13 comparison sequences obtained from NCBI. All the sequences were aligned and trimmed to the same length. Phylogeny analysis was performed using neighbor-joining (Saitou & Nei, 1987) with a bootstrap of 1,000 replications using the same application (Kumar *et al*., 2018).

RESULT AND DISCUSSION

Result

Morphological characteristic

The observed shrimp has a semitransparent body and black spots on the carapace and the third, fourth, and sixth abdominal segments (Figure 1a). The rostrum of this species is straight, short, and doesn't reach the length of eyes (Figure 1b). The chela and carpus of the first pereiopod stouter and broader than the second pereiopod (Figure 1c, Figure 1d). Scaphocerite's length exceeds its width (Figure 1e). The third pereiopod is slender,

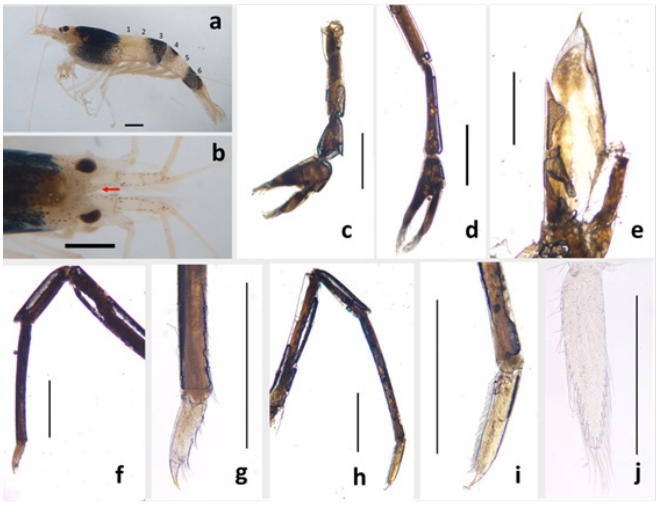

Figure 1. Morphology of *Caridina boehmei*. Whole body anatomy (a); shows the colorization and number of abdominal segments, a close-up photo of the cephalothorax and cephalic appendages show a short straight rostrum in red arrow (b); first pereiopod (c); second pereiopod (d); scaphocerite (e); third pereiopod (f); inner margin of propodus of third pereiopod (g); fifth pereiopod (h); dactylus of fifth pereiopod (i); endopod of first pleopod (j). Contrast-enhanced image (c-i). Scale bar = 1 mm.

terminating in one claw with five accessory spines (Figure 1f, Figure 1g). The fifth pereiopod is slender, terminating in one large claw with 53 small spinules (Figure 1h, Figure 1i). The endopod of the first pleopod elongated (Figure j).

PCR and sequencing

A 694 bp CO1 and 380 bp 18S rDNA fragments were successfully amplified by PCR and produced a single band (Figure 2). The CO1 sequencing produced fragments consisting of 25.84% adenine, 20.95% cytosine, 19.11% guanine, and 34.10% thymine. The frequencies of AT and GC contents were 59.94% and 40.06% respectively. The sequences of CO1 genes are deposited to the GenBank with accession number OR765944. Based on BLAST alignment results we found that the CO1 sequences of *C. boehmei* were similar to shrimp from genus. The similarities with other related CO1 genes were analyzed using multiple sequence alignment.

In addition, there are 14 nucleotide sequences including accession number, total score, query cover (%), and identity (%) in Table 1. The 18S rDNA amplification and sequencing produced 380 bp fragments consisting of 24.47% adenine, 26.05% cytosine, 28.16% guanine, and 21.32% thymine. The frequencies of AT and GC

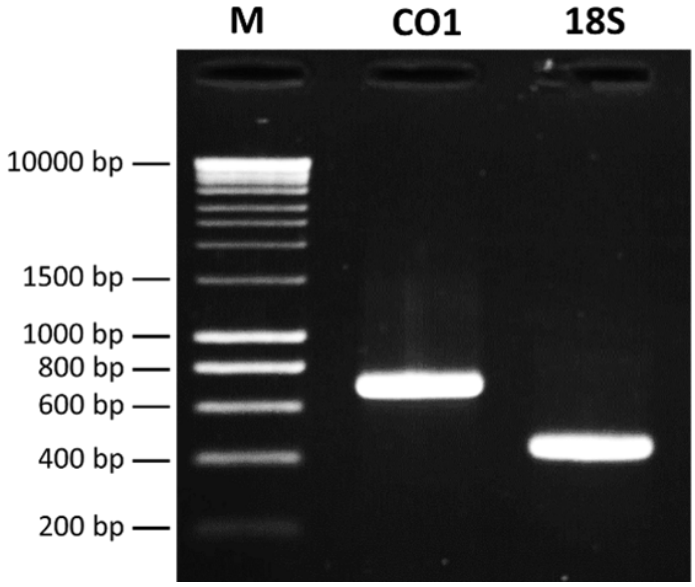

Figure 2. Visualization of product PCR of CO1 and 18S rDNA gene of *Caridina boehmei* on 1% agarose gel.

Table 1. Similarities of *Caridina boehmei* with other genera based on CO1 barcodes.

Sequence	Accession Number	Total Score	Query Cover (%)	Identity (%)
Atyopsis moluccensis isolate XI_2020	OM214470	599	100	83.18
Periclimenaeus arabicus voucher SNU-TW_TW65	MK598622	566	99	82.39
Atyopsis moluccensis isolate VI_2020b	OM214469	566	100	82.26
Neocaridina ketagalan isolate JG05d	MG734250	562	97	82.53
Atyopsis moluccensis isolate V_2020	OM214467	560	100	82.11
Caridina zhongshanica isolate 12	MN701598	558	95	82.83
Caridina sinanensis isolate 287	MT433964	553	94	82.74
Caridina variabilis isolate CA1205	MK190014	542	94	82.52
Caridina sp. n. Santo isolate CA1132	MK190012	542	94	82.48
Caridina formosae	AB300189	538	98	81.73
Caridina mariae isolate 145	MN701602	536	95	82.21
Troglocaris anophthalmus isolate MAND_307	FJ425984	536	94	82.29
Caridina brachydactyla isolate CA1131	MK190011	525	94	82.04
Acanthephyra purpurea voucher HBG5995	MH572597	516	99	80.98

contents were 45.79% and 54.65% respectively. The sequence of this gene was deposited to the GenBank with accession number OR775777. Based on BLAST alignment results we found that the 18S rDNA sequences of *C. boehmei* were identical to shrimp from other species. The similarities with other related 18S rDNA genes based on BLAST results are shown in Table 2.

Phylogenetic analysis

Phylogenetic analysis based on the CO1 gene placed *C. boehmei* in the same clade as *C. variabilis* MK190012, *C. variabilis* MK190014, and *C. brachydactyla* MK190011. In this phylogenetic tree, *C. boehmei* was separated from another three Caridina species (Figure 3). The analysis using 18S rDNA surprisingly showed no

Table 2. Similarities of *Caridina boehmei* with other genera based on 18S rDNA barcodes.

Sequence	Accession Number	Total Score	Query Cover (%)	Identity (%)
Caridina sp. *Suphan Buri-1*	MF622000	702	100	100
Caridina serratirostris voucher MNHN-IU-2013-11821	KP725708	702	100	100
Caridina multidentata voucher NTOU:M01002	JF346236	702	100	100
Caridina formosae	GQ131924	702	100	100
Caridina sp. Suphan Buri-2	MF622001	697	100	99.74
Atyoida pilipes voucher MNHN-IU-2013-11820	KP725696	697	100	99.74
Atyoida pilipes voucher MNHN-IU-2012-1051	KP725695	697	100	99.74
Atyoida bisulcata voucher KC2138	DQ079738	697	100	99.74
Atyoida bisulcata voucher PH_ref-103	MT656160	691	100	99.47
Atya scabra voucher CNCR EM17094	EU868722	689	100	99.47
Potimirim mexicana voucher CNCR EM17140	EU868732	689	100	99.21
Neocaridina denticulata	OP185352	651	100	97.63
Acanthephyra quadrispinosa voucher MNHN-IU-2008-14603	KP725677	425	100	86.96
Caridina sp. *Suphan Buri-1*	MF622000	702	100	100

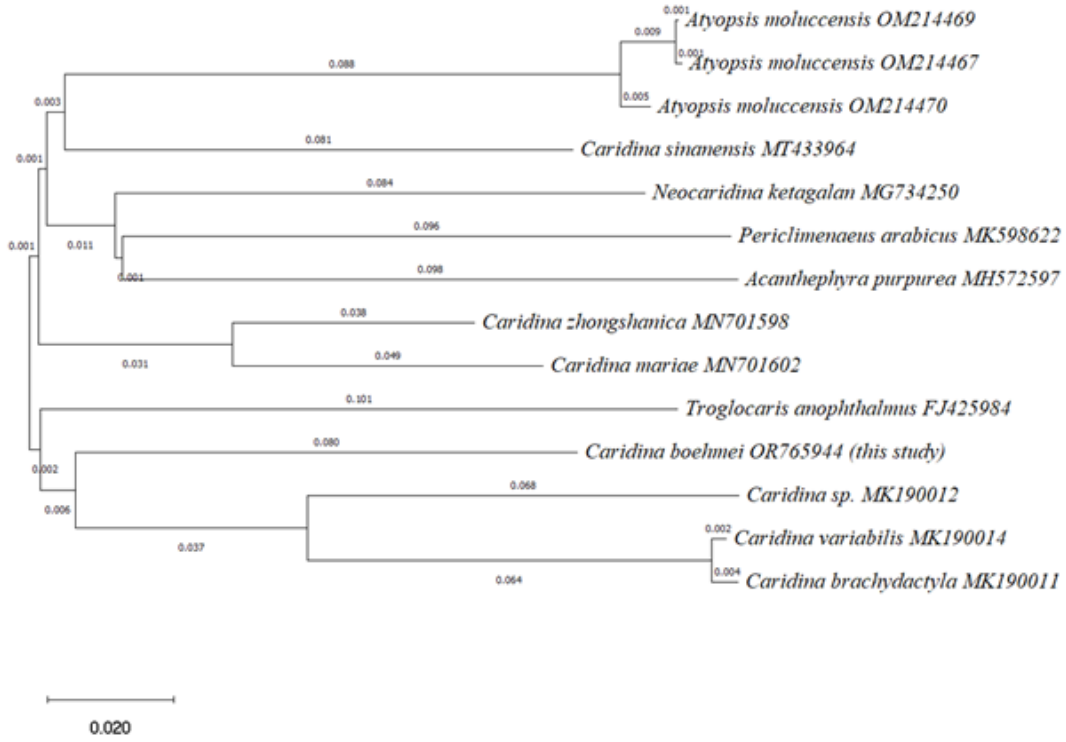

Figure 3. The phylogenetic tree of *Caridina boehmei* constructed using CO1 sequences.

genetic difference among *C. boehmei* and another five Caridina species (Figure 4) because the genetic distance among them was zero (Table 4).

Discussion

Based on morphological features of the cephalothorax, abdominal somites, scaphocerite, pereiopod, pleopods and coloration described by Klotz and Rintelen (2013) we found the sample is identified as *C. boehmei*. The morphological and live coloration features of the sample are identical except for the presence of additional blotch on the fourth abdominal segment. Tomas *et al.* (2020) reported the shelter and background color can change the coloration of *Neocaridina davidi*. In another study, the reflected light also influenced the coloration of peppermint shrimp, *Lysmata boggessi* (Calvo *et al*., 2016).

The CO1 and 18S rDNA genes that represent DNA sequences from mitochondria and chromosomes were first successfully isolated from the cultured Indonesian native_ornamental shrimp, *C. boehmei*. In this study, we used a universal CO1 primer pair for fish and a universal eukaryotic 18S rDNA designed from the *Saccharomyces cerevisiae* genome. PCR product visualization shows both primer pairs can amplify CO1 and 18S rDNA from *C. boehmei*. Sequencing result showed CO1 AT contents (59.94%) were higher than CG contents (40.06%), resulting in anti-G bias which is a mitochondrial gene characteristic (Karamat *et al*., 2021).

Table 3. Pairwise distances of *Caridina boehmei* with several populations based on the CO1 gene.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Caridina boehmei OR765944 (this study)														
Caridina zhongshanica MN701598	0.1574													
Caridina sinanensis MT433964	0.1730	0.1538												
Caridina variabilis MK190014	0.1828	0.1810	0.1966											
Caridina mariae MN701602	0.1683	0.0877	0.1648	0.1919										
Troglocaris anophthalmus FJ425984	0.1860	0.1732	0.1888	0.2097	0.1841									
Caridina sp. MK190012	0.1847	0.1830	0.1986	0.1348	0.1939	0.2116								
Caridina brachydactyla MK190011	0.1846	0.1829	0.1984	0.0067	0.1938	0.2115	0.1367							
Atyopsis moluccensis OM214470	0.1852	0.1661	0.1734	0.2088	0.1770	0.2010	0.2108	0.2107						
Periclimenaeus arabicus MK598622	0.1981	0.1789	0.1919	0.2217	0.1899	0.2139	0.2237	0.2235	0.2041					
Atyopsis moluccensis OM214469	0.1897	0.1706	0.1779	0.2133	0.1815	0.2055	0.2153	0.2152	0.0141	0.2086				
Neocaridina ketagalan MG734250	0.1844	0.1653	0.1783	0.2081	0.1762	0.2002	0.2100	0.2099	0.1905	0.1816	0.1950			
Atyopsis moluccensis OM214467	0.1901	0.1710	0.1784	0.2138	0.1819	0.2060	0.2157	0.2156	0.0146	0.2091	0.0017	0.1954		
Acanthephyra purpurea MH572597	0.1991	0.1800	0.1930	0.2228	0.1909	0.2150	0.2247	0.2246	0.2052	0.1939	0.2097	0.1827	0.2102	

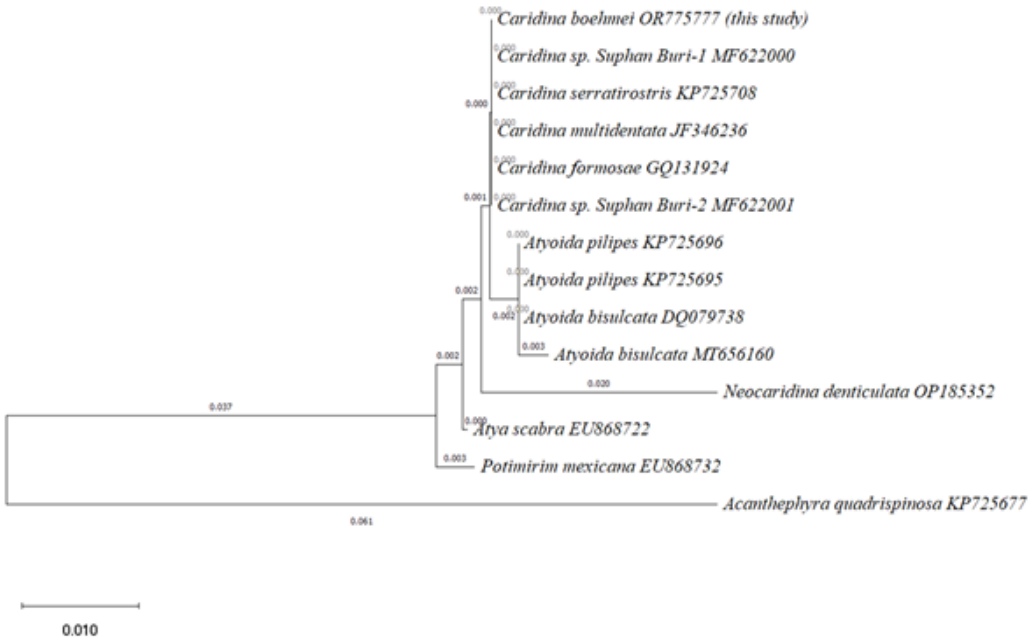

Figure 4. The phylogenetic tree of *Caridina boehmei* constructed using 18S rDNA sequences.

Table 4. Pairwise distances of *Caridina boehmei* with several populations based on the 18S rDNA gene.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Caridina boehmei OR775777 (this study)														
Caridina sp. Suphan Buri-1 MF622000	0.0000													
Caridina serratirostris KP725708	0.0000	0.0000												
Caridina multidentata JF346236	0.0000	0.0000	0.0000											
Caridina formosae GQ131924	0.0000	0.0000	0.0000	0.0000										
Caridina sp. Suphan Buri-2 MF622001	0.0000	0.0000	0.0000	0.0000	0.0000									
Atyoida pilipes KP725696	0.0026	0.0026	0.0026	0.0026	0.0026	0.0026								
Atyoida pilipes KP725695	0.0026	0.0026	0.0026	0.0026	0.0026	0.0026	0.0000							
Atyoida bisulcata DQ079738	0.0026	0.0026	0.0026	0.0026	0.0026	0.0026	0.0000	0.0000						
Atyoida bisulcata MT656160	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053	0.0026	0.0026	0.0026					
Atya scabra EU868722	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0053	0.0053	0.0053	0.0079				
Potimirim mexicana EU868732	0.0082	0.0082	0.0082	0.0082	0.0082	0.0082	0.0105	0.0105	0.0105	0.0131	0.0061			
Neocaridina denticulata OP185352	0.0212	0.0212	0.0212	0.0212	0.0212	0.0212	0.0235	0.0235	0.0235	0.0261	0.0223	0.0275		
Acanthephyra quadrispinosa KP725677	0.1025	0.1025	0.1025	0.1025	0.1025	0.1025	0.1048	0.1048	0.1048	0.1075	0.1005	0.1011	0.1218	

In contrast, 18S rDNA AT contents (45.79%) were lower than CG content (54.65%). A similar result found in the 18S rDNA of *Caridina serratirostris* voucher MNHN-IU-2013-11821 (Genebank accession number: KP725708) which is contains 48.3% AT and 51.7% CG (Aznar-Cormano *et al*., 2015). The similarities with other related CO1 genes were analyzed using multiple sequence alignment (MSA). The MSA of CO1 *C. boehmei* showed similarities with other shrimp genera Caridina (81.73-82.83%), Atyopsis (81.11-83.18%), Periclimenaeus (82.39%), Neocaridina (82.53%). Troglocaris (82.29%), and Acanthephyra (80.98%).

The most similar sequences from the shrimp *Atyopsis moluccensis* isolate XI-2020 (OM214470), *Periclimenaeus arabicus* voucher SNU-TW TW65 (MK598622), and *Atyopsis moluccensis* isolate VI-2020b (OM2144769) showed as top three high similarity with deduced sequence of CO1. Similarity under 98% indicates the sample is a different species because the genetic distance of mitochondrial DNA is less than 2% in many species (Hosein *et al.*, 2017; Dinh *et al*., 2019). The partial cds of CO1 sequences were compared with other closely related species and different genera such as Atyopsis, Troglocaris, Neocaridina, Periclimenaeus, and Acanthephyra. Phylogenetic analysis based on the CO1 gene revealed that *C. boehmei* was grouped with *C. variabilis* (MK190014), *C. brachydactyla* (MK190011), and *Caridina* sp. (MK190012).

Multiple sequence alignment of 18S rDNA *C. boehmei* showed similarities with other shrimp genera Caridina (99.74-100%), Atyoida (99.47-99.74%), Atya (99.47%), Potimirim (99.21%), Neocaridina (97.63%), and Acanthephyra (86.96%). The most similar sequences from the shrimp *Caridina* sp. *Suphan Buri-I* (MF622000), *C. serratirostris* voucher MNHN-IU-2013-11821 (KP725708), *C. multidentata* voucher NTOU:M01002 (JF346236) showed as top 3 similarities with a deduced sequence of 18S rDNA (Table 1). Phylogenetic analysis based on the 18S rDNA gene revealed that *C. boehmei* has zero genetic distance compared with *Caridina* sp. Suphan Buri-1 MF622000, *C. serratirostris* KP725708, *C. multidentata* JF346236, *C. formosae* GQ131924, and *Caridina sp.* Suphan Buri-2 MF622001. Zero genetic distance means they are genetically identical (Halah & Ammar, 2023). The constructed phylogenetic tree indicated that the 18S rDNA sequence of *C. boehmei* is clustered together and forms a separate group.

The phylogenetic based on CO1 and 18S rDNA includes ten and five main distinct branches with strong bootstraps respectively. The sequence analysis of CO1 and 18S rDNA from 14 nucleotide sequences showed the lowest-highest pairwise distance consecutive 0.0017-0.2247 (0.17-22.47%) and 0.0000-0.1218 (0-12.18%) as represented in Table 3 and Table 4. The basis of the phylogenetic tree reconstruction was based on the pairwise distance approach. The highest p-distance value means the widest kinship between the species or organism. Barcoding using CO1 gave no zero genetic distance, therefore it proves that CO1 barcode can distinguish *C. boehmei* from other species.

Several species in the Atydae family such as *Neocardina davidi*, *N. saccam*, *N. denticulata*, *N. koreana*, and *N. palmata* have been identified using CO1 markers (Jabłońska *et al*., 2018; Han *et al*., 2019). This is the first recorded sequence for *C. boehmei* CO1 in NCBI, therefore no BLAST result hits *C. boehmei*. The genetic distance between *C. boehmei* and other Caridina species is zero, indicating the sequences are identical and the primers pair for the V4 region of 18S rDNA cannot distinguish *C. boehmei* from other Caridina species. A previous study reported that 18S rDNA region V4 failed to describe the extant diversity for some major sub-division groups of eukaryotic compared with the V9 region (Choi & Park, 2020). Therefore, we conclude that using the only V4 region is not appropriate for barcoding genera Caridina.

CONCLUSION

DNA barcoding of CO1 and 18S rDNA genes that represent DNA sequences from mitochondria and chromosomes was successfully isolated from the Indonesian native ornamental shrimp, *C. boehmei.* The CO1 sequencing produced fragments consisting of 25.84% adenine, 20.95% cytosine, 19.11% guanine, and 34.10% thymine. The 18S rDNA amplification and sequencing produced 380 bp fragments consisting of 24.47% adenine, 26.05% cytosine, 28.16% guanine, and 21.32% thymine. Phylogenetic analysis based on the CO1 gene revealed that *C. boehmei* was grouped with *C. variabilis* (MK190014), *C. brachydactyla* (MK190011), and *Caridina* sp. (MK190012). Phylogenetic analysis based on the 18S rDNA gene revealed that *C. boehmei* was identical to *Caridina* sp. Suphan Buri-1 MF622000, *C. serratirostris* KP725708, *C.*

multidentata JF346236, *C. formosae* GQ131924, and *Caridina sp.* Suphan Buri-2 MF622001. The lowest-highest pairwise distance based on CO1 and 18S rDNA was consecutively 0.0017-0.2247 (0.17-22.47%) and 0.0000-0.1218 (0-12.18%).

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