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Clarification of freshwater shrimp species with DNA barcoding

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Abstract

Morphological character identification for the determination of freshwater shrimp species can only be found in adult and dominant males, so its application is only on a few specimens. Based on the above problems allows researchers to experience errors in identifying if only morphologically. Morphological identification errors occurred due to the phenomenon of cryptic or siblings species. This study applies the DNA barcoding techniques for the determination of several species of freshwater shrimp. Fifty-eight specimens were morphologically identified, consisting of two families, nine species of freshwater shrimp. However, there are two groups of freshwater shrimp species that are morphologically dubious. So do the molecular identification. Once done molecularly coded using COI, it can be ascertained the identification of *Macrobrachium malayanum*, and *M. pilimanus*. One target sample species of *M. Malayanum_AJ201*, *M. pilimanus_B1201* corresponding to GenBank data.

Keywords: Freshwater shrimp, DNA barcoding, macrobrachium

Introduction

Morphological character identification for the determination of freshwater shrimp species can only be found in adult and dominant males, so its application is only on a few specimens. Based on the above problems allows researchers to experience errors in identifying if only morphologically. Morphological identification errors can be caused by the phenomenon of species cryptic as well as species siblings^[1].

Barcoding DNA techniques can provide a standardized "biological barcode" of short order D to recognize a species^[4]. The idea of using barcoding is intended to distinguish species and identify unrecognized specimens, such as larval phases, cuts of organs and materials undergoing processing, using fairly short gene sequences^[5]. The cytochrome oxidase subunit 1 gene known as COI, is one of the genes in the mitochondrial genome (mtDNA) sequentially used as a barcode.

The COI gene in Crustacea is about 1500 bp and used as a barcode in animals is generally about 700 bp, otherwise this COI gene segment can be used to identify almost all animals [6]. The technique of barcoding DNA plays an important role as a taxonomic tool for exposing genetically distinct and separate species quickly and accurately. This study aims to apply barcode DNA techniques to the determination of some species of freshwater shrimp.

Materials and Methods**Material**

Freshwater prawn samples were preserved in 70% ethanol and 10 mM EDTA.

Sort and Identify Samples

The sorted sample was then identified using the identification key^[7, 3].

Extraction and Isolation of DNA

The shrimp tissue for DNA extraction comes from swimming legs. The preservative alcohol washing process was performed by immersing about 50 mg of muscle pieces in sterile aquades, then homogenized in the STE buffer (NaCl 1M, Tris-HCL 10mM, EDTA 0.1mM, pH 8). The muscle tissue was destroyed then dilisis using K protein of 0.125 mg / ml and sodium dodecyl sulphate 1%. Separation and purification of DNA from other organic materials then follow the extraction kit instructions Genomic DNA mini kit for animal tissue (Geneaid Biotech Ltd)

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Amplification and Visualization of DNA Fragments

The mitochondrial genomic COI gene segment was then amplified using primary barcoding DNA for crustaceans namely AF286 forward primer (5'-TCTACAAAYCATAAAGAYATYGG) and reverse AF287 primer (5'- GTGGCRGANGTRAARTARGCTCG). PCR reaction using PCR Kit Kappa 2 G Fast, with annealing temperature 55 ° C. The success of PCR was observed using a 6% polyacrilamide gel electrophoresis (PAGE) method run at 200 V for 50 min followed by silver sensitive dye [2].

Amplikon and DNA Analysis

PCR for sequencing prints are amplicons of a single tape quality using the big dye terminator and primary methods similar to the initial amplification. The results of manual nucleotide sequencing were edited manually based on chromatogram then used as input in the search for gene similarity using BLAST (Basic Local Alignment Search Tool) (<http://www.ncbi.nlm.nih.gov/>).

The nucleotide sequences of all samples and the homologous results of BLAST were aligned with Clustal W version 2.0 contained in the MEGA program version 5.00 (Tamura *et al.*, 2011). Nucleotide uniform analysis was performed to determine species using the Number of differences model and phylogenetic analysis using Neighbor Joining (NJ) method

Table 2: The results of morphological identification and barcode

Sampel	Morphological identification	Ordo	Family	Identifikasi Barcode
1	M.malayanum_AJ201	Decapoda	Palaemonidae	<i>M.malayanum</i>
2	M.pilimanus_B1201	Decapoda	Palaemonidae	<i>M.pilimanus</i>

After molecular barcoding using COI, there can be ascertained only two species: *Macrobrachium malayanum*, *Macrobrachium pilimanus*.

DNA Amplification and Visualization

The target COI gene amplified using the AF286-AF287 primary pair is about 600bp. The optimum temperature of the primary attachment at the time of amplification is 55 ° C. A total of two samples whose target DNA bands read clearly (Figure 1) were molded in PCR for sequencing. After the target DNA was purified and molded in PCR for sequencing, we obtained two samples whose nucleotide traces clearly on the chromatogram.

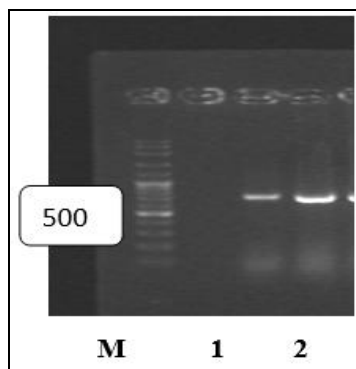


Fig 1: Amplikon of COI gene above PAGE 6%. Description M = marker, 1 = *M. malayanum_AJ201*, 2 = *M. Pilimanus_B1201*

DNA analysis and phylogeny

The length of the macrobrachium malayanum_AJ201 COI gene segment The length of the COI *M. malayanum* and *M. pilimanus* gene segments that can be analyzed and compared

with bootstrap 1000x.

Results and Discussion

Sort and Identify Samples

Sorting is done by various phases and sizes on freshwater shrimp. Rough sorting is done based on the difference of external morphological appearance covering body shape and specific characteristic of specimen such as karapaks form, path form, and body size obtained by six variations of morphology. Each morphology was taken twice to replicate molecular barcodes. A total of three samples were successfully amplified and yielded a single band while visualizing the 6% acrylamide gel. However, there are only two samples whose size of DNA bands fit the target (600-700 pb). Two successfully amplified samples yielding targeted DNA bands (600-700 pb) were then further identified morphologically by identification key [7,3]. Results consist of two families and six species Table 1.

Table 1: Results of morphological identification of freshwater prawn specimens

Sampel	Morphological identification	Family
1. <i>M. Malayanum_AJ 201</i>	<i>M.malayanum</i>	Palaemonidae
2. <i>M. pilimanus_B1201</i>	<i>M. pilimanus</i>	Palaemonidae

with the reference CO 2 gene sequence is 538 nucleotides. Compared with the database that *M. malayanum* is the same species with a nucleotide trace equivalent of 88%, whereas *M. pilimanus* is the same species with a nucleotide trace sequence of 99%.

Table 3: The result of the analisis homology of COI gene DNA in *Macrobrachium malayanum* and *M. pilimanus*.

No	Sampel	Speises di GenBank	BLAST-N	Acc Number	Identitas
1	<i>M. Malayanum_AJ201</i>	<i>M. malayanum</i>	88%	FM 958074	
2	<i>M. pilimanus_B1201</i>	<i>M. pilimanus</i>	99%	KP759429	

Table 4: Genetic distance of CO1 genes between types of freshwater shrimp.

No Sampel	1	2	3	4	5	6	7	8
<i>M.lanchesteri_C1201</i>								
<i>M.malayanum</i>	0,249							
<i>M.pilimanus</i>	0,296	0,233						
<i>Macrobrachium_lar</i>	0,245	0,244	0,292					
<i>Macrobrachium_latidactylus</i>	0,245	0,244	0,292	0,000				
<i>Palaemon_floridanus</i>	0,245	0,244	0,292	0,000	0,000			
<i>M.malayanum_AJ201</i>	0,249	0,000	0,233	0,244	0,244	0,244		
<i>M.pilimanus_B1201</i>	0,296	0,233	0,000	0,292	0,292	0,292	0,233	
<i>M.trompii</i>	1,237	1,249	1,370	1,181	1,181	1,181	1,249	1,370

The largest genetic distance was found between *M. pilimanus* and *M. lanchesteri* 0.296 the lowest genetic distance with a value of 0.000 occurred between *M. lar* and *M. latidactylus*, *M. latidactylus* and *P. Floridanus*, *M. malayanum_Aj201* and *M. pilimanus*, and *M.pilimanus* and *M. pilimanus_B1201*.

The results of morphological identification grouped three samples into one family and three species. After molecular

barcoding using the COI gene, there can be ascertained only two species, *Macrobrachium malayanum* and *Macrobrachium pilimanus*. The largest genetic distance was found between *M. pilimanus* and *M. lanchesteri* 0.296. the lowest genetic distance with a value of 0.000 occurred between *M.lar* and *M.latidactylus*, *M.latidactylus* and *P. floridanus*, *M. Malayanum_AJ201* and *M. pilimanus*, and *M. pilimanus* and *M. pilimanus_B1201*. After comparing the data from GenBank, the target sample of *M. malayanum_AJ201* proved as *M.malayanum* and *M.pilimanus_B1201* species proved to be the corresponding *M.pilimanus* species of barcode DNA using the COI gene can be applied to determine the species of freshwater shrimp, Palaemonidae, it is proved that. the target sample of *M. Malayanum_AJ201* proved to be as *M. Malayanum* and *M.pilimanus*-B1201 species as proven *M.pilimanus* species corresponding to GenBank data.

The determination of species by applying DNA barcode in this study can be used as a reference in aquaculture development as well as freshwater shrimp conservation..

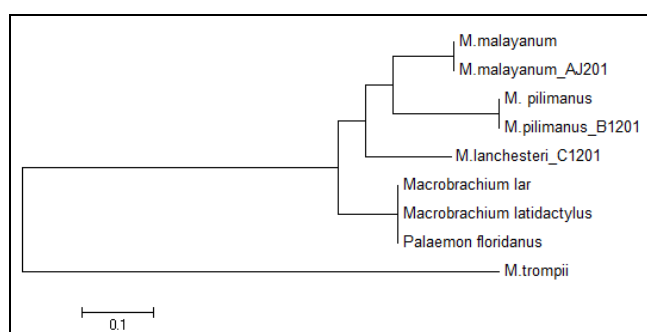


Fig 2: Reconstruction of freshwater prawn phylogenetic tree based on mtCOI with NJ

The phylogenetic tree based on DNA sequences by the NJ method indicates that the ingroup group is separate from the outgroup *M. trompii*. The target sample of *M. malayanum_AJ201* and *M. malayanum* were grouped into one group with bootstrap value of 88%. In the target sample group of *M. pilimanus_B1201* and *M. pilimanus* grouped into one group with a bootstrap value of 99%, whereas *M. Lanchesteri_C1201* formed a separate branch of both. It can be proven that the ingroup types *M. malayanum* and *M. Pilimanus* in alignment are correctly different genera with *M.lar*. identification in species of different sizes in the indistinguishable phase of life. The development of a barcode library can be a means of identification to the level of species with a high degree of truth. The base segments of the COI gene mutate quickly enough to distinguish almost similar species [5].

Barcoding techniques can also help the disclosure of species that are cryptic or siblings as occurs in the phenomenon of dimorphism Type *M. Malayanum* in this study.

Conclusion

Results of DNA barcoding analysis of the sample, namely *M. malayanum* and *M. pilimanus*. Based on the results of reconstruction of Samples *M. malayanum_AJ201* and *M. malayanum* into one group, *M. pilimanus_B1201* and *M. pilimanus* into one group.

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