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DNA Barcoding for Identification Freshwater Shrimp from Jambi Province, Sumatra, Indonesia.

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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 occured due to the phenomenon of cryptic or siblings species. swimming legs are used for DNA extraction and purification. Amplification of COI gene was done by PCR method. Nucleotide sequencing is done by sequencing method. The sequence of COI gene obtained in the BLAST analysis on the NCBI website and followed by phylogenetic reconstruction using the Neighbor JoiningProgram and the MEGA 7.0 Program. 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. Te results showed that identification of *M.malayanum*_AJ201 in morphologically have the highest similarity percentage with *M. malayanum*[FM 958074](88%). While the gene sequences *M. pilimanus*_B1201 each show the highest similarity with *M. pilimanus*[KP759429] (99%).

Keywords: freshwater shrimp, DNA Barcoding, Macrobrachium

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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 sibligs [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 [7]. The use of DNA barcoding to reveal phylogeographic pattern proved to be useful, as previous research successfully revnled population genetic structure using COI 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 CO1 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 [8]. 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

Sample Collection

Specimens were captured using electrofishing and hant net. The minimum number of individual per site was set to three specimens per species. All the specimens were photographed, individually labeled were preserved in a 96% ethanol solution.The sorted sample was then identified using the identification key [9,3].

Genetic Analyses

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)

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 7.00 [6]. Nucleotide uniform analysis was performed to determine species using the Number of differences model and phylogenetic analysis using Neighbor Joining (NJ) method with boostrap 1000x.

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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 [9,3]. 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. M. Malayanum_AJ 201	M.malayanum	Palamonidae
2. M. pilimanus_B1201	M. pilimanus	Palamonidae

Table 2: The results of morphological identification and barcode

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

After molecular barcoding using COI, there can be ascertained only two species: Macrobrachiummalayanum, Macrobrachiumpilimanus.

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 seven samples whose nucleotide traces clearly on the chromatogram.

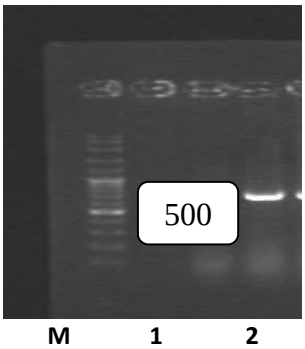

Figure 1: Amplikon of COI gene above PAGE 6%. Description M = marker, 1 = M. malayanum_AJ201, 2 = M. Pilimanus_B1201

DNA analysis and phylogeny

The length of the Macrobrachium malayanum_AJ201 COI gene segment The length of the COI M. malayanumand M. pilimanus gene segments that can be analyzed and compared 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 analisys homology of COI gene DNA in Macrobrachiummalayanum and M. pilimanus.

		BLAST-N		
No	Sampel	Speises di GenBank	Acc Number	Identitas
1	M. Malayanum_AJ201	M. malayanum	FM 958074	88%
2	M. pilimanus__B1201	M. pilimanus	KP759429	99%

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

No Sampel	1	2	3	4	5	6	7	8
M.lanchesteri_C1201								
M.malayanum	0,249							
M._pilimanus	0,296	0,233						
Macrobrachium_lar	0,245	0,244	0,292					
Macrobrachium_latidactylus	0,245	0,244	0,292	0,000				
Palaemon_floridanus	0,245	0,244	0,292	0,000	0,000			
M.malayanum_AJ201	0,249	0,000	0,233	0,244	0,244	0,244		
M.pilimanus_B1201	0,296	0,233	0,000	0,292	0,292	0,292	0,233	
M.trompii	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. latidacylus, M. latidactylus and P. Floridanus, M. malayanum_Aj201 and M. pilimanus, and M.pilimanus and M. pilimanus_B1201.

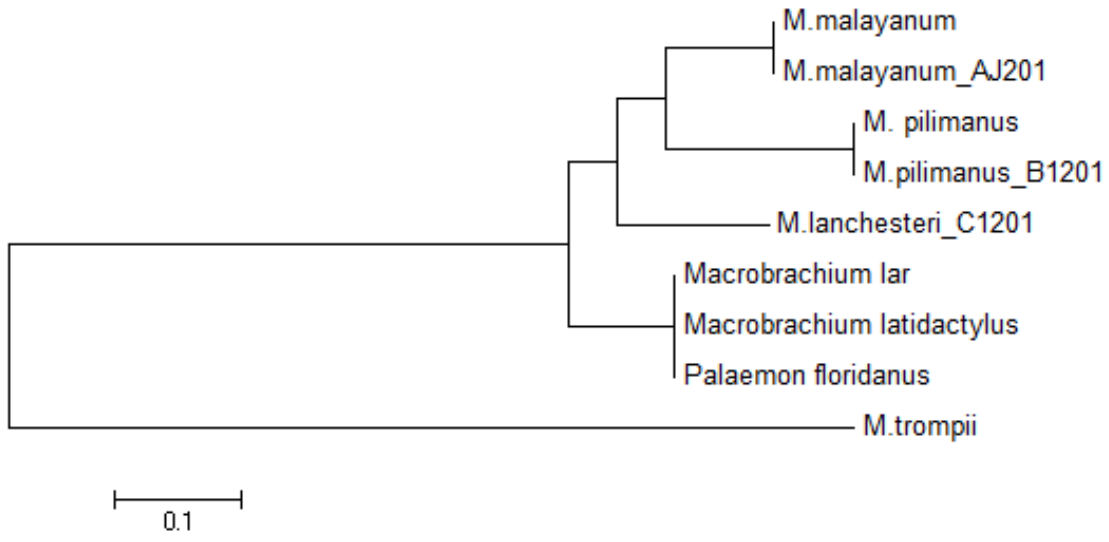

Figure 2: Reconstruction of freshwater prawn phylogenetic tree based on mtCO1 with NJ

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 [7].

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.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 Megalobum 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..

CONCLUSION

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

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