Genetic variation of sand crab *Albunea symmysta* (Crustacea: Albuneidae) from Enggano Island and adjacent region

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Abstract. The sand crab *Albunea symmysta* is widespread in the Pacific, including Indonesia. Its lifestyle of burying its body in the sand in the intertidal area near to the river estuaries means that this albuneid crab is rarely found and studied. This research aimed to elucidate the genetic diversity of albuneid crab in the intertidal area of Enggano Island, which is characterized by calcaerous sand based on DNA barcoding of the CO1 gene. We conducted DNA sequencing of the CO1 gene of *A. symmysta* samples caught in Enggano (n=14), North Bengkulu (n=11), And South Bengkulu (n=6). All DNA sequence data were combined with data obtained from previous studies (n=36) and *Albunea* spp (n=18, four species). The result showed that in Enggano, two species of albuneid crab, namely *A. symmysta* and *Albunea* sp1 (NJ genetic distance 17.3%), were determined. The genetic distance of *A. symmysta* Enggano to several other albuneid crab species (*Albunea* sp2, *Albunea* sp3, *Albunea* sp4) ranges from 14.9% to 20.2%. *A. symmysta* Enggano is closest to different locations, ranging from 0.2% to 6.6%. We found six haplotypes in Enggano out of 26 haplotypes in Indonesia. DNA barcoding revealed the existence of a cryptic species of albuneid crab in Enggano and another region in Indonesia. That way, we have to work more to determine the species of albuneid crab other than *A. symmysta* which was found in Enggano (n=2).

1 Introduction

Sand crab *Albunea symmysta* is a part of the Albuneidae family and is identifiable by the flattened and broad carapace with grooves on its surface [1]. This species' distribution spanned from the east coast of India and along the Pacific coast to Taiwan and Australia. In Indonesia, its record has been found in Sumatra, Java, Molucca, and Papua [2, 3]. Aside from

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A. symmysta, two other species, *A. microp* and *A. holthuisi,* have also been reported in Indonesia [4].

A. symmysta lives in the intertidal to the subtidal zone of sandy beaches [2,4]. Enggano Island is one of Indonesia's outermost islands. More precisely, Enggano Island is in the Indian Ocean on the west side of the island of Sumatra. Because of the island's relatively small size, the beach is one of Enggano's main ecosystems, and these beaches are characterized by its calcareous sand [5]. The coastal area has almost no sedimentary mud carried by large rivers entering the coast through the estuary as on the beaches on the west side of Sumatra. These sandy beaches make Enggano's coast a suitable habitat for albuneid crabs like *A. symmysta*. They act as detrivores in food chains that are a bridge between marine and terrestrial ecosystems. Connectivity between hippoid crab populations is bridged by pelagic larvae that follow ocean currents. In coastal locations suitable for settlement, larvae will develop into juveniles and adults [2,3].

Albuneid crab's lifestyle includes burying and dwelling in the sand, making it hard to find and study. The existence of cryptic species also makes traditional identification based on morphological characteristics harder to do. Cryptic species are two or more distinct species with highly similar physical characteristics. Therefore, a genetic approach is needed to distinguish these species. This research aims to elucidate the genetic diversity of albuneid crabs in the intertidal zone of Enggano Island based on DNA barcoding of the cytochrome c oxidase subunit 1 (CO1) gene.

2 Material and Method

2.1 Sample collection and identification

Samples were collected from June 2023 until February 2024 in Enggano Island (n=14), North Bengkulu (n=11), and South Bengkulu (n=6). The samples were fixed in 70% ethanol in the field, and moved into 96% ethanol for long storage. Species identification was conducted using morphological characteristics based on groove pattern of carapace, dactylus of pereopod IV and shape and size of telson [1, 2]. Samples identified morphologically as *A. symmysta* were used in the molecular analysis.

2.2 DNA extraction

DNA extraction was performed using GeneAid Genomic DNA mini kit, following the instructions from the manufacturer. The tissue sample was taken from the leg muscle tissue to minimize the possibility of contamination from other animals, such as symbionts.

2.3 Gene amplification

Gene amplification was done using the PCR method to amplify partial sequence of the CO1 gene. The primer pair used was AF286 (5'-TCTACAAAYCATAAAGAYATYGG-3') as forward primer and AF287 (5'-GTGGCRGANGTRAARTARGCTCG-3') as reverse primer. PCR conditions applied include a pre-denaturation step at 94°C for 1 minute, followed by 30 cycles of repeated stages consisting of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute, and elongation at 72°C for 1 minute. Subsequently, a post-elongation step was conducted at 72°C for 2 minutes and a finalization step at 15°C for 10 minutes. PCR products were then confirmed using electrophoresis on 1% agarose gel stained with Florosafe dye at 100 V for 18 minutes. The electrophoresis result was checked under UV light in the Geldock

instrument. Samples with clear and single band results were sent to the 1st Base, a sequencing service company, for Sanger sequencing.

2.4 Data analysis

Data analysis was conducted using all sequencing results and additional data from previous studies. Species confirmation was first done to all sequencing results using BLASTN in NCBI. All sequences were then aligned and trimmed in MEGA11 software [5]. Genetic distance computation using Kimura-2 parameter was done in MEGA11 between groups of species and locations. Haplotype composition was analyzed using DnaSP6 [6]. and a median joining haplotype network was built using PopART [7].

3 Result and Discussion

3.1 Result

3.1.1 Albuneid crab species in Enggano

DNA barcoding shows two distinct species of albuneid crab in Enggano Island, *A. symmysta* and *Albunea* sp1 based on Kimura-2 parameter genetic distance (> 0.162, Table 1). Both species have similar morphology characteristics and initially identified as *A. symmysta*.

3.1.2 Genetic distance between population

Based on genetic distance analysis using Kimura-2 Parameter, *A. symysta* and *Albunea* sp1 from Enggano shows a 17.3% distance. Genetic distance between *A. symmysta* Enggano and other *Albunea* species from other regions ranges from 14.9% to 21.1%. Meanwhile, the genetic distance between *A. symysta* Enggano and *A. symysta* from other regions ranges from 0.2% to 6.6%.

Table 1. Kimura-2 parameter genetic distance of Indonesian albuneid crab

		1	2	3	4	5	6	7	8	9	10	11
1	*A. symmysta* Manokwari											
2	*A. symmysta* Bengkulu	0.065										
3	*A. symmysta* Carita	0.067	0.003									
4	*A. symmysta* Cilacap	0.065	0.002	0.003								
5	*A. symmysta* Enggano	0.066	0.002	0.003	0.002							
6	*A. symmysta* Jayapura	0.002	0.066	0.067	0.066	0.066						
7	*A. symmysta* Jember	0.081	0.016	0.018	0.016	0.017	0.081					
8	*A. symmysta* South Bengkulu	0.065	0.002	0.003	0.002	0.003	0.066	0.017				

		1	2	3	4	5	6	7	8	9	10	11
9	A symmsyta Pelabuhan Ratu	0.068	0.004	0.005	0.003	0.004	0.068	0.018	0.004			
10	A symmsyta Bali Strait	0.072	0.008	0.010	0.008	0.009	0.072	0.023	0.009	0.008		
11	*Albunea* sp1 Enggano	0.162	0.174	0.174	0.174	0.173	0.162	0.192	0.174	0.176	0.184	
12	*Albunea* sp2 Madura	0.153	0.150	0.149	0.150	0.149	0.152	0.162	0.149	0.151	0.157	0.172
13	*Albunea* sp3 Madura	0.177	0.186	0.186	0.186	0.185	0.177	0.199	0.185	0.187	0.195	0.079
14	*Albunea* sp4 Tegal	0.211	0.212	0.212	0.211	0.211	0.210	0.224	0.211	0.213	0.217	0.199

3.1.3 Haplotype composition of albuneid crab in Indonesia

Haplotype analysis shows there are 26 haplotypes of *A. symmysta* in Indonesia. The population in Enggano consists of 4 specific haplotypes and 2 shared haplotypes with populations from Sumatra and Java (Table 2). Haplotype network construction shows that all *A. symmsta* populations clustered into one big group and separated from all other *Albunea* spp. Meanwhile, both samples of *Albunea* sp1 from Enggano also clustered into one group with only one nucleotide difference (Fig. 1).

Table 2. Haplotype composition of Indonesian albuneid crab

Haplotype	Number of individuals	Specimen label	Accession number
Hap_1	7	*A. symmysta* Amban Manokwari Am1	OP873019
		A. symmysta Amban Manokwari Am2	OP873020
		A. symmysta Amban Manokwari Am4	OP873022
		A. symmysta Amban Manokwari Am6	OP873024
		A. symmysta Jayapura AJ2	OP873014
		A. symmysta Jayapura AJ3	OP873014
		A. symmysta Jayapura AJ6	OP873018
Hap_2	3	*A. symmysta* Amban Manokwari Am3	OP873021
		A. symmysta Amban Manokwari Am8	OP873026
		A. symmysta Jayapura AJ1	This research
Hap_3	2	*A. symmysta* Amban Manokwari Am5	OP873025
		A. symmysta Amban Manokwari Am7	OP873025
Hap_4	21	*A. symmysta* Bengkulu Bn 100	This research
		A. symmysta Bengkulu Bn 101	This research
		A. symmysta Bengkulu Bn 104	This research
		A. symmysta Bengkulu Bn 106	This research
		A. symmysta Bengkulu Bn 99	This research

Haplotype	Number of individuals	Specimen label	Accession number
Hap_4	21	*A. symmysta* Carita Aca	OP873027
		A. symmysta Cilacap C1	OP873004
		A. symmysta Cilacap C4	OP873007
		A. symmysta Cilacap C5	OP873008
		A. symmysta Cilacap C8	OP873011
		A. symmysta Cilacap C9	OP873012
		A. symmysta Enggano Eng 03	This research
		A. symmysta Enggano Eng 04	This research
		A. symmysta Enggano Eng 10	This research
		A. symmysta Enggano Eng 13	This research
		A. symmysta Enggano Eng 28	This research
		A. symmysta Enggano Eng 32	This research
		A. symmysta Kaur Kau 08	This research
		A. symmysta Kaur Kau 13	This research
		A. symmysta PLRatu PR2	OP873033
		A. symmysta SBali 13a	This research
Hap_5	1	*A. symmysta* Bengkulu Bn 102	This research
Hap_6	12	*A. symmysta* Bengkulu Bn 105	This research
		A. symmysta Bengkulu Bn 108	This research
		A. symmysta Bengkulu Bn 96	This research
		A. symmysta Carita Ca2	OP873028
		A. symmysta Cilacap C10	OP873013
		A. symmysta Cilacap C2	OP873005
		A. symmysta Cilacap C3	OP873006
		A. symmysta Enggano Eng 06	This research
		A. symmysta Enggano Eng 30	This research
		A. symmysta Kaur Kau 09	This research
		A. symmysta PLRatu PR1	OP873032
		A. symmysta PLRatu PR3	OP873034
Hap_7	1	*A. symmysta* Bengkulu Bn 97	This research
Hap_8	1	*A. symmysta* Bengkulu Bn 98	This research
Hap_9	1	*A. symmysta* Carita Ca1	OP873027
Hap_10	1	*A. symmysta* Carita Ca3	OP873029

Haplotype	Number of individuals	Specimen label	Accession number
Hap_11	1	*A. symmysta* Carita Ca4	OP873030
Hap_12	1	*A. symmysta* Cilacap C6	OP873009
Hap_13	1	*A. symmysta* Cilacap C7	OP873010
Hap_14	1	*A. symmysta* Enggano Eng 01	This research
Hap_15	1	*A. symmysta* Enggano Eng 12	This research
Hap_16	1	*A. symmysta* Enggano Eng 15	This research
Hap_17	1	*A. symmysta* Enggano Eng 27	This research
Hap_18	1	*A. symmysta* Jayapura AJ4	OP873016
Hap_19	1	*A. symmysta* Jayapura AJ5	OP873017
Hap_20	1	*A. symmysta* Jember JB1	This research
Hap_21	1	*A. symmysta* Kaur Kau 10	This research
Hap_22	1	*A. symmysta* Kaur Kau 11	This research
Hap_23	1	*A. symmysta* Kaur Kau 12	This research
Hap_24	2	*A. symmysta* PLRatu 11	This research
		A. symmysta SBali 15a	This research
Hap_25	1	*A. symmysta* PLRatu PR4	This research
Hap_26	1	*A. symmysta* SBali 14a	This research
Hap_27	1	*Albunea* sp Enggano Eng 17	This research
Hap_28	1	*Albunea* sp Enggano Eng 26	This research
Hap_29	1	*Albunea* sp Madura 11a	This research
Hap_30	1	*Albunea* sp Madura 12a	This research
Hap_31	2	*Albunea* sp Tegal 7a	This research
		Albunea sp Tegal 8a	This research

4 Discussion

DNA barcoding shows two species of albuneid crabs on Enggano Island, even though they are morphologically similar. These two species have a 17.3% genetic distance. In crustaceans, the threshold commonly used for species delimitation using the CO1 gene is 16% genetic distance [8]. This further confirms the existence of two distinct species in Enggano. Meanwhile, the intraspecies distance between *A. symmysta* in Enggano and other regions has the highest value of 6.6%, which are with the populations from Manokwari and Jayapura. This distance could be affected by the low rate of gene flow between locations [3].

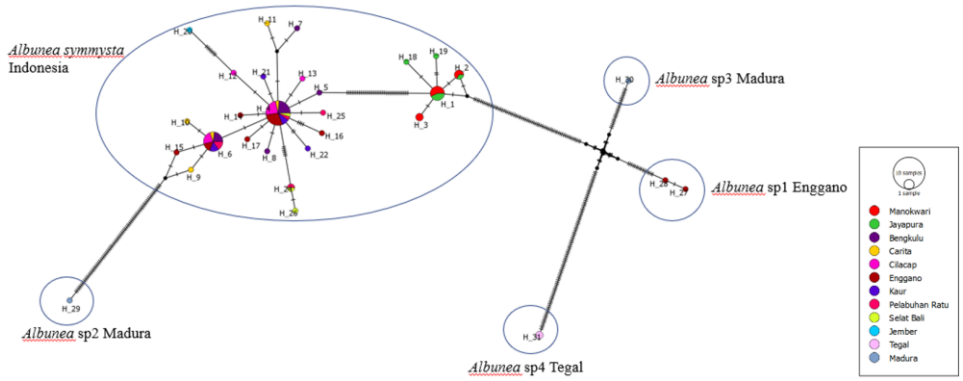

Fig. 1. The haplotype network between haplotype A symnysta from Enggano Island and several other regions in Indonesia. The network also involves haplotypes from *Albunea* sp.

DNA barcoding applications have been proven to accelerate the discovery of new species. At a certain level of difference, DNA barcode portion are able to differentiate between two species that are morphologically very difficult to distinguish, known as cryptic species. DNA barcoding is an initial stage for studying the species boundaries of less well-studied species. Once confirmation of the species is obtained, further studies can be carried out to analyze morphological characters that may be easily distinguished. In addition, nucleotide sequence data that was originally obtained to determine species can also be used to analyze several population phenomena, ranging from genetic diversity, population connectivity to phylogenetic relationships.

Haplotype network construction also shows that all 26 haplotypes of *A. symmysta* in Indonesia, including from Enggano, are clustered into one group. The *Albunea* sp1 from Enggano are clustering together and separated from other *Albunea* spp. Both haplotypes of *Albunea* sp1 have only one nucleotide difference, suggesting that they are from the same species. The existence of two different species in Enggano means that the albuneid crab can be cryptic. Therefore, more studies on the unidentified *Albunea* spp are needed to determine the species of albuneid crab on Enggano Island.

5 Conclusion

There are 26 haplotypes of *A. symmysta* found in Indonesia. DNA barcoding reveals two distinct and cryptic species of albuneid crab in Enggano Island. Further research regarding these cryptic species of albuneid crabs is needed.

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