https://doi.org/10.1051/bioconf/202517101013

Genetic mixing between distribution areas of *Birgus latro* based on maternal lineage molecular markers

Achmad Farajallah[1*], *Achmad* Alfiyan[1], *Yusli* Wardiatno[2], and *Sulistiono* Sulistiono[2]

[1]Department of Biology, Faculty of Mathematics and Natural Science, IPB University, Bogor 16680, Indonesia
[2]Departement of Aquatic Resources Management, Faculty of Fisheries, IPB University, Bogor 16680, Indonesia

Abstract. The distribution of *Birgus latro*, commonly known as the coconut crab, is influenced by its planktonic larvae, which migrate along ocean currents. The larvae's planktonic phase, facilitates genetic exchange among *B. latro* subpopulations, particularly between geographically proximate islands. This study investigates the genetic mixing across *B. latro* distribution ranges in the Pacific and Indonesia. DNA was extracted from hemolymph voucher specimens collected between 2017 and 2018. A total of 32 samples from four subpopulations - Derawan Island (n = 9), Morotai Island (n = 2), Ternate (n = 11), Yoi Gebe Island (n = 10) - were analyzed. The mitochondrial CO1 gene was amplified via PCR and sequenced. These sequences were then aligned with publicly homologous data from various Pacific locations. The reconstruction of phylogeographic tree based on the NJ grouping did not find any grouping supported by bootstrap values >50%. Genetic diversity within subpopulations (0.0113) is much greater than between subpopulations (0.0017). From a total of 102 sequence data grouped into 49 haplotypes. There is no specific haplotype that correlates with a specific location. This strengthens the suggestion that the longer age of pelagic larvae will cause genetic mixing between subpopulations.

1 Introduction

Birgus latro Linnaeus (1767) (=coconot crab - Eng.; kenari crab - Ina.) is a member of the Coenobitidae family (Decapoda: Arthropoda) that adapts to life on land with the largest body size among arthropods. The natural distribution of the *B. latro* population is in the tropical zone of the Pacific islands, starting from the east coast of Africa, Indonesia, Australia, several islands in the Pacific to Japan [1]. In Indonesia, *B. latro* is reported as a crab that is spread across the islands in Eastern Indonesia. In western Indonesia, its occurence has never been reported except on Derawan Island which is on the Wallacea line [2], [3]. The population of this crab is categorized as vulnerable due to over harvesting for exotic food and several changes in the management of the allocation of the coastline [3],[4]. As other members of the

* Corresponding author: achamad@apps.ipb.ac.id

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

crab, the life cycle of *B. latro* has a planktonic larval phase or pelagic larvae that are unable to swim. Its migration between islands in the Pacific is highly dependent on ocean currents [5], [6]. The age of planktonic larvae is quite long, ranging from 36 – 42 days, causing genetic mixing between subpopulations. Several reports mention the existence of a factor of changes in the coastline after the maximum ice age ended around 18,000 years ago as the cause of the formation of their subpopulations [7].

After the sea level rose about 100-120 m after the ice age ended, there was a change in the appearance of the coastline and several new coastlines were formed [8]. The previously established population structure underwent dramatic changes. For Indonesia, there is the Wallacea Line boundary that separates the Greater Sunda Shelf in the West and the Lesser Sunda Island in Eastern Indonesia. To study population structure, genetic markers of the CO1 gene of the mitochondrial genome can be used. Initially, this marker was popularized as a DNA barcode. In line with applications involving broader taxa, especially in Crustacea, the consistency of the mutation rate is neutral so that it can be used to study population structure [9]. The aim of this study is to investigates the genetic mixing across *B. latro* distribution ranges in the Pacific and several islands in Indonesia.

2 Materials and methods

This study used *B. latro* hemolymph samples stored in alcohol 96% as voucher specimens from several studies in 2017-2018. Hemolymph was bled using needle no. 25 from the joints of the limbs (**Fig. 1**). The total number of samples used was 32 hemolymph samples from Derawan Island (n = 9), Morotai Island (n = 2), Ternate Island (n = 11), and Yoi Gebe Island (n = 10).

Hemocyte cells were precipitated and then soaked in diluted TE (80% of 1xTE) buffer for 30 minutes to remove alcohol. DNA molecules were extracted from homocyte cells using the Geneaid™ DNA Isolation Kit. Amplification of the CO1 gene flanked by primer pairs AF215 (5'-T TCA ACA AAT CAT AAA GAT ATT GG) and AF216 (5'-TA AAC TTC AGG GTG ACC AAA AAA TCA) was carried out using GoTaq® Flexi DNA Polymerase (Promega) with an annealing temperature of 550C. PCR amplicons showing a single and thick band on the agarose gel were selected as templates in PCR for sequencing using ABI BigDye terminator mix followed by electrophoresis in an ABI Prism 310 Genetic Analyzer (Applied Biosystem) at 1stBASE - Apical Scientific Sdn Bhd (https://base-asia.com/services/sanger-sequencing-services/). The sequences were corrected manually with the program CHROMAS version 2.6.6 (Technelysium Pty Ltd, Queensville, Australia) and aligned by MUSCLE embedded in program MEGA version 7.0 [10]. The alignment processes guided by the translated codon for the invertebrate mitochondrial genome.

The genetic distance between samples and between subpopulations was analyzed using the Kimura-2 parameter model with 1000 bootstraps in program MEGA version 7.0 [10]. The haplotype identification for all DNA sequences were processed using DnaSP V.6.12.03 under default option coding region of mitochondrial genome [11]. To visualize the relation between identified haplotypes of *B. latro* subpopulation, a haplotype network was constructed using the Median-Joining Network in PopArt [12].

Fig. 1. Collection of *B. latro* hemolymph using a sterile 25G needle, inserted into the limb joints.

Fig 2. Primer positions of AF215 and AF216 to the CO1 gene (1534 bp) of the mitochondrial genome of *B. latro* (GenBank no. ACC. MN480450) with the PCR amplicon size of 658 bp.

3 Results and discussions

3.1 Results

The length of the CO1 gene segment amplified using the AF215 and AF216 primer pairs is 658 bp (**Fig. 2**). This segment is in the first half of the 5' end of the CO1 gene. After alignment was carried out involving homologous data stored in the public repository, the length of the aligned data was 465 bp. From the aligned CO1 gene segment, the genetic diversity value for all samples in each subpopulation was 0.0097-0.0017. This genetic diversity value is composed of diversity within each subpopulation of 0.0113-0.0019 and between subpopulations of 0.0017-0.0005 (**Table 1**). The genetic diversity between subpopulations is much smaller than within each subpopulation, indicating that each member of the subpopulation is not a member that is closely related genetically.

Genetic diversity in each subpopulation and genetic distance between subpopulations are presented in **Table 3**. In Indonesia, the greatest genetic diversity was found in the Derawan subpopulation (0.031), followed by Ternate (0.017), Morotai (0.015), and Yoi-Gebe (0.008). The position of Derawan Island on the Wallacea Line is thought to be the cause of the high genetic diversity of B. latro. In the Pacific Ocean further north, genetic diversity is very small, namely in Okinawa at 0.004 and on several islands in China at 0.005.

Haplotype 19 was the dominant haplotype with 20 sample members, followed by haplotypes 11 (12 samples), 12 (12 samples) and 14 (8 samples), respectively (**Table 3**). The four haplotypes above were represented by samples covering all subpopulations except Derawan (**Fig. 4**).

Table 1. Estimates of the mean diversity within subpopulations, inter-subpopulations and entire subpopulation. The Christmas Island subpopulation was not included in the calculations because it was only a single sample.

	Mean diversity					
	within subpopulation		Inter-subpopulation		entire subpopulation	
	D	SE	D	SE	D	SE
Diversity	0.0113	0.0019	0.0017	0.0005	0.0096	0.0017

Table 2. Estimates of genetic diversity of *B. latro* (bold, on diagonal), genetic distance between subpopulation (below diagonal with standart error after 1000x bootstrap (above diagonal).

No.	Subpopulation	1	2	3	4	5	6	7	8	9
1	Derawan	**0.031**	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005
2	Morotai	0.027	**0.015**	0.003	0.003	0.003	0.003	0.003	0.003	0.005
3	Ternate	0.027	0.017	**0.017**	0.003	0.002	0.003	0.002	0.002	0.005
4	Yoi-Gebe	0.024	0.013	0.013	**0.008**	0.002	0.002	0.002	0.002	0.005
5	Halmahera Utara	0.023	0.010	0.011	0.006	**0.004**	0.002	0.002	0.002	0.005
6	Halmahera Selatan	0.023	0.011	0.012	0.007	0.004	**0.006**	0.002	0.002	0.005
7	Pacific Ocean	0.023	0.011	0.012	0.007	0.004	0.005	**0.005**	0.002	0.005
8	Okinawa	0.022	0.010	0.011	0.006	0.004	0.004	0.004	**0.004**	0.005
9	Chrismast	0.026	0.018	0.019	0.016	0.014	0.015	0.014	0.013	**-**

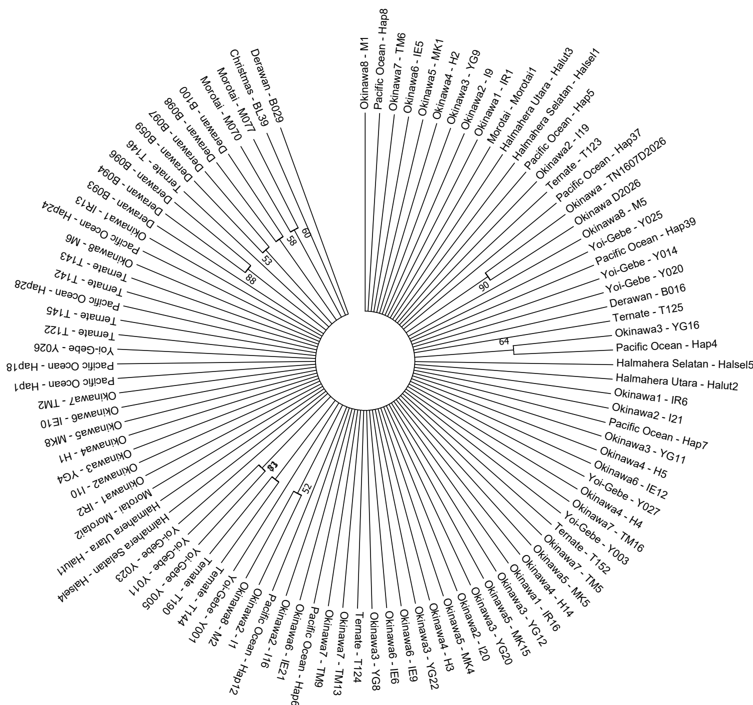

Fig. 3. Phylogeographic tree of Minimum Evolution with NJ grouping method, Kimura-2 Parameter substitution model with 1000x bootstrap. The numbers on the branches show the bootstrap value >50%

https://doi.org/10.1051/bioconf/202517101013

Table 3. Haplotype number of *B. latro* represented by more than one sample, from 49 identified haplotypes. The remaining haplotype numbers each consisting of one sample are not shown.

No. Haplotype	n	Subpopulation	Sample Id.
11	12	Halmahera_Selatan	Halsel1
		Halmahera_Utara	Halut3
		Morotai	Morotai1
		Okinawa1	IR1
		Okinawa2	I9
		Okinawa3	YG9
		Okinawa4	H2
		Okinawa5	MK1
		Okinawa6	IE5
		Okinawa7	TM6
		Okinawa8	M1
		Pacific_Ocean	Hap8
12	12	Halmahera_Selatan	Halsel4
		Halmahera_Utara	Halut1
		Morotai	Morotai2
		Okinawa1	IR2
		Okinawa2	I10
		Okinawa3	YG4
		Okinawa4	H1
		Okinawa5	MK8
		Okinawa6	IE10
		Okinawa7	TM2
		Pacific_Ocean	Hap1
		Yoi-Gebe	Y026
14	8	Halmahera_Utara	Halut2
		Okinawa1	IR6
		Okinawa2	I21
		Okinawa3	YG11
		Okinawa4	H5
		Okinawa6	IE12
		Pacific_Ocean	Hap7
		Yoi-Gebe	Y027
17	2	Okinawa	TN1607
		Okinawa	D2026
19	20	Okinawa1	IR16
		Okinawa2	I20
		Okinawa3	YG12
		Okinawa3	YG20
		Okinawa3	YG22
		Okinawa3	YG8
		Okinawa4	H14
		Okinawa4	H3
		Okinawa5	MK15
		Okinawa5	MK4
		Okinawa5	MK5
		Okinawa6	IE6
		Okinawa6	IE9
		Okinawa7	TM13
		Okinawa7	TM5
		Okinawa7	TM9
		Pacific_Ocean	Hap6
		Ternate	T124
		Ternate	T152
		Yoi-Gebe	Y003
20	2	Okinawa2	I1
		Okinawa8	M2
23	2	Okinawa3	YG16
		Pacific_Ocean	Hap4
28	2	Okinawa8	M6
		Pacific_Ocean	Hap24
47	2	Yoi-Gebe	Y014
		Yoi-Gebe	Y020

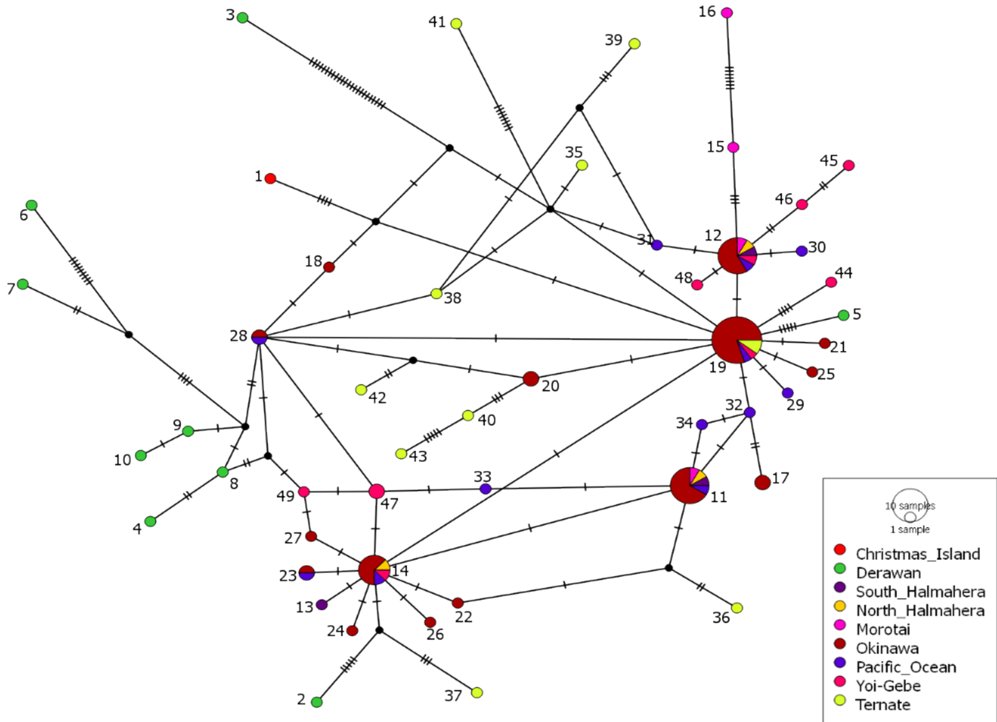

Fig. 4. Haplotype network of *B. latro* in generated based on median-joining network. Each line between points represents a single mutational step.

These indicate that the subpopulations of *B. latro* in the Pacific are genetically mixed. Although the 9 samples from the Derawan subpopulation are in the peripheral part of the network, there are at least two haplotype groups. The first group consists of 8 samples that have a substitution pattern of the CO1 gene segment that can be traced to the Pacific Islands subpopulation adjacent to the east coast of China and then connected to Okinawa, and 1 sample (= haplotype 3) is connected to the Ternate subpopulation. (**Fig. 4**). The very high haplotype diversity strengthens the initial suggestion that the subpopulations are interconnected because the age of the planktonic larvae reaches 46 days.

3.2 Discussion

The phenomenon that genetic diversity within a population is much greater than genetic diversity between subpopulations is commonly found in Crustacea (9) and other marine biota [10]. Connectivity between subpopulations through the sea is bridged by migration when the larval phase is passively involved in the sea. The choice of habitat for larval settlement and then moving towards land is determined by the availability of food resources and protection. Although it has adapted to the terrestrial environment, *B. latro* is often seen entering the sea, especially to reproduce. None of the branches of the phylogeographic tree are supported by adequate bootstrap values (<50) (**Fig. 3**).

The phylogeographic tree confirms the suggestion that there is no genetic differentiation between *B. latro* subpopulations, except for the Derawan and Christmas Island subpopulations. In the case of Derawan, it seems that there is an effect of the ocean current

that forms the Wallacea Line that cannot be passed by the movement of larvae perpendicular to the direction of the ocean current [5], [7]. While in the case of Christmas Island, the geographical distance is too far and is only represented by one sample. All clades formed contain mixed samples from the Morotai, Halmahera, Ternate, Okinawa and Pacific Ocean subpopulations.

A clearer pattern of connectivity and genetic mixing between subpopulations can be seen in the haplotype network (**Fig. 4**). The mutation pattern of the Derawan subpopulation resembles that of the Pacific Ocean which includes Taiwan and several islands on the east side of the Chinese Coastline, then continues to the islands of Okinawa. Only one Derawan sample (Haplotype 3) has a mutation pattern similar to Ternate and Halmahera. This is enough to suggest that the population of *B. latro*, although limited by the flow of the Wallace Line, has experienced genetic mixing. Of course, if the number of samples used in the study is increased, this suggestion will be more robust.

Acknowledgement

This study used specimen vouchers from SUL's (Prof Sulistiono) research. He conceptualized and co-wrote the article. FAR and WAR also conceptualized the study, analyzed the data, and wrote the article. ALF conducted DNA labwork and created haplotype network illustrations.

References

1. DecaNet eds., DecaNet., *Birgus latro* (Linnaeus, 1767). Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=208668 on 2025-01-20
2. M. Takeda, S.T. Ahyong, N. Ohtsuchi, H. Komatsu. Crabs (Crustacea, Decapoda) from the Seas of East and Southeast Asia Collected by the RV Hakuhō Maru (KH-72-1 Cruise). 4. South China Sea. Bull. Natl. Mus. Nat. Sci., Ser. A, **48**:147 (2022). https://doi.org/10.50826/bnmnszool.48.4_147
3. Sulistiono, R. Affandi, D.M. Wildan, D. Jusadi, S. Samson, S.G. Akmal, Yon vitner, J. Patoka, Artificial Feeding of *Birgus latro* (L., 1767) (Anomura: Coenobitidae) As an Alternative to Natural Diet: Perspective for Conservation Breeding. Sci. Agric. Boh., **53**:33 (2022). https://doi.org/10.7160/sab.2022.530203
4. [IUCN] International Union for Conservation of Nature and Natural Resources, Coconut crab. https://www.iucnredlist.org/species/2811/126813586 accessed on 2025-01-20
5. D. Veliz, N. Rojas-Hernández, C. Vega-Retter, C. Zaviezo, I. Garrido and L. Miguel Pardo, Spatial and temporal stability in the genetic structure of a marine crab despite a biogeographic break. Sci. Reports, **12**:14192 (2022). https://doi.org/10.1038/s41598-022-18368-5
6. P. Greenaway, Terrestrial adaptations in the Anomura (Crustacea: Decapoda), Memoirs of Museum Victoria. **60**:13 (2003). doi:10.24199/j.mmv.2003.60.3
7. S. Lavery, C. Moritz and D.R. Fielder, Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*, Mol. Ecol. **5**:557 (1996). https://doi.org/10.1046/j.1365-294X.1996.00125.x
8. V.W. Putri, K.A. Widayati, Y. Wardiatno and A. Farajallah, Genetic Diversity and Differentiation of Sand Crab (*Albunea symmysta*) (Crustacea: Decapoda: Hippoidea) from Java and Papua, Indonesia, Hayati Journal of Biosci., **31**:671 (2024).

https://doi.org/10.4308/hjb.31.4.671-677

9. N.I. Syuhada, A. Farajallah and A. Mashar, Distribution of moon crab Matuta victor (Fabricius, 1781) from the western and eastern of Java coastline and the characteristic of COI mitochondria among the locations, IOP Conf. Series: Earth and Environmental Science 1359 (2024) 012088. https://doi.org/10.1088/1755-1315/1359/1/012088
10. S. Kumar, G. Stecher and K. Tamura, MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. for Bigger Datasets. Mol. Biol. Evol., **33**:1870 (2015). https://doi.org/10.1093/molbev/msw054
11. J. Rozas, A. Ferrer-Mata, J.C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S.E. Ramos-Onsins, A. Sánchez-Gracia, DnaSP 6: DNA Sequence Polymorphism Analysis of Large Datasets. Mol. Biol. Evol., **34**: 3299 (2017). https://doi.org/10.1093/molbev/msx248
12. J.W. Leigh and D. Bryant, popart: full-feature software for haplotype network construction, Methods in Ecol. Evol., **6**:1110 (2015). https://doi.org/10.1111/2041-210X.12410