# Genetic diversity of octopus (*Octopus cyanea*) in the Northern Waters of Aceh

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**Abstract.** The octopus exhibits highly developed defensive adaptations, including the ability to camouflage itself to deceive predators. It undergoes two larval stages, during which it experiences wide dispersal influenced by ocean currents and oceanographic characteristics. One region with complex current dynamics is the northern waters of Aceh, specifically around Pulau Aceh, which are influenced by the currents of the Indian Ocean and the Andaman Sea. The objective of this study is to assess the genetic diversity of *Octopus cyanea* by comparing the Aceh population with those from Sri Lanka and Japan, which are the closest geographically. The analysis was conducted using the COI gene with HCO and LCO primers. Results indicate that the Aceh population exhibits the highest genetic diversity, while the Sri Lanka population shows the lowest diversity. Populations with higher genetic diversity tend to have better survival prospects, suggesting that the *Octopus cyanea* population in Aceh is in a healthy condition. Furthermore, the study reveals that the Aceh population shares a close genetic relationship with the Japanese population, with a similarity value of 0.4. These two populations share haplotypes, indicating genetic connectivity. The mixing of haplotypes in the Japanese population is likely due to larval dispersal and varying current circulation, which influences species diversity.

## 1 Introduction

Pulo Breuh is a small island located off the northern coast of Sumatra Island, in the coastal village area of Pulo Aceh. The waters of Pulo Aceh, uniquely situated between the Andaman

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Sea and the Indian Ocean, provide an ideal habitat for coral reef ecosystems. Pulo Breuh has high fishery resource potential, making it one of the fishing zones for fishermen from Banda Aceh and Aceh Besar [1].

One of the abundant commodities in these waters is the octopus species *Octopus cyanea*. Commonly known as the day octopus, *Octopus cyanea* inhabits benthic habitats, particularly shallow waters surrounded by supporting ecosystems that provide shelter. This octopus has a life cycle that includes pelagic larvae whose distribution is influenced by ocean currents and other geographical factors. The broad geographical range of these larvae determines the population structure of the octopus and its adaptation to the surrounding environment [2]. This, in turn, influences population differentiation between various groups. Information about the geographical range of these larvae can be obtained by analyzing genetic diversity and connectivity between populations [3].

Genetic diversity measures the extent of genetic variability within a population [4]. This diversity directly impacts the stability and resilience of the population. A decrease in genetic diversity reduces a species' ability to adapt to environmental changes. Additionally, genetic diversity information is critical for the conservation and sustainable management of these resources [5]. To contextualize the genetic diversity and connectivity of *Octopus cyanea* populations in Pulo Breuh, this study compares the findings with populations in Japan and Sri Lanka. These two locations were chosen because of their distinct oceanographic conditions and proximity to major ocean current systems that may influence larval dispersal. Japan is influenced by the Kuroshio Current, a warm ocean current, while Sri Lanka lies near the Indian Ocean Gyre, characterized by unique current patterns. Both regions represent critical points in understanding how regional oceanographic processes affect the genetic connectivity of octopus populations.

The implications of genetic diversity for adaptive capacity and resilience to environmental changes are profound. High genetic diversity can enhance a population's ability to respond to stressors such as climate change, habitat degradation, and overfishing. In contrast, reduced genetic diversity may limit adaptive potential, leaving populations vulnerable to environmental shifts. Therefore, understanding genetic diversity is essential for designing effective conservation strategies and ensuring the long-term sustainability of octopus fisheries in Pulo Breuh and beyond. Genetic diversity studies on octopuses have never been conducted in Sumatra’s waters, particularly in Aceh. Therefore, this research serves as essential groundwork for the management of octopus fisheries. The findings will provide valuable insights into the connectivity and genetic health of *Octopus cyanea* populations, contributing to their sustainable management and conservation.

## 2 Research methods

This research was conducted from February to May 2024. Samples of the octopus *Octopus cyanea* were collected from Pulo Aceh, Aceh Besar Regency, Aceh (Figure 1). The samples were processed for genetic analysis following standard procedures at the Genetics and Acoustic Biodiversity Laboratory, Faculty of Marine and Fisheries, Universitas Syiah Kuala. Additionally, sequences from Sri Lanka and Japan were retrieved from GenBank to confirm phylogenetic relationships and connectivity.

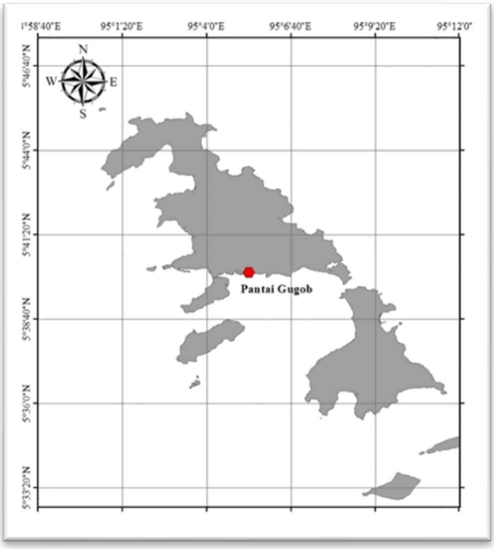


**Fig. 1.** Map of the research location for genetic diversity of *Octopus cyanea* in the Northern Aceh Waters.

## 2.1 Sample collection and handling

Samples of the octopus species *Octopus cyanea* were obtained from catches along the coast of Gampong Gugob. A total of five samples were collected. These samples were first identified morphologically and then preserved for subsequent molecular analysis.

## 2.2 DNA extraction

DNA extraction was performed using a modified Cetyltrimethyl Ammonium Bromide (CTAB) protocol. CTAB is a surfactant used for DNA isolation [6]. The extraction process followed the steps outlined in the modified CTAB protocol [7]. The extracted samples were taken from the tentacle tissues of the octopus.

## 2.3 DNA amplification

DNA amplification was conducted using the Polymerase Chain Reaction (PCR) method, targeting the Cytochrome Oxidase Subunit 1 (COI) locus. The primers used were forward LCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and reverse HCO-2198 (5′ TAAACTTCAGGGTGACCAAAAAATCA-3′) [8]. The PCR mixture consisted of 2 µL template DNA, 8.5 µL ddH$_2$O, 1 µL primer, and 12.5 µL Master Mix Red. The PCR process was carried out using a Sensoquest LabCycler Basic machine, with the following thermal cycling conditions: an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes [9].

### 2.4 DNA translation

DNA translation was performed to convert nucleotide sequences into corresponding peptide sequences. The DNA sequence (nucleotides) was translated into a protein sequence (amino acids) to identify the presence of stop codons. DNA sequencing was conducted to determine the identity and function of genes or other DNA segments by comparing the sequence with known DNA sequences [10]. In this study, DNA sequencing was performed at First BASE Laboratory, Malaysia, using the Sanger method. This method is widely used in DNA sequencing, where the Sanger sequencer [11] generates chromatograms (electropherograms) that provide the resulting DNA sequence [12].

## 3 Results and discussion

### 3.1 Results

#### *3.1.1 COI* gene implification of octopus (*Octopus cyanea*)

A total of five octopus (*Octopus cyanea*) samples were successfully collected during this study, with a base length of 651 bp (base pairs). Additionally, five sequences were downloaded from the NCBI website (Table 1).

**Table 1.** Sekunder sequences.

| Sequence Code | Location | Reference |
|---|---|---|
| GBCPH991-09 | Japan | [9] |
| GBCPH992-09 | Japan | |
| AB191280.1 | Japan | |
| MN989942.1 | Sri Lanka | Unpublished |
| MN989947.1 | Sri Lanka | |

Identification of Octopus (*Octopus cyanea*) Using BLAST and BOLD SYSTEMS BLAST on the NCBI website and BOLD SYSTEMS can be used to match sequencing results with existing databases in GenBank. The E-Value from this study was 0.0, and the identity values were high, ranging from 99% to 100% (Table 2).

**Table 2.** Results of *Octopus cyanea* Identification Using BLAST and BOLD SYSTEM.

| No | Sample Code | Species | | Similarity | |
|---|---|---|---|---|---|
| | | Morphology | DNA Barcode | BLAST | BOLD |
| **1** | GPA – 02 | *Octopus cyanea* | *Octopus cyanea* | 98,60% | 99,17% |
| **2** | GPA – 13 | *Octopus cyanea* | *Octopus cyanea* | 98,60% | 99,17% |
| **3** | GPA – 14 | *Octopus cyanea* | *Octopus cyanea* | 98,60% | 99,17% |
| **4** | GPA – 15 | *Octopus cyanea* | *Octopus cyanea* | 98,60% | 99,17% |
| **5** | GPA – 18 | *Octopus cyanea* | *Octopus cyanea* | 98,60% | 99,17% |

### *3.1.2 Phylogenetic tree reconstruction of octopus (Octopus cyanea)*

The phylogenetic relationship of *Octopus cyanea* can be explained through the reconstruction of a phylogenetic tree using 10 mitochondrial COI DNA sequences, consisting of one species (5 sequences from this study and 5 sequences from GenBank). Phylogenetics represents the relationship between species based on DNA sequence composition, resembling a tree structure, and aims to provide information about the evolutionary processes of species in the past. The phylogenetic tree construction of the COI gene for *Octopus cyanea* is divided into two clades and an outgroup.

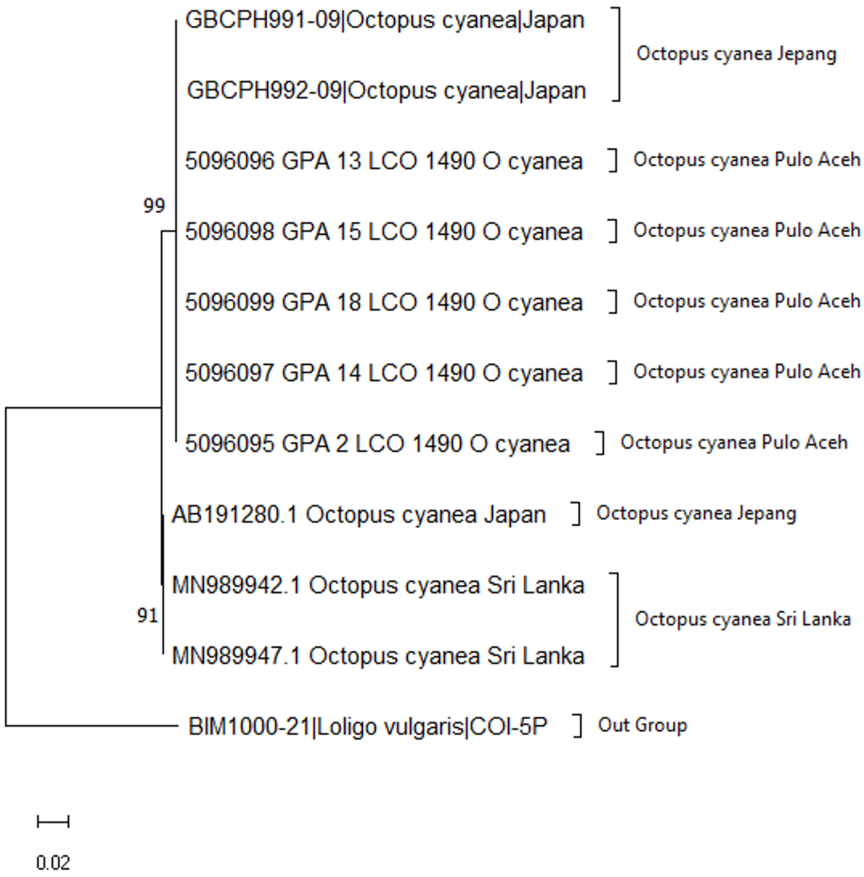


**Fig. 2.** Phylogenetic tree of 10 sequences of Octopus (*Octopus cyanea*) using the maximum likelihood method with 1000 bootstrap replicates.

### *3.1.3 Nucleotide composition of* Octopus cyanea

The nucleotide base length of the COI gene was 651 bp (base pairs) with an average nucleotide composition of T = 35.95%, C = 19.63%, A = 28.64%, and G = 15.76% (Table 4.2). The average GC composition was 35.40%, while the average AT composition was higher at 64.59%. The GC composition in the first, second, and third codon positions showed a decrease. Furthermore, the results from DAMBE indicated that the analyzed data did not show evidence of saturation effects (Table 3).

**Table 3.** Summary of Statistical Distribution of Nucleotide Frequencies for the COI Sequences of *Octopus cyanea* Samples.

| Base Persentage (%) | Average | Minimum | Maximum |
|---|---|---|---|
| T% | 35,95 | 35,79 | 37,17 |
| C% | 19,63 | 17,66 | 19,96 |
| A% | 28,64 | 28,11 | 29,03 |
| G% | 15,76 | 15,51 | 17,05 |
| AT% | 64,59 | 64,2 | 65,28 |
| GC% | 35,4 | 34,71 | 35,79 |
| GC% Codon Pos 1 | 47,46 | 44,23 | 47,92 |
| GC% Codon Pos 2 | 42,6 | 41,93 | 42,85 |
| GC% Codon Pos 3 | 16,12 | 15,66 | 17,05 |

Note: A = Adenine, T = Thymine, G = Guanine, and C = Cytosine.

### *3.1.4 Haplotype distribution of* Octopus cyanea *across three populations*

10 of *Octopus cyanea* samples, a total of three haplotypes were identified, originating from three water populations: Pulo Aceh, Japan, and Sri Lanka. One common haplotype was found to be shared among all three populations: Pulo Aceh, Japan, and Sri Lanka.

**Table 4.** Haplotype frequency distribution in COI (3 populations).

| Haplotype | Pulo Aceh | Jepang | Sri Lanka |
|---|---|---|---|
| 1 | 5 | 1 | - |
| 2 | - | 1 | - |
| 3 | - | 1 | 2 |

The results of the AMOVA analysis showed an Fst value of 0.64, which falls into the low range of 0.6–1.00.

**Table 5.** Fst values between and within populations.

| Species | Variation Source | d.b. | Variation(%) | FST |
|---|---|---|---|---|
| *Octopus cyanea* | Between population | 2 | 64,92 | 0,64 |
|  | Within population | 7 | 35,08 |  |

Note: d.f = Degrees of Freedom, FST = Pairwise Distance Value

### *3.1.5 Genetic distance of* Octopus cyanea

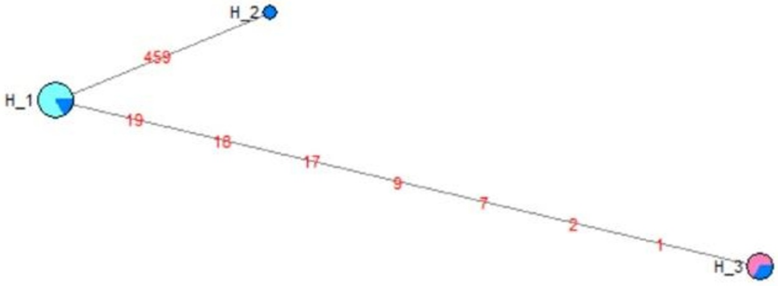
The analysis results indicate the genetic distances between populations (interspecific) and within the same species population (intraspecific). The highest genetic distance between populations was found in the Japan population, at 0.01, while the highest genetic distance within a population was observed in the Sri Lanka population, at 1.08.

**Table 6.** Genetic distance between (bolded) and within populations of *Octopus cyanea* based on the mitochondrial COI gene.

| No | Population | 1 | 2 | 3 |
|---|---|---|---|---|
| 1 | Pulo Aceh | **0.00** | | |
| 2 | Jepang | 0,41 | **0,01** | |
| 3 | Sri Lanka | 1,08 | 0,78 | **0.00** |

### *3.1.6 Population connectivity*

The connectivity between *Octopus cyanea* populations in the waters of Pulo Aceh, Japan, and Sri Lanka was analyzed to observe genetic patterns using haplotype networks and maps (Figure 3). The haplotypes identified from the 10 sequences of *Octopus cyanea* species amounted to 3 haplotypes: Haplotype 1 was found in the waters of Pulo Aceh and Japan, Haplotype 2 was present in the waters of Japan, and Haplotype 3 was found in the waters of Japan and Sri Lanka.



Legend: ● Pulo Aceh ● Jepang ● Sri Lanka

**Fig. 3.** Haplotype network of three *Octopus cyanea* populations.

## 3.2 Disscusion

The sequence analysis of *Octopus cyanea* using the COI locus on 10 sequences resulted in a COI nucleotide length of 651 bp (base pairs) with the following average nucleotide composition: Adenine (A) = 28.64%, Cytosine (C) = 19.63%, Thymine (T) = 35.95%, and Guanine (G) = 15.76% (Table 3). The average GC content was 35.40%, while the AT content was higher at 64.59%. The GC content decreased from the first to the third codon positions. A higher AT base composition indicates a high degree of similarity among species due to independent parallel substitutions, causing them to cluster and share similar nucleotide base compositions. Nucleotide substitutions can be categorized into two types: transitions and transversions. Transitions involve changes between purine bases (A-G) or pyrimidine bases (C-T), whereas transversions involve changes between purine and pyrimidine bases (A-T, C-A, C-G, and T-G). Each nucleotide substitution can indicate distinguishing characteristics of a species. During inheritance from ancestors, some traits remain unchanged, while others mutate, leading to genetic mutations [13].

Based on the analysis of 10 samples from three different populations—Pulo Aceh, Japan, and Sri Lanka—the *Octopus cyanea* sequences were analyzed and used to construct a phylogenetic tree. The outgroup utilized was *Loligo vulgaris*, whose role in a phylogenetic tree is to facilitate predictions of genetic distances between populations [14]. A phylogenetic tree is a diagrammatic representation illustrating the evolutionary relationships between various organisms or species. It demonstrates how organisms evolved from a common ancestor and their evolutionary interconnections. Phylogenetics represents relationships between species based on the composition of DNA base sequences, forming a tree to estimate past evolutionary processes [15]. The results of the phylogenetic tree reconstruction yielded main branches, or clades, as shown in Fig. 3.1. This figure illustrates the evolutionary relationships between species from different populations, which are distinct from the outgroup. The branches of the phylogenetic tree include bootstrap values, indicating the confidence level of the genetic or morphological sequence results. If the bootstrap value is ≥95%, the branching is highly accurate, while values ≥70% are considered significant [16]. The phylogenetic reconstruction (Fig. 2) is supported by genetic distance values and genetic relatedness within each clade, influenced by the oceanographic conditions of the population's location [17]. Based on the phylogenetic tree and genetic distance values, the populations of Pulo Aceh, Japan, and Sri Lanka exhibit distinct evolutionary relationships. Species with different ancestral relationships form separate clades. Significant differences in anatomy, physiology, and genetics reflect their adaptations to aquatic environments and predation. Analyzing samples from different clades in the phylogenetic tree serves as a powerful tool for understanding the evolution and diversification of life on Earth. The outgroup functions as a comparison to identify the ingroup, consisting of all *Octopus cyanea* samples.

Genetic diversity was analyzed using molecular marker methods, specifically mitochondrial DNA [18]. Genetic diversity refers to variations arising from ecological, behavioral, or physical isolation. The haplotype diversity (Hd) values for the populations were as follows: Pulo Aceh at 1.000, Japan at 0.333, and Sri Lanka at 1.000. Haplotype diversity values indicate the probability that two random individuals within a population have different haplotypes, ranging from 0 (no diversity) to 1 (all haplotypes are different). Populations with more haplotypes exhibit higher diversity. For instance, Japan's haplotypes are distributed across all individuals, resulting in low haplotype diversity, reflecting lower nucleotide variation. Populations with high genetic diversity have a greater chance of surviving and adapting to environmental changes [19]. Increased genetic diversity is associated with greater resilience against exploitation [20]. The Japanese population exhibited the lowest genetic diversity, while Pulo Aceh and Sri Lanka had the highest values, at 1.000, indicating a species' ability to adapt to their environments. Conversely, the low genetic diversity in Japan may impact adaptive capacity, potentially leading to species extinction.

Genetic distance information can be used to determine individuals or populations selected as breeding material. Genetic distance measures the ratio of genetic differences between species or populations [19]. The greater the genetic distance of a population, the higher the heterosis effect when crossbreeding is conducted. High levels of genetic diversity are indicated by the number of haplotypes and haplotype diversity [21]. Populations with low genetic distance exhibit closer genetic relationships, while populations with high genetic distance show more distant relationships [22]. The genetic distance values for *Octopus cyanea* between populations ranged from 0.00–0.01, while within populations, they ranged from 1.08–0.78, with the highest value observed in the Sri Lankan population and the lowest in Pulo Aceh. Smaller genetic distances indicate populations with greater similarities, while geographic factors also influence genetic distance [23]. The Pulo Aceh population, which is still within Indonesian waters, exhibited lower genetic variation compared to the Japanese and Sri Lanka populations. Previous research demonstrated that genetic distances across seven species ranged from 0.091 to 0.178, where closer genetic distances indicate greater similarity in their COI genes.

The pairwise distance test (Fst) analysis of *Octopus cyanea* showed an Fst value of 0.64, which is considered high (0.6–1.0) [24]. A high Fst value indicates the need for a specific conservation strategy for that population. Overall, the genetic relationships among the three populations can be seen in the phylogenetic tree, which illustrates the kinship of the species. Connectivity between the populations of Pulo Aceh, Japan, and Sri Lanka was analyzed using sequence data to observe the network patterns and haplotype map (Fig. 3). A total of 3 haplotypes were generated from 10 *Octopus cyanea* sequences. Haplotype 1 originated from Pulo Aceh, haplotypes 1, 2, and 3 originated from Japan, and haplotype 3 was found in Sri Lanka. The haplotype found in the Japanese population shows mixing caused by larval dispersal carried by ocean currents [19], which affects the species' diversity in that population.

## 4 Conclusion

The identification results from the Gugob octopus showed that it is *Octopus cyanea*, with a query cover of 99.17%. The Fst values from the three populations were 0.64, indicating that the three populations are moderately structured. This is due to geographic barriers that limit the dispersal of octopus larvae, resulting in a relatively high FST value. The Sri Lankan population exhibited the highest genetic diversity and genetic distance, while the lowest genetic diversity was found in the Pulo Aceh population, with the lowest genetic distance in the Japanese population. Connectivity between populations indicates gene flow, leading to similar haplotypes across different populations.

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