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Morphological, morphometric, and molecular identification of *Octopus cyanea* in the Waters of Kaur District, Bengkulu Province, Indonesia

AHMAD PARIANSYAH, RATIH IDA ADHARINI♥, DINI WAHYU KARTIKA SARI, EKO HARDIANTO
Laboratory of Aquatic Resources Management, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada. Jl. Flora, Gedung A-4 Bulaksumur, Sleman 55281, Yogyakarta, Indonesia. Tel./fax.: +62-274-551218, ♥email: ratih.adharini@ugm.ac.id

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Abstract. *Pariansyah A, Adharini RI, Sari DWK, Hardianto E. 2025. Morphological, morphometric, and molecular identification of* Octopus cyanea *in the Waters of Kaur District, Bengkulu Province, Indonesia. Biodiversitas 26: 2640-2652.* Octopus is a high-value fishery resource abundant in the waters of Kaur District, Bengkulu Province. This study aimed to identify octopus species inhabiting Kaur waters through integrated morphological, morphometric, and molecular analyses. A total of 68 octopus specimens were collected (20 males and 48 females) with a sex ratio of 1:2.4. Field identification confirmed all specimens as *Octopus cyanea*, characterized by oval mantles, W-shaped funnels, and two rows of suckers per arm. Males possessed larger suckers and a hectocotylized third right arm. The body displayed brown and white coloration, false eye spots (*ocelli*), black stripes, white spots along he arms, and four small *papillae*. Molecular analysis using the Cytochrome c Oxidase Subunit I (COI) gene showed a 100% match with *O. cyanea* sequences in GenBank, confirming species identity. Morphometric analysis revealed that females had relatively larger body ratios, particularly MW/TL, ML/MW, FuL/PA, and WD/AL. Phylogenetic and genetic distance analyses demonstrated close relationship between *O. cyanea* from Kaur and populations from Jayapura, Buton, Wakatobi, Madagascar, India, and Japan, indicating a broad geographic distribution. These results confirm the presence of *O. cyanea* in Kaur waters and highlight its morphological and genetic similarity to other populations, providing a foundation for future conservation and sustainable fishery practices in the region.

Keywords: Cephalopoda, DNA barcoding, genetic distance, mitochondrial marker, species identification

INTRODUCTION

Kaur District, located in the southernmost region of Bengkulu Province, Indonesia, has a coastline of 108.6 kilometers, significantly contributing to its marine resource potential (Kaur Regency Fisheries Service 2022). One of the primary strengths of the region's marine sector is its seafood production, which reaches a minimum of 330 tons per year to meet both export demands and local consumption (Hestiawan et al. 2022). Among the various seafood products, octopus has emerged as a prominent commodity dominating the local waters (Kaur Regency Fisheries Service 2022). The abundance of octopus in Kaur District has positively affected the local economy. In addition to being marketed fresh, octopus is also sold dried by fishermen. Furthermore, octopus from Kaur has driven the growth of various octopus-based food processing businesses, adding economic value to the local community (Hafiza et al. 2024). As a result, it has become one of Kaur District's strategic assets in the fisheries and marine sectors. Globally, cephalopod populations, including octopuses, have exhibited a significant upward trend over the past six decades (Sauer et al. 2019). This increase was influenced by factors such as climate change and the declining population of finfish due to overexploitation (Pauly and Zeller 2016).

In 2020, the global export value of cephalopod commodities reached USD 10.2 billion, accounting for 6.8% of the total global fisheries export value (FAO 2022). As one of the countries with the largest octopus resources, Indonesia has become a major contributor to this global trade. Consequently, octopus serves as one of Indonesia's significant sources of foreign exchange (Alim et al. 2023). Indonesian octopuses are highly sought after in various international markets. In Asia, key consumer countries include Japan, South Korea, Vietnam, Hong Kong, and Malaysia. Additionally, European markets such as the Netherlands, Spain, Italy, Greece, France, and Cyprus have shown strong demand for Indonesian octopuses. The United States and Australia are also important markets for national octopus exports (Ministry of Marine Affairs and Fisheries of Republic of Indonesia 2023). The increasing trend in export value each year has further strengthened Indonesia's position as a major player in the global octopus trade (Tarigan et al. 2020).

Scientific studies have identified various octopus species distributed in Indonesian waters, including *Octopus cyanea* (Gray, 1849) (big blue octopus), *O. laqueus* (Kaneko & Kubodera, 2005) (white striped octopus), *O. marginatus* (Taki, 1964) (coconut octopus), *O. vulgaris* (Cuvier, 1797) (common octopus) *Hapalochlaena fasciata* (Hoyle, 1886) (blue lined octopus), *H. lunulata* (Quoy & Gaimard, 1832) (greater blue ringed octopus) *Amphioctopus aegina* (Gray, 1849) (marbled octopus), *A. marginatus* (Taki, 1964) (coconut octopus), *Abdopus aculeatus* (d'Orbigny, 1834) (algae octopus), and *Callistoctopus ornatus* (Gould, 1852) (ornate octopus) (Faskanu 2019; Hakim et al. 2020; Kholilah et al.

2021). These species reflect the high biodiversity of Indonesian waters, including those in Kaur District.

However, there remains a considerable data gap in the Kaur District, where scientific publications focusing on the morphology, morphometry, and molecular characteristics of *O. cyanea* are still very limited. This absence of data hinders a comprehensive understanding of the distribution and biological characteristics of this species in the southern Sumatra region. Moreover, the increasing intensity of octopus fishing activities in Kaur driven by high market demand combined with environmental changes due to climate change and human activities, may affect the morphological and genetic structure of octopus populations. Integrated morphological and molecular studies are therefore crucial not only for species identification but also for understanding potential ecological and evolutionary impacts. These scientific approaches provide essential tools for monitoring population health and adaptation. Despite its strategic importance, there is currently no comprehensive data on *O. cyanea* in Kaur District. This research is thus necessary to fill empirical gaps related to species composition, theoretical gaps in biodiversity distribution in southern Sumatra, and practical gaps in providing reliable baseline data for fisheries management. Hence, this study aimed to identify octopus species, particularly *O. cyanea*, through morphological, morphometric, and molecular analysis using the Cytochrome c Oxidase Subunit I (COI). The results are expected to provide foundational data to support conservation strategies, sustainable octopus fisheries, and evidence-based policymaking in Kaur District.

MATERIALS AND METHODS

Study area

This research was conducted from April to November 2024 at the Fish Auction Site located at Linau Port, Linau Village, Maje Sub-District, Kaur District, Bengkulu Province, Indonesia (4°50'34"S, 103°24'46"E). Linau Port is located on the western coast of Sumatra Island, facing the Indian Ocean. The area is characterized by a tropical coastal ecosystem

with sandy substrates and serves as an active landing site for small-scale fisheries, particularly those targeting reef-associated species such as octopuses. This site was selected due to its consistent year-round landings of octopus, making it a representative and strategic location for sampling and data collection. This site was selected due to its consistent year-round octopus landings, which reflect the typical fishing activity and ecological characteristics of the broader coastal region. The fishing practices, target species, and environmental conditions at this site are comparable to those found in neighboring areas, thereby ensuring that the data collected can be generalized to represent the wider region. Consequently, this location is considered both representative and strategic for sampling and data collection.

Data collection

Octopus specimens were collected from the catches of traditional fishers using handline fishing gear specifically designed for octopus capture, with all sampling taking place during daylight hours. A total of 68 specimens were obtained from the coastal waters of Kaur District, consisting of 20 males and 48 females, resulting in a male-to-female ratio of 1:2.4. Morphological assessments and morphometric measurements were performed immediately at the Fish Auction Site in Linau Port (Figure 1). Morphological assessment involved the visual examination of key external features, while morphometric measurements were conducted using standardized protocols with a measuring tape to ensure accuracy. These procedures aimed to capture detailed physical characteristics of the octopus specimens for subsequent analysis.

For molecular analysis, a tissue sample was excised from one of the octopus arms and preserved in a 100 mL vial containing 96% ethanol for subsequent analysis. The molecular analysis was then conducted at the Laboratory of Aquatic Resources Management, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia.

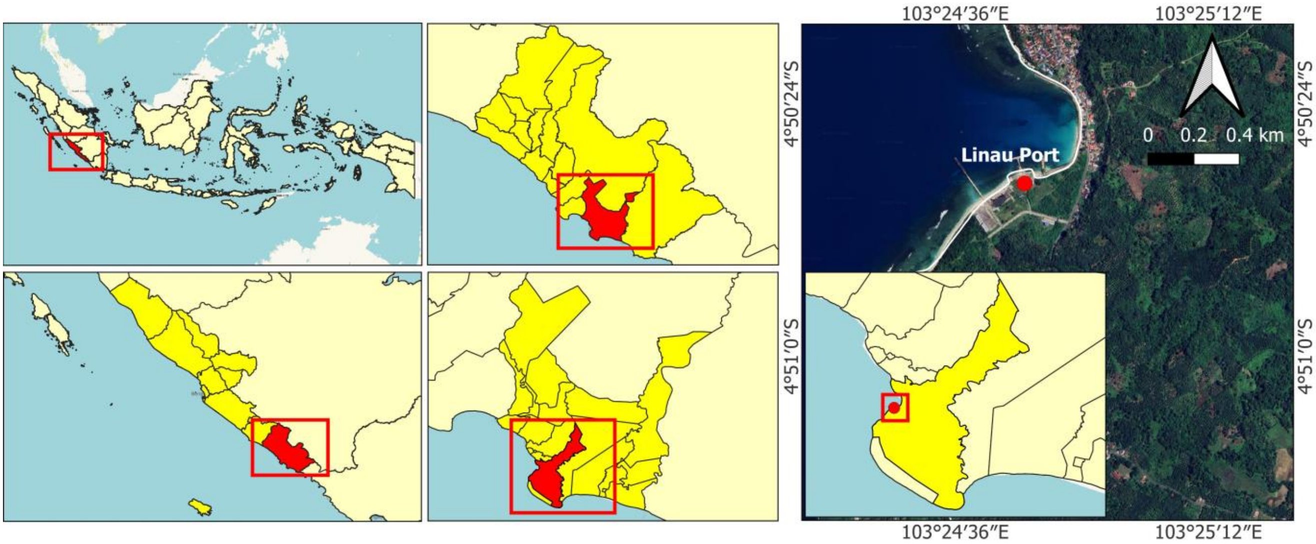


Figure 1. Research site for octopus sampling at Linau Port, Linau Village, Maje Sub-District, Kaur District, Bengkulu, Indonesia

Morphological and morphometric identification

The data collected comprised 15 morphological characters of octopus body sections, modified according to the measuring methodologies established by Jereb et al. (2016). These morphometric measurements included: Total Length (TL), Arm Length (AL), Arm Sucker Count (ASC/n), Web Depth (WD), Arm Sucker Diameter normal (ASD), Arm Sucker diameter enlarged (ASe), Ligula Length (LL), Calamus Length (CaL), Mantle Length (ML), Mantle Width (MW), Head Width (HdW), Head Length (HdL), Eye Office diameter (EO), Funnel Length (FuL), and Pallial Aperture extent (PA). Each parameter was measured using tape measure to the nearest 0.1 mm, ensuring consistency and precision across all samples. The examination of morphological and morphometric characteristics for species comparison followed the guidelines outlined in the FAO Species Catalogue for Fishery Purposes No. 4, Vol. 3: Cephalopods of the World, An Annotated and Illustrated Catalogue of Cephalopod Species Known to Date—Octopods and Vampire Squids (Jereb et al. 2016).

Molecular identification and analysis

Muscle tissue from octopus arms was dissected into small fragments, each weighing approximately 20-25 mg (Treleven et al. 2024). DNA extraction was performed using the Favorgen Tissue Genomic DNA Extraction Mini Kit, following a standardized protocol comprising four essential steps: lysis (to disrupt cellular structures), DNA binding (to adhere DNA to a silica membrane), washing (to eliminate impurities), and elution (to recover purified DNA). This method produced high-quality genomic DNA suitable for subsequent molecular analyses.

Amplification of the Cytochrome c Oxidase subunit I (COI) gene was carried out using the universal primers LCOI 1490 (Forward: 5'-GGTCAACAAATCATAAAGATAT TGG-3') and HCOI 2198 (Reverse: 5'-TAAACTTCAGGGTG ACCAAAAAATCA-3'), as described by Folmer et al. (1994). The Polymerase Chain Reaction (PCR) was set up in a 50 µL reaction volume, comprising 25 µL of PCR master mix (Favorgen), 1 µL of each primer, 1 µL of DNA template, and 23 µL of nuclease-free water. The amplification was conducted using a T100™ Thermocycler (Bio-Rad, Germany), with thermal cycling conditions as follows: initial denaturation at 94°C for 5 minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing at 48°C for 30 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 1 minute.

The amplified products were purified and submitted to PT. Genetics Science Indonesia for DNA sequencing. The resulting sequences were aligned and compared with those in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool for Nucleotides (BLAST-N). Species identification was based on sequence similarity percentages and identity scores, following the approach of Yu et al. (2019).

Multiple sequence alignment of the COI gene was conducted using the CLUSTAL W algorithm to identify conserved and variable regions. Phylogenetic analysis was subsequently performed using the Neighbor-Joining method in MEGA 11 software (Tamura et al. 2021), with genetic distances calculated using the Kimura 2-Parameter (K2P) model. Node confidence was assessed with 1000 bootstrap replications to ensure the reliability of the phylogenetic relationships inferred.

RESULTS AND DISCUSSION

Morphological characteristics

Based on observations of morphological characteristics, the octopus species found in the waters of Kaur District was suspected to be *Octopus cyanea* (Figure 2). The morphological documentation from this study, compared with reference sources, is presented in Table 1.

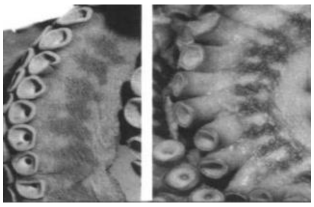
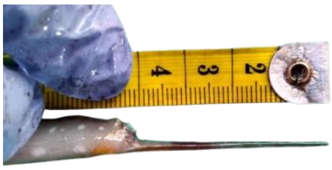
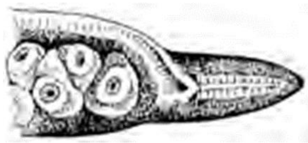
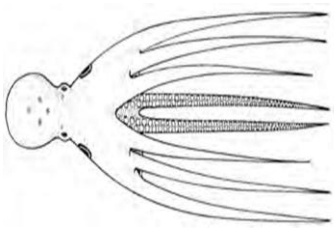
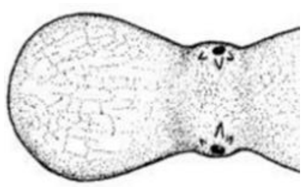
Based on Table 1, the octopus species found in the waters of Kaur District was morphologically suspected to be *Octopus cyanea*. In addition to the characteristics listed in Table 1, the octopus exhibits other distinguishing morphological features. The body (mantle) is oval-shaped, with a medium-sized head and relatively small eyes. The funnel (siphon) is W-shaped. Male individuals possess two rows of larger suckers on their arms. The structure of the arm membrane is classified as type "1A," while the hectocotylus is located on the third right arm, featuring a small ligula and calamus. The octopus's skin color is predominantly dark brown and white, adorned with false eyes (ocelli), black stripes, and scattered white spots on the arms. Additionally, four small papillae protrusions are present on its body surface, further contributing to its morphological identification.

Morphometric characteristics

The specimens were identified as *Octopus cyanea* based on morphological characteristics. Subsequent to the morphological examination, the collected data were systematically processed using Microsoft Excel and subjected to Principal Component Analysis (PCA) to rigorously assess the morphometric characteristics of octopus specimens from Kaur waters.

Figure 2. *Octopus cyanea* from Kaur waters

Table 1. Morphological characteristics of octopuses from Kaur waters compared with *Octopus cyanea* based on references

Morphological characteristics of octopuses from Kaur waters	**Description**	**Morphological characteristics of *O. cyanea* based on references**	**Description**
	Complete morphology of the octopus from Kaur waters		*O. cyanea* (Jereb et al. 2016)
	Black spots (zebra pattern) on the arms		Zebra pattern on the arms of *O. cyanea* (Jereb et al. 2016)
	Hectocotylus present on the third arm of the male		Hectocotylus of the male *O. cyanea* (Jereb et al. 2016)
	Four papillae protrusions on the mantle		Four papillae protrusions on *O. cyanea* (Jereb et al. 2016)
	Three spots near the eyes		Three spots near the eyes of *O. cyanea* (Jereb et al. 2016)
	Presence of false eyes (ocelli)		Ocelli on *O. cyanea* (Jereb et al. 2016)

Most of the specimens were female, with a male-to-female ratio of approximately 1:2.4. This disparity in sex ratio is likely attributed to differences in behavioral patterns and habitat preferences between the sexes. Female octopuses tend to be more sedentary and are often found inhabiting shallow coastal areas for extended periods, particularly during the brooding phase, which increases their likelihood of being captured during sampling. In contrast, male octopuses are generally more mobile and may occupy deeper or less accessible habitats, resulting in lower capture rates. Figure 3 illustrates a schematic representation of the measured morphometric parameters, encompassing 15 anatomical regions of the octopus (see Table 2). This analysis provides insight into the variability in body size among the sampled individuals. Octopuses are gonochoric animals, meaning they have separate sexes and do not undergo sex changes throughout their lifespan. The sex of an octopus can be distinguished by examining the arms, where males possess a specialized reproductive structure known as the hectocotylus, located on the third arm on the right side of the body.

Morphometric analysis of 15 anatomical features of octopuses from Kaur waters indicated distinct variability in body size among individuals within the population. The greatest range was observed in Total Length (TL), implying a high degree of variation in arm development, which is a prominent morphological characteristic in octopuses. Conversely, Arm Length (AL) appeared relatively uniform, suggesting lower variability across specimens. The count of Arm Suckers (ASC/n) demonstrated substantial fluctuation, which may be attributed to ontogenetic stages or environmental influences.

Mantle characteristics displayed moderate levels of variation, highlighting their crucial role in facilitating movement and shielding internal organs. The diameters of both typical and enlarged suckers (ASD and ASe) exhibited limited variation, indicating consistency in this trait among individuals. Head dimensions—specifically Head Width (HdW) and Head Length (HdL)—also showed relatively stable proportions within the studied population. Moreover, the sizes of reproductive structures, such as the Ligula (LL) and Calamus (CaL), were notably consistent, supporting their functional association with reproductive maturity. In summary, the population demonstrated a mixture of stable and variable morphometric features. Differences in body length, sucker count, and mantle measurements may reflect environmental adaptation or developmental dynamics, while the observed uniformity in other anatomical structures may point to functional or evolutionary constraints.

Table 2. Morphometric measurements of the Kaur aquatic octopus (n = 68)

Acronym	Mean±standard deviation	Description
ML (Mantle Length)	7.5±2.5 cm	Measured from the midpoint between the eyes to the posterior end of the mantle
AL (Arm Length)	45.5±4.0 cm	Measured from the beak to the tip of the arm
ASe (Arm Sucker diameter enlarged)	0.6±0.3 cm	Diameter of the largest sucker
ASD (Arm Sucker Diameter normal)	0.4±0.3 cm	Diameter of a typical sucker
ASC/n (Arm Sucker Count)	63.3±19.8	Number of suckers along a specific intact arm (up to the tip)
CaL (Calamus Length)	3.8±2.1 cm	Length from the distal-most sucker to the distal tip of the calamus
EO (Eye Orifice diameter)	0.3±0.2 cm	Diameter of the eye opening
FuL (Funnel Length)	3.0±1.2 cm	Length of the funnel from the anterior opening to the posterior border, measured along the ventral midline
HdL (Head Length)	2.6±1.6 cm	Curvature diameter along the body's anterior-posterior axis
HdW (Head Width)	4.1±1.4 cm	Greatest width of the head at the level of the eyes
LL (Ligula Length)	3.8±1.9 cm	Length from the distal-most sucker to the tip of the hectocotylus arm
MW (Mantle Width)	6.2±2.3 cm	Greatest straight-line (dorsal) width of the mantle
PA (Pallial Aperture extent)	4.8±1.7 cm	Distance between the two points of mantle attachment to the head along the ventral margin
TL (Total Length)	57.0±17.7 cm	Length from the tip of the longest arm to the posterior end of the mantle
WD (Web Depth)	8.2±3.8 cm	Depth of the deepest web sector, measured from the beak to the midpoint of the sector

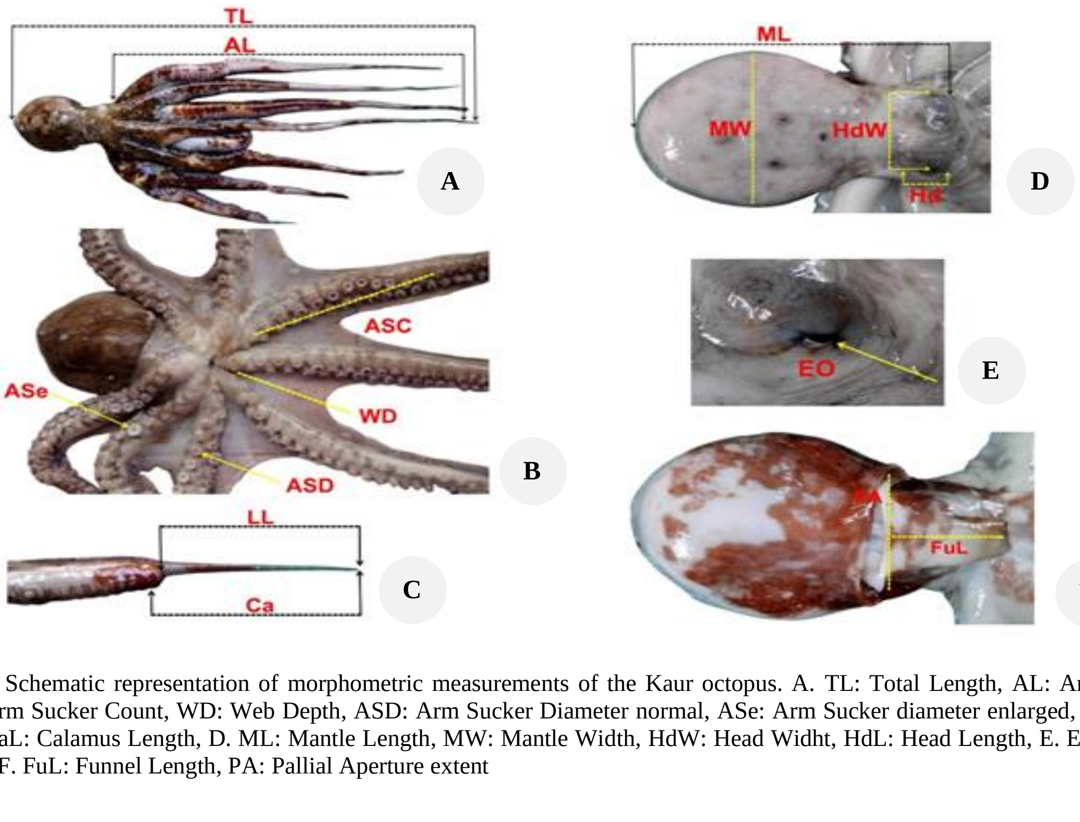


Figure 3. Schematic representation of morphometric measurements of the Kaur octopus. A. TL: Total Length, AL: Arm Length, B. ASC/n: Arm Sucker Count, WD: Web Depth, ASD: Arm Sucker Diameter normal, ASe: Arm Sucker diameter enlarged, C. LL: Ligula Length, CaL: Calamus Length, D. ML: Mantle Length, MW: Mantle Width, HdW: Head Widht, HdL: Head Length, E. EO: Eye Office diameter, F. FuL: Funnel Length, PA: Pallial Aperture extent

The morphometric parameter ratios of male and female octopuses from Kaur waters are summarized in Table 3. Notable differences and similarities between sexes were observed. For example, females tend to have a relatively wider mantle compared to total length, which may be related to reproductive adaptations such as increased space for egg development and energy storage. Mantle length relative to total length was consistent across sexes, indicating conserved structural features likely essential for physiological functions. The presence of the ligula in males, absent in females, aligns with its reproductive role. Variations in funnel length to pallial aperture extent and web depth to arm length ratios suggest potential sexual dimorphism influenced by ecological and biological factors. These differences might also be shaped by environmental conditions, such as food availability and predation pressures, which affect morphological development and sex-specific adaptations.

PCA of the morphometric data from fifteen body parts of octopuses (Figure 4) shows that male octopuses are represented by the light blue area, while female octopuses are represented by the orange area. Two principal components, Dim1 and Dim2, account for 66.6% and 13.3% of the data variation, respectively, explaining 79.9% of the total variation.

Dim1 indicates that most of the variation in the dataset was influenced by primary body size measurements, such as Ligula Length (LL), which is part of the reproductive organ in male octopuses, as well as Mantle Length (ML) and Mantle Width (MW). In contrast, Dim2 reflects additional variation, most likely associated with Head Length (HdL) and Eye Diameter (EO). The biplot shows that most samples are concentrated around the center of the coordinate graph, indicating a strong similarity in morphometric size among the majority of male and female octopuses. However, some samples, such as numbers 49 and 68, are positioned far from the main cluster, suggesting distinct morphometric differences from the rest of the samples. This suggests that there may be greater individual variation within the population, or that environmental factors could be influencing their growth. The possibility of environmental adaptations, such as variations in habitat complexity, prey availability, or microhabitat conditions, may contribute to these outliers.

The blue vectors in the graph represent the contribution of each variable to the principal components. Variables such as Ligula Length (LL) and Calamus Length (CaL) contribute significantly to Dim1, whereas Eye Diameter (EO) and Head Length (HdL) have a greater influence on Dim2. The relationships between variables can be observed through the angles between the vectors. For instance, nearly parallel vectors, such as those for Mantle Length (ML) and Mantle Width (MW), indicate a strong positive correlation, while variables with angles close to 90° suggest a weak correlation. Samples positioned close together on the graph reflect similar morphometric sizes, whereas those more dispersed indicate significant variation.

These findings suggest that the morphometric variation of octopuses in the waters of Kaur was primarily determined by overall body size, with additional factors such as head or eye shape playing a lesser role. Samples separated from the main cluster tend to have larger body sizes than the majority. Further analysis is needed to determine the underlying causes whether related to adaptation to specific environmental conditions or other influencing factors. Overall, the PCA results indicate that the morphological variation of octopuses in the waters of Kaur District was largely influenced by differences in morphometric size.

Electrophoresis results

Figure 5 presents the electrophoresis results of the Cytochrome Oxidase Subunit I (COI) gene from *O. cyanea* following PCR amplification. The amplified DNA fragments appear as single, distinct bands in each sample. These bands range from approximately 600 to 700 base pairs (bp) in length, aligning with the expected amplification target when using Folmer universal primers (LCO1490 and HCO2198). The uniform band size and intensity across all lanes indicate successful amplification of the COI gene, with no additional bands or smearing—typically associated with DNA degradation, contamination, or inefficient amplification. This result confirms the high purity of the extracted DNA, making it suitable for further molecular analysis, specifically DNA sequencing to determine nucleotide sequences. The COI gene is commonly used in DNA barcoding, which aids in species identification and the study of genetic diversity within populations. By comparing COI sequences from different populations of *O. cyanea*, population-specific variations can be identified and genetic differences assessed, potentially revealing adaptations to varying environmental conditions. Furthermore, COI gene analysis can be employed to determine phylogenetic relationships, providing insights into the evolutionary history of the species in relation to other species within the same genus or family.

Sequencing analysis results

The BLAST analysis confirmed that the octopus samples from Kaur waters exhibited 100% genetic similarity to *O. cyanea*. Based on reference databases, these findings strongly support the identification of the specimens as *O. cyanea*. This analysis demonstrates the effectiveness of DNA barcoding using the COI gene for octopus species identification, particularly in samples collected from the study site. The results further confirm that the octopus species analyzed belong to *O. cyanea*. Table 4 summarizes the species identification results based on BLAST analysis.

Table 3. Ratio of morphometric parameters of male and female octopuses from Kaur waters

Morphometric parameter ratio	Male octopuses	Female octopuses
MW/TL	0.10	0.11
ML/TL	0.13	0.13
MW/ML	0.79	0.82
AL/TL	0.80	0.79
FuL/PA	0.6	1.00
WD/AL	0.17	0.18
LL/AL	0.25	0.00

Note: MW: Mantle Width, TL: Total Length, ML: Mantle Length, AL: Arm Length, FuL: Funnel Length, PA: Pallial Aperture Extent, WD: Web Depth, LL: Ligula Length

Table 4. BLAST-based species identification of *Octopus* samples from Kaur waters

Sampling location (Beach)	Sample code	Identified species	Sequence size (bp)	Query cover (%)	Percent identity (%)	Accession number	Accession number reference
Kaur	K1	*Octopus cyanea*	631	100	100	PQ967972	MW556237 (Jayapura, Indonesia)
Kaur	K2	*Octopus cyanea*	631	100	100	PQ967973	MW556233 (Buton, Indonesia)
Kaur	K3	*Octopus cyanea*	631	100	100	PQ967974	MW556231 (Wakatobi, Indonesia)

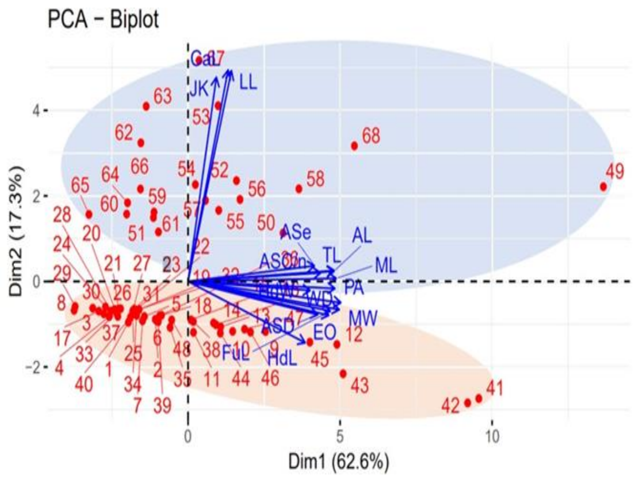


Figure 4. PCA morphometric analysis of 15 body parts of male and female of *Octopus cyanea* from Kaur waters

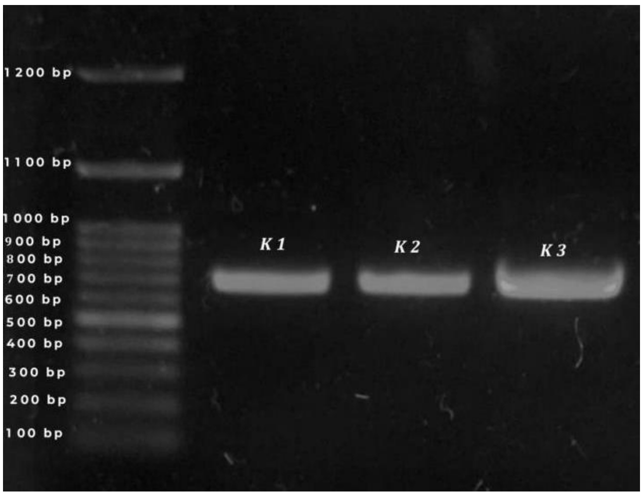


Figure 5. Electrophoresis results of *Octopus cyanea* samples from Kaur waters

Analysis of the COI gene sequences revealed that the three octopus samples from Kaur waters K1, K2, and K3 were successfully identified as *O. cyanea*. Each sample exhibited a sequence length of 631 base pairs (bp), with a Query Cover value of 100%. The Percent Identity value also reached 100%, indicating a perfect match with the reference sequences in GenBank. Sample K1 matched accession number PQ967972, corresponding to MW556237 from Jayapura, Indonesia. Sample K2 exhibited similarity to accession number PQ967973, corresponding to MW556233 from Buton, Indonesia. Sample K3 matched accession number PQ967974, corresponding to MW556231 from Wakatobi, Indonesia.

These genetic similarities suggest that the *O. cyanea* population in Kaur waters shares a close phylogenetic relationship with *O. cyanea* specimens from various regions of Indonesia. Furthermore, this finding supports the notion that *O. cyanea* is a dominant species in tropical and subtropical marine ecosystems. The high Query Cover and Percent Identity values also highlight the robustness of the molecular approach in achieving accurate species identification.

Analysis of *Octopus cyanea* genetic distance from Kaur waters

Genetic distance analysis was conducted using the COI gene-based pairwise distance approach to determine the genetic proximity or divergence of octopus specimens from Kaur waters in comparison to those from other locations. Genetic distance measurements indicate the degree of genetic similarity or dissimilarity between two specimens. This information can be used to assess kinship relationships, genetic distribution patterns, and potential population differences. Table 5 presents the results of the genetic distance analysis for *O. cyanea* specimens from Kaur waters.

Based on the genetic distance analysis in Table 5, the *O. cyanea* samples from Kaur District (Kaur 1 Indonesia, Kaur 2 Indonesia, and Kaur 3 Indonesia) exhibited a genetic distance value of 0.000, indicating that these three samples share identical genetic sequences without any nucleotide variation. This finding suggests that all three samples originate from the same haplotype. The consistency of these results indicates an absence of genetic differentiation within the local population, which may be attributed to limited genetic variation or a genetically homogeneous population in the study area. When compared to *O. cyanea* samples from other regions, such as Jayapura (Indonesia), Buton (Indonesia), Wakatobi (Indonesia), Madagascar, Osaka (Japan), and Kerala (India), the genetic distance values ranged from 0.000 to 0.004. These low values suggest a close genetic relationship, indicating that the *O. cyanea* haplotypes from these locations were highly similar or identical to those found in Kaur District. The widespread similarity of these haplotypes might be attributed to the high larval dispersal potential facilitated by ocean currents, allowing for genetic exchange among *O. cyanea* populations across different regions.

Table 5. Genetic distance of *Octopus cyanea* from Kaur waters based on the COI gene pairwise distance method

No.	Sample names	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	PQ967972 *Octopus cyanea* (Kaur 1, Indonesia)																			
2	PQ967973 *Octopus cyanea* (Kaur 2, Indonesia)	0.000																		
3	PQ967974 *Octopus cyanea* (Kaur 3, Indonesia)	0.000	0.000																	
4	MH289829 *Octopus tetricus* (Tasmania, Australia)	0.131	0.131	0.131																
5	MK187267 *Octopus insularis* (Gulf Mexico)	0.126	0.126	0.126	0.117															
6	KP976310 *Octopus vulgaris* (Wuhan, China)	0.144	0.144	0.144	0.041	0.130														
7	KF489450 *Octopus cyanea* (Kerala, India)	0.000	0.000	0.000	0.131	0.126	0.144													
8	MK593402 *Octopus cyanea* (Madagascar)	0.004	0.004	0.004	0.135	0.130	0.149	0.004												
9	AB191280 *Octopus cyanea* (Osaka, Japan)	0.000	0.000	0.000	0.131	0.126	0.144	0.000	0.004											
10	MW556231 *Octopus cyanea* (Wakatobi, Indonesia)	0.000	0.000	0.000	0.131	0.126	0.144	0.000	0.004	0.000										
11	LC553345 *Amphioctopus aegina* (Cirebon, Indonesia)	0.109	0.109	0.109	0.117	0.143	0.135	0.109	0.113	0.109	0.109									
12	LC553363 *Hapalochlaena fasciata* (Tuban, Indonesia)	0.147	0.147	0.147	0.198	0.184	0.193	0.147	0.152	0.147	0.147	0.148								
13	LC552307 *Octopus laqueus* (Karimun Jawa, Indonesia)	0.084	0.084	0.084	0.126	0.130	0.143	0.084	0.088	0.084	0.084	0.121	0.174							
14	MW556237 *Octopus cyanea* (Jayapura, Indonesia)	0.000	0.000	0.000	0.131	0.126	0.144	0.000	0.004	0.000	0.000	0.109	0.147	0.084						
15	MW559642 *Octopus sp.* (Bangka Belitung, Indonesia)	0.080	0.080	0.080	0.139	0.130	0.148	0.080	0.084	0.080	0.080	0.143	0.165	0.081	0.080					
16	MW556233 *Octopus cyanea* (Buton, Indonesia)	0.000	0.000	0.000	0.131	0.126	0.144	0.000	0.004	0.000	0.000	0.109	0.147	0.084	0.000	0.080				
17	MW556224 *Octopus cyanea* (Wakatobi, Indonesia)	0.000	0.000	0.000	0.131	0.126	0.144	0.000	0.004	0.000	0.000	0.109	0.147	0.084	0.000	0.080	0.000			
18	MW556220 *Octopus cyanea* (Lombok, Indonesia)	0.000	0.000	0.000	0.131	0.126	0.144	0.000	0.004	0.000	0.000	0.109	0.147	0.084	0.000	0.080	0.000	0.000		
19	MW556216 *Octopus cyanea* (Anambas, Indonesia)	0.004	0.004	0.004	0.135	0.130	0.149	0.004	0.007	0.004	0.004	0.113	0.152	0.088	0.004	0.084	0.004	0.004	0.004	

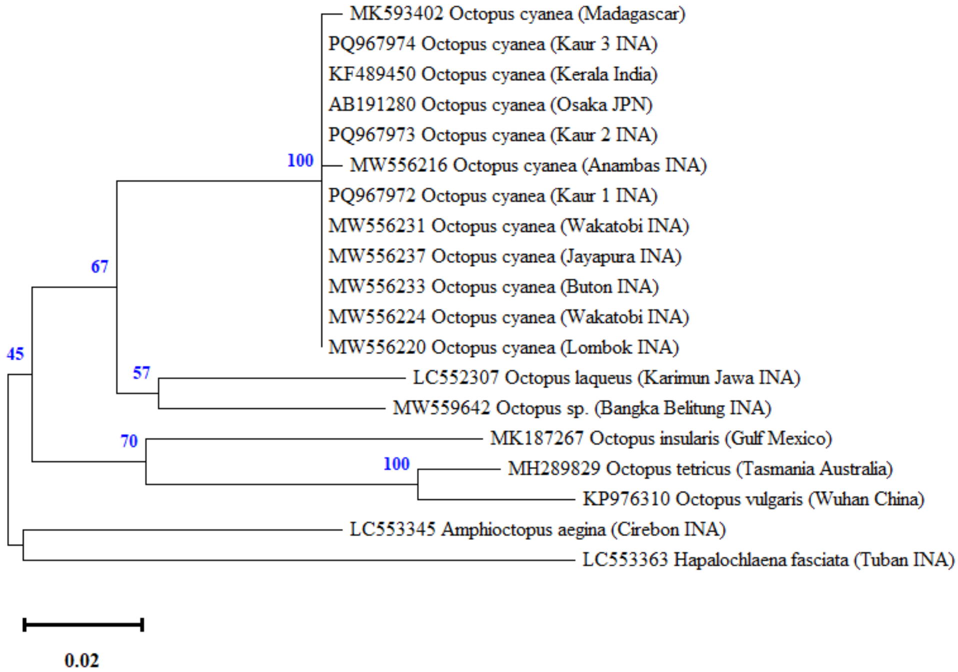


Figure 6. Phylogenetic tree of *Octopus cyanea* from Kaur waters

This high level of genetic similarity, especially among Indonesian populations such as those from Jayapura, Buton, and Wakatobi, suggests the presence of significant gene flow facilitated by oceanographic connectivity. The Indonesian Throughflow and regional current systems may act as natural conduits, enabling the widespread dispersal of *O. cyanea* larvae and promoting genetic mixing across distant coastal regions. As such, the observed low genetic distances are likely a reflection of both historical and ongoing gene flow between populations. These findings carry important implications for the conservation and management of *O. cyanea* in Indonesia and beyond. The genetic connectivity between populations suggests that conservation strategies should adopt a regional or ecosystem-based management approach, rather than treating populations as isolated units. Effective management plans should consider the interconnectedness of habitats and account for the role of ocean currents in sustaining genetic diversity. Additionally, the existence of shared haplotypes across geographically distant areas highlights the need for collaborative, cross-regional policies to ensure the sustainable exploitation and long-term viability of *O. cyanea* resources.

In contrast, significantly higher genetic distances were observed between *O. cyanea* and other species, such as *Octopus tetricus* (Gould, 1852) (Tasmania, Australia), with a genetic distance of 0.131, and *Octopus vulgaris* (Wuhan, China), with a genetic distance of 0.144. These values reflect substantial haplotype differences and indicate strong genetic divergence between species. This considerable genetic distance aligns with taxonomic distinctions at the species level, further emphasizing the role of genetic distance in supporting molecular identification and species classification. Overall, the genetic distance and haplotype analysis suggest that the *O. cyanea* population in Kaur District belongs to a widely distributed haplotype with extremely low genetic variation.

Phylogenetic analysis

Phylogenetic trees illustrate that *Octopus cyanea* from Kaur waters is closely related to *O. cyanea* samples from Wakatobi, Jayapura, Buton, Lombok, and Anambas (see Figure 6). The three samples from Kaur—Kaur 1, Kaur 2, and Kaur 3—formed a well-supported clade with a bootstrap value of 100, indicating a high degree of confidence in their genetic relationship. This group also exhibits genetic proximity to samples from outside Indonesia, including Madagascar, Kerala (India), and Osaka (Japan). This kinship suggests that *O. cyanea* from Kaur shares a common lineage with other populations across the Indo-Pacific region. The findings indicate the possibility of widespread genetic distribution in this species, likely influenced by environmental factors and oceanic currents in the Indo-Pacific.

However, the phylogenetic tree also reveals substantial genetic divergence between *O. cyanea* from Kaur and other *Octopus* species, such as *Octopus laqueus* from Karimunjawa, *Octopus* sp. from Bangka Belitung, *Octopus insularis* from the Gulf of Mexico, and *Octopus tetricus* from Tasmania (Australia). The high bootstrap values in some clades indicate a greater evolutionary distance, suggesting significant genetic differentiation. Additionally, species such as *A. aegina* from Cirebon and *H. fasciata* from Tuban are positioned at the base of the tree, further highlighting their evolutionary distinctness from *O. cyanea*. Overall, the phylogenetic tree demonstrates substantial genetic diversity within the *O. cyanea* species. While there are clear genetic links between populations in Indonesia and some regions beyond, considerable variation still exists in their relatedness compared to other *Octopus* species.

Discussion

This study describes the aquatic environment of Kaur as a tropical habitat that supports high biodiversity, including octopus populations. Morphologically, the octopus specimens found exhibit characteristics similar to *O. cyanea*. This species is among the most found octopus species in tropical and subtropical waters (Jereb et al. 2016) and among the most widespread octopus species in Indonesian waters (Noegroho et al. 2023). Previous studies have reported the morphological identification of *O. cyanea* in the Makassar Strait and Bone Bay (Junedi et al. 2020), as well as in the waters of Sikka District, East Nusa Tenggara (Lamalelang et al. 2024). Additionally, molecular studies have confirmed the presence of *O. cyanea* in the waters of West Java (Bagaskoro 2018) and North Aceh (Ramadhaniaty et al. 2025). The species has also been recorded in the Anambas Islands, Lombok, Buton, Wakatobi, and Jayapura (Kholilah et al. 2021).

The octopus samples obtained from Kaur waters comprised 68 individuals, consisting of 20 males and 48 females, with a sex ratio of 1:2.4. Similarly, a study conducted by Amir et al. (2021) on *O. cyanea* from Bone Bay reported that, out of 1,647 individuals, 263 were males and 1,384 were females, resulting in a sex ratio of 1:6.26. The primary morphological difference between male and female octopuses lies in the presence of a specialized reproductive organ in males, known as the hectocotylus (Hutagaol et al. 2019), which is modified into a copulatory organ located on the third right arm. The hectocotylus functions to transfer sperm into the female's body cavity. These findings align with previous studies (Guard and Mgaya 2002; Raberinary and Benbow 2012), which reported that *O. cyanea* populations consist of both males and females, similar to other cephalopods. The sex ratio of this species varies across different regions and often deviates from the theoretical 1:1 ratio, with females predominating in many populations. This trend has been observed in waters off Tanzania, Australia, and Madagascar. Hamad et al. (2025) further support these findings, reporting a sex ratio of 1:1.4 for *O. cyanea* caught in Zanzibar, with males being relatively fewer than females.

Octopus cyanea is one of the most commonly found diurnal octopus species in coral reef areas across the tropical Indo-Pacific Ocean (Hanlon 2024). This species belongs to the cephalopod group, functioning as both a predator and prey in various marine ecosystems (Toha et al. 2015) and represents a high-value fishery resource for many countries (Clark 2019). Its most prominent morphological characteristics include eight long, flexible arms, suckers arranged in two rows, a hectocotylus in males, and distinct body color patterns such as dark stripes and white spots at the tips of the arms (Hochner 2008). These unique biological traits make octopuses highly relevant models for research across various disciplines (O'Brien et al. 2018).

Octopuses can squeeze through narrow crevices, using their eight arms equipped with suckers for various activities such as swimming, capturing prey, opening shells, digging, and building nests (Hochner 2008). Their suckers perform multiple extraordinary functions, including anchoring to substrates, grasping, manipulating, and exploring objects (Kuba et al. 2006). The mantle and siphon facilitate respiration, enabling octopuses to swim rapidly and expel water jets to deter predators. In threatening situations, octopuses can release ink into the water to obscure their location from predators and hide in crevices or holes in substrates with diameters of 10-30 cm at depths of up to 45 meters (Yarnall 1969). They also utilize natural crevices or holes as shelters or gather rocks and shells to build nests (Hanke and Kelber 2020). Octopuses exhibit rapid growth rates and relatively short lifespans. Males die after mating, while females die shortly after their eggs hatch. During the brooding period, females can lose 30-60% of their initial body weight (Estefanell et al. 2010) and lay eggs only once in their lifetime before dying (Ivey 2007). Furthermore, octopuses possess several unique and fascinating biological characteristics, including high adaptability, large brains, and advanced sensory organs. These features enable cephalopods to adapt quickly to environmental challenges, both natural and anthropogenic, making them resilient organisms capable of thriving amid changes in marine ecosystems (Di Cosmo et al. 2021).

A morphometric analysis of 68 octopus samples from the waters of Kaur indicated that female octopuses exhibit larger body proportions than males in several aspects. These differences were observed in multiple parameters, including the ratio of Mantle Width to Total Length (MW/TL), the ratio of Mantle Length to Mantle Width (ML/MW), the ratio of Funnel Length to Funnel Aperture (FuL/PA), and the ratio of Web Depth to Arm Length (WD/AL). Environmental factors play a crucial role in determining the length, morphometric size, body weight, and numerical abundance of the octopus population (Roura et al. 2019). Morphometric measurements provide insights into stock composition, aiding in estimating octopus size availability, mortality rates, growth, reproduction, and life cycles. This information is essential for determining the appropriate size range of octopuses for capture in a given aquatic environment (Tarigan et al. 2020).

Molecular identification using DNA barcoding of the COI gene through GenBank NCBI confirmed that the octopus species found in the waters of Kaur District is *O. cyanea*, with Query Cover and Percent Identity values reaching 100%. This finding indicates that the octopus samples from Kaur waters exhibited a very high degree of similarity with reference data available in GenBank NCBI. The high degree of similarity observed may be attributed to the fact that *O. cyanea* is a widely distributed species, commonly found in tropical and subtropical waters, including several regions of Indonesia. The consistency of its genetic sequences across various geographical locations, including the waters of Kaur, suggests a stable genetic profile for this species in the region. Moreover, this high degree of similarity may indicate the genetic stability of *O. cyanea* populations in Indonesian waters, where environmental conditions and ecological factors likely promote the persistence and uniformity of its genetic composition. Query Cover and Percent Identity are used to measure sequence alignment with the database, supporting species identification (Ratnasingham and Hebert 2007). In

O. cyanea found in the waters of Kaur, oceanographic factors such as prevailing currents and the species' planktonic larval phase are believed to facilitate genetic exchange among populations across various regions. This results in limited genetic differentiation at the COI gene level, leading to nearly identical sequences across populations, including those found in the waters of Kaur.

Molecular methods provide greater accuracy than morphological approaches, particularly in differentiating octopus species with high morphological similarity. The use of molecular markers has been widely applied for precise species identification, with DNA barcoding being the most commonly used method (Böhme et al. 2019). DNA barcoding allows species-level identification even when morphological characteristics are no longer distinguishable (Schindel and Miller 2005). Therefore, molecular identification based on COI gene DNA barcoding serves as a crucial genetic marker for distinguishing closely related species in marine ecosystems (Gebhardt and Knebelsberger 2015). Studies conducted by Ritschard et al. (2019), Yalla and Mohanraju (2019), Kholilah et al. (2021), Petrić et al. (2023), and Mulyani et al. (2024) support these findings. They state that DNA barcoding using universal primers LCO1490 and HCO2198 (Folmer 1994) on COI sequences demonstrated high accuracy in octopus species identification. This establishes DNA barcoding as a highly useful method for compiling a baseline dataset for future octopus DNA barcoding research and development.

Genetic distance and phylogenetic analyses revealed that *O. cyanea* from Kaur waters shares a close genetic relationship with specimens from other Indo-Pacific marine regions, including Jayapura (Indonesia), Buton (Indonesia), Wakatobi (Indonesia), Madagascar, Osaka (Japan), and Kerala (India). The genetic distance values ranged from 0.000 to 0.004, indicating that the haplotypes of *O. cyanea* from these locations are highly similar and, in some cases, identical to those found in Kaur waters. In contrast, significantly greater genetic distances were observed between *O. cyanea* and other species, such as *Octopus tetricus* from Tasmania (Australia) (0.131) and *Octopus vulgaris* from Wuhan (China) (0.144). A lower genetic distance signifies a higher similarity between populations, reflecting close genetic relationships among them. Phylogenetic tree reconstruction illustrates the evolutionary relationships among species, tracing their divergence from a common ancestor and highlighting evolutionary linkages. This phylogenetic analysis is based on comparisons of DNA base sequences, which generate a tree estimating past evolutionary processes (Ramadhaniaty et al. 2025). Geographic factors also significantly influence the genetic distance of octopuses. The life cycle of *O. cyanea* includes a pelagic larval stage, during which its distribution is shaped by ocean currents and other geographical factors. The broad dispersal range of larvae plays a key role in determining population structure and the species' adaptability to its habitat (Andriyono et al. 2022).

The findings of this study provide insights into the genetic distribution of *O. cyanea* in the Indo-Pacific marine region, emphasizing the importance of both morphological and molecular approaches in supporting the sustainable management of octopus stocks. Species identification through these approaches is crucial for stock assessment, providing essential data for the sustainable management of octopus resources (Bagaskoro 2018). However, knowledge regarding octopus population ecology and habitat preferences remains limited, both in the Indian Ocean and globally. Expanding this knowledge base is critical for effective conservation efforts (Bhandari 2020).

The findings of this study indicate that the octopus specimens collected from the waters of Kaur District belong to *Octopus cyanea*, as supported by distinctive morphological features, including an oval-shaped mantle, a W-shaped funnel, two rows of suckers on the arms, and the presence of ocelli and papillae on the body, which serve as key diagnostic traits for the species. Morphometric analysis reveals that female individuals tend to have larger proportions than males in several key measurements, such as Mantle Width to Total Length (MW/TL), Mantle Width to Mantle Length (MW/ML), Funnel Length to Funnel Aperture (FuL/PA), and Web Depth to Arm Length (WD/AL). Molecular analysis of the COI gene from three samples further substantiates this identification, with a Query Cover and Percent Identity of 100% when compared to reference data in the GenBank NCBI database. The morphometric analysis also reveals sexual dimorphism, with females generally exhibiting larger body proportions in several parameters. Phylogenetic analysis indicates that *O. cyanea* from Kaur shares a close genetic relationship with populations from various regions in Indonesia and shows genetic similarity with populations from Madagascar, India, and Japan. These results suggest that the *O. cyanea* population in Kaur District is part of a broader genetic network across Indo-Pacific marine regions, highlighting the distribution dynamics and genetic connectivity of populations across tropical and subtropical ecosystems. The waters of Kaur feature a sandy seabed substrate, with water temperatures ranging from 28.0 to 32.0°C, water clarity reaching 100%, Dissolved Oxygen (DO) levels between 5.0 and 8.8 mg/L, salinity ranging from 32 to 35 ppt, and a pH level between 7.2 and 8.1. Additionally, nitrate and phosphate concentrations were recorded at 0.07-0.1 mg/L and 0.006-0.1 mg/L, respectively (Munru et al. 2023). Environmental factors such as water temperature, salinity, seabed substrate, and food availability play a critical role in supporting and maintaining healthy octopus populations in the region, while also influencing their distribution patterns. Therefore, the integration of these environmental parameters into population models is essential for achieving a comprehensive understanding of *O. cyanea* ecology, predicting shifts in distribution patterns, and enhancing conservation strategies aimed at ensuring the long-term sustainability of octopus populations in Kaur District and the broader Indo-Pacific marine area.

Overall, this study provides valuable baseline data on the morphology, morphometrics, and molecular characteristics of *O. cyanea* in the waters of Kaur District. These findings can serve as a reference for future research, inform conservation strategies, and support the development of fishery management plans to secure the long-term viability of this important cephalopod species within the Indo-Pacific marine ecosystem.

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