Morphology and molecular identification on octopus (Genus *Octopus* Cuvier, 1798) caught in Palabuhanratu, Sukabumi, West Java

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**Abstract.** Octopus (genus *Octopus*) is a member of family Octopodidae with two valid species in Indonesia. From fishery point of view octopus utilization has started to increase, although, the effort to catch it is still limited. This research was aimed to identify octopus (genus *Octopus*) collected from Palabuhanratu Bay morphologically, with validation by means of molecular technique. This research was done from January to June 2017. Thirty-seven individuals were obtained, and were analyzed morphologically and molecularly. Morphological identification showed that samples have a characteristic similarity to *Octopus cyanea*. Arm Suckers Count (ASC) was the most diverse morphometrical character. Gene characteristics of the samples have 99% similarity to *Octopus cyanea* gene that stored in NCBI site’s database. Molecular analysis obtained similarity on genetic characteristics for all samples. Based on morphology characteristic and molecular validation, all samples were determined as *Octopus cyanea*.

**1. Introduction**

Octopus (Genus *Octopus*) is a member of family Octopodidae. The name of this genus comes from the Greek language: *octo* (eight) and *podos* (legs). The numbers of genus Octopus species member reach 301 species [1]. There are two valid genus *Octopus* species in Indonesia: *Octopus cyanea* [2] and *Octopus pyrum* [3]. The octopus has eight arms attached at head, has a beak-shaped mouth, has two rows of suckers under each arm, and has hectocotylus arm in male species [4]. The octopus can be
found in oceans around the world [5], with rocky and sandy bottom habitat. The octopus has a habitat distribution range from intertidal to depth water up to 1,000 m (continental slope) [6].

Octopus resources have been the target in Indonesian fishery recently. The octopus production value of Indonesia in 2010 is about 10,860 tons, the fourth largest in Asia [7]. On May 2017, Indonesia exported octopus for about 30 tons to Japan [8]. Indonesian octopus potential is quite high and can be developed further as an export commodity.

As fishery resources octopus has become a potential commodity to develop in the future. However, to get best sustainable management of the resources, some biological information of the octopus is still limited, such as the species richness of octopus, habitat, distribution, etc. In addition, the octopus catch is still subsistence to small-scale commercial fisheries [7], so the octopus production is lower compared to other fishery resources. As a consequence, the octopus production is still under demand in the domestic market, especially due to its unappealing morphological shape [9]. On top of that, there is no program from the Ministry of Marine Affairs and Fisheries of Indonesia to promote and manage octopus resources particularly.

As mentioned above, management is needed to balance the recent octopus utilization effort with its development potential. One of the needed biological information for effective resources management is species certainty [10]. Therefore, identification is needed to describe the existing species as basic important information for octopus resources management. This research was aimed to identify octopus (genus Octopus) in Palabuhanratu Bay morphologically, with validation by means of molecular technique.

2. Materials and methods

2.1. Time and location
This research was conducted from January to June 2017 and the samples were collected from Palabuhanratu Bay, Sukabumi Regency, West Java (Figure 1). Morphological characters description and molecular analysis were performed in Molecular Biology Laboratory, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, IPB University.

![Figure 1. Map of Palabuhanratu Bay, Sukabumi, West Java where the octopus samples were collected.](image)
2.2. Morphological analysis
A total of 37 individual samples were collected from local fishermen. The samples were stored in frozen condition and were transported to the laboratory for morphology, morphometric, and molecular analysis. Morphological identification was conducted by observing the external morphology with an identification key for Octopod [7] (Figure 2 & 3). Octopus morphology was described according to Roper and Voss [11]. Morphometric data was collected by measurement of 15 characters [12], i.e. total length (TL) and eye orifice diameter (EO) (Figure 4a); web depth (WD), arm length (AL), and arm sucker diameter (ASD) (Figure 4b); ligula length (LL) and calamus length (CaL) (Figure 4c); mantle length (ML), head length (HdL), and head width (HdW) (Figure 4d); pallial aperture extend (PA) and funnel length (FuL) (Figure 4e, Table 1). The measurement was conducted using the standard ruler with accuracy 0.5 mm.

![Figure 2. Morphological scheme of octopus.](image)

![Figure 3. Web types of octopus.](image)

| Code | Character                          | Definition                                                                 |
|------|------------------------------------|--------------------------------------------------------------------------|
| ML   | Mantle length                      | Length from the midpoint between eyes to the posterior end of the mantle. |
| AL   | Arm length                         | Arm length measured from beak to tip of the arm.                         |
| ASE  | Arm sucker diameter enlarged       | Diameter of arm suckers enlarged.                                        |
| ASD  | Arm sucker diameter normal         | Diameter of arm suckers normal.                                          |
| ASC  | Arm sucker count                   | Number of suckers along entire intact designated arm (to arm tip).       |
| CaL  | Calamus length                     | Length from last (distal-most) sucker to calamus distal tip.             |
| EO   | Eye orifice diameter               | Diameter of the opening of the eye.                                      |
| FuL  | Funnel length                      | The length of the funnel from the anterior opening to the posterior border measured along the ventral midline. |
| HdL  | Head length                        | Diameter of orbits along the anteroposterior axis of the body.          |
| HdW  | Head width                         | The greatest width of head at the level of eyes.                         |
| LL   | Ligula length                      | Length from distal-most sucker to the tip of hectocotylized arm.         |
| MW   | Mantle width                       | Greatest straight-line (dorsal) width of the mantle.                     |
| PA   | Pallial Aperture Extent            | Length between the points of attachment of the mantle to the head along the ventral margin of the mantle. |
| TL   | Total length                       | Length from end of the longest arm to the posterior end of the mantle.   |
| WD   | Web depth                          | Length of deepest web sector from beaks to the midpoint of the sector.   |
2.3. Molecular analysis

The muscle from the base of the arm was cut off with weight of 60 mg. The muscle sample was split into two, one part was used for analysis and the other part was stored in ethanol 96% as a reserve. DNA Extraction was conducted using DNA Extraction Kit for tissue (Gene Aid). There were four steps in DNA extraction: lysis, DNA binding, DNA washing, and DNA elution by following manual procedure from extraction kit.

The 16S rRNA gene was amplified using Polymerase Chain Reaction (PCR) technique. The amplification required 5 µl of DNA sample, 3 µl of 16S rRNA primer (Butet, unpublished), 12.5 µl of KAPA Taq HotStart, and 4.5 µl of ddH₂O. The PCR was processed with sequential steps, i.e. pre-denaturation at 94 °C for 3 min, denaturation at 94 °C for 45 sec, annealing at 46 °C for 1 min, elongation at a 72 °C for 1 min and 15 sec, post-elongation at 72 °C for 5 min. Denaturation, annealing, and elongation steps were repeated for 30 cycles. PCR product was sent to DNA sequencing service company for sequencing analysis using Sanger method.

2.4. Data Analysis

2.4.1. Morphology data. The morphology identification was conducted by comparing morphological characteristic on octopus sample with reference species with known morphological description. The
The reference species was octopus species member from genus *Octopus* in Indonesian waters, i.e. *O. cyanea* and *O. pyrum*.

The Principal Component Analysis (PCA) was constructed from morphometric measurement data using software R [13]. The PCA analysis was created to reveal morphometric character with the highest diversity. Every morphometric character was paired with each other, then the covariant value was calculated from each pair of morphometric characters. Next, the eigenvalue and eigenvector value were determined from the calculated covariant value. The eigenvalue and eigenvector value were used to determine the order of the principal component. Last, the data transformation showed the order of morphometric character diversity.

2.4.2. *Molecular data.* Molecular identification was conducted by matching the gene sequence data with the database from the National Center for Biotechnology Information (NCBI) with Basic Local Alignment Search Tool-Nucleotide (BLAST-N). This result would be showed the most similar gene characteristic between gene sequence from sample and database. Nucleotide base was aligned with the Clustal W method [14] in MEGA 7 software [15]. Genetic distance was determined by Pairwise Distance method. The grouping of 16S rRNA gene sequence data was established by the Neighbor-Joining method with Pairwise Distance Kimura 2 Parameter (K2P) substitution model option and 1000 times bootstrap.

3. Results and Discussion

3.1. Results

3.1.1. *Morphological description of octopus sample.* The morphological characteristic of octopus samples was described at eight body parts. The observed body parts were mantle, head, funnel, arm, sucker, web, hectocotylus, and integument. The morphological description of the eight parts was shown in Table 2.

| Morphological Character | Description |
|-------------------------|-------------|
| Mantle                  | Oval-shaped, composed of thick muscles, does not have a rigid structure. |
| Head                    | Medium-shaped, slightly rigid structure at dorsal part, small eyes. |
| Funnel                  | W-shaped funnel, large-sized, attach at pallial aperture extend. |
| Arms                    | Long and narrow size, two rows of sucker on the ventral part. |
| Suckers                 | Enlarged suckers present at male species, the size of suckers shrink toward the tip of the arm. |
| Web                     | Type 1 web shape. |
| Hectocotylus            | *Hectocotylus* present at the third arm on the right side of the body (arm 3R), has tiny ligula and calamus at the tip of the arm. |
| Integument              | Dark chocolate brown color at the dorsal part and white color at the ventral part, a pair of ocelli present, dark black bars along the lateral part of the arms and white spots at the tip of the arm present, four small papillae at the dorsal part of the mantle forms diamond arrangement, has patch and groove texture on the dorsal part and smooth texture on the ventral part. |

3.1.2. *Morphological identification on samples.* The morphological comparison showed that octopus samples had more characteristic similarity with *O. cyanea* species rather than *O. pyrum* species (Table 3). Characteristic similarities with *O. cyanea* were found in the mantle, head, funnel, arms, suckers, webs, hectocotylus, and integument. Meanwhile, characteristic similarities with *O. pyrum* were found only in the mantle, funnel, and arms. Therefore, based on the character similarity, octopus sample was morphologically identified as *Octopus cyanea*.
3.1.3. Morphometric analysis. The most diverse morphometric character was arm sucker count (ASC); then followed by total length (TL) and arm length (AL) (Figure 5). High diversity morphometric character could not be used as a character in morphometric identification.

3.1.4. Molecular sample identification. Molecular identification showed that Octopus sample was identified as *O. cyanea*. Gene characteristics on samples showed 99% similarity with *O. cyanea* gene data from NCBI site. This molecular analysis validates the morphological identification result.

![Figure 5. The Principal Component Analysis (PCA) from morphometric measurement of the octopus samples collected from Palabuhanratu Bay, West Java.](image)

### Table 3. Morphology comparison between octopus sample with *Octopus cyanea* and *Octopus pyrum*.

| Morphological Character | *Octopus cyanea* [7]                                      | *Octopus pyrum* [3]                                      | Octopus sample                                      |
|-------------------------|----------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------|
| Mantle                  | Oval-shaped                                              | Oval-shaped                                              | Oval-shaped                                         |
| Head                    | Medium-sized, small eyes                                 | Medium-sized, large eyes                                 | Medium-sized, small eyes                             |
| Funnel                  | W-shaped                                                 | W-shaped                                                 | W-shaped                                            |
| Arm                     | Two rows of suckers                                       | Two rows of suckers                                       | Two rows of suckers                                 |
| Suckers                 | Enlarged suckers on male species                         | No enlarged suckers on male species                       | Enlarged suckers on male species                     |
| Web                     | Type 1A                                                  | Type 2                                                   | Type 1A                                             |
| Hectocotylus            | Position in arm 3R, tiny ligula and calamus              | Position in arm 3R, large ligula and calamus              | Position in arm 3R, tiny ligula and calamus          |
| Integument              | Dark chocolate brown and white color; Ocelli present, dark black bars and white spots on arms; has 4 small papillae sculpture. | Orange and cream color; numerous scatter leucophores and chromatophores present; has numerous papillae sculpture. | Dark chocolate brown and white color; Ocelli present, dark black bars and white spots on arms; has 4 small papillae sculpture. |

3.1.5. Genetic distance analysis of 16S rRNA gene. The genetic distance of interspecies and with outgroup species showed in Table 4. All samples had a very close genetic proximity with distance of 0.0000, except the sample with code GBP 28.1 (0.002). Meanwhile, the genetic distance of *Octopus* samples with outgroup species *O. vulgaris* had a distance of 0.501 and with *Abdopus aculeatus* has a distance of 0.132-0.135.

3.1.6. Phylogenetic analysis. Phylogenetic tree construction illustrated that octopus samples were grouped in one branch (Figure 6). *A. aculeatus* and *O. vulgaris* species made a separate branch because of the
higher genetic distance than the samples. *A. aculeatus* showed closer proximity with sample species rather than *Octopus vulgaris*.

Table 4. The genetic distance of interspecies and with outgroup species.

| No | Octopus Species          | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|----|--------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1  | *O. cyanea* (GBP 19.1)   | -   |     |     |     |     |     |     |     |     |     |     |     |
| 2  | *O. cyanea* (GBP 23.1)   | 0.000 | -   |     |     |     |     |     |     |     |     |     |     |
| 3  | *O. cyanea* (GBP 25.1)   | 0.000 | 0.000 | -   |     |     |     |     |     |     |     |     |     |
| 4  | *O. cyanea* (GBP 27.1)   | 0.000 | 0.000 | 0.000 | -   |     |     |     |     |     |     |     |     |
| 5  | *O. cyanea* (GBP 28.1)   | 0.002 | 0.002 | 0.002 | 0.002 | -   |     |     |     |     |     |     |     |
| 6  | *O. cyanea* (GBP 29.1)   | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | -   |     |     |     |     |     |     |
| 7  | *O. cyanea* (GBP 30.1)   | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | -   |     |     |     |     |     |
| 8  | *O. cyanea* (GBP 31.1)   | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | -   |     |     |     |     |
| 9  | *O. cyanea* (GBP 32.1)   | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | -   |     |     |     |
| 10 | *O. cyanea* (GBP 34.2)   | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | -   |     |     |
| 11 | *O. cyanea* (GBP 35.1)   | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | -   |     |
| 12 | *O. vulgaris* (LC063337.1) | 0.501 | 0.501 | 0.501 | 0.501 | 0.501 | 0.501 | 0.501 | 0.501 | 0.501 | 0.501 | 0.501 | 0.515 |
| 13 | *A. aculeatus* (GQ900717.1) | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 | 0.135 | 0.155 |

**Figure 6.** Phylogenic constructions according to 16S rRNA gene on octopus samples and outgroup species.

3.2. Discussion

Octopus samples have eight arms at the oral part of the body, with length with four to six times from the length of mantle [7]. Webs are located between each arm with width 15-23% of the arm length. Each arm has two rows suckers with the number vary from 450 to 500 suckers. Male reproductive organ is a hectocotylus arm, located at the third arm on the right side of the body, with tiny ligula and calamus. The Octopus samples has a dark brown color, with dark bars pattern along the lateral side of the arm, and white spots at the tip of the arm. This species has a pair of ocelli (a dark large spot pattern) on the base dorsal part of the third arm. Four papillae with diamond formation presented at the dorsal part of the mantle. According to the characteristic, octopus sample was identified as *Octopus cyanea* (Figure 7).
The distribution of *Octopus cyanea* area was at tropical Indo-West Pacific ocean from the east coast of Africa to Hawaii, and from southern Japan to northern Australia [7]. *Octopus cyanea* had the habitat range from intertidal area to 22-meter depths. This species could be found in the holes and crevices in coral reefs, seagrass beds, and across rock, sand, or mud bottom areas of the ocean [16]. This species was active in the daytime with the highest activity in the dawn and dusk. This species could be found in Indonesia and had higher population than other species [17].

The morphometric analysis showed that the most diverse morphometric character was arm suckers count (ASC). However, the ASC character could not be used in identification because it has wide data spread could not be compared with the available literatures. The morphometric character that could be used as a character in identification process must have low diversity.

Molecular analysis showed similarities in 16S rRNA gene characteristics of all samples. The result showed that the octopus population in Palabuhanratu bay is only one population. When a nucleotide sequence from two or more different organism had similarities, it could be suspected that these organisms were inherited from a common ancestor sequence [18].

Phylogenic tree construction displayed that Octopus sample (*O. cyanea*) had close proximity with *A. aculeatus* rather than *O. vulgaris*. The sample had similarities in morphology feature with *O. vulgaris* in male reproductive organ structure and suckers but had genetic proximity with *Abdopus* subgenus [19]. This condition was caused by a polyphyletic condition in family Octopodidae [20] and genus *Octopus* [21]. *Octopus cyanea* species had proximity with clade *Abdopus* rather than clade *Octopus vulgaris* [19].

Species information is important for management of octopus resources in Palabuhanratu bay. Species information was needed to understand if the exploited octopus resources came from the same species or from the same management unit (stock) [22]. Stock was the smallest management unit and could be a population conspecific individual species or sub-species with a low degree of connectivity, and stock should be monitored and managed separately [23]. Therefore, morphological and molecular identification was important to understand octopus stock in Palabuhanratu bay.

4. Conclusions
Based on morphology characteristic and molecular validation, all samples were identified as *Octopus cyanea*. Arm Suckers Count (ASC) was the most diverse morphometric character. Molecular analysis indicated the similarity of genetic characters for all samples.

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