

Morphological and molecular identifications of ectoparasites infesting the gills of scalloped spiny lobster *Panulirus homarus* Linnaeus, 1758

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Abstract. Kismiyati, Hidayat YT, Amin M. 2024. Morphological and molecular identifications of ectoparasites infesting the gills of scalloped spiny lobster *Panulirus homarus* Linnaeus, 1758. *Biodiversitas* 25: 372-378. Ectoparasite infestation has been reported to cause stress, weaken the immune system, and disturb respiratory systems, which lead to death in cultured spiny lobsters. Many lobster farmers have reported an infestation of ectoparasites in cultured scalloped spiny lobsters in West Nusa Tenggara, Indonesia. However, fewer studies have been conducted to identify the ectoparasite-infesting scalloped spiny lobster cultured in West-Nusa Tenggara Province. Thus, using morphological and molecular approaches, the present study aimed to identify ectoparasites infesting scalloped spiny lobsters (*Panulirus homarus* Linnaeus, 1758). A total of 30 scalloped spiny lobsters were collected from lobster farmers at Ekas, East Lombok, West Nusa Tenggara, Indonesia, and ectoparasites were observed and identified morphologically under a microscope as well as confirmed using CO1 gene sequences. The results showed that the ectoparasites were morphologically identified as *Octolasmis angulata* Aurivillius, 1894 and *Octolasmis lowei* Darwin, 1852. Similarly, based on the mtDNA sequence of the COI region (~721 bp length), the ectoparasite was also referred to *O. angulata* (95.65% similarity) and *Octolasmis lowei* (~683 bp length) with a similarity of 98.75%. These results suggest that ectoparasite has become one potential disturbance in lobster aquaculture; therefore, preventing or curing strategies should be developed.

Keywords: Ectoparasite, lobster, molecular, morphology, *Octolasmis*

INTRODUCTION

Due to their high economic value, spiny lobsters (*Panulirus* spp.) are one of Indonesia's most important fisheries commodities. According to data from the Ministry of Marine Affairs of Indonesia, Indonesia produced 12,438.2 tons of spiny lobster valued at US\$ 257,620 in 2021 (KKP 2022). This number has increased by 22.35% compared to 2020, with a value of more than US\$ 315,198. Previously, the lobster supply was provided mostly from wild catch by fisherman catches. However, the lobster supply has been provided by aquaculture industries for the last few years (Amiri et al. 2022). One of the Lobster aquaculture centers in Indonesia is Ekas Bay, located in the southern part of Lombok Island, West-Nusa Tenggara Province, Indonesia. These waters have relatively calm water conditions with a very large expanse of coral reefs. Lobster fishing in this area is quite intensive. Scalloped spiny lobster (*Panulirus homarus* Linnaeus, 1758) is a type of lobster that fishermen often obtain as a catch in the East Lombok area, West Nusa Tenggara. However, according to the Ministry of Marine Affairs, Indonesia's scalloped spiny lobster export decreased during 2021-2023. From 1,959 tons in 2021 to 1,469 tons in 2022, sharply decreasing to only 281.608 kg in 2023. Preliminary studies indicated that diseases, including ectoparasites, caused the decrease. Previous studies also showed that ectoparasite infestations

may cause stress, weaken host immunity, and affect the respiratory system (gills) to death.

Several authors have reported ectoparasites that infested scalloped spiny lobster, and a preliminary study was previously identified mostly until genus level, which was *Octolasmis* spp. (Barzakh et al. 2022). *Octolasmis* is a Class Maxillopoda parasite often found in lobsters, crabs, and shrimps (Suherman and Arsad 2020). Other studies suggest that ectoparasite is a potential bioindicator of water pollution, not only for the risks contained within the contaminated area but also for sensitivity to environmental changes. Therefore, it is important to identify more specifically what possible species serve as specific bioindicators of heavy metals. In Malaysia, a study by Ihwan et al. (2014) identified ectoparasites, which are frequently infested in scalloped spiny lobster, and found two *Octolasmis* species (*Octolasmis lowei* Darwin, 1852 and *Octolasmis warwicki* Gray, 1825) on mud spiny lobster (*Panulirus polyphagus* Herbst, 1793) from Mersing, Johor Malaysia. According to Nur et al. (2021), *Octolasmis* pose a threat to host populations and even cause death. However, such studies have not been performed on lobster farmers in Ekas Bay until now; therefore, such studies should be performed.

Ectoparasite identification methods are divided into two types, phenotypic and molecular identifications. According to Lim et al. (2010), the phenotypic identification of parasitic species can be performed by observing

morphological and anatomical characteristics through a light microscope. Several key identifiers were the number of capitular plates, scuta, terga, and carina (Suherman and Arsad 2020). Molecular profiling of *Octolasmis* ectoparasites can be done through the analysis of short fragments of mitochondrial DNA (mtDNA) sequences of the *Cytochrome C Oxidase subunit I* (COI) region (Shen et al. 2016). According to Maulid et al. (2016), COI has specific characteristics such as mitochondrial DNA (mtDNA), which has no introns, and maternal vertical inheritance. In addition, the amplification process is easy, and the sequence results are much better. Therefore, it is necessary to conduct exploratory research to identify ectoparasite-infesting spiny lobster in East Lombok, West Nusa Tenggara, Indonesia.

MATERIALS AND METHODS

Sample collection

A total of 30 scalloped spiny lobsters 5-20 cm in length or 200-300 g of weight were collected from Lobster farmers at Ekas, East Lombok, West Nusa Tenggara Province, Indonesia. Then, the gills of each scalloped spiny lobster were examined for the presence of ectoparasites (Figure 1). Parasites found in the gills of scalloped spiny lobsters were collected in microtubes containing different fixative solutions, 95% ethanol for molecular analysis purposes or 10% formalin for morphological analysis purposes.

Morphological identification

The ectoparasites were identified based on the identification key by Mathison and Pritt (2014). In brief, specific morphological characteristics were observed, including the presence or absence of tergum, scutum, carina, cirri, peduncle, and capitulum. In addition, the shape and size of the capitulum, the presence or absence of a calcareous plate (and its variations), and morphometrics to the color of certain organs are the other identification keys.

Molecular Identification

DNA extraction

Ectoparasite DNA was extracted according to a protocol of Amin et al. (2023a) with some modifications. Firstly, the ectoparasite tissue sample was cut into small pieces with a tissue rupturer and put in a 1.5 mL sterile tube. Then, 180 µL ATL buffer and 20 µL Proteinase K were added, vortexed, and incubated at 56°C for 1-3 hours. Then, 200 µL of AL buffer was added and mixed thoroughly by a vortex for 15 sec, followed by incubation at 70°C for 10 min. After that, 200 µL of absolute ethanol was added and vortexed for 15 sec. The mixture was transferred to a QIAamp Mini spin column and centrifuged at 6,000× *g* for 1 min. The QIAamp Mini spin column was placed in a new 2 mL sterile tube, followed by the addition of 500 µL of AW1 buffer. The tube was centrifuged at 6,000 × *g* for 1 min, continued by adding AW2 buffer. The next step was to centrifuge at 13,000× *g* for 3 min. After that, the QIAamp Mini spin column was placed in a new 1.5 mL sterile tube, followed by the addition of 200 µL of

AE buffer and incubated at room temperature for 1 min, centrifuge at 6,000× *g* for 1 min to elute the DNA. The quality of the extracted DNA was determined by visualizing the DNA using a blue light transilluminator.

Amplification of COI gene

The amplification stage was performed with the Polymerase Chain Reaction (PCR) technique using a thermal cycler machine according to a protocol of Folmer et al. (1994) with some modifications. The total PCR reaction was 50 µL reactants consisting of 10 µL of template DNA, 1.5 µL Primer F, 1.5 µL R each, 23 µL dH₂O, 5 µL 10× PCR Buffer, 5 µL of 2 mM dNTPs, 3 µL of 25 mM MgSO₄ and 1 µL KOD-Plus-Neo. The PCR mixture was then run under the following conditions: initial 94°C for 3 min, 30 cycles (denaturation 94°C for 45 sec, 46°C annealing for 2 min, and 72°C extension for 1 min), and final extension 72°C for 5 min. The primers used were universal primers covering the COI region, namely Primer LCO1490:(5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198:(5'-TAAACTTCAGGGTGACCAAAAAATCA-3').

Gel electrophoresis

The electrophoresis stage needs to be done to confirm the amplified DNA sample. At this stage, 1% agarose gel electrophoresis is used in 1× TBE solution. The agarose solution was allowed to warm up and poured into an electrophoresis mold that had added 2 µL of ethidium bromide as a DNA dye. Next, 5 µL of PCR DNA was mixed with 1 µL loading dye and inserted into the well slowly. The comb is attached to the end of the electrophoresis bath and allowed to stand until the agarose solution hardens (gel). Furthermore, the electrophoresis process (running) was carried out with a voltage of 100 volts for 30 minutes (Sundari and Priadi 2020). Then, visualization was carried out on a UV transilluminator, and documentation was conducted using GelDoc. The thickest band was selected as a sample for the sequence stage. The sequence stage was optimized with temperature according to the sequence kit reagent instructions. Samples of PCR sequence results were re-purified before proceeding to the sequencing machine. The purification results in second amplicons were added with a special buffer, namely Hi-DiTM Formamide, and sequenced using ABI PRISM®310 Genetic Analyzer (Yudarana 2022).

Sequencing

The sequence of amplified gene can determine a species identity of octolasmis samples through primer reads. One database that can be used to compare COI sequences is GenBank (NCBI). Several sequences of *Octolasmis* species have deposited in the National Center for Biotechnology Information (NCBI). There are several database search techniques, namely <https://www.ncbi.nlm.nih.gov/> (United States GenBank website), selecting "BLAST" on the home menu, then selecting Nucleotide Blast as the analysis program, and after entering the DNA sequence in the program by blocking the sequence data (in FASTA format) and duplicating it on the available form, then selecting

"RUN BLAST." The results will appear after the loading process (Rukmana 2015).

Phylogenetic analysis

The relationship between *Octolasmis* ectoparasites was established using the Maximum Likelihood method based on COI DNA sequences. Maximum Likelihood is a character-based statistical method that compares all sequences in an alignment to calculate the likelihood value for each tree (Bromham 2016). This method considers all possible numbers of changes or mutations in the sequence for each tree; therefore, it is suitable for constructing phylogenies with a small number of sequences. The phylogenies in bootstrap methods were used repeatedly to observe the tree arrangement's validity level with several replications of 1000× to shorten the construction time (Dharmayanti 2011).

RESULTS AND DISCUSSIONS

Morphological identification

Moreover, 5 colonies of parasites were found mostly on the gills of the lobsters. Based on organ character shape, morphological analysis was identified as *Octolasmis angulata* Aurivillius, 1894 and *O. lowei*. *O. angulata* has a sprout-like shape. Its body parts consist of cirri (Ci), scutum (Sc), carina (Ca), capitulum (C), and peduncle (P). The color of *O. angulata* tends to be pale brown, especially on the capitulum. The scutum was thin and inverted L-shaped. The carina was thin and wide in the horizontal part. The anterior part consists of an oval-shaped capitulum without tergum and is protected by several limestone skeletal plates in certain parts. The plate consists of a pair of scutum and carina. There is an elongated peduncle on the posterior part. The morphological observation of *O. angulata* can be seen in Figure 2.

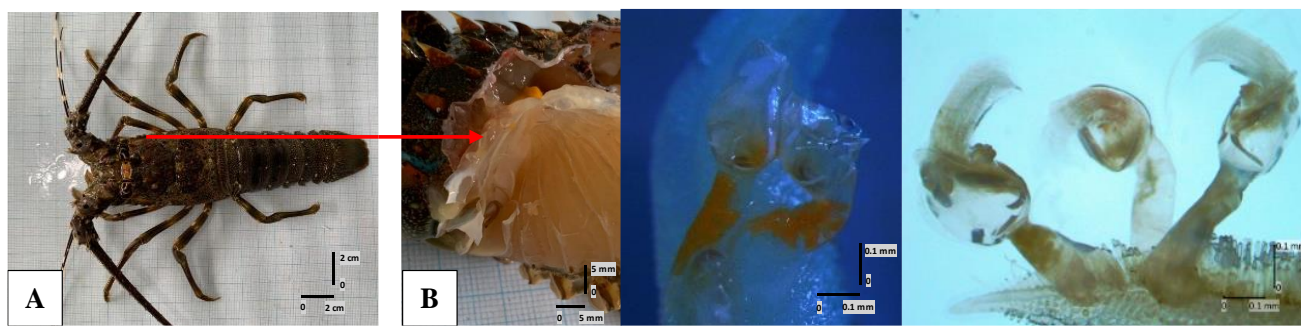


Figure 1. Cross-section of the sample: A. Scalloped spiny lobster; B. Colonies of *Octolasmis* ectoparasites attached to lobster gill lamellae macroscopically and microscopically

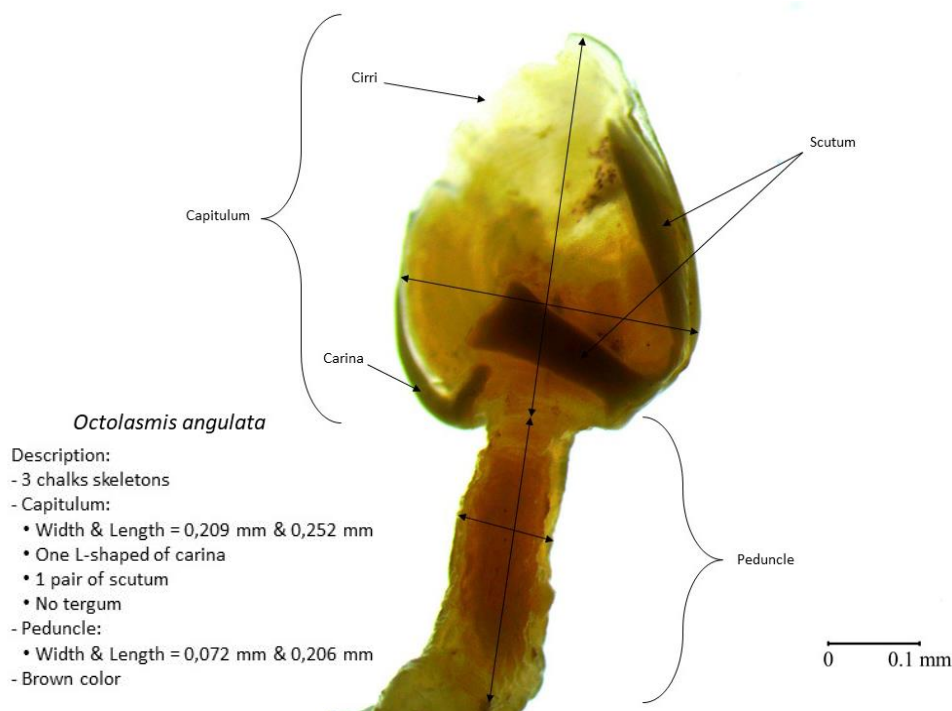


Figure 2. Morphological cross-section of *Octolasmis angulata* through *Optilab Viewer 4.0.0* software on a binocular stereo microscope, and through Lucida camera on a 1x magnification binocular stereo microscope and its parts: C: Capitulum; Ca: Carina; Ci: Cirri; P: Peduncle; Sc: Scutum. Scale = 0.1 mm

The *Octolasmis* specimens found were also measured on several organs, such as the capitulum and peduncle. The capitulum of *O. angulata* has three plates or consisted of three sections. Morphometrics aims to determine the average size of organs that characterize each specimen found; therefore, based on the results of measurements, the morphometric data of organs such as the capitulum and peduncle is obtained. *O. angulata* observed in this study has a capitulum length and width of 3.2 mm and 2.1 mm, and the length and width of the peduncle are 2.5 mm and 1.1 mm, respectively.

Based on the identification of *Octolasmis*, another ectoparasite specimen found besides *O. angulata* is *Octolasmis lowei*. This *Octolasmis* has shape characteristics similar to other species of the genus *Octolasmis*, which is like a swan's neck. The capitulum (C) is located in the anterior part, and the peduncle or stalk is in the posterior part. The cirri organ is not yet visible in this species.

The capitulum is oval and pale yellow with white plates. On the posterior part, there is an elongated peduncle (P). Several organs have morphological characteristics in the capitulum, including the presence of an inverted U-shaped tergum (T), an L-shaped carina (Ca), and a pair of scutum (Sc), which is inverted L-shaped with a blunt posterior end. The morphological observations of *O. lowei* can be seen in Figure 3.

Based on the measurement results, morphometric data of organs such as the capitulum and peduncle were obtained. *Octolasmis lowei* observed in this study had a peduncle length and width of 4.1 mm and 1.0 mm and a capitulum length and width of 3.2 mm and 2.1 mm, respectively. In addition to morphometrics, there are meristic data on one of the organs of the *Octolasmis*

ectoparasite. *Octolasmis lowei* observed in this study has a capitulum with five plates or five sections.

Molecular identification

Electrophoresis of PCR products of COI gene amplified using primers LCO1490 and HCO2193 with 1% agarose showed a single band at 721 bp. The qualitative test results of *O. angulata* PCR products show that the amplification of these samples has good results. This is characterized by a band of approximately 721 bp with a thick and visible DNA band. The electrophoresis band of the *O. angulata* sample can be seen in Figure 4.

Based on COI sequences, octolasmis sample found on the gills of wild-caught scalloped spiny lobster (*P. homarus*) in Ekas Waters, East Lombok, West Nusa Tenggara was identified as *O. angulata* with 95.65% similarity to NCBI data. COI sequences were translated with the help of a sequencing machine and sequence scanner software. The total sequence of *O. angulata* COI ectoparasite is 1-721 bp.

Moreover, the PCR electrophoresis process successfully detected DNA bands on *O. lowei*. The results obtained in the qualitative test showed that the COI gene was amplified at a base length of 683 bp with a fairly thick and visualized band quality. The electrophoresis band of the *O. lowei* sample is shown in Figure 5. Based on 683 bp DNA sequence of *O. lowei* found in the gills of wild-caught scalloped spiny lobster (*P. homarus*) in Ekas waters, East Lombok, West Nusa Tenggara, the octolasmis showed 98.75% to *O. lowei* sequence deposited in NCBI. In the electrophoresis results, no smear was found to indicate DNA quality; the more there is no or little smear, the better the quality of DNA.

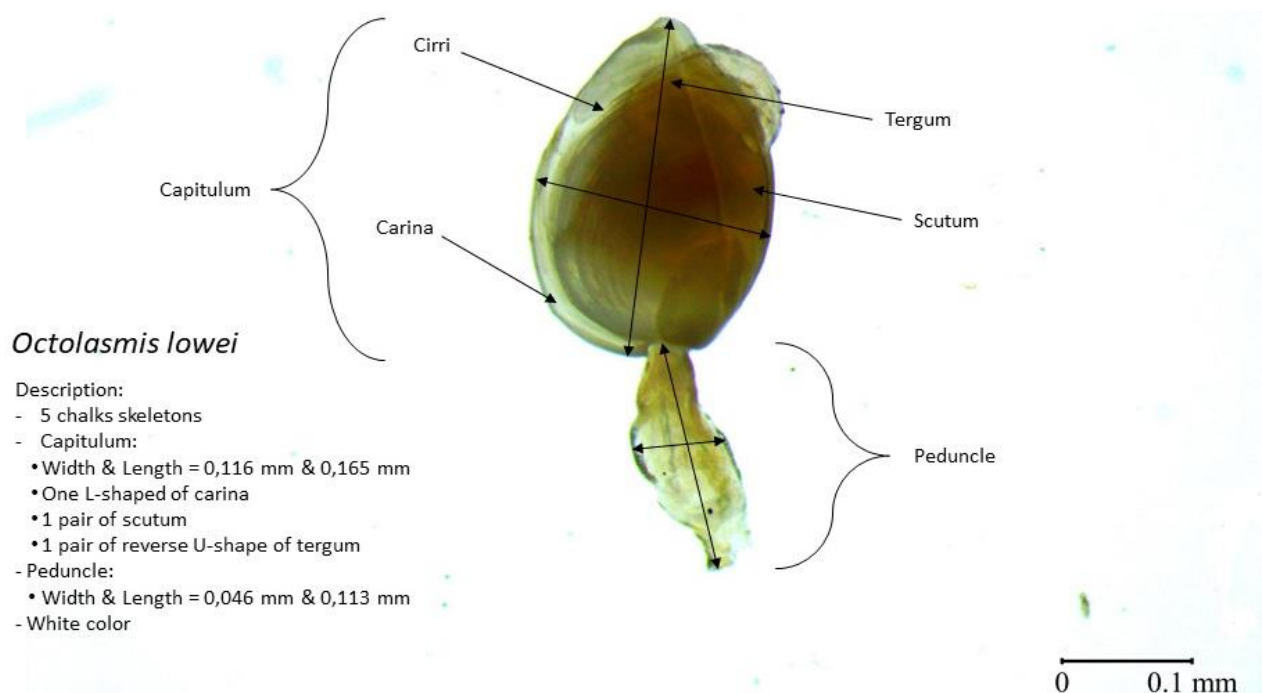


Figure 3. Morphological cross-section of *Octolasmis lowei* through *Optilab Viewer 4.0.0* software on a binocular stereo microscope, and through Lucida camera on a binocular stereo microscope at 1x magnification and its parts: C: Capitulum; Ca: Carina; P: Peduncle; Sc: Scutum; T: Tergum. Scale = 0.1 mm

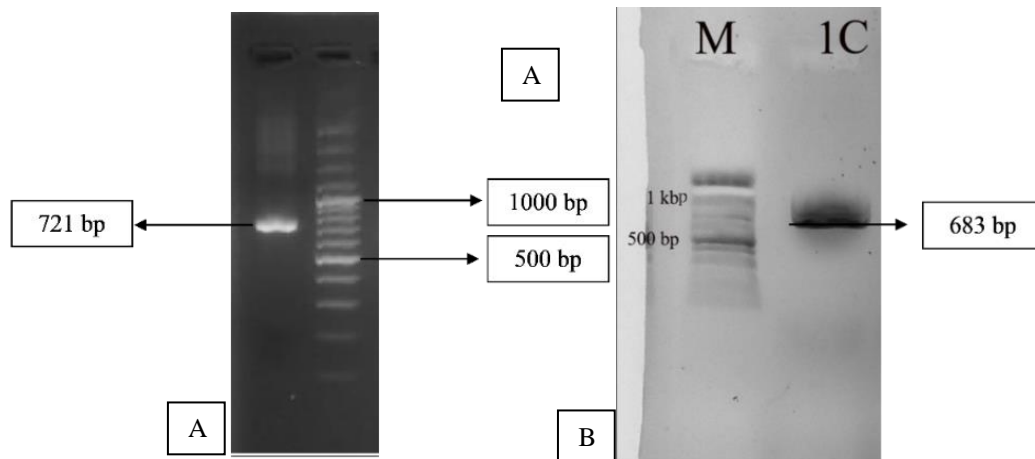


Figure 4. A. Electrophoresis results of *O. angulata* PCR products indicated by a single band. Description (M: Marker; 1C: Sample). B. Electrophoresis results of *O. lowei* PCR products indicated by a single band. Notes: M: Marker; 1C: Sample)

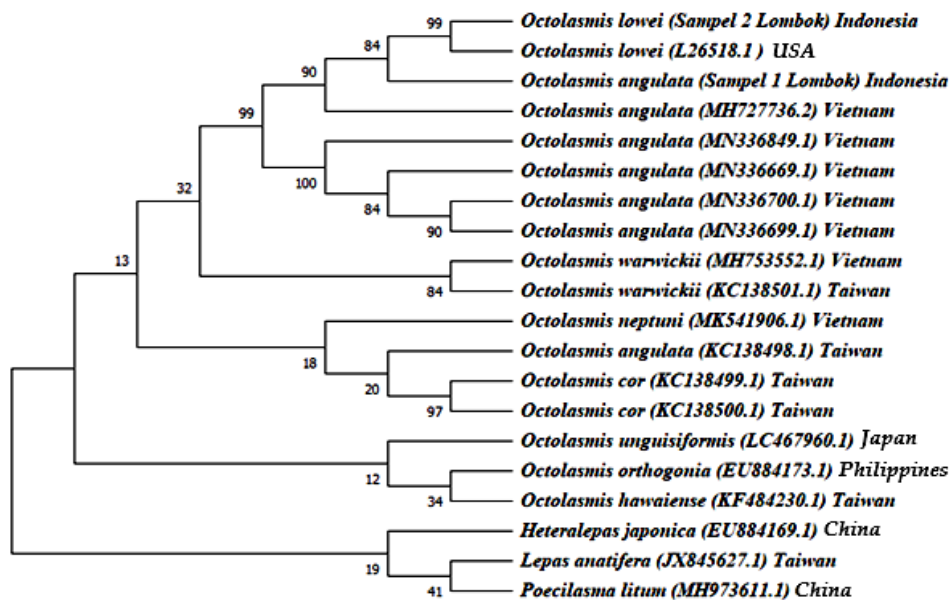


Figure 5. Phylogenetic tree analysis (bootstrap consensus tree) of *Octolasmis* ectoparasites from Ekas Waters, East Lombok, and West Nusa Tenggara with 11 other species from GenBank

The comparison between these samples' DNA sequences by BLAST to the data on NCBI shows that *Octolasmis* infesting the gills of wild-caught scalloped spiny lobster in Ekas Waters, East Lombok, West Nusa Tenggara are identified as *O. angulata* and *O. lowei* with an identification percentage of 95.65% and 98.75% respectively. Molecular identification is an advanced development stage for confirmation of morphological identification. Molecular identification is better used when morphological identification is difficult to distinguish on their characteristics, so it can use molecular or DNA barcoding. After performing morphological identification, the specimen was tested for the correctness of the species type by aligning it to the NCBI website (<https://www.ncbi.nlm.nih.gov/>). In addition, it uses the

Basic Local Alignment Search Tool Nucleotide (BLASTN) program to evaluate the percentage of identification or their similarity level. Therefore, a phylogenetic tree was created by combining several sequences of *Octolasmis* samples infesting the gills of wild-caught scalloped spiny lobster in Ekas Waters, East Lombok, West Nusa Tenggara compared to other sequences from the NCBI GenBank. The reconstruction of the phylogenetic tree is shown in Figure 5.

Discussion

The present study identified two species of ectoparasites that infested scalloped spiny lobster, which are *O. angulata* and *O. lowei* morphologically and confirmed with COI sequence. These species were found

attaching on the gill filaments and the inner layer of the scalloped spiny lobster *carapace*. This follows a previous study by Jeffries et al. (2005), who reported that *O. angulata* has a preference in the gill chamber of the host families Calappidae, Palinuridae, and Portunidae. Similar characteristics of *O. angulata* have been previously described by Khattab (2018) and Leung and Jones (2000), including thin and inverted L-shaped scutum.

Morphologically, *O. lowei* was pale yellow in the capitulum with white plates. The oval-shaped capitulum on the anterior part was protected by five limestone skeletal plates consisting of a pair of scuta, a pair of terga, and a carina. Posteriorly, there is an elongated peduncle. This also follows Jeffries et al. (2005) that *O. lowei* has five lime skeletal plates consisting of two *scuta*, two *terga*, and a carina that support and protect vital organs such as feeding apparatus. Mathison and Pritt (2014) added that *O. lowei* has a U-shaped tergum and L-shaped carina and scutum. Molecularly, the sequence analysis results by BLAST showed 95.65% similarity to *O. angulata* and 98.75% to *O. lowei*. These ectoparasites have been reported to be distributed in tropical to subtropical oceans, covering areas in several countries such as Taiwan, Japan, the Malay Archipelago, Australia, Indo-Pacific (Mathison and Pritt 2014). The ectoparasite attack causes stress, weakening host immunity and affecting the respiratory system (gills), followed by death (Radhakrishnan and Kizhakudan 2019). In addition, parasitic pathogens rarely cause sporadic disease outbreaks but, at high attack intensities, cause significant economic losses due to the death results.

The distribution of the two *Octolasmis* species is likely due to several factors; first, Indonesia has a tropical climate, so it has an advantage for both *Octolasmis* species and hosts in breeding and distributing larvae quickly. This follows Suherman and Arsad (2020) that *Octolasmis* was often found symbiotic with Crustaceans, namely lobsters and crabs, in waters worldwide with temperate, subtropical, and tropical climates. Second, Indonesian waters are located in the equatorial zone, with more sunlight, causing them to be rich in natural food sources such as plankton, which are the main producers in the food chain (Jeffries and Voris 1996). These advantages were utilized by *Octolasmis* as filter feeding and benefitted from meeting its food needs (Ewers-Saucedo et al. 2019). Third, both *Octolasmis* species are cosmopolitan and often infest Crustaceans in several countries including Thailand and Vietnam. According to Jeffries et al. (2005), *O. angulata* is a species of the genus *Octolasmis*, which is cosmopolitan because it is often found in several countries infesting the gills of Decapoda hosts such as Palinuridae, Portunidae, Scyllaridae, Calappidae, Glyphocrangidae, Hepatidae, Leucosiidae, Mithracidae, Phartenopidae, Menippidae, Pisidae, Raninidae and Xanthidae.

The PCR amplification results show a band measuring 721 bp for the COI mtDNA region of *O. angulata* and 683 bp for *O. lowei*. Samples with high DNA concentration show the results of DNA strands that look brighter and thicker on the electrophoresis gel. The different length was previously also reported by Dang et al. (2021), which was 642 bp. The differences in the length of the DNA band

might be due to the difference in the type of primer used. Primer selection affects the polymorphism of the resulting band because each primer has its attachment site; as a result, the polymorphic DNA bands produced by each primer become different, both in the size of the number of base pairs and the number of DNA bands. The intensity of the amplified DNA band on each primer is strongly influenced by the purity and concentration of DNA mold (Gusmiaty et al. 2016). Therefore, Sunandar and Imron (2018) state the success of PCR amplification is determined by the presence or absence of DNA or attachment sites; the primer will attach to genomic DNA with a nucleotide base arrangement that complements the primer base arrangement. DNA quality and quantity greatly affect the PCR process's success; one of the qualities observed is the DNA genome purity and size. Low purity, for example, due to secondary metabolites, will inhibit the attachment of primers to the base sequence and DNA chain (Farrar and Wittwer 2015).

Moreover, Amin et al. (2023b) state phylogenetic trees can estimate taxonomic relationships between species. Phylogenetic trees aim to determine taxonomic relationships between species and intraspecies, populations, individuals, or genes of species by DNA sequences. The clade or branch group on the phylogenetic tree shows a fairly high bootstrap value. Furthermore, Kress et al. (2002) state the validity of phylogenetic tree topology is explained by the *bootstrap* value category, which is classified as high (>85%), moderate (70-85%), weak (50-69%), and very weak (<50%) categories. Other DNA sequences from NCBI can also be used to compare taxonomic relationships from previous studies. The phylogenetic tree shows *Octolasmis* species intraspecies. Sequences were obtained from COI of *O. angulata* and *O. lowei* from the sample sites plus some sequences of other species in other places, especially other countries. *O. angulata* sequences samples are in a group with *O. angulata* from Vietnam with code MH727736.2 having a *bootstrap* value of 84%. In addition, *O. lowei* sample sequences are in a group with *O. lowei* from America with code L26518.1 with a *bootstrap* value of 99%. This is thought to be because it is still in a close water area rather than with other countries. The relatively close geographical location also resulted in samples from the location being in the same group (Wilujeng 2018). The results of the phylogenetic tree are the maximum possible with 1000 *bootstrap* replicates, resulting in several levels of similarity.

This was confirmed by Pigot and Tobias (2013) that differences in the geographic habitat of each species often cause differences in species proximity. In addition to geographical differences, species proximity values also occur due to differences in genetic sources (Butet et al. 2019). Another study by Wirdateti et al. (2016) stated that differences in geographical location can affect genetic conditions. Phylogeny trees are built from genetic distance values, which can then describe taxonomic relationships between species and subspecies. The phylogeny tree's confidence level is determined based on the number of bootstraps, which is 100 to 1000. A high bootstrap value

causes the reconstructed tree results to have a high topological confidence value.

In conclusion, *Octolasmis* species found in the gills of scalloped spiny lobster were identified through morphological methods and classified as *O. angulata* and *O. lowei*. Sequence analysis of the mtDNA COI region showed a nucleotide length of 721 bp with a similarity percentage of 95.65% in *O. angulata* and 683 bp with a similarity percentage of 98.75% in *O. lowei*. The taxonomic relationship of *O. angulata* with the *O. angulata* reference sequence (MH727736.2) is 84%. In comparison, *O. lowei* with the *O. lowei* reference sequence (L26518.1) has a taxonomic relationship of 99%, which means that the sequences are closely related to the same ancestor.

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