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MORPHOLOGICAL COMPARISON OF *Octolasmis* sp. ECTOPARASITES INFESTING THE GILLS OF SCALLOPED SPINY LOBSTER (*Panulirus homarus*) WITH DIFFERENT FIXATIVE SOLUTIONS

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Abstrak

Octolasmis merupakan salah satu jenis ektoparasit yang banyak ditemukan pada insang lobster. Octolasmis juga dapat menjadi ancaman bagi populasi lobster bahkan dapat menyebabkan tingkat mortalitas yang tinggi. Penelitian ini bertujuan untuk mendeteksi pengaruh larutan fiksatif yang berbeda terhadap karakter morfologi Octolasmis yang menginfestasi insang lobster pasir (Panulirus homarus). Sampel Octolasmis dikumpulkan dari insang lobster pasir hasil tangkapan nelayan di Lombok Timur, Nusa Tenggara Barat. Sampel direndam secara terpisah dalam dua larutan fiksatif yang berbeda yaitu larutan etanol absolut atau larutan formalin. Setelah 7 hari, analisis morfologi Octolasmis sp. dianalisis menggunakan mikroskop stereo binokuler terintegrasi optilab dan kamera lusida. Hasil penelitian menunjukkan bahwa terdapat perbedaan yang signifikan pada kondisi morfologi Octolasmis sp. yang meliputi warna kulit, kenampakan organ dan ukuran Octolasmis sp.. Secara umum, hasil penelitian menunjukkan bahwa formalin merupakan larutan fiksatif yang lebih baik.

***Kata Kunci**: Etanol 95%, Formalin 10%, Morfologi, Octolasmis sp., Optilab*

Abstract

Octolasmis is a type of ectoparasite which is often found on the gills of lobsters. Octolasmis can also be a threat to lobster populations and can even cause high mortality rates. This study aims to detect the effect of different fixative solutions on the morphological characters of Octolasmis infesting the gills of scalloped spiny lobsters *(Panulirus homarus). Samples of Octolasmis were collected from the gills of wildly-caught* scalloped spiny lobsters *in East Lombok, West Nusa Tenggara. The samples were immersed separately in two different fixative solution which are either absolute ethanol or formalin solution. After 7 days, morphological analysis of Octolasmis was analysed using an integrated optilab stereo binocular microscope and a lucida camera. The result showed that there were significant differences in the morphological condition of Octolasmis* sp. *including skin colour, organ appearance and size of the Octolasmis. In general, the result showed that formalin is better fixative solution.*

***Keywords**: 95% Ethanol, 10% Formalin, Morphology, Octolasmis* sp., *Optilab*

1. INTRODUCTION

Lobster (*Panulirus* spp.) is one of the leading commodities with high economic value, so it is one of the targets for fishermen to catch (Djayanti *et al.*, 2021). This leading fishery commodity has the potential to be developed in Indonesia because it is designed for consumption and sale by import and export (Anggraini *et al.*, 2021). It has been proven that in a year, 78.5 tons of lobsters are produced worth IDR 55.25 billion and the economic value of selling the seeds alone reaches IDR 16 billion per year (Hilal and Fachri, 2016).

According to DJPT data (2015) the fluctuating increase in production of Indonesian scalloped spiny lobster

(*Panulirus homarus*) during the 2005-2014 period from 6,648 tons in 2005 to 16,482 tons in 2013, but then decreased by around four tons in 2014 and continues to decline to date. The decline in the catch of scalloped spiny lobsters can certainly reduce fishermen's income. Some of the obstacles often faced by fishermen are attacks by ectoparasites which cause stress, weakening the host's immune system, affecting the respiratory system (gills) to death. As a result, it has an impact on decreasing the selling price of lobsters. The parasite that is often found in scalloped spiny lobsters (*Panulirus homarus*) is *Octolasmis* sp. (Barzakh *et al.*, 2022).

Octolasmis sp. is a type of parasite from the class Maxillopoda which is often found in lobsters, crabs and shrimp (Suherman and Arsad, 2020). Several previous studies related to the identification of *Octolasmis* sp. have been carried out including the discovery of two *Octolasmis* sp. species (*O. lowei* and *O. warwickii*) in mud spiny lobsters (*Panulirus polyphagus*) from Mersing, Johor Malaysia (Ihwan *et al.*, 2014). In addition, *O. lowei* and *O. angulata* were found on scalloped spiny lobsters (*Panulirus homarus*) in KJA Sape, Bima, NTB (Yusgita *et al.*, 2019).

Octolasmis sp. ectoparasites can be a threat to the host population and can even cause high mortality rates. However, on the other hand, with the development of science and technology, findings related to *Octolasmis* sp. exploration emerged. These ectoparasites have been studied as potential bioindicators of water pollution. It is proven by the results of Nur *et al.* (2021) that the concentration of the heavy metal type mercury (Hg) in *Octolasmis* sp. was significantly higher than the concentrations observed in the tissue of the host crab and its surrounding habitat.

According to Lim *et al.* (2010) that parasite identification by observing morphological and anatomical characters can be obtained through a light microscope. Currently the identification key for *Octolasmis* sp. is only seen from the number of capitular plates, scuta, terga and carina (Jeffries *et al.*, 2005; Chan *et al.*, 2009). In the morphological identification process, a suitable fixation is needed to preserve the specimen. However, until now, there is no ideal fixative solution that is a safe fixative that maintains cellular morphology perfectly but does not change the composition of the specimen so as not to change the reactivity of the chemical parts in it for further detection. (Haque *et al.*, 2020).

In general, there are two types of fixation for biological specimens, namely physical fixation and chemical fixation. Physical fixation uses very low temperatures (cryo-fixation) or very high (boiling and microwave). Chemical fixation is an important process to prevent autolysis and tissue degradation, while preserving cellular morphology and detail for microscopic evaluation (Panzacchi *et al.*, 2019) Chemical fixatives are most commonly used for preparations viewed under a microscope. Many factors affect the fixation process including solution concentration, duration and others (Musyarifah and Agus, 2018).

Several types of existing fixative solutions such as ethanol and formalin have been used in the last 10 years. Ethanol and formalin are fixative solutions which have different characters. Therefore, it is important to compare the morphological characters of *Octolasmis* sp. ectoparasites after being fixed using different types of fixative solutions. This study aims to detect the effect of using different fixative solutions on the morphological characters of *Octolasmis* sp. ectoparasites that infest the gills of the scalloped spiny lobster (*Panulirus homarus*).

2. RESEARCH METHOD

2.1 Research Time and Place

This research was conducted in May-August 2022. Samples of *Octolasmis* were collected from the gills of wildly-caught scalloped spiny lobsters in East Lombok, West Nusa Tenggara. Examination and

storage of *Octolasmis* sp. ectoparasites in fixative solutions were carried out at the Laboratory of the Fish Quarantine Center for Quality Control and Safety of Fishery Products in Mataram. *Octolasmis* sp. ectoparasites were identified morphologically at the Laboratory of Biotechnology, Laboratory of Microbiology and Fish Diseases and Laboratory of Anatomy and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya.

2.2 Tools and Materials

The equipment used in this study was a sectio set (surgical scissors, tweezers, scalpel), pipette, tissue, petri dish, label paper, 2ml microtube, ImageRaster 3.0.0 software, binocular stereo microscope equipped with a lucida camera and integrated with Optilab Viewer 4.0.0 software. The materials used in this study were *Octolasmis* sp. ectoparasites on scalloped spiny lobster gills, 95% ethanol and 10% formalin.

2.3 Procedures and Data Analysis

Scalloped spiny lobsters were caught by fishermen in East Lombok, West Nusa Tenggara. Sampling locations were determined by random sampling method. Examination of scalloped spiny lobsters was carried out by weighing and measuring the body. The size criteria for the scalloped spiny lobster samples taken were a total length of 15-20 cm, a weight of 200-300 grams/head. Parts of the scalloped spiny lobster gills were later documented. Gills were taken directly from live lobsters.

The gills that were positively infested by *Octolasmis* sp. were separated from the host's body and stored using two different fixative solutions in different microtubes. The sample preservation time is seven days. Identification and comparison of ectoparasites were carried out under a binocular stereo microscope natively with 1x magnification with the help of optilab software and drawn with the help of a lucida camera. Morphometric measurements using ImageRaster 3.0.0 software.

The *Octolasmis* sp. ectoparasites found were identified based on special morphological features that appeared when observing the presence or absence of several organs such as the tergum, scutum, carina, cirri, peduncle, and capitulum. In addition, morphometrics to meristics of several organs are the key to identification. *Octolasmis* sp. identification based on identification key by Chan *et al.* (2009). Analysis of the results of the research data is presented in the form of tables and pictures and is explained descriptively.

3. RESULTS AND DISCUSSION

3.1 Identification of *Octolasmis* sp.

The results showed that *Octolasmis* sp. ectoparasites were found from the gills of wildly-caught scalloped spiny lobsters (*Panulirus homarus*) in East Lombok, West Nusa Tenggara (Table 1). A cross-section of a sample of lobsters as well as their gills infected with *Octolasmis* sp. can be seen in Figure 1.

Table 1. Identification of *Octolasmis* sp. Ectoparasites on Scalloped Spiny Lobster (*Panulirus homarus*) Gills with Different Fixative Solutions.

No.	Ectoparasites	Types of Fixative Solutions
1.	*Octolasmis* sp.	95% Ethanol
2.	*Octolasmis* sp.	10% Formalin

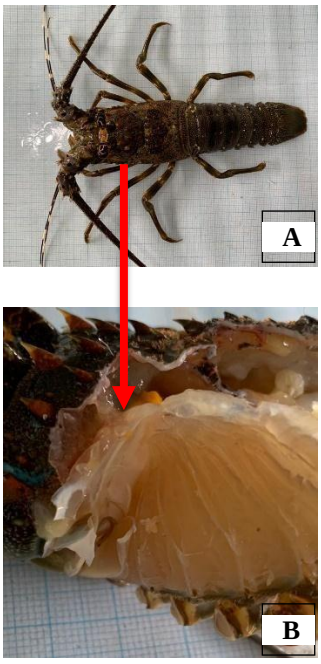

Figure 1. Cross-section of the sample: (A) *Panulirus homarus*, (B) *Octolasmis* sp. ectoparasites attached to the gill lamellae of *Panulirus homarus*.

Based on the observations made by the two *Octolasmis*. *Octolasmis* sp. has a sprout-like shape. The body parts consist of cirri (Ci), scutum (Sc), carina (Ca), capitulum (C) and peduncle (P). The *Octolasmis* sp. observed in this study had capitulum length and width of 3.2 mm and 2.1 mm, respectively. and a peduncle length of 1.1 mm.

According to Chan *et al.* (2009) *Octolasmis* sp. has a capitulum length of up to 13.9 mm. thin L-shaped scutum, carina thin and wide in horizontal section. *Octolasmis* sp. tends to be brown in color. The anterior portion consists of a capitulum which is oval in shape and is protected by several plates of calcareous framework at certain points. The plate consists of a pair of scutum and carina. There is also a peduncle that is elongated posteriorly (Figure 2).

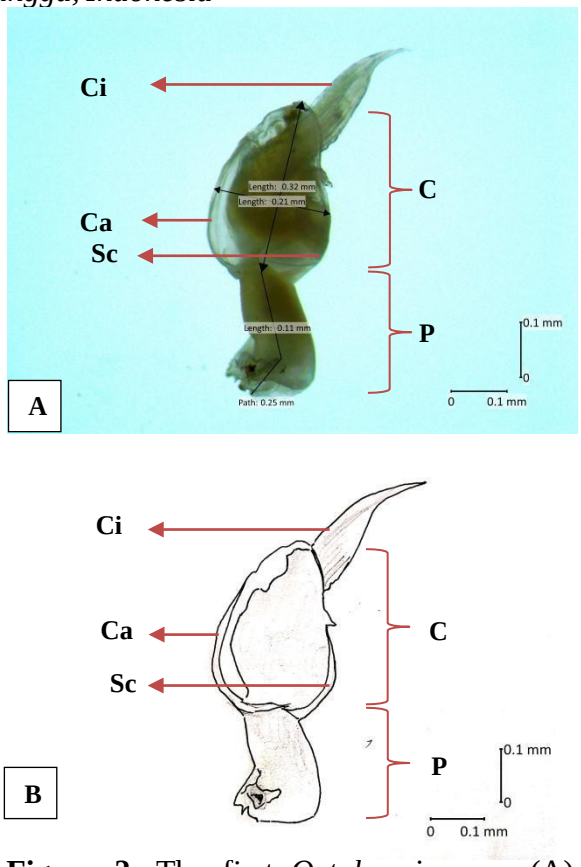

Figure 2. The first *Octolasmis* sp. : (A) through the Optilab Viewer 4.0.0 software, (B) through the lucida camera on a 1x magnification binocular stereo microscope.

Octolasmis sp. belongs to the class Maxillopoda Crustacea, with the characteristics of having a body with a neck like a swan with a capitulum in the anterior and a peduncle or stalk in the posterior. There are several organs that have morphological features within the capitulum as follows: an L-shaped carina, and a pair of inverted L-shaped scutum with a blunt anterior end. There is also a pair of tergums (T) which are inverted U-shaped (Figure 3).

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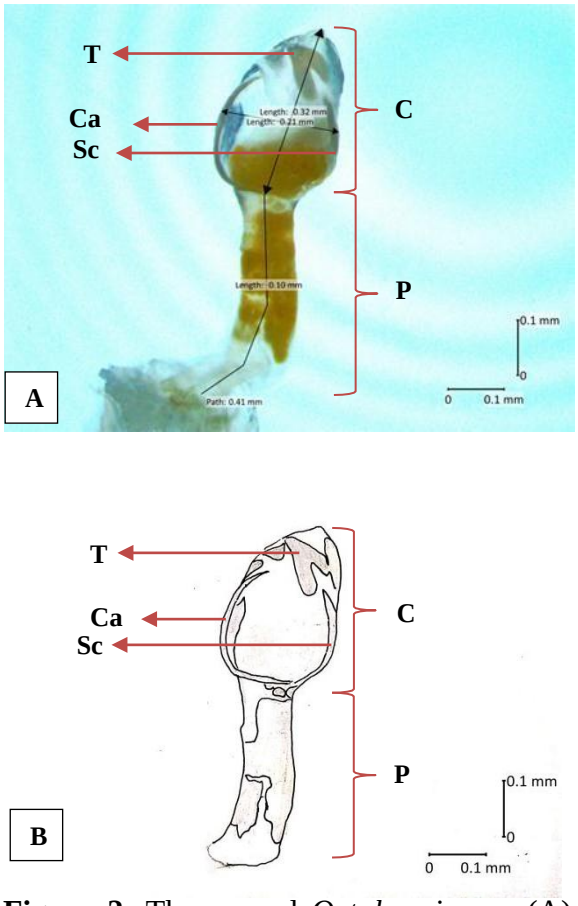

Figure 2. The second *Octolasmis* sp.: (A) through the Optilab Viewer 4.0.0 software, (B) through the lucida camera on a binocular stereo microscope with 1x magnification.

The second *Octolasmis* sp. has a yellowish-white color. The capitulum is oval anteriorly, and is covered by a five calcareous skeleton consisting of a pair of scutum, tergum, and carina. Posteriorly there is an elongated peduncle. This is also in line with the statement of Jeffries *et al.* (2005) stated that *Octolasmis* sp. has five limestone skeletons consisting of two scutums and a tergum, as well as a carina which functions as a support and protector for vital organs such as cutlery. (Chan *et al.*, 2009) This *Octolasmis* sp. has a U-shaped tergum, carina and L-shaped scutum.

3.2 Comparison of Morphology with Different Fixative Solutions

Based on the morphological observations of *Octolasmis* sp. ectoparasites using two different types of fixative solutions, it can be concluded that there are significant differences (Table 2).

Table 2. Comparison of *Octolasmis* sp. Morphology with Different Fixative Solutions

Types of Fixative Solutions	Microscope Image (Optilab)	Information
95% Ethanol		- The body parts are self-explanatory - Whole form - Pale color - Size shrink (red circle) - Can be identified morphology
10% Formalin		- Clear body parts - Whole form - Clear color - Fixed size - Can be identified morphology

The fixation used in this study was 10% formalin and 95% ethanol. The use of formalin is because it is easier to prepare and the formalin fixative will preserve the tissue

structure very well. The fixation process of more than 24 hours can cause tissue hardening (Waheed, 2012). In this study, sample fixation was carried out for seven days after the collection process on the host gills. This is in accordance with the statement of Bruce *et al.* (2002) that fixation with formalin takes about three to seven days. When tissue is fixed in 95% ethanol it can deform significantly, which complicates external morphological analysis and taxonomic identification, especially for specimens with soft aqueous bodies (Gordeeva *et al.*, 2019).

4. CONCLUSIONS AND SUGGESTION

Octolasmis sp. ectoparasites were identified as infesting the gills of the scalloped spiny lobster (*Panulirus homarus*). Different types of fixative solutions affect the morphological quality of *Octolasmis* sp. which includes skin color, organ appearance and *Octolasmis* sp. morphometrics. There were significant differences in the morphological comparison of *Octolasmis* sp. ectoparasites using two different fixative solutions (10% formalin and 95% ethanol). In general, formalin is a better fixative solution for morphological analysis. Suggestion for research related to morphological analysis are expected to use formalin as a fixative solution.

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