Assessment of tunicate distribution and *Polycarpa* sp. tunic color variation in the Ambon Island waters

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Abstract. Updated information regarding tunicates distribution and diversity in the waters around Ambon Island is scarce if any. We carried out an assessment using the underwater census approach in June 2018 to obtain the said information. Tunicates' composition and distribution were described based on six sampling station observations. Their average density in five of six stations was less than one individual per meter square. Predominant genera observed were *Didemnum* and *Polycarpa*. Tunicates belonging to *Polycarpa* sp. had 12 color variations on its tunic. Those sampled in Htu had the most variations (8 variants) compared to all the other four stations. *Didemnum* sp. was highly abundant in three stations (Morella, Htu, and Larike). Those identified as *Polycarpa* sp. were predominant only in two stations (Mamala and Wakal). Further studies are needed to investigate the anthropogenic pressure effect on these tunicates, as they were known as biomarkers in ecotoxicological studies.

Keywords: tunicates, Banda Sea, ecology, interspecific variation

1. Introduction
Tunicates are grouped with Hemichordata and Cephalochordata as member of the Protochordata [1]. They are varied in forms and were divided into solitary and colony based on their social organisation. Solitary living species are most easily recognized because they have a relatively large size and usually have two siphons that are easily visible even though their bodies are covered by other organisms that grow on their surface, whereas colonial species consist of small individual units, called zooids, which can amount to thousands in a large colony [2].

Despite their soft bodies, tunicates have a wide distribution and can be found in diverse marine environments [3] from rocky substrates in the intertidal and shallow subtidal zones, and inhabiting hard substrates such as corals, rocks and shells, especially in shaded areas [4–6] to the deep sea [1]. Some species are invasive organisms and their existence poses a threat to the indigenous organisms [5]. However, some tunicates species are beneficial to humans, for example as a source of protein in some countries [7]. Other than that, they are reported to contain bioactive compounds that could be useful in the pharmaceutical field (cf. [8] and references therein).

*Didemnum* sp. and *Polycarpa* sp. are two ascidian genera commonly found in tropical coral reefs area. In some areas, *Didemnum* sp. grows to the size of a fist, still flaccid, and they have deep green *Prochloron* algae as symbionts wich easily visible. *Polycarpa* sp. is probably the most distinctive species and it has a variety of tunic colors [2]. Both genera presence is also frequently observed in eastern Indonesian coral reef ecosystems [9]. Research on tunicates distribution, composition, and diversity in Indonesia were carried out in several locations by various teams, such as in Barranglombo...
Island waters (33 species) [10], Samalona Island waters (18 species) [11], Punuktukan waters (9 species) [12] and Berau, East Kalimantan waters (49 species) [13].

A preliminary survey done in 2017 indicated *Polycarpa* sp. tunic color variation found in the northern area of Ambon Island waters [9]. The current study was thus designed as a follow up of this initial observation. Assessment using photographic data is an accessible tool for monitoring purpose that allows rapid identification of tunicates until genera level, based on the tunic morphology and color variation. Tunic color variation is one of many variation across species (interspecific variation). The ecological importance of variation across species is established by now, where it affects the interaction dynamic between individuals across species and with their surrounding environment [14].

Waters surrounding Ambon Island, the Banda Sea, is included in the Coral Reef Triangle Initiatives (CTI) area, an area known to be the global center of marine biodiversity. However, many marine biotas in the Banda Sea are still understudied ecologically, among them are tunicates. There was only one report published in 2018 about an initial attempt to rear these tunicates in a laboratory setting [9]. Because of this gap of information, the objectives of this study are thus: 1) to assess the tunic color variation of *Polycarpa* sp. in the waters around Ambon Island, and 2) to obtain preliminary information about tunicates (*Didemnum* sp. and *Polycarpa* sp.) density and distribution at the said location.

2. Material and Methods

2.1 Sampling locations

There were six observation locations namely Morella, Mamala, Hitu, Wakal, Kalauli, and Larike. The first five stations were situated in the northern part of Ambon Island and one station (Larike) in the southwest part (Figure 1).
We used a modified underwater census method (UWC) [15]. A 50-m linear transect was used as the range of reference where the data (in the form of images) were taken. The photographs were taken in the 1 m area on the right and on the left side of the transect, so the total observation area per station was 100 m² (Figure 2). Data were retrieved at 3 to 6 m deep using SCUBA diving equipment. Retrieval of field data was carried out in parallel with the coastline.

![Figure 2. Underwater census (UWC) method used for data collection.](image)

Transect positions were recorded per location using GPS on a smartphone. The photos taken were then grouped according to the location of the collection. Observation of tunicate density was only carried out for two genera, namely *Polycarpa* and *Didemnum*, whose identification was based on tunic morphology and tunic color variation (tcv) according to available guidelines [16–18]. Population density was expressed by the number of individuals per area of the sampling unit (individual.m⁻²).

2.2. Data Analysis

We tried to quantify the tcv using the same principal as looking into the diversity. The number of tcv in each location was expressed as the first level of color variation (CV1), or the equivalent of alpha diversity [14]. The second level (CV2) or the equivalent of beta diversity was defined as the variation observed between two stations, using the following equation modified from [15]:

\[
CV_2 = (tcv1 - c) + (tcv2 - c)
\]

with tcv1 is the number of tcv in the first station, tcv2 is the number of tcv in the second station, and c is the number of tcv common to both stations. The third level (CV3), or the equivalent of gamma diversity is the number of *Polycarpa* sp. tcv from all stations.

3. Results and Discussions

3.1. Sampling location profile

The intertidal seabed area of Morella and Mamala stations stretched to about 50 m seaward, and a steep slope is observed afterward [9]. The sampling site was known as a fishing ground, notably for small pelagic and coral reef fishes. Local fishermen used homemade bombs in the sampling area to fish (ca. 2005). Local authorities have intervened by educating them on the importance of keeping the coral reef ecosystem unharmed. This intervention resulted in the discontinuation of the blast fishing method in recent years. One of Morella’s beach (Tanjung Setan) was popular locally as a recreational site, notably for swimming, snorkeling, and diving. Fragments of branched coral found were mostly due to anchoring and tourism activities. Overall, Morella coral reef ecosystem was in better condition compared to those in Mamala at the time of observation. Morella station was closed to the shore, where local inhabitant houses were built along the shoreline.

The coral reef condition of this area, in general, was relatively good visually during the day on the sampling period. The depth was between 4 to 6 m (Table 1).

3.2. Polycarpa sp. tunic color variations

Tunic color variations of ascidians are confusingly common [16]. *Leptoclinides echinatus* has yellow, orange, or violet tunics, while *Phallusia mignardii* have black or dark brown color on their tunics [19]. In colonial species with tunic spicules and/or algal symbionts, colony color is determined by the density and distribution pattern of tunic pigment cells, spicules, and algal cells [20]. Some of these tunics have special functions and are associated with protection; phagocytosis to prevent infection, acid storage to prevent predation and pigmentation to protect against solar radiation [21].
Table 1. The profile of six sampling stations

| Stations | Station profile Position coordinates (Latitude; Longitude) | Approximate depth (m) | Transparency (%) | Dominant substrate          |
|----------|-------------------------------------------------------------|-----------------------|-----------------|----------------------------|
| Morella  | 3.516550; 128.22364                                        | 5                     | 100             | Coral reef                 |
| Mamala   | 3.516749; 128.223160                                       | 4                     | 100             | Coral reef                 |
| Hitu     | 3.582170; 128.172563                                       | 4-6                   | 50-75           | Muddy sand                 |
| Wakal    | 3.587526; 128.158637                                       | 3                     | 100             | Sand                       |
| Kalauli  | 3.588280; 128.057364                                       | 3-4                   | 100             | Sand and rocks             |
| Larike   | 3.734999; 127.907858                                       | 5                     | 100             | Coral reef and Rocks       |

As benthic invertebrates, tunicates can only grow optimally in habitats with good water quality, which is characterized by currents that are strong enough to guarantee regular water mass exchange. Tunicates were only found in five of the six stations surveyed. The absence of two tunicate genera surveyed at the Kalauli station was probably caused by substrate conditions that were not conducive for growth. This area was dominated by branch corals and sandy substrates with many coral fragments. Unstable substrates such as these do not allow tunicate larvae to adhere themselves [22,23] hence decreasing their density [16] to the point of abandoning the location, as in the case here.

Twelve tcv were recorded in this study (CV3). Ten variants are presented in figure 3. The highest color variation was observed at Hitu station (9 variants) followed by Morella ones (8 variants), while in Wakal station only 5 variants were observed (Table 2). The lowest variation was recorded in Mamala and Larike (4 variants). Yellow with purple stripes tunicates were predominant in Morella waters (9 variants), as well as in Mamala station (14 variants). At Hitu Station and Larike, more yellow and white with purple stripes (9 and 14 variants, respectively) were recorded, while at Wakal stations purple and yellow variations were more abundant (5 variants). Morella Station was the most populated by both genera and had the highest tcv.

Figure 3. Some color variations on tunic of Polycarpa sp.: a-brown; b-yellow and white purple stripes; c-irregular mix of purple and yellow with purple stripes; d-yellow with purple stripes; e-yellow with white stripes; f-green with purple stripes; g- (left) orange and (right) purple-green with purple stripes; h-yellow; i-yellow and white; j-green and yellow.

Strong currents could be one of the factors that influence tunicates’ presence and abundance in Morella. Polycarpa is an animal with an adaptable body shape that can live in water with strong current conditions and, by obtaining food with a filter feeder [2], this is beneficial for them. However further studies need to be done to see if there are environmental influences on Polycarpa sp. tunic color variation.
3.3. Distribution and Density of Didemnum and Polycarpa

This study documented the distribution and abundance of tunicate in the waters around Ambon Island, especially two types commonly found in this waters, namely Didemnum and Polycarpa. (Figure 4). Both types of tunicate are reported to live at a depth of 5 - 30 m (Didemnum sp.) and 5 - 35 m (Polycarpa sp.) [24], yet in this study, both were found even at 3 m depth (Table 1). This was presumably due to the availability of habitat which was already conducive to adhere to and breed, as adhering substrate is one of the factors that play an important role in the distribution and abundance of tunicate [2].

| Variation                        | A  | B  | C  | D  | E  |
|----------------------------------|----|----|----|----|----|
| Yellow with purple stripes       | 9  | 14 | 8  | 4  | 8  |
| Yellow–white with purple stripes | 6  | 5  | 9  |    | 15 |
| Yellow and white                 | 2  | 1  | 1  |    |    |
| Green and yellow                 | 2  |    |    |    |    |
| Yellow                           | 3  | 2  |    |    | 1  |
| Orange                           | 2  |    | 1  |    |    |
| Green                            | 1  |    | 1  |    |    |
| Purple                           |    | 5  | 5  | 1  |    |
| Purple-green with purple stripes | 3  |    |    |    |    |
| Purple and yellow                | 6  | 4  | 5  | 4  |    |
| Brown                            |    | 1  |    |    |    |
| Yellow with white stripes        |    |    |    |    | 3  |
| CV1                              |    | 8  | 4  | 8  | 5  | 4  |
| CV2                              | a vs. b: 8 | a vs. c: 3 | a vs. d: 7 | a vs. e: 7 | b vs. c: 7 |
| CV3                              | b vs. d: 5 | b vs. e: 4 | c vs. d: 6 | c vs. e: 5 | d vs. e: 5 |

Polycarpa sp. is very common in almost all reef areas where there is a hard surface that can be used as a place to adhere. Didemnum sp. commonly found on coral reefs and rocky areas in the Indian Ocean and Pacific and this species is the most abundant found on almost every Pacific coral reef [2].

Figure 4. Co-existing Didemnum sp. and Polycarpa sp. in Morella station, northern waters of Ambon Island.
At Morella, Hitu, and Larike stations, *Didemnum* sp. density was higher than *Polycarpa* sp. On the contrary, *Polycarpa* sp. density was higher at Mamala, followed by Wakal station (Figure 5). Different living preference (colony or solitary) could be the cause of the density difference. Overall the density of *Didemnum* sp. was higher than *Polycarpa* sp. in Ambon Island waters. By living in colonies, *Didemnum* sp. can reach an abundant amount in one location [2]. *Polycarpa* sp. has solitary life type, even though several individuals were found growing in proximities to each other. In general, colony-forming ascidians are more abundant in tropical waters compared to the solitary ones [25].

![Figure 5. The tunicate density in each station.](image)

The high density of the two types of tunicate at Morella and Larike might be related to the seabed topography and water quality. *Polycarpa* is often found in the edge of the steep slope area, as in the case of Morella station. The current was strong and the water quality was appropriate for *Polycarpa* sp. and *Didemnum* sp. growth. Tunicates distribution and growth are influenced by environmental conditions such as salinity, temperature, and light intensity. Natural or anthropogenic pressure in coastal waters can affect the presence and distribution of tunicates [26,27]. Other than that, substrate is one of several ecological factor that could affect their distribution [27]. Morella station is one of the touristic sites in northern Ambon Island, where snorkeling and diving activities are frequent. The local community tries to maintain the coral reef ecosystems conditions and organisms living on it. Whereas in Wakal, there were lots of small recovered corals found in this area. It indicates that the coral reef ecosystem in this area was conducive to support the growth of these two types of tunicates.

Other factor that could play a role was the location of sampling. At Morella and Larike stations, the sampling locations were not too close to the residential areas on coastal areas, while in other locations (Mamala, Hitu, Wakal), the sampling stations were very close to residential areas. This explains the sighting of loads of rubbish trapped on the reef or floating on the surface.

In regards to the sampling method, we suggest that to assess the density of colony-type tunicates such as *Didemnum* sp., it is best to use a quadrant transect. This would facilitate easier observation because of their high density and relatively small size. Solitary types such as *Polycarpa* sp. were easily observed using line transect because of their relatively larger size compared to *Didemnum* sp. in the waters around Ambon Island and the amount in one particular area was not as much as a colony like *Didemnum* sp. Further monitoring effort should include in-depth identification using the combination of representative number of individual specimen body tissues examination and molecular technique. Both techniques will be necessary to avoid misidentification that could happen when based solely on the tunic morphology and color variation, and to deepen our understanding of tunicates ecology in Ambon Island waters.
4. Conclusion
The lack of available data on tunicate especially in *Didemnum* and *Polycarpa* genera in Ambon Island waters makes this study one of the few future references to marine resources monitoring in the local coral reef ecosystem. This study represents the first ever assessment on the interspecific tunic color variation of the solitary-living *Polycarpa* sp found in Ambon Island waters. Density monitoring carried out showed an average density of five stations of less than 1 individual per square meter, with tunicates of the social type (*Didemnum* sp.) dominating three of the five stations where tunicates were found. Twelve tunic color variations were observed on *Polycarpa* sp. This might be due to water quality and substrate condition where the monitoring was carried out. Further studies are needed to confirm this initial assessment results, and to understand the ecological implication of the *Polycarpa* sp. tunic color variation on their environmental adaptation and survival, notably against the ever-increasing anthropogenic pressure in this area.

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Author contributions
All authors have reviewed the final version of the manuscript and approved it for publication. Conceived and designed the study: YT; performed research, analysed data, and wrote/reviewed the paper: YT, FR.

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