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FULL LENGTH ARTICLE

Color diversity and distribution of sea urchin *Tripneustes gratilla* in Cenderawasih Bay ecoregion of Papua, Indonesia

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Abstract *Tripneustes gratilla* is a sea urchin with a variety of colors, which is associated with adaptability to the environment. The color diversity of sea urchin in the Cenderawasih Bay ecoregion has not been studied yet. Therefore, we investigated the habitat influences on color pattern and distribution of *T. gratilla* at Cenderawasih Bay. One hundred and seven individuals of *T. gratilla* were randomly collected in 5 different locations in Cenderawasih Bay. There are 35, 30, 13, 20 and nine individuals from Manokwari, Wasior, Biak, Nabire, and Yapen, respectively. The result showed the test (skeleton), spine, and tube feet color of *T. gratilla* in Cenderawasih Bay have 31 different colors. Tangency analysis indicated that the color diversity was associated with location site of *T. gratilla* at Cenderawasih Bay. Black-white is dominant test color of *T. gratilla* and occurs in all populations, whereas dark purple-orange only occurs in Wasior population. Five spine colors of *T. gratilla* were not uniformly distributed in all populations. The white color spine is dominant and occurs at all locations while reddish orange occurs only in Wasior. Moreover, the color of tube feet is very influenced by the environment.

Introduction

Tripneustes gratilla is one of the three sea urchin species of genus Tripneustes while two other species are *Tripneustes ventricosus* and *Tripneustes depressus*. These species are distributed in the ocean worldwide, such as West Pacific, East Africa, Southern Islands, Australia, and South Japan (Kroh, 2015). *T. gratilla* is also found in the Indonesia waters (Wainwright et al., 2013) including Cenderawasih Bay (Toha et al., 2012).

T. gratilla shows several color patterns and the color polymorphism is correlated with natural selection and habitat adaptation (Growns and Ritz, 1994; Boudouresque and Verlaque, 2001; Calderon et al., 2010; Vardaro, 2010). Color variations are beneficial for opportunity enlargement to survive and reproduce, such as selection against predation through camouflage (Bond, 2007). The color diversity also occur in snail (Johannesson and Ekendahl, 2002; Manriquez

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et al., 2009), gastropod (Miura et al., 2007), bivalve (Todd et al., 2006), sea star (Harley et al. 2006), and crab (Palma and Steneck, 2001).

Habitually, color diversity studies used for species identification (Brueggeman, 1998; Calderon et al., 2010) and taxonomy (Jensen, 1974). Nevertheless, the color diversity also associated with adaptation to the environment; then we want to examine the habitat influences on the color pattern of *T. gratilla* at Cenderawasih Bay ecoregion. Moreover, the color diversity of sea urchin in the bay also has not been studied yet. Further, this study intended to examine the distribution of *T. gratilla at* Cenderawasih Bay based on the color pattern.

Materials and method

As many as 107 individuals of *T. gratilla* were randomly collected in 5 different locations in Cenderawasih Bay ecoregion (Fig. 1). There are 35, 30, 13, 20 and nine individuals from Manokwari, Wasior, Biak, Nabire, and Yapen, respectively. Species identification based on morphology and short DNA sequences of Cytochrome Oxidase I gene (COI). Color pattern of *T. gratilla* was observed visually and recorded in fresh or alive condition, especially test (skeleton), spine, and tube feet. The color was categories into a single color, bi-color and tri-color of the test, spine and tube feet of *T. gratilla*. The single color was described as black, dark purple, brown, orange, reddish orange, white. The bi-color was classified into black-brown, black-orange, black-white, dark purple-brown, dark purple-orange, brown mixed with white, clear white, clear white-white, white-grey. Whereas the tri-color was categorized into grey-brown-white, grey-black-white, grey-white-grey, clear-grey-white, clear-brown-white, clear-black-white, brown-white-grey, etc. The colors were then sorted from clear to dark in each color category based on visual accuracy, and grouped it in an extensive category based on localized color pattern on each part of *T. gratilla*. Qualitative data of the color of the test, spine and foot tube of *T. gratilla* were transformed into quantitative data. The quantitative data were analyzed using contingency analysis, and descriptive statistic analyzed to obtain mean, standard deviation and percentage values.

Results

The *T. gratilla* were identified based on morphology and, representation of each location was analyzed using DNA Barcode. The phylogenetic tree showed that sea urchin samples were clustered into *T. gratilla* from a gene bank (Fig. 2). All samples shows 99–100% similarity with the COI sequences of *T. gratilla* (accession number KF012821.1–KF012824.1.) from Southwest Pacific (Liggins et al., 2014). The data indicated that the samples were collected from Cenderawasih Bay ecoregion is *T. gratilla* species.

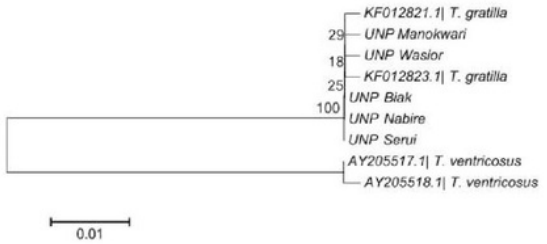

Figure 2 Neighbor-Joining tree of the COI gene data for Genus Tripneustes (with 1000 bootstrap). UNP code was COI sequences (592 bp) of *T. gratilla* from five sites in this study while KF and AY were sequences from GenBank.

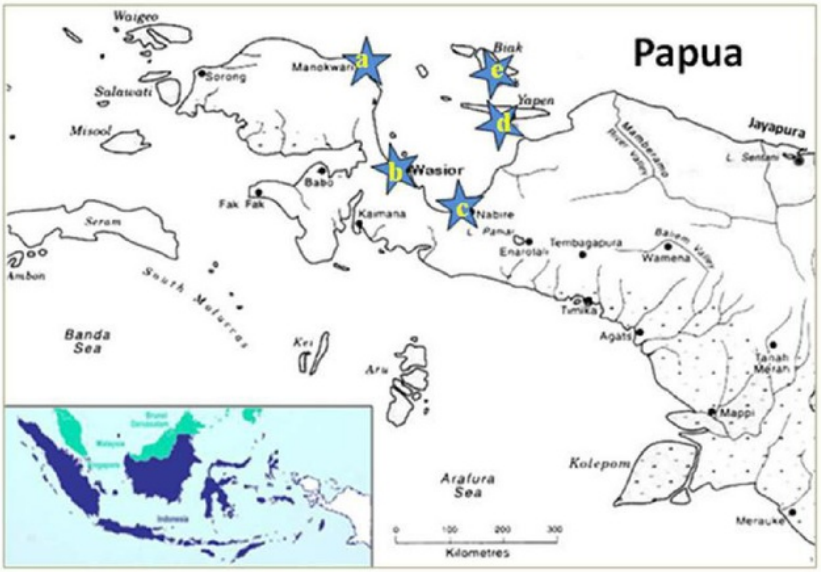

Figure 1 Sampling sites of *T. gratilla* in Manokwari (a), Wasior (b), Nabire (c), Yapen (d) and Biak (e).

Color pattern of T. gratilla

The test, spine and tube feet of the *T. gratilla* have 31 colors consisting of four single colors, nine bi-colors, and 18 triple colors. The test and spines have a lower diversity than the color tube feet. The color diversity of tube feet has a color cast wide and almost has shades of color according to the habitat (sampling site). This data indicates that the color of tube feet is very influenced by the environment (Fig. 3).

The test of *T. gratilla* have six multiple colors, i.e., black-white colored (43.27%), brownish purple (23.08), black-orange (12.5%), dark purple-orange (9.61%), black-brown (7.69%), and dark purple-orange (3.85%). The result of contingency analysis showed that the test color frequency of *T. gratilla* from all study sites was significantly different from the location (χ^2 = 2.80E−24, df = 24, $P < 0.0001$). Black-white was the only test color recorded at all five study sites. Dark purple-orang was identified in *T. gratilla* shells from Biak, Manokwari, and Nabire. Black-brown occurred only in Biak and Wasior, black-orange from Nabire and Yapen; dark purple-brown in *T. gratilla* test from Manokwari and Nabire; and dark purple-orange (reddish) only in *T. gratilla* tests from Wasior.

Coincident with test color diversity, contingency analysis indicated that spine and tube feet of *T. gratilla* also have color different pattern at each location with significant value, i.e. χ^2 = 5.65E−17, db = 16, $P < 0.0001$ and χ^2 = 1.07859E−23, df = 80, $P < 0.0001$, respectively. *T. gratilla* spines were dominantly white-colored (43.27%) that found in all 5 study sites, followed by brown-white mixture (23.08%), orange (22.12%), brown (n7.69%) and then orange (reddish) (3.85%). Whereas tube feet color variation was white-black-grey (21.15%); grey-brown-white (20.19%); clear-grey-white (5.77%), clear-brown-white and black-brown-clear (4.81%); grey-black-white, clear-white, clear-white-black, black-white-clear, and white (3.85%); and other colors (< 3%).

Color distribution of T. gratilla

The test color distribution of *T. gratilla* at Biak was black-brown (53.85%), black-white (30.77%) and dark purple-orange (15.38%). While Manokwari was dark purple-brown (57.14%), black-white (25.71%) and dark purple-orange (11.76%). Those of Nabire *was* black-orange (35.29%), black-white (29.41%), dark purple-brown (23.53%) and dark purple-orange (11.76%). *T. gratilla* from Wasior had 3 test bi-colors, i.e., black-white (83.33%), black-brown (3.33%) and dark purple-orange (14%). Moreover, the test color from Yapen had only 2 test bi-colors, black-orange (77.78%) and black-white (22.22%). Black-white is dominant test color of *T. gratilla* (43.3%) and occurs in all populations, whereas dark purple-orange (reddish) only occurs in Wasior population (Fig. 4).

Five spine colors of *T. gratilla* were not uniformly distributed in all populations (Fig. 3). In Manokwari, the spine colors of *T. gratilla* consisted of the brown-white mixture (57.14%), orange (17.14%), and white (5.71%). While from Biak and Nabire were brown (53.85% and 23.53%, respectively), white (30.77% and 29.41%, respectively) and orange (15.38% and 47.06%, respectively). Those from Wasior consisted of white (83.33%), reddish orange (13.33%) and brown (3.33%). Yapen had *T. gratilla* with the orange spine (77.78%) and white spine (22.22%) only. The white color spine is dominant (43.3%) and occurs at all locations while reddish orange (3.8%) occurs only in Wasior. The spine color dominated by

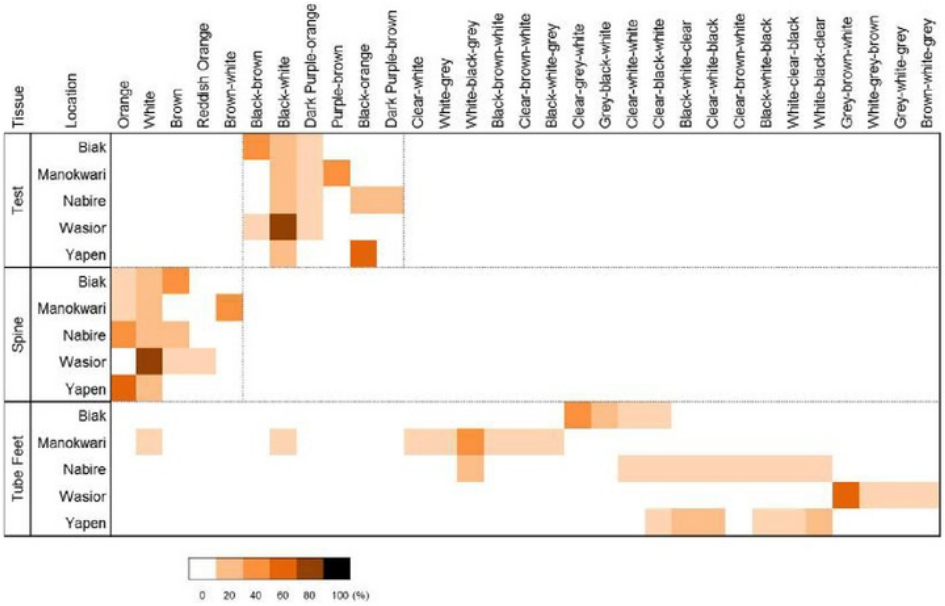

Figure 3 The color pattern of the test, spine and tube feet of *T. gratilla* at Cenderawasih Bay. The color variation might be influenced by its habitat or sampling site, especially the color of tube feet.

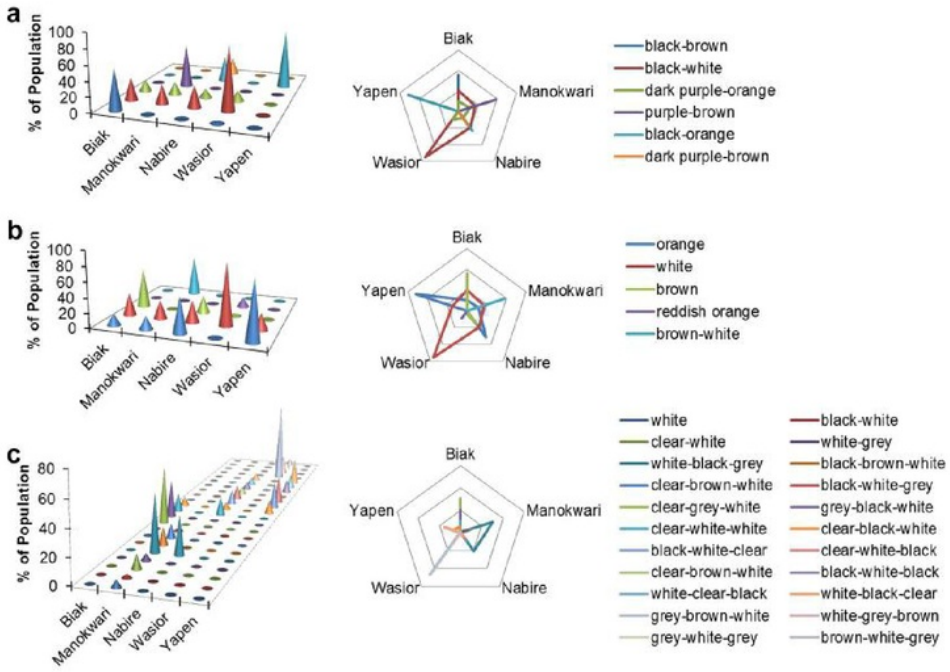

Figure 4 The color variation and distribution of the test (a), Spines (b) and Tube feet (c) of *T. gratilla* at Cenderawasih Bay (with all P value < 0.0001). The color of sea urchin shows distribute according to habitat or sampling site. The test color at Wasior, Yapen, Biak, Manokwari, and Nabire were dominated by black-white, orange, brown, purple-brown and orange, respectively. While Spine color at Wasior, Yapen, Biak, Manokwari, and Nabire were dominated by white, orange, brown, brown-white and orange, respectively. While the tube feet Wasior, Yapen, Biak, Manokwari, and Nabire were dominated by grey-brown-white, white-black-clear, clear-grey-white, and white-black-grey, respectively.

white, followed by brown-white mixture, orange, brown and orange (reddish), which is in line with Kroh's (2015) report.

The tube feet color of *T. gratilla* in Manokwari had 8 tube feet colors, i.e., white-black-grey (45.71%), black-brown-white (14.29%), clear-brown-white and clear-white (11.43%), white and white-grey (5.71%) and black-white and black-white-grey (2.86%). Biak had 4 tube feet colors, clear-grey-white (46.15%), grey-black-white (30.77%), clear-white-white (15.38%), and clear-black-white (7.69%). Nabire had 9 tube feet colors, white-black-grey (35.29%), white, black-white-clear, clear-white-black (11.76%), clear-brown-white, clear-black-white, black-white-black, white-clear-black, white-black-clear, each only occur in 5.88% in population. Wasior had four tube feet colors, grey-brown-white (70%), white-grey-brown (16.67%), grey-white-grey and brown-white-grey (6.67%). Yapen had tube feet in white-black-clear, black-white-clear, clear-white-black, each 22.22% in population, and clear-black-white, black-white-black, and white-clear-black, each in 11.11% in population. White-black-grey is dominant tube feet color of *T. gratilla* (21.2%) while the least colors of the tube feet are black-white and black-white-grey (each holds 1.0%).

Discussion

Color variations of *T. gratilla* appear in tests, spine, and tube feet colors that create a complex polymorphism. This data correspond with Growns and Ritz (1994), and Coppard and Campbell (2004) stated that sea urchin have color polymorphism due to the ability changing color intensity (Jensen, 1974). This color polymorphism also often occurs in the snail, *Cepaea nemoralis* (Burke, 1989; Vicario et al., 1988), *Nucella lapillus* (Etter, 1988), and *Littorina* spp (Johannesson and Ekendahl, 2002). The color variation of sea urchin was controlled by environment such as sunlight exposure (Stoletzki and Schierwater, 2005), food (Tlusty and Hyland, 2005) and gene (Medioni et al., 2001; Pérez-Portela et al., 2007; Hizi-Degany et al., 2007; Prada et al., 2008; Pleijel et al., 2009). The variation is accumulation of selection, ecological and physiological adaptation (Sokolova and Berger, 2000; Palma and Steneck, 2001; Tollrian and Heibl, 2004).

Other study suggested that the color variation of sea urchin possible due to the presence of carotenoid and naphthoquinone pigment (Tsushima and Matsuno, 1990; Matsuno and Tsushima, 2001). Carotenoid gives colors including red, orange and yellow (Kelly and Symonds, 2013). While purple, green and red colors are derived from naphthoquinone (Wise, 2011). Although animals could not synthesis carotenoid, the compound can be absorbed by another organism (Shahidi et al., 1998). Besides that, sea urchin might possess variations chromophore brings on different spine colors. As an example red sea urchin contains chromophores type spinochrome B, echinochrome A and spinochrome E (Amarowicz et al., 1994).

Five locations in Cenderawasih Bay ecoregion has variation in test color highly. Two locations in Manokwari and Biak

showed a tendency of has a dark color of the test. The other place (Nabire and Wasior) exhibited more dominant dark to light color mixture. While Yapen showed dark-light color. The test color variation of *T. gratilla* seems to be related to sunlight exposure, alga, and substrate. The white proportion of *T. gratilla* increases in location abundance of alga that suggested the algae camouflage visualization of sea urchin predator. Whereas High intensity of sunlight stimulated pigmentation of sea urchin, so the test color tends to be darker. According to Wise (2011), the lighter-colored test could raise the fitness than the dark color.

Conclusion

The test, spine and tube feet of the *T. gratilla* have high colors diversity. The color diversity was associated with location site or habitat of *T. gratilla* at Cenderawasih Bay ecoregion. The color diversity in Manokwari, Wasior and Nabire were lower than Biak and Yapen.

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