

Genetic Diversity and Connectivity of Sea Urchin *Tripneustes gratilla* in Region Surrounding Cenderawasih Bay, Papua-Indonesia and Indo-Pacific

Abdul Hamid A. Toha^{1*}, Ambariyanto Ambariyanto², Widodo Widodo³, Luchman Hakim³, Sutiman B. Sumitro³, Agustina L. N. Aminin⁴

¹Fisheries Department, University of Papua, Manokwari, Indonesia

²Department of Marine Science, University of Diponegoro, Semarang, Indonesia

³Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia

⁴Chemistry Department, University of Diponegoro, Semarang, Indonesia

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ABSTRACT

Cenderawasih Bay has extremely high biodiversity with an enormous amount of endemic species due to geography isolation. *Tripneustes gratilla* is one of the species that is abundant in the bay. The species has ecological value that is suitable for bio-indicators of the environmental condition. Since the Bay relatively isolated area, then we examined the impact of the geographical conditions on genetic diversity and connectivity of *Tripneustes gratilla* among populations in the region surrounding Cenderawasih Bay based on the mitochondrial cytochrome c-oxidase-1 gene. Results of the study showed that genetic variation of the species within the population is high; despite the genetic variation among populations was low. The data suggested that the entire population of *T. gratilla* were closed connected, homogeneous, and shared polymorphic profile. Then we assumed the gene flow occurred for a long time among populations without geographical barriers. This information is a warrant to develop an effective strategy to maintain biodiversity in the Cenderawasih Bay.

1. Introduction

Cenderawasih Bay is coral ecoregion in Papua-Indonesia, which has extremely high biodiversity and an enormous amount of endemic species (Veron *et al.* 2009; Starger *et al.* 2015). Over 50 different species of marine organisms have been found in the Cendrawasih Bay (Carpenter *et al.* 2011). The bay was isolated since 14 million years ago that facilitated evolution and developing endemic species (Allen and Erdmann 2008; Allen and Erdmann 2009; Alonso *et al.* 2011). The condition caused some species viz. Mantis shrimp and starfish were isolated in the bay (Barber *et al.* 2006; Crandall *et al.* 2008). Sea urchin found evenly distributed in the Cenderawasih bay (Toha *et al.* 2012, 2015). Then we anticipated that the existence of sea urchin in the bay was also isolated and has low genetic diversity.

Tripneustes gratilla is one of over 1,000 described species of sea urchins (WorRMS 2020) that is widely distributed (Toha *et al.* 2017; Kroh and Mooi 2019, 2020). The habitat ranges from Pacific to African coast of the Indian Ocean, including Indonesia (Toha *et al.* 2012; Lawrence and Agatsuma 2013; Wainwright *et al.* 2013; Parvez *et al.* 2018; Wainwright *et al.* 2019). The *T. gratilla* has economic and ecological value (Casilagan *et al.* 2013; Brink *et al.* 2019, Onomu *et al.* 2020; Nane and Paramata 2020) that warrant for use of bio-indicators of marine environmental conditions. Then understanding the genetic diversity of the species is paramount information to promote long-term sustainable management (Toha *et al.* 2014) in Cenderawasih Bay. Since the bay relatively isolated area, then we examined the impact of the geographical conditions that led to isolation of *T. gratilla* in the region.

Lessios *et al.* (2003) and Liggins *et al.* (2014) examined phylogeography and genetic patterns of *T. gratilla* in the Indo-Pacific region based on nucleotide

* Corresponding Author

E-mail Address: h.toha@unipa.ac.id

sequence of the mitochondrial cytochrome c-oxidase-1 (COI mtDNA). They reported that *T. gratilla* were closed connected, homogeneous, and shared polymorphic profile. Meanwhile, in Indonesia, the population genetic of *T. gratilla* were revealed as evidence of genetically distinct clusters and structure based on microsatellite loci (Wainwright *et al.* 2019). However, *T. gratilla* remains genetically uncharacterised in Cenderawasih Bay. In the present study, we determined the genetic diversity and connectivity among populations of *T. gratilla* in the area surrounding Cenderawasih Bay based on the COI gene sequences. We also compared the data from this region with other the Indo-Pacific region to examine the impact of the geographical condition to the broader region.

2. Materials and Methods

2.1. Sampling and DNA Sequencing

T. gratilla was collected from Cenderawasih Bay, i.e. Wasior (3 samples), Nabire (11 samples) and Yapen or Serui (11 samples). We also collected samples from the out region of Cenderawasih Bay, such as Manokwari (34 specimens) and Biak (17

specimens). Sequences from GenBank AY205373-455 (Lessios *et al.* 2003), JX661089-167 (Casilagan 2012), KF012802-824 (Liggins *et al.* 2014) were also used to complement our 76 sequences of *T. gratilla* from five locations (Figure 1). The tube feet samples were preserved in 95% ethanol and stored at room temperature until to be used for genomic DNA extraction. The Genomic DNA were extracted from tube feet tissue using Chelex (Walsh *et al.* 2018). The COI gene was amplified using primers Trip2F (5'CCTGCAGGAGGAGGAGAYCC3') (Jacobs *et al.* 1988) and CO1TR1 (5'GGCATTCCAGCTAGTCTCTARAA3') (Lessios *et al.* 2003). Polymerase chain reaction (PCR) was conducted with thermocycling parameters 95°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec, for 38 cycles. Five microliters of double-stranded PCR fragments were purified by adding 0.5 units of shrimp alkaline phosphatase, and five units of exonuclease, then incubating at 37°C for 30 min and 80°C for 15 min. The cleaned PCR fragments were sequenced on an ABI 377 automated sequencer using BigDye (Applied Biosystems, Foster City, CA) terminator chemistry. The forward and reverse sequences were proofread in Sequencher (Gene Codes Corp., Ann Arbor, MI).

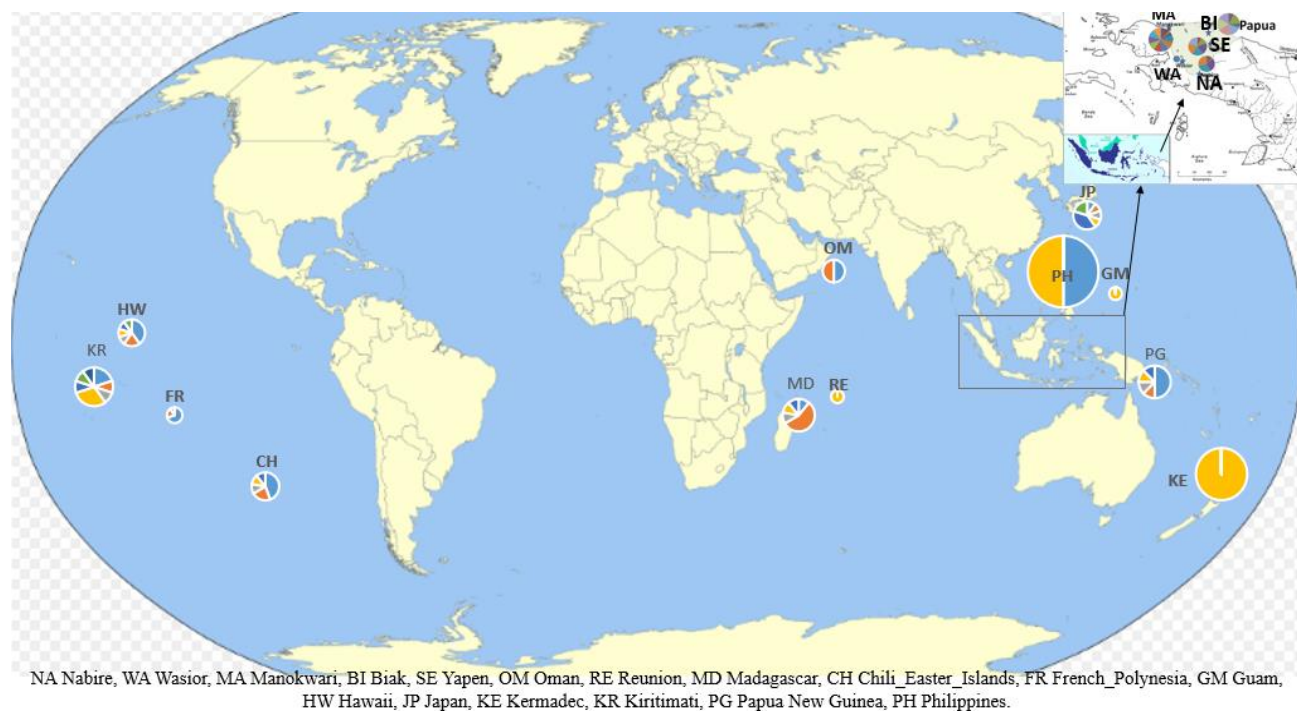


Figure 1. Map of study locations and their haplotypes frequency. Sampling location of *T. gratilla* from inside of Cenderawasih Bay (Wasior, Yapen and Nabire) and outside of the bay (Manokwari and Biak)(insert). Pie indicated the size of the haplotypes frequency at each location. Every color in the pie indicated the number and kind of haplotype. Dash line insert is a barrier hypothesis that isolates between inside and outside of the Cenderawasih Bay

2.2. Sequence Analyses

The numbers of variable sites were assessed with MEGA6 (Tamura *et al.* 2013). Polymorphic site and haplotype were calculated using the DnaSP software version 5 (Librado and Rozas 2009). We also created a haplotype network using the program Network 4.6.0.0 to examine the distribution of haplotypes among populations (Bandelt 1994). Genetic structure of the population was tested by Analyzing Molecular Variance (AMOVA), used Arlequin version 3.5 (Excoffier and Lischer 2010). The test was done at three hierarchical, i.e. among groups (inside and out the region of the Cenderawasih Bay), among populations within groups, and within populations levels. The samples were grouped into two, i.e. inside (Nabire, Wasior, and Yapen population) and out region (Manokwari and Biak populations) of the bay. Phylogenetic reconstruction of *T. gratilla* was done with neighbor-joining method using MEGA6. Bootstrap resampling (n = 1,000) was performed to test the robustness of the dendrogram topologies using MEGA6 (Tamura *et al.* 2013).

3. Results

The COI mtDNA sequences of the *T. gratilla* obtained in this study have been deposited at GenBank under accession No. KX598982 to KX599057. A total of 592 bp of CO1 mtDNA were sequenced from 76 samples of five populations.

The 28 polymorphic sites, including 19 singletons and nine parsimoniously informative sites were found from the sequences. In total polymorphism were 43 transitions (10.17 + 5.43), one transversions (0.17 + 0.37) and 44 substitutions (10.33 + 5.50). Despite all samples having 25 haplotypes but only five haplotypes were shared among all populations (Table 1, Figure 1). The haplotype network reveals that most common haplotype (No. 5) found in 14 individuals that occurred in all populations. Haplotype 1 was found in 29 individuals, which is the share in four populations. Besides haplotype 2 and 4 are found in five and four samples, respectively, in three populations in Papua.

Haplotype diversity (Hd) was moderate to high in all populations (0.84 in Biak, 0.75 in Manokwari, 0.80 in Nabire, 0.87 in Yapen, and 0.67 in Wasior). Therefore the nucleotide diversity (π) was low for all samples (0.003), with values from 0.002 (Wasior and Manokwari) to 0.003 (Nabire, Biak and Yapen). According to Tajima's D, Fu and Li's D statistical tests indicated that hypotheses of selective neutrality among the population were rejected (Tajima's D = 2.233, P <0.01; Fu and Li's D = -4.278, P <0.02). Furthermore, we use AMOVA to determine the variation of the species' populations. Result of the analysis showed that the source of variation (SV) of the total sequence divergences in *T. gratilla* was not differ significantly (Table 2). Genetic variation within the population nearly 95% (high), but the

Table 1. Summary of included data and genetic diversity statistics for five studied location and 12 other locations from GenBank AY205373- 455 (Lessios *et al.* 2003), JX661089-167 (Casilagan 2012), KF012802-824 (Liggins *et al.* 2014) as a comparison: number of sequences (n), polymorphic sites (θ), number of haplotypes (H), haplotype diversity [Hd (SD)], nucleotide diversity [π (SD)], Tajima's D statistic and significance (P, no correction). Source (Src) of the CO1 data: *= present study, a = Lessios *et al.* (2003), b = Casilagan (2012), c = Liggins *et al.* (2014)

Location	Code	n	θ	H	Hd	π	Tajima's D	P	Scr
Biak	BI	17	1	9	0.84	0.003	2.05808	0.00600	*
Manokwari	MA	34	12	11	0.75	0.002	-1.77741	0.01600	*
Nabire	NA	11	8	6	0.80	0.003	-1.05273	0.17000	*
Yapen (Serui)	SE	11	4	5	0.87	0.003	-0.44419	0.35500	*
Wasior	WA	3	2	2	0.67	0.002	0.00000	0.93100	*
Chile_Easter Island	CH	8	5	5	0.8	0.003	-0.83938	0.23700	a
French_Polynesia	FR	9	8	3	0.5	0.004	-1.36919	0.09600	a
Guam	GM	2	0	1	0	0	0.00000	1.00000	a
Japan	JP	10	6	6	0.8	0.004	-0.49593	0.34500	a
Kiribati_Kiritimati	KR	10	7	7	0.9	0.004	-0.24147	0.41700	a
Madagascar	MD	8	7	5	0.7	0.004	-1.35929	0.09400	a
Oman	OM	2	1	2	1	0.002	0.00000	1.00000	a
Papua New Guinea	PG	7	7	5	0.8	0.003	-1.52412	0.03100	a
Philippines	PH	91	62	69	0.9	0.006	-2.45365	0.00000	a, b
Reunion	RE	5	0	1	0	0	0.00000	1.00000	a
USA_Hawaii	HW	10	7	6	0.8	0.003	-1.38265	0.07900	a
Kermadec Islands	KE	23	62	22	0.9	0.006	-1.92094	0.01300	c

variation among populations in the Cenderawasih bay was low (2.80%). Besides the variation between inside and outside populations of the bay was low (2.35%). Fixation indices of F-statistics (F_{ct}, F_{sc}, F_{st}) values were close to zero, which is indicated the genetic among populations was not different.

The Tajima D statistic was also significantly negative at most sites for all populations in Cenderawasih Bay and Indo-Pacific regions, indicating departures from neutral expectations for the number of recent mutations. *T. gratilla* in all locations were not significant, most likely due to the admixture of a few divergent clades. These results are expected in the case of past population expansion. These results suggested that the *T. gratilla* from the inside and outside of Cenderawasih Bay and Indo-Pacific were connected to each other (Figure 2).

The data is consistent with the results from neighbor-joining tree analysis of the COI sequences of *T. gratilla* (Figure 3). The phylogeny clearly revealed

that all samples were considered a single clade due to low bootstrap support for identified clades. The phylogeny and median-joining haplotype networks also show that individuals among locations have strong connectivity with each other.

Using AMOVA to partition genetic variation into Indo-Pacific (including Cenderawasih Bay), we found evidence of low genetic structure in all *T. gratilla* (Table 3).

There is strong genetic connectivity among the population of *T. gratilla* in Cenderawasih Bay and between *T. gratilla* in Cenderawasih and Indo-Pacific regions. Assuming no a priori structure in *T. gratilla*, F_{st} = 0.051 (P >0.006). There was not significant structure between Indian Ocean (Oman, Reunion, and Madagascar), Pacific Ocean (Chili Easter, French Polynesia, Gua, Hawaii, Japa, Kermadec, Kiritimati, PNG, and Philippines), and Cenderawasih Bay (Wasior, Nabire, Yapen, included Manokwari and Biak) regions in the study area (F_{ct} = -0.001, P >0.385).

Table 2. AMOVA based on COI mtDNA gene of *T. gratilla* in Cenderawasih Bay

Source of variation	df	Sum of squares	Variance components	Fixation indices	P value
Among groups	2	1.46	0.010	F _{CT} = 0.023	0.371
Among populations within groups	2	1.11	0.012	F _{SC} = 0.029	0.163
Within populations	70	27.71	0.396	F _{ST} = 0.052	0.052
Total	74	30.28	100.00		

AMOVA calculation by 1023 permutations. Degree of freedom (d.f), probability (P), Fixation indices of samples from group level (F_{CT}), Fixation indices of samples from populations (F_{SC}), Fixation indices of samples within populations level (F_{ST}). Groups are divided into two regions namely region in bay (consist of Nabire, Wasior, and Yapen population) and out the bay (consist of Manokwari and Biak populations)

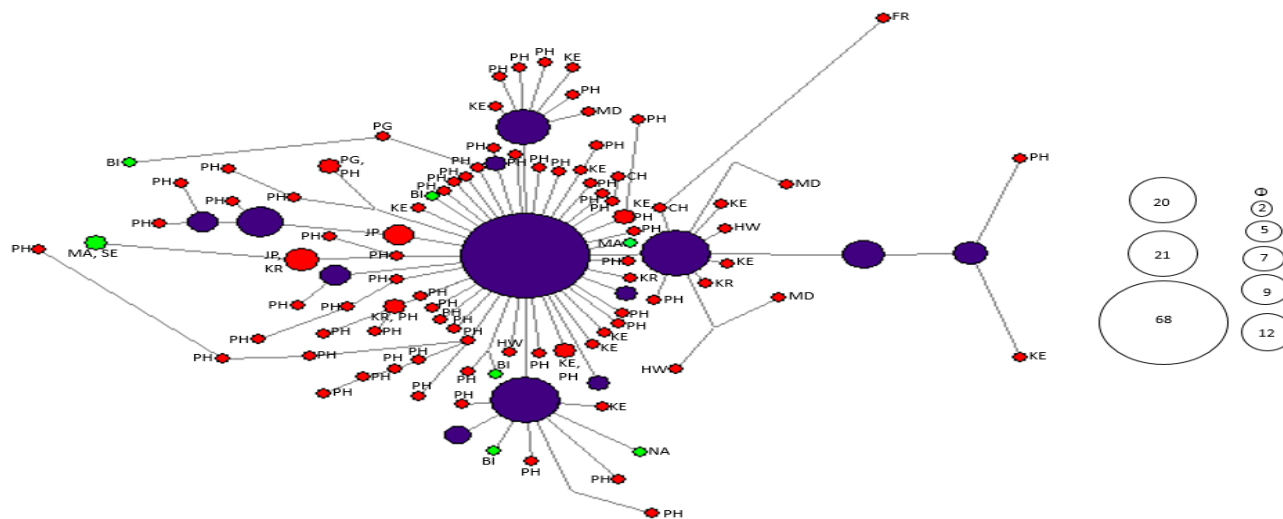


Figure 2. Median-joining haplotype networks for *T. gratilla* displaying which haplotype is found in the Cenderawasih Bay, Papua (green tone), which haplotypes are shared among locations in Indo-Pacific region and found in the Papua (purple tone), and haplotypes that are not found in the Papua (red tone, also with location code indicated). The frequency of each haplotype is indicated by size (see key, right). NA Nabire, WA Wasior, MA Manokwari, BI Biak, SE Yapen, OM Oman, RE Reunion, MD Madagascar, CH Chili_Easter_Islands, FR French_Polynesia, GM Guam, HW Hawaii, JP Japan, KE Kermadec, KR Kiritimati, PG Papua New Guinea, PH Philippines

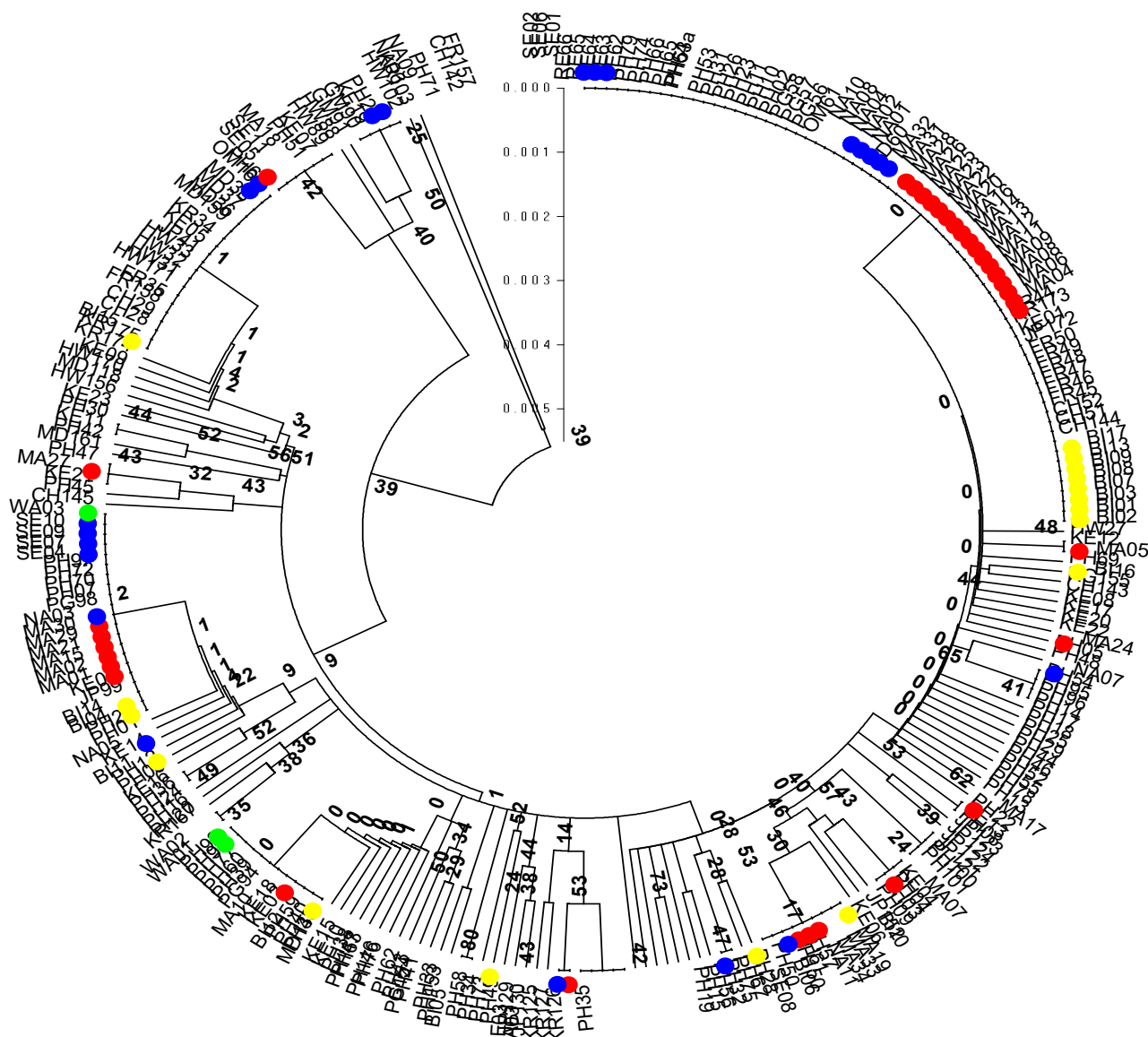


Figure 3. Neighbor-joining tree of the COI sequences of *T. gratilla* from Cenderawasih Bay and GenBank, based on Kimura 2-parameter distance. Color circles represent locations of sequence from Cenderawasih Bay ecoregion i.e. yellow (Biak), red (Manokwari), blue (Nabire), black (Yapen or Serui) and green (Wasior). The numbers at the nodes are bootstrap percent probability values based on 1,000 replications. NA Nabire, WA Wasior, MA Manokwari, BI Biak, SE Yapen, OM Oman, RE Reunion, MD Madagascar, CH Chili_Easter_Islands, FR French_Polynesia, GM Guam, HW Hawaii, JP Japan, KE Kermadec, KR Kiritimati, PG Papua New Guinea, PH Philippines

Table 3. AMOVA based on COI mtDNA gene of *T. gratilla* in Cenderawasih Bay and Indo-Pacific

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices	P value
Among groups	2	1.46	0.010	3.20	$F_{CT} = 0.023$	0.371
Among populations within groups	2	1.11	0.012	5.33	$F_{SC} = 0.029$	0.163
Within populations	70	27.71	0.396	91.47	$F_{ST} = 0.052$	0.052
Total	261	119.62	0.469	100.00		

AMOVA calculation by 1023 permutations. Degree of freedom (d.f), probability (P), Fixation indices of samples from group level (F_{CT}), Fixation indices of samples from populations (F_{SC}), Fixation indices of samples within populations level (F_{ST}). Groups are divided into three regions namely region in bay (consist of Nabire, Wasior, Manokwari, Biak, and Yapen locations), Hindia (Oman, Reunion, and Madagascar) and Pacific (consist of Chili_Easter_Islands, French_Polynesia, Guam, Hawaii, Japan, Kermadec, Kiritimati, PNG, Philippines)

4. Discussion

The number of haplotypes of *T. gratilla* in each population at Cenderawasih Bay was moderate to high. This results similar to other invertebrates, i.e. *Tridacna maxima* (Nuryanto and Kochzius 2009), *T. crocea* and *T. maxima* (DeBoer *et al.* 2014), *Haptosquilla pulchella* (Barber *et al.* 2002). Liggin *et al.* (2014) determined 23 haplotypes of COI gene of *T. gratilla* from Kermadec Island (Southwest Pacific), now this species under the name *Tripneustes kermadecensis* (Bronstein *et al.* 2017). The analyzing of 82 COI gene fragment sequences of *T. gratilla* from 11 locations in Indo-Pacific accessed from the GenBank data (Lessios *et al.* 2003) resulted in 34 haplotypes. These results are similar with allelic richness using microsatellite markers in previous studies of *T. gratilla* in South African coast (Brink *et al.* 2018), in the Philippines (Casilagan *et al.* 2013), the Philippines and the Indonesian Archipelago (Wainwright *et al.* 2013), and in Hawaii (Carlson and Lippé 2007).

Despite all populations having high haplotype diversity, the nucleotide diversity was low. This combination was similar to *T. gratilla* population in the Indo-Pacific (Lessios *et al.* 2003; Liggins *et al.* 2014), and many others marine taxa (Rocha *et al.* 2002 on Bowen *et al.* 2001), such as *H. pulchella* (Barber *et al.* 2002) and *T. crocea* (Kochzius and Nuryanto 2008; DeBoer *et al.* 2014). The high levels of haplotype diversity and low levels of nucleotide diversity indicated either a long stable evolutionary history or secondary contact among differentiated lineages (Väli *et al.* 2019). According to Plough (2016), this combination has frequently been attributed to expansion after a period of small effective population size, retaining new mutations and related to episodes of marine level oscillations (e.g. Barber *et al.* 2002).

The haplotypes were spread evenly inside and out the region of Cenderawasih Bay, suggesting that gene flow between those areas have occurred. This condition is supported by AMOVA tests that are unable to differentiate genetic variation between populations from inside and outside of the bay and also between Cenderawasih Bay and Indo-Pacific. Population within the Indo-Pacific appears to be panmictic as there are no statistically significant differences. Mitochondrial sequence variation showed very weak regional divergence of *Tripneustes* populations across the Indo-Pacific region despite the significant local differentiation among the populations in this region (Casilagan *et al.* 2013).

According to Mora *et al.* (2011), marine species often have high dispersal potential influencing their genetic diversity as well as connectivity can be maintained

in peripheral populations by immigration. Then, the influence of immigration and local reproductive success, often determined by ecological circumstances, can promote patterns of genetic diversity and differentiation that do not conform to the expectations for a peripheral population according to the Core-Periphery Hypothesis (Liggins *et al.* 2014). The CPH also known as the “central-marginal hypothesis,” predicts that these genetic patterns at the edge-of-range are a consequence of reduced population size and connectivity toward a species range periphery. López-Márquez *et al.* (2019) stated connectivity among populations, and patterns of dispersal and gene flow, are primarily determined by the physical characteristics of the landscape occupied by a species and the biological life-history traits of that species.

The result was parallel with phylogeny analysis and haplotype network that all samples for *T. gratilla* were considered a single clade and connected each other. Therefore, the result suggested that all populations of *T. gratilla* in Cenderawasih Bay had the same evolutionary history and connected with each other including with Indo-Pacific. The phenomenon occurs through dispersal of *T. gratilla* during larvae period. The species has a larval stage for 20 to 52 days under culture conditions (Shimabukuro 1991; Lawrence and Agatsuma 2013) and thus have potential to disperse relatively large distances. Taken together, overall genetic analysis suggested that the entire population of *T. gratilla* were closed connected, homogeneous, and shared polymorphic profile (Lessios *et al.* 2003; Liggins *et al.* 2014). The condition is indicating that gene flow occurred high and for a long time among the populations. This implies that *T. gratilla* in this region belongs to a large *Tripneustes* metapopulation (Lessios *et al.* 2003).

Since *T. gratilla* has no endemism in Cenderawasih Bay, and then we assumed that the geography of the bay is not able to isolate the species spread to other places. These conditions were similar to whale shark *Rhincodon typus* population in the Indo-Pacific including in the Cenderawasih bay ecoregion (Toha *et al.* 2016; Toha *et al.* 2020). The hypothesis is supported by the previous report on *T. gratilla* from along the South African coast (Brink *et al.* 2018) and the Philippines (Casilagan *et al.* 2013) and other species of sea urchin, such as *Eucidaris* (Lessios *et al.* 1999), *Diadema paucispinum* and *D. setosum* (Lessios *et al.* 2001) in Indo-Pacific.

These results were contrary to other report that explained mantis shrimp (Barber *et al.* 2002, 2006), starfish (Crandall *et al.* 2008), *Astreopora* (Scleractinia: Acroporidae) (Wallace *et al.* 2011), *Linckia laevigata* (Crandall *et al.* 2008), *T. maxima* (Nuryanto and

Kochzius 2009), *T. crocea* and *T. maxima* (DeBoer *et al.* 2014), *Gonodactylinus viridis* (Barber *et al.* 2006), and *Protoreaster nodosus* (Crandall *et al.* 2008) were isolated in Cenderawasih Bay (see Allen and Erdmann 2008; Allen and Erdmann 2009; Alonso *et al.* 2011). In these cases, connectivity may be restricted due to the relatively sheltered nature of the bay, limiting water and larval movement to outside populations. Wainwright *et al.* (2019) proposed that the observed genetic differentiation of *T. gratilla* is the result of habitat loss during periods of glacial maxima and differences in physical oceanographic properties throughout Indonesia.

4.1. Consequences for Conservation

According to Bertocci *et al.* (2018), the high demand for Uni has led to overexploitation and depletion of many urchin populations in their natural habitat. Consequently, the world sea urchin fishery is now in a state of decline (Stefánsson *et al.* 2017; FAO 2020). Characterising genetic diversity and structure of populations is essential for effective conservation of threatened species (Väli *et al.* 2019). According to Goodall-Copestake *et al.* (2012) estimates of genetic diversity represent a valuable resource for biodiversity assessments and could be used to guide conservation and management programs. Wainwright *et al.* (2019) stated the documentation of cryptic diversity will aid in the identification of hot spots of biodiversity and ultimately its conservation.

The maintenance of genetic diversity within species is critically important as insurance for the ability of populations to survive and reproduce through global climate change. This diversity gives populations and species a better chance that at least some individuals bear the traits necessary to ensure environmental changes. Genetic diversity represents the very building blocks of adaptation and natural selection, and serves as a primary buffer against extirpations and even extinction. To minimize marine extinctions, Indonesia's national marine conservation and MPA strategy and Papua was overwhelmingly ranked the top marine biodiversity conservation priority in Indonesia and must include a focus on maintaining genetic diversity (Huffard *et al.* 2012).

Results from this study clearly indicated that *T. gratilla* populations from Cenderawasih Bay in Papua-Indonesia have strong genetic connectivity. The results also show strong connectivity among *T. gratilla* populations from Cenderawasih Bay and Indo-Pacific, indicating a general pattern of panmixia among these regions. According to Brink *et al.* (2018), the panmixia observed within these natural populations of *T. gratilla* indicated that they could be managed as a single genetic stock. The establishment of a large

transboundary marine protected area has existed for the region namely Cenderawasih Bay National Park covers 1,453,500 hectares (Mangubhai *et al.* 2012).

Existing infrastructure and capacity in Cenderawasih Bay National Park should serve as a sound expansion point for conservation, which should include the creation of a network of MPAs running north to south and creating a "connectivity corridor" along the Cenderawasih Ecoregion. Integral role in connectivity and larval dispersal suggested an effective strategy to maintain biodiversity in the Cenderawasih Bay is to connect of the two actively managed of Marine Protected Area (MPAs) viz., Taman Nasional Teluk Cenderawasih covers 1,453,500 hectares; and Padaido Islands Marine Tourism Park covers 183,000 hectares (Mangubhai *et al.* 2012) and others. The network of seven MPAs in the Raja Ampat region (Papua-Indonesia) is an excellent example of this approach (Starger *et al.* 2015).

In conclusion, haplotype diversity of *T. gratilla* was moderate to high and spread evenly across the Cenderawasih bay. The entire populations of *T. gratilla* in Cenderawasih Bay were closed connected and shared polymorphic profile. The phenomenon is indicating that gene flow occurred high and for a long time among populations including Indo-Pacific regions.

Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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