

GENETIC ASPECTS OF THE COMMERCIALY USED SEA URCHIN *Tripneustes gratilla*

A REVIEW

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ABSTRACT

Tripneustes gratilla is economically important, supports small-scale, commercially important, ecological values, a prospect as a biological control agent and also considered as the commercially traded sea urchin. We review genetic aspects of *T. gratilla* for understanding the status to the sustainable use in the future. In GenBank, there are 267 nucleotide sequences related with *T. gratilla*. Most of the sequences (189 sequences) are COI gene of *T. gratilla* from Indo-Pacific Ocean. Study on molecular genetics mentioned that there is no genetic structure for *T. gratilla* distribution in Indo-Pacific Ocean including in Indonesia waters.

Key words: *T. gratilla*, sea urchin, Indo-Pacific Ocean, genetic, COI gene.

INTRODUCTION

T. gratilla (Linnaeus 1758) is one of three species of sea urchin clustered into genus *Tripneustes*. Two other species in the same genus are *T. ventricosus* (Lamarck 1816) and *T. depressus* (Agassiz 1863) (Lawrence & Agatsuma 2007, Pena et al., 2010). Taxonomic classified *T. gratilla* into kingdom Animalia, phylum Echinodermata, subphylum Echinozoa, class Echinoidea, subclass Euechinoidea, infraclass Carinacea, superordo Echinacea, ordo Camarodonta, infraordo Echinidae, superfamily Odontophora, family Toxopneustidae and genus *Tripneustes* (L. Agassiz 1841)(Kroh, 2015).

T. gratilla spreads out throughout the world ocean including Indonesia. This has been supported with numerous both local and common name dedicated to this species found in different countries such as common names of Pfaffenhutseeigel (German); sea egg, tuxedo urchin, priest-hat urchin and collector urchin (British and U.S), hairy sea urchin (Australia) and bulu babi (Indonesia). Some local names of *T. gratilla* are Swaki, Santol-santolan, Maritangtnag, and Kuden-kuden in Phillipines, hawa'e maoli in Hawaii (personal comm. with Gustav Paulay). This species have some local name in Indonesian region such as Taeo and Tihe (Bau-Bau and Tomia, Buton districts), Asarwae, Soroaki, Isarwae, Soroaku, Sarwae, Ansam (Fakfak, Biak, Serui-Papua), Barange (Wakuru-Muna), Katiri (Bima, NTB), Sahoaki (Tagulandang-Sulawesi Utara), and Saroa (Bacan-Maluku Utara).

As most of other littoral echinoids, *T. gratilla* poses important economic and ecological values and has been categorized as primary herbivore in various marine habitats (Unsworth et al., 2010). *T. gratilla* is also reported to own a prospect as a biological control agent (Stimson et al., 2007) which can produce a peditoksin and bioactive

compound usefull for drug discoveries and pharmacological research (Takei et al., 1991, Nakagawa et. al., 2003). Its gonad has also been reported as an important economic commodity and alternative livelihood source for most people living in coastal areas in many countries (Williams, 2002, Juinio-Meñes et al., 2001). In addition to that in Japan this species is also considered as the commercially traded sea urchin (Rahman et al., 2009).

This paper covers comprehensive information on genetic of this species. This we hope can provide important information to improve human welfare through the advancement of science development, technology and environmental science.

GENOME ORGANIZATION

The mitochondrial DNA (mtDNA) of animal is generally a small genome (about 15-20 kb) containing 37 genes (Boore, 1999). The genes are 13 protein-coding genes which encode proteins involved in the oxidative phosphorylation machinery: cytochrome oxidase subunits 1, 2, and 3 (CO1 to CO3); cytochrome b subunit (CytB), NADH dehydrogenase subunits 1, 2, 3, 4, 4L, 5, and 6 (ND1 to ND6, ND4L), and ATPase subunits 6 and 8 (ATP6 and TP8). The mt genome also contains 2 ribosomal RNA genes (16S and 12S) and 22 transfer RNA genes (Luo et al., 2011).

The COI gene of *T. gratilla* is located between LrRNA and R genes. It codes the protein cytochrome oxidase unit I involved in oxidative phosphorylase of *T. gratilla*. The fragment sequence of the gene was determined, for instance, Zigler & Lessios (2003) and Hoareau & Boissin (2010). The gene of 15S rRNA codes the polypeptide of 15S RNA ribosome. This polypeptide, as polypeptides coded by the 23S rRNA gene, has structural

roles acting as scaffolding, determines the ribosomal protein position, binds to the initial codon upstream of AUG on mRNA, interacts with the 23S, helps binding two ribosome subunits (50S +30 S), and stabilizes the codon-anticodon pairing on A side, through formation of hydrogen bonds between N1 atom of residual Adenine 1492 and 1493 residue and 2'OH group of the mRNA backbone. The nucleotide sequence of this gene was determined by Oohara et al. (2004).

The MT-ND1 or ND1 mtDNA gene codes proteins called the NADH dehydrogenase 1. This protein is part of active large enzyme complex in the mitochondria. The MT-ND2 gene codes proteins called the NADH dehydrogenase 2. This protein is a nucleic subunit of the mitochondrial chain NADH dehydrogenase respiratory membrane (complex I) believed to have minimal assembly needed for catalysis. The second sequence of the fragment or this gene was determined by Kinjo et al. (2008). Some nucleotide sequence of the nucleic DNA (cytoplasm), such as the nucleotide partial sequence of the nucleic DNA, such as the microsatellite clone Tgr-A11, was shown by Carlon & Lippe (2007). The nucleotide complete sequence of beta-catenin mRNA gene of the nucleic DNA possesses 3061 bp. The data can be accessed from the GenBank at the accession number of L10354.1 (Rosenthal, 1993). The nucleotide complete sequence of protein 217g coding gene of the nucleic DNA consists of 4702 bp. The data were accessed from the GenBank at the accession number of M22207.1 (Dolecki et al., 1988).

The nucleotide partial sequence of beta dynein heavy chain mRNA gene can be accessed from the GenBank at the accession number of S57977. Number of nucleotides of this gene was only 1-51 bp (Gibbons et al., 1991). The nucleotide partial sequence of actin gene Cyl=Tg616 can also be accessed from the GenBank at the accession number of S74059, with nucleotide sequence of 1-5277 bp (Wang et al., 1994). The nucleotide complete sequence of laminin-binding protein mRNA gene with 34/67kD, can be accessed from the GenBank at the accession number of U02371. Number of nucleotides was 1-1212 bp (Rosenthal & Wordeman 1995). The nucleotide partial sequence of dynein heavy chain isotope 3A (DYH3A) mRNA gene was accessed from the GenBank at the accession number of U02391, with nucleotide sequence of 1-3213 bp (Gibbons et al., 1994).

The nucleotide partial sequence of dynein heavy chain isotope 4 (DYH4) mRNA gene of *T. gratilla* was accessed from the GenBank at the accession number of U03973, with the nucleotide sequence of 1-3408 bp (Gibbons et al., 1994). The nucleotide partial sequence of rRNA 18S gene of *T. gratilla* was accessed from the GenBank at the accession number of Z37134, with the nucleotide sequence of 1-1764 bp (Littlewood & Smith, 1995). The nucleotide complete sequence of toposome mRNA gene of *T. gratilla* was accessed from the GenBank at the accession number of AY026514, with the nucleotide sequence of 1-4741 bp

(Noll et al., 2007). The nucleotide complete sequence of TgHBox4 homeodomain protein mRNA gene of *T. gratilla* can also be accessed from the GenBank at the accession number of AF254953 with a nucleotide length of 3634 bp (Vansant & Humphreys, 2000).

DNA sequence studies have shown that the homeobox sequence is well represented and highly preserved in invertebrates, mollusks, arthropods and vertebrates (Holland & Hogan, 1986). In *T. gratilla*, there are not less than five box genes containing the homeo, at least one is transcribed during the blastula and gastrula stages (Dolecki et al., 1986). Since the embryo in this case was not segmented, it is possible that this gene possess different developmental roles from that of the conventional sent to the box containing other homeo genes. This also apparently becomes cases for several homeo box genes of fruit flies, *Drosophila melanogaster*. Thus, in gene S67 case (=caudal), the transcription, particularly in the anterior and posterior tissues of the embryo, the gene is probably involved in the identity of the antero-postero position (Hoey et al., 1986). In gene S60 (=zen), on the other hand, the transcription activity is mostly limited to the embryo dorsal tissue and this gene works in the dorso-ventral differentiation of the embryo (Doyle et al., 1986).

T. gratilla possesses the Hox gene. It is one of the gene families recently paid attention due to their benefits for evolution and animal shape development (Carroll, 1995). This gene belongs to transcription factor coding homeobox genes during the expression development setting of various downstream genes (Arnone et al., 2006). Hox gene was firstly identified in Hawaiian *T. gratilla* in 1986 (Dolecki et al., 1986). The Nucleotide partial sequence of homeo box gene was accessed from the GenBank at the accession number of M19709, with nucleotide sequence of 1-589 bp (Dolecki & Humphreys, 1988).

NUCLEOTIDE GENE DATA

Nucleotide sequences are completely shown (mRNA toposome, homeodomain protein, ND2, laminin binding mRNA protein, protein 217g coding gene, and beta-catenin mRNA), and the others are partial nucleotide sequences (such as COI gene, dynein heavy chain, binding gene, 16S rRNA gene, and mitochondrial ND1). The length of the gene nucleotide or gene fragment recorded ranged from 69 bp (beta dynein heavy chain partial mRNA, with accession code of AF254953.1) to 7542 bp (retrovirus-like element, with accession code of M75723.1). The sequences possessing many samples from COI partial group (189 partial sequences) and the least from ectodermal regulating gene sequence group, retroviral elements, toposome mRNA, beta-catenin mRNA, 16S rRNA mtDNA gene, mRNA laminin binding protein, bond morphogenetic protein, NDI gene, ND2, 18S rRNA gene, actin Cyl, and mRNA homeodomain protein each of which possesses 1 sequence.

The use of the nucleotide sequence varied, such as for morphological and molecular phylogeny (Littlewood &

Smith, 1995), marker organization (Carlson & Lippe, 2007), primary design for barcoding (Hoareau & Boissin, 2010), phylogeny and expression (Gibbons et al., 1994), mtDNA phylogeny (Zigler & Lessios, 2003), retrovirus-like element (Springer et al., 1991), evolutionary history of larval skeleton morphology (Kinjo et al., 2008), and others.

Search for the nucleotide sequence in NCBI (<http://www.ncbi.nlm.nih.gov/nucleotide>) found 267 nucleotide sequences. These sequences originate from mtDNA and nDNA nucleotides.

Table 1. Gene or DNA sequences of *T. gratilla* from GenBank

Sequence	No. sequences	Fragment/gene size	GenBank accession number
Homeodomain protein gene	5	341, 420, 869, 3061, 589	X13146.1-X13148.1, X14508.1, M19709.1
Ectoderm regulating gene through development	1	6649	X544889.1
Beta-dynein heavy chain partial mRNA (complete)	12	13799, 13230, 4374, 51, 18, 3213, 420, 126, 1152, 3234, 3342, 183, 3408, 3375, 3669, 3942, 3750, 69	X59603.1, UO3969.2, Z21941.1, S57977.1, S57975.1, U03970.1-U03972.1, U03974.1, U03977.1, U03978.1, U03981.1, U03973.1, U03975.1, U03976.1, U03979.1, U03976.1, AF254953.1
Microsatellite clone Tgr	11	430-1083	EF382854.1-EF382864
Protein 217g coding gene	1	Lengkap, 4702	M22207.1
Retrovirus-like element	1	7542	M75723.1
Partial gen pengikatan	6	1608-1616	AF520207.1-AF520209.1, AF520214.1-AF520216.1
Partial COI gene	189	579, 607-639	GU480557.1-GU480558.1, AY205373.1-AY205455.1, KF012802- KF012824. JQ341159.1.
Complete toposom mRNA	1	4741	AY026514.2
Complete beta-catenin mRNA	1	3061	AY026514.2
mtDNA gene for partial rRNA 16S	1	551	AB154279.1
Complete mRNA laminin binding protein	1	1212	U02371.1
Partial 2/4mRNA homologous bond morphogenetic protein	1	897	AF133305.1
Mitochondrial ND1 gene for partial NADH dehydrogenase subunit 1	1	819	AB178503.1
Mitochondrial ND2 gene for complete NADH dehydrogenase subunit 2	1	1059	AB178518.1
Gene for rRNA 18S	1	1764	Z37134.1
Genomic Actin Cyl genomic of the embryo	1	5277	S74059.1
Complete mRNA homeodomain protein	1	3634	AF254953.1

GENETIC DIVERSITY

Liggin et al. (2014) published 23 haplotypes of COI gene of *T. gratilla* from Kermadec Island, Southwest Pacific to the GenBank at the accession number of KF012802- KF012824. While the analyzing of 82 COI

gene fragment sequences from 11 locations accessed from the GenBank data (Lessios et al., 2003) resulted in 34 haplotypes with the haplotype diversity (Hd) of 0.90, with average difference number of 2.39 and nucleotide diversity of 0.004. Each sequence has 577 bp.

Table 2. Genetic differentiation of COI gene fragments from 11 locations of the GenBank (NCBI) data.

Genetic differentiation	Chile Easter	French Polynesia	Guam	Japan	Kiribati Kiritimati	Madagascar	Oman	PNG	Philippines	Reunion	USA-Hawaii
No. of sequences	8	9	2	10	9	8	2	7	12	5	10
No. segregation side, S	5	8	0	7	7	10	1	6	14	0	7
No. haplotypes, h	6	3	1	6	7	6	2	5	9	1	6
Haplotype diversity, Hd	0.93	0.56	0	0.84	0.94	0.89	1	0.86	0.95	0	0.84
Average number of differences, K	1.75	2.06	0	2.07	2.33	2.68	1	1.71	3.47	0	1.67
Nucleotide diversity, Pi	0.003	0.003	0	0.003	0.004	0.004	0.002	0.003	0.006	0	0.003

Source: Analyzed the Gene Bank data at the accession number of AY205373.1-AY205455.1 (Lessios et al. 2003)

There were 81 mutation sequences, Eta, of the 82 sequences. Also, the average pairwise difference (k) was 3.401. Based on 577 bp analyzed from 11 populations above, there were 78 varied sides (polymorphic). Twenty of these were parsimony informative sites and 58 were singleton variable sites. Singleton variable sites (two variants) consisted of 56 sides and parsimony informative sites (two variants) of 19 sides. Singleton variable sites (three variants) comprised 2 sides and parsimony informative sites (three variants) of 1 side.

GENETIC STRUCTURE

Study on molecular genetics of the sea urchin also mentioned that high gene flow up to more than hundreds kilometers is standard characteristic of all sea urchin species possessing planktonic larval stage (Palumbi & Wilson, 1990; Palumbi et al., 1997; Lessios et al., 1999; Lessios et al., 2001). Zigler & Lessios (2003) studied genetic structure variations of *T. gratilla* based upon gamed recognizing molecules, binding, and there is no geographic structure in the allele binding distribution of *T. gratilla*.

Based on 82 sequences of COI gene fragment gained from the GenBank, it is also apparent that *T. gratilla* does not have geographic structure. Samples collected from Guam, Japan, Papua New Guinea, USA-Hawaii, Reunion, Oman, Philippine, Marquesas-Polynesia France, Easter Island-Chile, Madagascar, and Kiribati-Kiritimati possessed

genetic relationship of one and another. This indicates that there is genetic information flow of *T. gratilla* from different waters. Liggins et al. (2014) showed the same genetic pattern for *T. gratilla* from around the Kermadec Islands.

T. gratilla from Indonesian waters also reflects similar structural patterns. Forty-two samples of COI gene fragment of *T. gratilla*, collected from 5 locations in Central Indonesia waters (Lembah, Molas, Togian, Donggala, and Derawan) showed that there was no geographic structure from all locations. Randomly mixed samples of each population group indicated that there was gene flow from one area to the others. This result also reflects that there is no distribution boundary of *T. gratilla* despite on evolutionary line (Wallace line) between Derawan and four other waters (Lembah, Molas, Donggala, and Togian) (Toha et al. in preparation).

The COI sequence samples of Indonesian *T. gratilla*, compiled with the COI sequence of *T. gratilla*, from other waters in the world (Guam, Japan, Papua New Guinea, USA-Hawaii, Reunion, Oman, Philippine, Marquesas-Polynesia France, Easter Island-Chile, Madagascar, Kiribati-Kiritimati) shows similar result. Indonesian *T. gratilla* possesses genetic relationship with that from non-Indonesia waters. As a whole, it is apparent that *T. gratilla* do not have geographic structures based upon COI gene fragment.

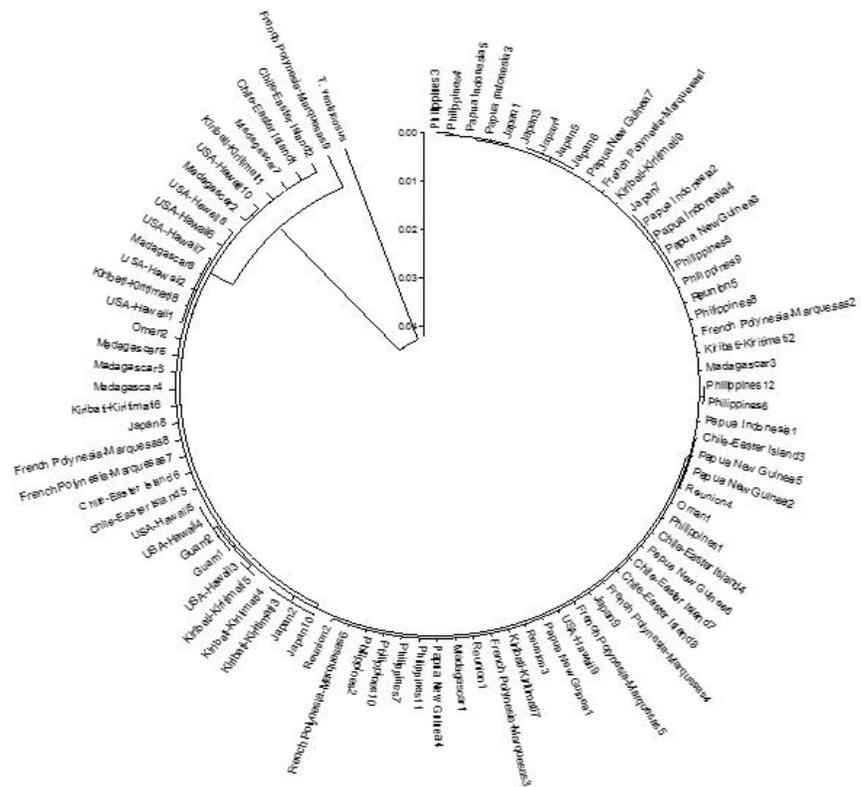


Figure 1. *T. gratilla* Phylogenetics from various world waters, including Indonesia waters (represented by Papua waters)

The COI *F_{st}* values among 11 populations of *T. gratilla* was 0.21. The *F_{st}* value between Chile Easter and 10 other populations ranged from -0.22 (with Oman) to 0.59 (with France Polynesia). The *F_{st}* values between France Polynesia and 9 other populations ranged from -0.19 (with Oman) to 0.58 (with Guam). The *F_{st}* values between Guam population and 8 other populations ranged from 0.36 (USA Hawaii) and 1 (Reunion). The *F_{st}* values between Japan population and 7 others ranged from 0.08 (Philippine) and 0.33 (USA Hawaii). The *F_{st}* values between Kiribati Kiritimati population and 6 others ranged from -0.11 (Oman) and 0.19 (Reunion). The *F_{st}* values between Madagascar population and 5 others ranged from -0.13 (Oman) and 0.28 (Reunion). The *F_{st}* values between Oman population and 4 others ranged from -0.02 (USA Hawaii) and 0.07 (Philippines). The *F_{st}* values between Papua New Guinea population and 3 others ranged from 0.00 (Reunion) and 0.34 (USA Hawaii). The *F_{st}* values between Philippine population and 2 others ranged from -0.09 (Reunion) and 0.29 (USA Hawaii). The *F_{st}* value between Reunion population and USA Hawaii was 0.59.

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