GENETIC DIVERSITY AND ORIGIN OF LEATHERBACK TURTLE (Dermochelys coriacea) FROM SUMATRA

MASLIM

GRADUATE SCHOOL
BOGOR AGRICULTURAL UNIVERSITY
BOGOR
2016
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Bogor, March 2016

*Maslim*
G352140191
RINGKASAN

MASLIM. Keragaman Genetik dan Asal Penyu Belimbing (*Dermochelys coriacea*) di Sumatera. Dibimbing oleh ACHMAD FARAJALLAH dan NEVIATY P. ZAMANI.

Penyu belimbing (*Dermochelys coriacea*) adalah salah satu dari tujuh spesies penyu di dunia. Distribusi spesies ini tersebar di perairan tropis dan subtropis. Habitat mencari makan dan bersarang spesies ini dapat ditemukan di beberapa tempat di Indonesia, mulai dari Sumatera sampai Papua.


Berdasarkan hasil dari 763 bp control region, kami memperoleh 4 haplotipe dari 22 variasi. Haplotipe yang ditemukan menunjukkan bahwa pola konektivitas penyu belimbing Sumatera memiliki jalur migrasi ke Samudera Hindia dan Laut Cina Selatan. Haplotipe yang ditemukan di Sumatera yang sama dengan haplotipe yang ditemukan di Papua.

Populasi di Sumatera memiliki keragaman genetik yang tinggi dibandingkan dengan Papua, namun perbedaan genetic antar populasi di Sumatera kecil. Hasil dari 5 lokus mikrosatelit menunjukkan konektivitas antara populasi penyu belimbing di Sumatera. Oleh karena itu, daerah yang menjadi lokasi penghubung spesies ini perlu diperhatikan dengan lebih baik.


Kata kunci: genetik, keragaman, *Dermochelys coriacea*, Sumatera, DNA, mikrosatelit
SUMMARY

MASLIM. Genetic Diversity and Origin of Leatherback Turtle (*Dermochelys coriacea*) from Sumatra. Supervised by ACHMAD FARAJALLAH and NEVIATY P. ZAMANI.

The leatherback turtle (*Dermochelys coriacea*) is one of seven species sea turtle in the world. Distribution of this species spread in the tropics and subtropics water. Foraging and nesting habitat of this species can be found in several places in Indonesia including Sumatra and Papua.

Studies of Leatherback turtles from Sumatra need to be done. We need a variety data to support the management and protection of this species, so that the management and the protection of leatherback turtles can be done better. This study aimed to analyze the genetic diversity and connectivity patterns of leatherback turtles from Sumatra using mitochondrial and microsatellite markers.

Sample of leatherback turtles were collected from the nesting area of leatherback turtles in Panga (Aceh Jaya) and Lhoknga (Aceh Besar), northern part of Sumatra. Tissue of leatherback turtles were collected from flipper. DNA isolation used method phenol/chloroform. Amplification of control region from mitochondria was performed using Polymerase Chain Reaction (PCR). Primers for microsatellites were used loci B103, C102, L142, L143 and L145 for microsatellite.

Based on 763 bp control region in leatherback turtles, we obtained 4 haplotypes from 22 variable sites. The haplotypes showed that the connectivity patterns of Sumatran leatherback turtles have migration path to Indian Ocean and South China Sea. Haplotypes that were found in Sumatra were similar with haplotypes in Papua. Sumatra has a high genetic diversity compared to Papua, but the population in Sumatra have a little genetic differentiation. Five loci of microsatellites showed the connectivity between leatherback turtle populations from Sumatra. Therefore, the connecting location of these species need to be managed more specifically.

Sumatran leatherback turtle populations have high genetic diversity. Leatherback turtle populations from Sumatra have little genetic differentiation. The connectivity pattern showed that Sumatran leatherback turtles have the pathway to the Indian Ocean and South China Sea. Five microsatellite loci (B103, C102, L142, L143 and L145) were polymorphic in leatherback turtle populations from Sumatra.

Keywords: genetic, diversity, *Dermochelys coriacea*, Sumatra, DNA, microsatellite
GENETIC DIVERSITY AND ORIGIN OF LEATHERBACK TURTLE (*Dermochelys coriacea*) FROM SUMATRA

MASLIM

Thesis
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Title: Genetic Diversity and Origin of Leatherback Turtle (*Dermochelys coriacea*) from Sumatra

Name: Maslim
Student ID: G352140191

Approved by
Advisory Board

Dr Ir Achmad Farajallah, MSi
Supervisor

Dr Neviaty P. Zamani, MSc
Co-Supervisor

Endorsed by

Head of Animal Bioscience Program

Dr Ir RR Dyah Perwitasari, MSc

Dean of Graduate School

Dr Ir Dahrul Syah, MScAgr

Date of Examination: 17th March 2016
Date of Graduation: 06 APR 2016
PREFACE

Praise and gratitude to Allah SWT for all of his gifts so that the scientific work was successfully completed. The theme chosen in this study is to collect data related to the population of leatherback turtles in Sumatra.

The author wants to thank Dr. Ir Ahmad Farajallah, MSi and Dr. Neviaty P. Zamani, MSc as supervisor, as well as lecturers of Animal Biosciences Program. Thank you to all students of BSH 2014, the staff of Molecular Laboratory of Biology Department and all members of zoocorner who always give support. The author thank Aceh Government which has gave scholarship during study. In addition, the authors convey appreciation to Panga and Lhoknga sea turtle team, who has helped during data collection. Expressions of thanks are also extended to father, mother, and the whole family, for all the prayers and affection. Hopefully this thesis could be useful.

Bogor, March 2016

Maslim
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GENERAL INTRODUCTION

Background

The leatherback turtle (Dermochelys coriacea) is one of seven sea turtles in the world. Distribution of this species spread in the tropics and sub-tropics water (Vargas et al. 2008). Foraging and nesting habitat of this species can be found in several places in Indonesia. Some coastal areas in Indonesia are as the nesting habitat of this species. Papua and Sumatra are the center of nesting habitat of this species in Indonesia. The studies of leatherback turtles in Papua can be found in many subject. Instead, Sumatra as one habitat of this species has no data. This is due to the lack of research conducted at this location.

As one of the leatherback turtle habitat, Sumatra has an immense potential to be explored in deeper. It is essential to execute a study about leatherback turtle in Sumatra, so that the protection management could be developed. The decline in the population of leatherback turtles occurs constantly, demanding the protection of this species needs to be improved. Therefore, we need a variety data to support the protection management of this species.

Mitochondrial DNA (mtDNA) and Microsatellite markers have been used as a genetic markers on many animals including leatherback turtle. These tool are powerful to identify the phylogeography and connectivity of leatherback turtles. Genetic studies of the leatherback turtles and genetic assessment of the stock population on a global scale showed that the group that lodged separately are an independent population maternally (Limpus 2009).

Many Studies of leatherback turtle were using genetic markers, such as the genetic diversity of the leatherback turtle populations in the Pacific (the East Pacific (Mexico and Costa Rica), the Western Pacific (Dutton et al. 1999, 2007 and 2013), Indian Ocean (Andaman and Nicobar Islands) (Phillott and Gamage 2014). However, there is still no data from Sumatra. IUCN Red List 2013 (Wallace et al. 2013) puts populations in Sumatra in a category of data deficient. Study of leatherback turtles from Sumatra need to be done to get adequate data. Data of genetic diversity, and connectivity patterns are needed.

Objective

The objectives of these researches are:
1. To Determine the genetic diversity and analyse connectivity pattern of leatherback turtle populations from Sumatra using mitochondrial DNA.
2. To Determine polymorphisms of five microsatellites loci on leatherback turtles (Dermochelys coriacea) from sumatra

Advantages

The benefits of these researches can be used as basic information for:
1. Management of leatherback nesting beaches in Sumatra
2. Policies for the protection of leatherback turtles in Indonesia
3. As a reference in the assessment of the status of leatherback turtles in the Indian Ocean.
LEATHERBACK TURTLE (Dermochelys coriacea) POPULATIONS FROM SUMATRA: GENETIC DIVERSITY AND CONNECTIVITY PATTERN

Introduction

Leatherback turtle (Dermochelys coriacea) is a turtle species that can be found in the tropics and sub-tropics, including areas within Indonesian archipelago. Leatherback turtle populations in Indonesia are divided into two sub-populations, sub-populations of Papua (Western Pacific) and sub-populations of Sumatra (Northeast Indian Ocean) (Wallace et al. 2013). Sub-populations of Papua have nesting habitat centralized in Jamurba-Medi and Warmon beach (Hitipeuw et al. 2007). The movement of leatherback turtles in this area was reaching to the North America region (Benson et al. 2007). There is no report about populations of leatherback turtle in Sumatra, only some places in Indian Ocean (Nicobar island, Sri Lanka and South Africa) (Bowen and Karl 2007).

Indonesia is an area that is flanked by two oceans (DeBoer et al. 2008). Indonesia’s water has a chance as an interaction place of two leatherback turtle populations (West Pacific and Indian Ocean) (Bowen et al. 1998). The connectivity of these populations, migration path and interaction areas of these populations are important to be studied (Avise 2009). It requires further verification by analysing two populations of leatherback turtles in Indonesia (Sumatra and Papua), but data populations of Sumatra was unavailable, it needs more studies.

Studies about genetic diversity that have been performed in leatherback turtle are global phylogeography of leatherback turtle (Dutton et al. 1999), phylopatric (Stewart and Dutton 2011) and natal homing (Prosdocimi et al. 2014). All of these studies were performed in Atlantic, (Dutton et al. 2013), Pacific (Dutton et al. 2007), and Indian ocean (Phillott and Gamage 2014). IUCN Red List 2013 (Wallace et al. 2013) puts the sub-populations of Sumatra into data deficient category. Sub-populations of Sumatra require exploration to obtain adequate data. This study aims to determine the genetic diversity and analyse connectivity pattern of leatherback turtle populations from Sumatra using mitochondrial DNA.

Materials and Method

Sample Collection

Sample of leatherback turtles were collected from the nesting area of leatherback turtles in Panga (Aceh Jaya) and Lhoknga (Aceh Besar), Sumatra (Figure 2.1). Tissue of leatherback turtles were collected from flipper (Dutton and Stewart 2013). Fourteen samples from Panga and Lhoknga were collected and preserved in absolute alcohol.
DNA Isolation and Amplification

DNA isolation was using standard phenol/chloroform by modifying the method of Sambrook et al. (1989). DNA amplification was performed using Polymerase chain reaction (PCR). Primers used are LCM15382 (5’GCTTAACCCTAAAGCATTGG-3’) (forward) and H950g (5’GTCTCAGATTTAGGGGTTTG-3’) (reverse) to amplify 832 base pairs (bp) fragment of mtDNA control region (Abreu-Grobois et al. 2006). PCR reaction was performed at 25 μL using Gotaq Green Mix Master. PCR consists of initial denaturation 94 ºC for 5 min; 35 cycles of 94 ºC for 30 seconds (denaturation), 58 ºC for 30 seconds (annealing), and 72 ºC for 60 seconds (extension), and final extension 72 ºC for 9 min. The amplicons showed a single band on polyacrylamide gel were sequenced using previous primers.

Data Analysis

Alignment was conducted using Mega v 5.1 (Tamura et al. 2011). Arlequin 3.5 was used to calculate the haplotype diversity (h) and nucleotide diversity (π) (Excoffier and Lischer 2010). Superimposed of phylogeny tree to the geography map used Network 4.6.1.3 (http://www.Fluxus-engineering.com).

Results

Based on 763 bp control region in leatherback turtles, we obtained 22 variable sites from 4 haplotypes (Appendix 1). Haplotypes that were found in Sumatran leatherback turtle populations were the similar haplotypes in Pacific and Indian Ocean (Table 2.1) but 2 haplotypes were different. We found two new haplotypes Dc4.2 (GenBank accession no. KU234548) and Dc4.3 (GenBank accession no. KU234549).
Table 2.1 Variable sites of 4 haplotypes based on sequence (763) of mtDNA Control Region in Sumatran leatherback turtle populations

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Variable Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>053 092 093 115 157 168 199 203 212 292 312 430 537 588 616 673 720 721 725 738 739 741</td>
</tr>
<tr>
<td>Dc1.1</td>
<td>A C C A A A A A G A T T C A A G A C A A C C</td>
</tr>
</tbody>
</table>

*New

The highest value of haplotypes and nucleotides diversity found in the Sumatra populations was Lhoknga $h = 0.6$ and $\pi = 0.0078$ followed by Panga $h = 0.5$ and $\pi = 0.0026$ (Table 2.2). Papua populations (Jamursba Medi and Warmon) have a low diversity ($h = 0.187$ and $\pi = 0.0008$).

Table 2.2 Genetic diversity of Sumatran leatherback turtle populations

<table>
<thead>
<tr>
<th>Populations</th>
<th>n</th>
<th>h</th>
<th>$\pi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sumatera (Panga)</td>
<td>4</td>
<td>0.5</td>
<td>0.0026</td>
</tr>
<tr>
<td>Sumatera (Lhoknga)</td>
<td>6</td>
<td>0.6</td>
<td>0.0078</td>
</tr>
<tr>
<td>Papua Jamursba Medi*</td>
<td>31</td>
<td>0.187</td>
<td>0.0008</td>
</tr>
<tr>
<td>Papua Warmon*</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Dutton et al. 2007*
### Table 2.3 Haplotype frequencies, haplotype (h) and nucleotide (π) diversities for *Dermochelys coriacea* populations using 763 bp from Indonesian Populations compared with other worldwide populations (Dutton et al. 1999 and 2007)

<table>
<thead>
<tr>
<th>Populations</th>
<th>Haplotypes</th>
<th>n</th>
<th>h</th>
<th>π</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dc1.1</td>
<td>Dc2.1</td>
<td>Dc3.1</td>
<td>Dc4.1</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Indonesia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sumatera (Panga)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Sumatera (Lhoknga)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Papua Jamursba Medi</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Papua Warmon</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Atlantic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>9</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Florida</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>26</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Trinidad</td>
<td>16</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Suriname</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>St. Croix</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pacific</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNG</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Solomon island</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mexico</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Indian Ocean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malaysia</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

- h represents haplotype diversity.
- π represents nucleotide diversity.
Discussion

Genetic Diversity

Relatively high genetic diversity was found in Sumatra. These findings support several previous studies that showed the genetic diversity of leatherback turtles from Indian Ocean were higher than Pacific region (Dutton et al. 1999, and 2007). However, despite having a high genetic diversity, the number of populations in the Indian Ocean region, particularly Sumatra, are smaller than the Pacific region (Wallace et al. 2013).

Nucleotide and haplotype diversity that is found in Sumatra indicate that the population of leatherback turtles in the region still connect with populations from other regions. The genetic assessment of leatherback turtles from Atlantic showed different results (Rivalan et al. 2006 and Prodocimi et al. 2014). It supports the division of the leatherback turtles region which show that the populations in the Pacific region and the Indian ocean still can not be separated (Dutton et al. 1999, 2007 and 2013).

Genetic Connectivity of Sumatran Leatherback Turtle

The haplotypes show that the connectivity patterns of Sumatran leatherback turtles (Figure 2.2) have migration path to Indian Ocean and South China Sea. Haplotypes that were found in Sumatra were similar with haplotypes in Papua. This indicates that there is connectivity between leatherback turtles form Sumatra and Papua. This model was related with the phylogeography and genetic connectivity of boring giant clam (DeBoer et al. 2008), co-distributed stomatopods (Barber et al. 2006), and giant mottled eel (Ishikawa et al. 2004) in the Pacific and Indian Ocean.

South China Sea is the connecting location between Sumatra and Papua leatherback turtles. The connectivity pattern of Sumatran leatherback turtles was supported by the leatherback nesting beach that found in Terengganu (Malaysia). The migration path of leatherback turtles nesting in Papua also showed the movement to the North Pacific region and the South China Sea (Bailey et al. 2012). The availability of jellyfish in Malacca Strait and South China Sea (Omori and Nakano 2001) and ocean flow were also important factors for the movement of leatherback turtles. In addition, some public report that leatherback turtles were captured in the Malacca Strait area.

Indonesia as a country that is flanked by two oceans is a unique location to study about phylogeography and demographic history of leatherback turtles (Shrive and Hurlburt 1997). Indonesian leatherback turtle populations were centralized in the two large islands (Papua and Sumatra) (Wallace et al. 2013). Both of these islands are interpretations of the leatherback turtle populations from Pacific and Indian Ocean. Therefore, we found genetic mixing between the two populations indicated that the populations of leatherback turtles in the Pacific and India disable to separate genetically.
Origin of Indonesian Leatherback Turtle

Leatherback turtle populations in Indonesia (Papua and Sumatra) is the combination of leatherback turtle populations from Pacific and Indian Oceans.
Haplotypes diversity that were found in Indonesia were similar with Pacific and the Indian Ocean. It shows the origin of the leatherback turtle populations in Indonesia came from Indian Ocean and the Pacific region.

Based on the median joining network, Dc4.1 haplotype is the origin of haplotypes in the world (Table 2.3). Figure 2.3 shows haplotypes that were found in Atlantic came from Pacific-Indian haplotype. It shows that leatherback turtles in the world comes from the Pacific-Indian Ocean region. Then the distribution spread to the Atlantic, before finally separated genetically.

**Conclusion**

Sumatran leatherback turtle populations have higher genetic diversity than Papua populations. The connectivity pattern showed that Sumatran leatherback turtles have the pathway to the Indian Ocean and South China Sea.
POLYMORPHISMS OF FIVE MICROSATELLITES LOCI ON LEATHERBACK TURTLES (Dermochelys coriacea) FROM SUMATRA

Introduction

Leatherback turtle (Dermochelys coriacea) is the species with widespread distribution in the world (Vargas et al. 2008), migration path to tropical or subtropical beaches for nesting (Rivalan et al. 2006). Foraging and nesting habitat of this species can be found in several places in Indonesia. One of the nesting habitats of this species is in Sumatra (Sarong and Maslim 2013). Leatherback turtle is a pelagic species, it is able to migrate extensively (> 5000 km) between tropical nesting habitats and cold foraging habitats. Wide distribution and high capacity of migration also caused interaction with the other species such as pelagic marine fish and marine mammals (Dutton et al. 1999).

Study of intraspecific genetic variation using molecular markers was useful to analyse the structure of populations and phylogeography of sea turtle. Mitochondrial DNA has been used to identify the composition of origin, stock populations and conclude the migration path in sea turtle (Prosdocimi et al. 2014). DNA microsatellite was useful in analysing the structure of the populations in many organisms. Study of polyandry in green turtles in West Java (Purnama et al. 2013), multiple paternity in leatherback turtles in the Virgin Islands (Stewart and Dutton 2011) and analysing the structure of the populations of leatherbacks in Atlantic (Dutton et al. 2013) are few studies that used DNA microsatellite marker.

Information regarding the existence of leatherback turtle populations in Sumatra are unavailable. IUCN Red List 2013 puts leatherback turtle populations in Sumatra into the data deficient (Wallace et al. 2013). Therefore, it requires a lot of studies related to the leatherback turtle populations in Sumatra, specially using microsatellite marker. Data can be used as a basic information in making policy management and protection of leatherback turtles in Sumatra. The aim of this study is to determine polymorphisms of five microsatellite loci on leatherback turtles (Dermochelys coriacea) in sumatra.

Materials and Method

Sample Collection

Sample of leatherback turtles were collected from the nesting area of leatherback turtle in Panga (Aceh Jaya) and Lhoknga (Aceh Besar). Tissue of leatherback turtles were collected from flipper (Dutton and Stewart 2013). Fourteen samples from Panga and Lhoknga were collected and preserved in absolute alcohol.

DNA Isolation and Amplification

DNA isolation was using standard protocol following phenol/chloroform method (Sambrook et al. 1989). Amplification used Polymerase chain reaction (PCR) technique. Primers were used to amplify five loci are available in Table 3.1. PCR reaction was performed at 10 µL total volume consisted of 1 µL DNA template, 2,4 µL sterile water, 0,8 µL each of forward and reverse primer, and 5 µL Gotaq Green
Mix Master. PCR consist of initial denaturation 94 °C for 5 min; 30 cycles of 94 °C for 30 seconds (denaturation), 60 °C for 90 seconds (annealing), and 68 °C for 120 seconds (extension); and final extension 72 °C for 5 min.

PCR product performed by polyacrylamide gel electrophoresis (PAGE) 8%, (Byun et al. 2009). DNA bands were scored based on the migrating rate of DNA bands. Allele a was identified as the fastest allele (Appendix 2).

Table 3.1 Characteristics of five microsatellites loci were used in this study

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primary sequence</th>
<th>T (°C)</th>
<th>Alleles Size</th>
<th>Number of Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>B103</td>
<td>F: CAGTCCTTGTGTGGTTAGAGT</td>
<td>60</td>
<td>151 - 168</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>R: GTTTCATTTTTCCCTTTCTCTGTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C102*</td>
<td>F: TAAAAAGGCAGCCAAGTAAG</td>
<td>60</td>
<td>212 – 266</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>R: GTGCAAGAACACAGATAAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L142**</td>
<td>F: GGCCAACCTTTCTTTCTATTA</td>
<td>58</td>
<td>219 – 237</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>R: CTTCTGTCATCTGCAACCCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L143**</td>
<td>F: CCTATGGGCCTCTGCAATGACA</td>
<td>58</td>
<td>181 – 197</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>R: CAGCTGGAGGGATGCAAGATGT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L145**</td>
<td>F: GGCCTCCAACAAATAAATAAA</td>
<td>58</td>
<td>121 – 197</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>R: CATTCACCTTACGCAGAAGAA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Dutton & Frey (2009), ** Roden & Dutton (2011)

Data Analysis

Allele polymorphism, gene diversity and allele frequency were detected using Nei’s formula (1987). Population structure of leatherback turtle was showed through gene diversity in the total population (H_T), within subpopulations (H_S), between subpopulations (D_ST), and gene differentiation among subpopulations (G_ST). Analysis of fixation index used PopGen32 (Yeh et al. 1999).

Results

Results of genotype frequencies showed that the genotype distribution between the populations in Panga and Lhoknanga on all loci spread evenly (Table 3.2). Nonetheless, some genotypes were not shared between populations. The highest percentage among all loci were found in locus L142 (66.67%) in Panga and locus B103 (57.14%) in Lhoknanga.

Allele a, b, and c on locus B103 were found in both populations of leatherback turtles that observed. Allele b has a high frequency in both populations that observed, the value are 0.5 (Panga) and 0.4167 (Lhoknanga) (Table 3.3 and 3.4). The same thing happened in the three alleles (a, b & c) on locus C102. It was distributed in both populations were observed. Frequency of allele a (the highest frequency on Locus C102) in both populations showed the same value (0.5). Allele a, b and e on the Locus L142 were found in both populations that observed. Interestingly, alleles c was only found in Panga and allele d was only found in Lhoknanga. Both alleles were not shared between the populations, it shows the distribution of alleles has been separated.
Table 3.2 Genotype frequencies (%) of Loci B103, C102, L142, L143 and L145

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>B103 Panga</th>
<th>C102 Lhoknga</th>
<th>L142 Panga</th>
<th>L142 Lhoknga</th>
<th>L143 Panga</th>
<th>L143 Lhoknga</th>
<th>L145 Panga</th>
<th>L145 Lhoknga</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>42.86</td>
<td>33.33</td>
<td>0.00</td>
<td>66.67</td>
<td>14.29</td>
<td>0.00</td>
<td>0.00</td>
<td>16.67</td>
</tr>
<tr>
<td>AB</td>
<td>14.29</td>
<td>50.00</td>
<td>14.29</td>
<td>28.57</td>
<td>0.00</td>
<td>14.29</td>
<td>0.00</td>
<td>16.67</td>
</tr>
<tr>
<td>AC</td>
<td>0.00</td>
<td>33.33</td>
<td>14.29</td>
<td>0.00</td>
<td>14.29</td>
<td>0.00</td>
<td>0.00</td>
<td>16.67</td>
</tr>
<tr>
<td>AD</td>
<td>0.00</td>
<td>16.67</td>
<td>28.57</td>
<td>16.67</td>
<td>14.29</td>
<td>0.00</td>
<td>0.00</td>
<td>16.67</td>
</tr>
<tr>
<td>AF</td>
<td>28.57</td>
<td>0.00</td>
<td>14.29</td>
<td>0.00</td>
<td>28.57</td>
<td>16.67</td>
<td>16.67</td>
<td>0.00</td>
</tr>
<tr>
<td>BB</td>
<td>14.29</td>
<td>0.00</td>
<td>28.57</td>
<td>16.67</td>
<td>14.29</td>
<td>0.00</td>
<td>14.29</td>
<td>0.00</td>
</tr>
<tr>
<td>BC</td>
<td>57.14</td>
<td>33.33</td>
<td>14.29</td>
<td>16.67</td>
<td>14.29</td>
<td>0.00</td>
<td>28.57</td>
<td>16.67</td>
</tr>
<tr>
<td>BD</td>
<td>0.00</td>
<td>16.67</td>
<td>28.57</td>
<td>16.67</td>
<td>14.29</td>
<td>16.67</td>
<td>14.29</td>
<td>16.67</td>
</tr>
<tr>
<td>BE</td>
<td>42.86</td>
<td>16.67</td>
<td>14.29</td>
<td>0.00</td>
<td>14.29</td>
<td>0.00</td>
<td>16.67</td>
<td>0.00</td>
</tr>
<tr>
<td>CC</td>
<td>14.29</td>
<td>16.67</td>
<td>14.29</td>
<td>0.00</td>
<td>16.29</td>
<td>0.00</td>
<td>16.67</td>
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<tr>
<td>CD</td>
<td>0.00</td>
<td>33.33</td>
<td>14.29</td>
<td>0.00</td>
<td>16.29</td>
<td>0.00</td>
<td>16.67</td>
<td>0.00</td>
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<tr>
<td>CE</td>
<td>0.00</td>
<td>16.67</td>
<td>14.29</td>
<td>0.00</td>
<td>16.29</td>
<td>0.00</td>
<td>16.67</td>
<td>0.00</td>
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<tr>
<td>CG</td>
<td>0.00</td>
<td>16.67</td>
<td>14.29</td>
<td>0.00</td>
<td>16.29</td>
<td>0.00</td>
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<tr>
<td>DD</td>
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<td>14.29</td>
<td>0.00</td>
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<td>0.00</td>
<td>16.67</td>
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<td>EG</td>
<td>0.00</td>
<td>16.67</td>
<td>14.29</td>
<td>0.00</td>
<td>16.29</td>
<td>0.00</td>
<td>16.67</td>
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<tr>
<td>FF</td>
<td>14.29</td>
<td>16.67</td>
<td>14.29</td>
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<td>16.29</td>
<td>0.00</td>
<td>16.67</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 3.3 Alleles frequencies of population in Panga

<table>
<thead>
<tr>
<th>Allele</th>
<th>B103</th>
<th>C102</th>
<th>L142</th>
<th>L143</th>
<th>L145</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele A</td>
<td>0.0714</td>
<td>0.5000</td>
<td>0.1429</td>
<td>0.1429</td>
<td>0.2143</td>
</tr>
<tr>
<td>Allele B</td>
<td>0.5000</td>
<td>0.4286</td>
<td>0.5714</td>
<td>0.4286</td>
<td>0.0714</td>
</tr>
<tr>
<td>Allele C</td>
<td>0.4286</td>
<td>0.0714</td>
<td>0.0714</td>
<td>0.2857</td>
<td>0.1429</td>
</tr>
<tr>
<td>Allele D</td>
<td>0.1429</td>
<td>0.0714</td>
<td>0.1429</td>
<td>0.0714</td>
<td>0.1429</td>
</tr>
<tr>
<td>Allele E</td>
<td>0.2143</td>
<td>0.1429</td>
<td>0.2143</td>
<td>0.1429</td>
<td>0.2143</td>
</tr>
<tr>
<td>Allele F</td>
<td>0.2857</td>
<td>0.2857</td>
<td>0.2857</td>
<td>0.2857</td>
<td>0.2857</td>
</tr>
<tr>
<td>Allele G</td>
<td>0.0714</td>
<td>0.0714</td>
<td>0.0714</td>
<td>0.0714</td>
<td>0.0714</td>
</tr>
</tbody>
</table>

Table 3.4 Alleles Frequencies of population in Lhoknga

<table>
<thead>
<tr>
<th>Allele</th>
<th>B103</th>
<th>C102</th>
<th>L142</th>
<th>L143</th>
<th>L145</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele A</td>
<td>0.2500</td>
<td>0.5000</td>
<td>0.6667</td>
<td>0.1667</td>
<td>0.1667</td>
</tr>
<tr>
<td>Allele B</td>
<td>0.4167</td>
<td>0.4167</td>
<td>0.1667</td>
<td>0.1667</td>
<td>0.2500</td>
</tr>
<tr>
<td>Allele C</td>
<td>0.3333</td>
<td>0.0833</td>
<td>0.4167</td>
<td>0.0833</td>
<td>0.0833</td>
</tr>
<tr>
<td>Allele D</td>
<td>0.0833</td>
<td>0.2500</td>
<td>0.3333</td>
<td>0.3333</td>
<td>0.3333</td>
</tr>
<tr>
<td>Allele E</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
</tr>
<tr>
<td>Allele F</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
</tr>
<tr>
<td>Allele G</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
<td>0.0833</td>
</tr>
</tbody>
</table>

Four alleles on the locus L143 (a, b, c, d) were distributed in both populations. The highest frequency of alleles in population of Panga is allele b (0.43) and Lhoknga is allele c (0.4167). In contrast to the locus L143, locus L145 has an allele was only
found in one of the leatherback turtle populations were observed. Allele f on the locus L145 was only found in Panga. It shows the difference in the distribution of allele f in both populations.

Table 3.5 Estimates of heterozygosities of five polymorphic loci for populations of Leatherback turtles from Sumatra

<table>
<thead>
<tr>
<th>Locus</th>
<th>Panga</th>
<th>Lhoknga</th>
</tr>
</thead>
<tbody>
<tr>
<td>B103</td>
<td>0.6044±0.0680</td>
<td>0.7121±0.0700</td>
</tr>
<tr>
<td>C102</td>
<td>0.6044±0.0680</td>
<td>0.6212±0.0781</td>
</tr>
<tr>
<td>L142</td>
<td>0.6484±0.0741</td>
<td>0.5606±0.0956</td>
</tr>
<tr>
<td>L143</td>
<td>0.7473±0.0598</td>
<td>0.7727±0.0663</td>
</tr>
<tr>
<td>L145</td>
<td>0.8791±0.0412</td>
<td>0.8485±0.0553</td>
</tr>
<tr>
<td>H</td>
<td>0.6967±0.0622</td>
<td>0.7030±0.0731</td>
</tr>
</tbody>
</table>

Heterozygosity values in both populations were observed, are generally quite high (Table 3.5). The average value of heterozygosity (H) of Panga is 0.6967, and Lhoknga is 0.7030. Based on the average value of heterozygosity (H) of the five loci showed both populations have high allele diversity (H> 0.60). However Lhoknga population has a high allele diversity when compared with the Panga population.

Genetic differentiation aims to examine the genetic diversity within and between subpopulation (Nei, 1987). Genetic differentiation was obtained by calculating the average diversity in subpopulation (H_S), genetic diversity in the population total (H_T) and genetic diversity among subpopulations (D_ST). The results show the value of genetic diversity populations total (H_T) is 0.9653, the average diversity in subpopulation (H_S) is 0.8786, genetic diversity among subpopulations (D_ST) is 0.0867, and coefficient of genetic differentiation (G_ST) is 0.0898. Small genetic differentiation (8%) in both populations showed that the genetic differentiation of leatherback turtle populations from Sumatra (Panga and Lhoknga) is low. Fixation index (F_IS, F_ST, F_IT) was used to determine the breeding and the selection pattern of the populations. Nei (1987) stated that the value of F was also called coefficient breeding. The results showed the value of F_IS is 0.0168, F_ST is 0.0898, and F_IT is 0.1052.

Discussion

Genetic Diversity (Heterozygosity)

The diversity of alleles from two populations of leatherback turtles in Sumatra showed a high scores. It can be seen from the value of heterozygosity in both populations was greater than 0.6 (H> 0.60). The diversity that obtained indicate that these populations need special attention on the protection of this species. Genetic conservation needs to be done in a population of leatherback turtles from Sumatra. Results of studies that have been done on some marine species. Species that have a high genetic diversity, had a chance for the occurrence of genetic isolation (Hoffman et al. 2009).

Genetic differentiation of leatherback turtles from Sumatra showed a low value. This indicates that populations of leatherback turtles from Sumatra have a
genetic connectivity. Although being on different nesting sites, the low value of genetic differentiation can be interpreted that both of these populations still have a relationship. Therefore, it is necessary to conduct further study, related path connectivity of these populations to reduce the potential for bycatch by fishermen in the region. This study is also useful to execute how far away the migration location of leatherback turtles from Sumatra.

Benson et al. (2007) showed the movements of leatherback turtles from Jamursba Medi Papua to the northern America. This indicated that the distance of migration locations, increasing their chances to connect with other leatherback turtles from other locations. It can cause the genetic mixture between populations. The high genetic diversity and low genetic differentiation, indicated that there is connectivity among leatherback turtle populations from Sumatra. It can be assumed that leatherback turtles from Sumatra have the same migration path. It is necessary to conduct further study to prove it.

**Polymorphism**

Based on the 5 microsatellite loci, it is showed that all loci are polymorphism on leatherback turtles in Sumatra. Although the two loci (B103 and C102) has been tested on a green turtle (Dutton and Frey 2009), but these loci also showed polymorphism on leatherback turtles. It is showed that some of the microsatellites marker can still be used on other sea turtle species.

The highest allele was obtained on the locus L145 (7 alleles), and lowest one was on the loci B103 and C102 (3 alleles). The number of alleles that obtained on the locus L145 in accordance with the results of Rodden and Dutton (2012). However, on the loci B103 and C102 were only found three alleles, it was different with Dutton and Frey (2009). Their results gained 9 alleles on these loci. It was caused their research conducted on green turtles, while on leatherback turtles only gained 3 alleles.

The sharing of alleles showed the presence of genetic connectivity between the two populations. Although some alleles at 5 loci were not shared, but in general, alleles that were found in leatherback turtles from Sumatra were shared alleles. This supports previous results that show the low value of genetic differentiation on leatherback turtles from Sumatra. Despite having a high allelic diversity, but the number of each shared alleles in both populations showed a genetic flow in both populations.

**Genetic Differentiation**

The genetic structure of the leatherback turtle populations from Sumatra can be seen from the genetic differentiation and fixation index (Nei, 1987). The value of genetic differentiation between populations (Gst) obtained is low (0.0898), whereas based on the fixation index was known that in each subpopulation were crossbred (FIS = 0.0168), also occurs interbreed in all populations (FIT = 0.1052), and between subpopulations (FST = 0.0898). The low value of FST is proportional to genetic differentiation. The value of FST indicates little genetic differentiations (0 - 0.05) between Panga and Lhoknga. It showed leatherback turtle populations from Sumatra (Panga and Lhoknga) still have a close relationship. This is supported by studies conducted by Hitipeuw et a.l (2007) that showed leatherback turtles that have nesting beach on Jamursba medi, will be back again to spawn in the the next season, so that
the data of female leatherback turtles, can be used as one of the data to analyse the population structure of leatherback turtles.

The use of microsatellites as genetic markers to analyse population structure is useful. Microsatellite markers have also been used in analysing polyandry in population of Chelonia mydas (Purnama et al. 2013), multiple paternity in Dermochelys coriacea (Stewart and Dutton 2011), multiple paternity in Lepidochelys olivacea (Hoekert et al. 2002), effective population size in Natator depressus (Theissinger et al. 2009), and genetic structure in Carretta carretta (Carreras et al. 2007). Therefore, genetic differences in the two populations in Sumatra are low. This is also evidenced by Dutton et al. (2013) showed genetic differences between populations of leatherback turtles from Atlantic that close each other are still low.

**Conclusion**

Five microsatellite loci (B103, C102, L142, L143 and L145) were polymorphic on leatherback turtle populations from Sumatra. Leatherback turtle populations in Sumatra have high genetic diversity (alleles and genotypes) but little genetic differentiations.
GENERAL DISCUSSION

Leatherback turtle populations in the world have a strong structure (Dutton et al. 1999). Although this species has a wide range, but genetically the structure of the population of leatherback turtles can be identified based on breeding territory. The structure of the leatherback turtle populations divided into two major regions, Atlantic and Pacific-Indian (Bowen and Karl 2007). The identity of Indonesia leatherback turtle proves that Pacific-Indian region was inseparable as well as the ranges of these species.

Molecular approaches in analysing the spread of leatherback turtles are indispensable (Lee 2008). The results of genetic identification has been studied by Dutton et al. (1999, 2007, 2013), Molfetti et al. (2013), Prosdocimi et al. (2014), Phillott and Gamage (2014) showed the haplotypes diversity of leatherback turtles in the world. All of the studies showed that phylogeography of leatherback turtle has a genetic identity related to their nesting habitat.

The use of microsatellites as genetic markers to analyse population structure is useful. Microsatellite markers have been used in analysing polyandry in population of Chelonia mydas (Purnama et al. 2013), multiple paternity in Dermochelys coriacea (Stewart and Dutton 2011), multiple paternity in Lepidochelys olivacea (Hoekert et al. 2002), effective population size in Natator depressus (Theissinger et al. 2009), and genetic structure in Carreta carreta (Carreras et al. 2007). Therefore, genetic differences in the two populations in Sumatra are low. This is also evidenced by Dutton et al. (2013) showed genetic differences between populations of leatherback turtles adjacent to the Atlantic are still low.

Although Sumatra has a high genetic diversity compared with Papua, but the population in Sumatra have little genetic differences between the populations. This indicates the connectivity between populations of leatherback turtles in Sumatra. Therefore, the areas that are as the connecting place of these species need to be considered more specifically. The areas, as a fishing ground area, need to be disseminated to the fishermen to be more careful in the fish capturing in the connecting path of leatherbacks.

Protection of sea turtle species in Sumatra has been limited to the species protection. In fact, some areas that are as nesting and foraging habitat of this species also need a protection. Nesting habitats, foraging habitats and migration path of this species should be considered in the protection management of leatherback turtle, particularly in Sumatra Damage to the habitat is one that causes decline of this population. This indicates the need for special attention on the management of this species' habitat.
CONCLUSION

Sumatran leatherback turtle populations have higher genetic diversity than Papua populations. The connectivity pattern showed that Sumatran leatherback turtles have the pathway to the Indian Ocean and South China Sea. South China Sea, as the connecting location, is important place for leatherback turtles from Sumatra and Papua. Five microsatellite loci (B103, C102, L142, L143 and L145) were polymorphic in leatherback turtle populations in Sumatra. Leatherback turtle populations in Sumatra have little genetic differentiations.

RECOMMENDATION

Further research needs to be done, especially tagging and transmitter installation to prove the theory of connectivity of leatherback turtles in Indonesia. Leatherback turtle habitats (nesting and migration area) also need to be protected from bycatch and predator.
REFERENCES


Appendix 1. Haplotypes of leatherback turtle from Sumatra

```
#De1.1 CCOCAAAAACG GAATCTTTTTT AATTTAATCTA CCTCTGACA GACAAGATAT AACACTTTCTT TATTCTCTTC CGTGCCCCAA AAGAGCAATG TCCATAAGAC
#De2.1
#De2.2
#De2.3 T
#De3.1 AA
#De3.2
#De3.3
#De4.1 TAACCGCTATG TATATTCTGTG CATTCTTTAA TTTGCCAATG GCATAATATCT AGTAAATAATG CCGCTTTAATG TGCTTTAAAATA CATAATATAA TAAATATAAC
#De4.2
#De4.3 .G.
#De5.1.
#De5.2
#De5.3
#De6.1 ATAATAACTAGCAATAGAGAATAGAATAG AATCTAATCT AAATCATTAT TCTCAAACAT GAATATTTGCC
#De6.2
#De6.3
#De7.1 ACAGTATCGT GCTTTTATTATTT AATTTTATTATA ATACGCAAGA AATAGCAATCG ATGTTTAAAG AAGATACAATA TATACTAGTT CAGCCGATAT AACACTAAGAC
#De7.2 .G.
#De7.3
#De8.1 GTACATAACTG AATTTTATCT AGCTTGAAGG CTTCTATAGT TCTCTTAAAAG GGGCTCTAGG TTAATGAGTT
#De8.2 G
#De8.3
#De9.1 CTAATTTACTG AATTTTATGAGC CTGTTGCTATG TGTATGAAGG ACTATTTGCACT AGTTTAAAGG TCTCTTAAAAG GGGCTCTAGG TTAATGAGTT
#De9.2
#De9.3
#De10.1 TAACTAAAA CAGCTTGAAGGC ATGTTTGAAGG TCTCTTAAAAG GGGCTCTAGG TTAATGAGTT TTTTAAAAGG
#De10.2
#De10.3 .G.
#De11.1 CTAACATAAGG TAAATTTTAA AGTTTAAAAC TAAACACCCAT CATTCTTTCT AAATTTAATAG CCCC
#De11.2
#De11.3
#De12.1
```
Appendix 2. Gel of Acrylamide (a) B103 (b) C102 (c) L142 (d) L143 and (5) L145
BIOGRAPHY

The author was born in Rimo on September 23rd, 1991 as a first son from Misno and Salbiah. The author obtained a bachelor's degree education (S.Pd) from the Department of Biology Education, Faculty of Teacher Training and Education, University of Syiah Kuala in 2013. Afterwards, authors continued to Master of Science in Animal Bioscience Program, Graduate School of Bogor Agricultural University in 2014.

During the study the authors obtained a scholarship from Aceh government through LPSDM Aceh. The author was a member of Himpunan Mahasiswa Muslim Pascasarjana (HIMMPAS) IPB as head of entrepreneurship department in 2014.

Since 2012, the author was involved in turtle protection program in coastal area of Aceh. The author with UNESCO were involved in coastal care program for school children in Aceh Besar which called Sandwatch Program in 2013. The author with Prof M Ali Sarong and Mimi Saputri published a book “Wajah Pesisir Aceh” in 2014.