ANALYSIS 16S RDNA OF THE TURTLES IN FORECLOSURE CAGES AT PADEI LAUT, CENTRAL SULAWESI, INDONESIA

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ABSTRACT

Marine turtle is one of the protected aquatic animals as listed in CITES Appendix and IUCN red list. However, illegal fishing of marine turtle is still occurred Padei Laut Village, in Morowali Regency, Central Sulawesi Province, Indonesia. The research aims to study the population of marine turtle based on the carapace length and the genetic relationships. Data of carapace length was measured in-situ and genetic analysis was used mitochondrial DNA. The results showed that the carapace length was ranges between 42-102 cm; 91% of samples was immature and 9% was mature. Moreover, it also revealed that those turtles resembled by 99.98% of genetic similarity.

Keywords: Turtle; carapace; genetic; Sulawesi; Indonesia

INTRODUCTION

There is six of seven species of turtles that inhabited in Indonesian waters, namely green turtle (Chelonia mydas), hawksbill (Eretmochelys imbricate), olive ridley turtles (Lepidochelys olivacea) and leatherbacks (Dermochelys coriacea). A loggerhead (Caretta caretta) known nesting in Indonesia. While, flat turtle (Natator apex sp) nested only in Australia, but this species has been observed to foraging in waters of Indonesia (Shanker & Pilcher, 2003).

Sea turtles are protected faunas from extinction under protection status by the State with the category of Appendix I of CITES and IUCN red list, so that all forms of utilization of the turtle should be forbidden. Legal sanctions have been imposed to the perpetrators of turtle utilization activities (poachers, collectors, traders). However, the legal sanction seems ineffective to eliminate the sea turtle poaching, as happened in the Village of Padei Laut.

Nowadays, the population of green turtles in Indonesian water tends to be declined due to the illegal poaching on both eggs and meat for commercial purposes. The increasing disturbance by visitors during nesting period and habitat loss due to abrasion resulting the degradation of turtle nesting habitat (Nastiti & Wiadnyana, 2013). Noises generated from water traffic by fisherman, and even fishing gears used with nets opening 35-40 cm allowing to catch turtles (Aureggi & Delucia, 2013).

A Green turtle (Chelonia mydas) is the most common species of sea turtles and live in the tropical ocean. Therefore, the genetic analysis needed to determine the kinship of the population. The green turtle can be recognized from the shape of a small head and a blunt beak. Name of the green turtle is not because scales are green, but the colour of fat located under the green scales. The body colour could be grey, blackish or brownish, and heavy the maximum weight reaching 400 kg (Nuitja, 1992).

Morphological features of green turtle exposed by Hirt (1971) and Bustard (1972), showed the presence of a pair of prefrontal or scales on the head. The turtles have scales shield the back (dorsal shield) which does not impose each other, four pairs of side scales along the surface from the head to the tail (costal scute), wherein the first side scales pair does not touch a nuchal. At the edge of the carapace there are 12 pairs of marginal scute, front legs as paddles shaped flat, there is a toe nail on the large front (Cape et al., 2001).

Even turtles have properties “orientation homing” instinct means that the turtles are able to return to their origin place where they were born (Hirth in Nuintja, 1992).
One effort to reduce the declining of green turtle population is to provide guidance for the public to conserve the green turtle population, as in the Pangumbahan beach, Sukabumi (Wiadnyana & Nastiti, 2013; Nastiti & Wiadnyana, 2015). According to Akira et al. (2012), turtles can migrate hundreds and even thousands of kilometres of habitat to find the nesting habitat. During their migration turtles are often susceptible to the poachers as happened in Padei Laut Village, Central Sulawesi, where many turtles have been caught for commercial purpose. This research aims to determine the carapace length and kinship turtle populations from the foreclosures in Padei Laut village. The information of kinship is important to understand the population status.

**MATERIALS AND METHODS**

**Sample Preparation**

The observation conducted in Padei Laut Village, Menui District, Morowali Regency, Central Sulawesi that transferred to the cages in Malta Village, South East Sulawesi Province at position 3° 58’ 2.888 “S 122° 36’ 58.67” E (Figure 1). Data and information were collected during period 18 to 20 April 2016.

The carapace length of 68 turtles was measured for morphometric data. For genetic analysis, 10 samples were collected randomly (Table 1). Muscle (1 x 1 cm²) sized was stored in tube contain in absolute alcohol. The DNA preparation was conducted in the Research Institute for Inland Fisheries Indonesia. The genetic data from Gen Bank was sequenced in Macrogen, Inc., South Korea. Genetic analysis is done based primer 16S rDNA gene.

**Table 1.** The samples of genetic study showing code, and length

<table>
<thead>
<tr>
<th>No.</th>
<th>Tagging Code</th>
<th>Carapace Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9/KKP/IV/16</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>4/KKP/IV/16</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>1/KKP/IV/16</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>11/KKP/IV/16</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>19/KKP/IV/16</td>
<td>44</td>
</tr>
<tr>
<td>6</td>
<td>2/KKP/IV/16</td>
<td>43</td>
</tr>
<tr>
<td>7</td>
<td>3/KKP/IV/16</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>6/KKP/IV/16</td>
<td>42</td>
</tr>
<tr>
<td>9</td>
<td>13/KKP/IV/16</td>
<td>66</td>
</tr>
<tr>
<td>10</td>
<td>18/KKP/IV/16</td>
<td>49</td>
</tr>
</tbody>
</table>

Figure 1. Location of foreclosure and shelters green turtle: Padei Laut Village, Menui Island District, Morowali Regency and Floating net cage to accommodate the green turtle (Malta Village).
**Extraction and Isolation of mtDNA**

Genomic DNA isolation was performed using DNA mini kit for blood (Geneaid) modified. The muscle cells of samples stored in 70% alcohol were washed with distilled water twice and then suspended in STE buffer (1M NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, pH 8) to a volume of 350 μl. The muscle cells were analyzed by SDS 1% and proteinase K 0.125 mg/ml (25 μL) at 55°C for 12 hours. The DNA extraction method followed the instructions Genomic DNA mini kit for fresh blood (Geneaid).

**Amplification and Visualization Fragments of mtDNA**

Amplification of 16S rRNA gene fragment portion used a universal primer Palumbi (1991) 16Sar - 5’ CGCCTGTTTTACTCAAACACAT and 16Sbr-3’ CCGGTTCGAACTGATCAGTACGT. The composition of the PCR reaction was carried out with a final volume of 50 μL of sample DNA consists of 5 μL, 16 μL sterile DW, each primer 2 μL and 25 μL Taq ready mix. PCR reactions were performed using a machine thermocycler applied Biosystems with the following conditions: pre-denaturation 95°C for 10 minutes, the second phase was consisting of 35 cycles, each of which includes a stage of denaturation 94°C for one minute, annealing on temperature of 48°C for one minute, extension at 72°C for 1.5 minutes and the last phase is the elongation of the final extension at 72°C for 7 min. The PCR products were tested using PAGE 6% in 1x TBE buffer (10 mM Tris-HCl, 1 M boric acid, and 0.1 mM EDTA) were run at 200 Mv conditions for 30 minutes. Furthermore, sensitive DNA stained by silver.

**Tracking PCR Products**

PCR products on polyacrylamide gels in size are in accordance with the primary design purified by the method of spin-column DNA purification kit. PCR products were purified and used in PCR for sequencing using the same primer pairs with initial amplification.

**Data Analysis**

Results tracing nucleotide were edited manually by the chromatogram. Nucleotide sequences that have been edited using the Clustal W were embedded in 4.0 MEGA (Molecular Evolutionary Genetics Analysis) (Tamura et al., 2007).

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Analysis of Phylogeny (kinship)

Analysis of phylogeny Neighbour Joining (NJ) was done using the MEGA 4.0 (Tamura et al., 2007), based on the model of nucleotide substitution Kimura-2-parameter with bootstrap 100 times.

**The Genetic Distance**

Genetic distance was analyzed based on the formula proposed by Nei (1987), and performed using MEGA 4.0 (Tamura et al., 2007):

$$I = \frac{J_{ai} J_{bi}}{J_{ai} J_{bi}}$$

were;

- \(i\) = Haplotype to i
- \(a_i\) = Haplotype frequencies to-i from A population.
- \(b_i\) = Haplotype frequencies to-i from B population.
- \(J_{ai}\) = Multiplication frequency haplotype to - i of the population A and frequencies haplotype to - i in B population \(j\) \(j\) is the average \(J_{a}\), \(j\) for all haplotype

**RESULTS AND DISCUSSION**

Result

The carapace lengths of turtles are raging between 42-102 cm. Based on the size and characteristics, turtles are divided into four categories such as: young hatchling, hatchlings, young and adult sea turtles. In this observation, most turtles are as young hatchling and hatchling (91% of 68 turtles) with sizes of 40-80 cm, while adult sea turtle were only 6 animals (9%). In those ranges of size, it seems to be difficult to identify the species correctly, except the adults were recognized as green turtles.

Amplification of the 16S rDNA gene produced 600 bp fragments at position 2,954-3,547 that all the observed sample were green turtle (*Chelonia mydas*) (Figure 2).

![Figure 2. 16S rDNA Partial sequence mtDNA of *C. mydas* (528 bp). SNP (Single Nucleotide Polymorphism) showed by bold and underlined text.](image-url)
Discussion

After comprehensive analysis, 70 examined green turtles from the Padei Laut Village, were confirmed as a child or young (young hatchling and hatchling) green followed the criteria given by Nuitja, (1992) and Nastiti et al. (2008). In nature, young hatchling and hatchling turtle, live in the sea water area that abundance with Sargassum seaweed as their main feed. According to Bjorndal (1997), beside seaweed, the turtle also consumes other organisms such as fish jelly and sponge. In the Gulf of California, green turtle dives in 1.3 to 7 m water depth (Seminoff et al., 2006). Swimmer et al. (2007), mentioned that the survival of turtle much influenced by physiological and behavioural aspect. Allison et al. (2009) stated that the injury suffered by the turtle will affect the ability of the turtle to migrate, escape from predators and reproduction ability. The genetic distance or intra-specific variation from samples (Figure 3).

![Figure 3](image-url)  
Figure 3. The genetic distance or intra-specific variation from samples and reference species from Gen bank.

Results of phylogram based on 16S r-DNA gene nucleotide inform that the analysed samples are Chelonia mydas with the level of 99.98% genetic similarity (Figure 4). The genetic variation was existing in the transition, the pattern A (adenine at position 2,174 bp) found in four samples together with the referent from Gen Bank. The pattern G (guanine at position 2,174 pb) was found in another 6 samples.

![Figure 4](image-url)  
Figure 4. Neighbour Joining parameter phylogram from fish collection and the reference of species.

According to Nastiti et al. (2008) genetic analysis carried out on green turtles on the south coast of West Java, Indonesia (Citireum, Pangumbahan, Cipatuah and Legok Java) have a common origin in the history of evolution, due the long distance of turtle migration (high mobility) (Spotila, 2004) (Figure 5). It is assumed that the turtle population in a region derived from the same parent stock.
Fuentes et al. (2013) stated that the fitness of turtle populations will support diversity, and geographical distribution in the world. Even though, wide distribution of green exist in the world, they still need suitable region for certain phase of their life such as to breed and grow out at early life history, such as Indonesian waters.

Previous study on the population of the green turtle from 7 different nest site in Indonesia (Sukamade, Pangumbahan, Sangalaki Island, Derawan, Piai Island, Enu Island, and Paloh) showed they were different each other’s enabling to be used as population stock (Dermawan et al., 2009). Among the population observed, only the population of the turtles from Paloh beach has closely relationship to the turtles from Sarawak (Moritz et al., 2002).

The study of post-nesting migration green turtles in Indonesia have been conducted in several nesting sites, such as Raja Ampat in Papua (Gearheart, 2005), Misol in Papua (Adnyana & Jayaratha, 2009), Berau in East Kalimantan (Adnyana et al., 2008) and Sukamade in East Java (Dermawan et al., 2009). The results showed that only a small percentage of turtles in the feeding area comes from the near spawning areas. Most of the turtles migrate to the new areas for grow out within thousands of kilometres distance from the spawning sites. This phenomenon showed a consistency of pathway and destination. In supporting of the management of sea turtles along the coast of Sulawesi, Indonesia, it would be urgent to study bi-ecology, socio-economic and institutional responsibility as the basis for green turtle conservation. That information could be used to setup management measures of nesting habitat as well as to develop the climate change mitigation for turtle.

CONCLUSION

The current investigation confirmed that the sea turtles observed are the green turtles (Chelonia mydas) based on 16S rDNA analysis. Moreover, it revealed that the green turtle have 99.98% genetic similarity.

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