Leatherback turtle (*Dermochelys coriacea*) populations in Sumatra: genetic diversity and connectivity pattern

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Abstract. Sumatra is part of Indian Ocean population of leatherback turtles. Data of leatherback turtles from Sumatra is unavailable. This study aims to determine the genetic diversity and analyze connectivity pattern of Sumatran leatherback turtle populations based on haplotype variation of control region mtDNA. We used 14 samples from 2 locations in Sumatra (Lohknga and Panga). Haplotypes have been determined by DNA sequencing. Four haplotypes were found from Sumatra. Interestingly, two of four haplotypes are new haplotypes that only found in Sumatra. Sumatra population that has high genetic diversity is Lhoknga, h=0.6 and π=0.0078 followed by Panga h=0.5 and π=0.0026. Connectivity pattern of Sumatran leatherback turtles showed that migration path of this populations reaching to Indian Ocean and South China Sea. South China Sea is important location as interaction place for leatherback turtles from Sumatra and Papua, Indonesia. Sumatran Leatherback turtle is important population of leatherback turtle. It needs good management to protect these populations. Sumatran Leatherback turtles need further studies to obtain the annual population data.

Key Words: migration, new haplotypes, DNA sequencing, mtDNA, haplotype.

Introduction. Leatherback turtle (*Dermochelys coriacea*) is the turtle species can be found in the tropics and sub-tropics, including areas within Indonesian archipelago (Vargas et al 2008). Leatherback turtle populations in Indonesia are divided into two sub-populations, sub-population 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 & 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 analyzing two populations of leatherback turtles in Indonesia (Sumatra and Papua), but data for 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 & 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 & Gamage 2014). IUCN Red List 2013 (Wallace et al 2013) puts the sub-populations of Sumatra into deficient category data. Sub-populations of Sumatra require exploration to obtain adequate data. This study aims to determine the genetic diversity and analyze connectivity pattern of leatherback turtle populations in Sumatra using mitochondrial DNA.

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

DNA isolation used standard phenol/chloroform by modifying the method of Sambrook et al (1989). DNA amplification was performed using polymerase chain reaction (PCR). Primers used were LCM15382 (5 'GCTTAACCCTAAAGCATTGG-3') (forward) and H950g (5'GTCTCGGATTTAGGGGTTTG-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 94ºC for 5 min; 35 cycles of 94ºC for 30 seconds (denaturation), 58ºC for 30 seconds (annealing), and 72ºC for 60 seconds (extension), and final extension 72ºC for 9 min. The amplicons that showed a single band on polyacrylamide gel were sequenced using previous primers. Sequencing was performed by 1st Base (DNA Sequencing service).

Alignment was conducted using Mega v 5.1 (Tamura et al 2011). Arlequin 3.5 used to calculate the haplotype diversity (h) and nucleotide diversity (π). Analysis of molecular variance (AMOVA) was also calculated to determine the population structure (Excoffier & Lischer 2010). Superimposed phylogeny on the geography map was performed using Network 4.6.1.3 (www.Fluxus-engineering.com).

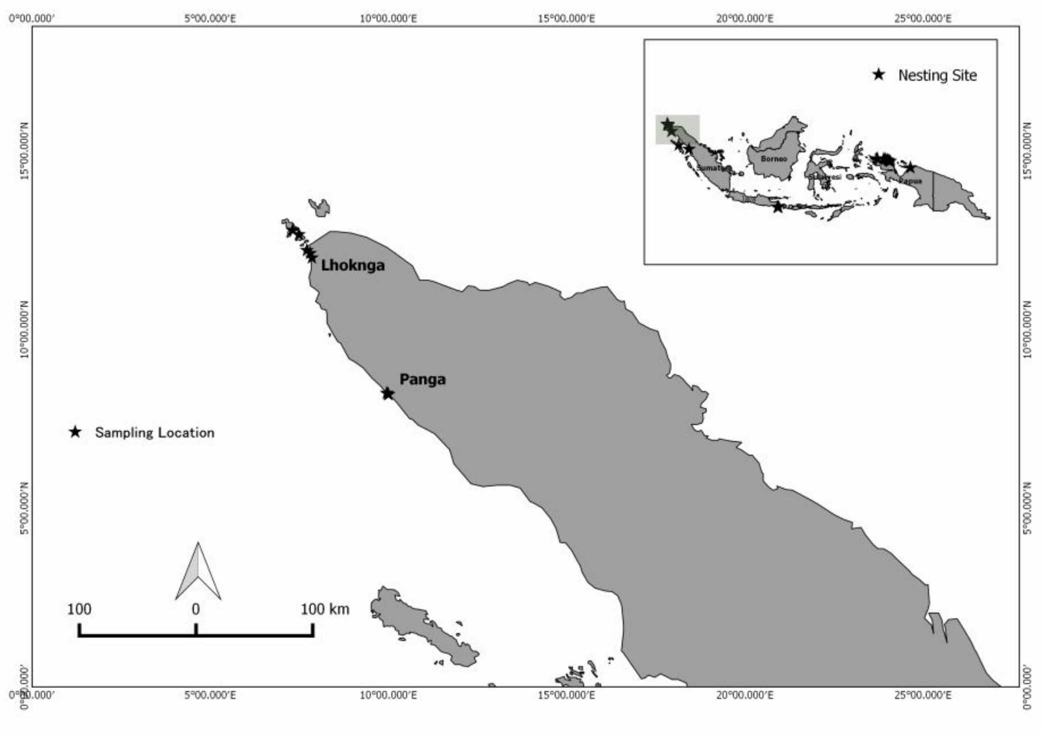

Figure 1. Sampling location in Sumatra (original).

Results and Discussion. Based on of 763 bp control region in leatherback turtles, we obtained 22 variable sites from 4 haplotypes. Haplotypes that found in Sumatran Leatherback turtle populations were the same haplotypes that found in Pacific and Indian Ocean (Table 1) but 2 haplotypes were different. We found two new haplotypes Dc4.2 (GenBank accession no. KU234548) and Dc4.3 (GenBank accession no. KU234549) from nesting site in Panga.

Table 1

Variable sites of 4 haplotype based on sequence (763) of mtDNA control region in Sumatran leatherback turtle populations

Haplotypes	*Variable sites*																					
	053	*092*	*093*	*115*	*157*	*168*	*199*	*203*	*212*	*292*	*312*	*430*	*537*	*588*	*616*	*673*	*720*	*721*	*725*	*738*	*739*	*741*
Dc1.1	A	C	C	A	A	A	A	A	G	A	T	T	C	A	A	G	A	C	A	A	C	C
Dc4.1	.	.	.	.	.	.	G	.	A	G	.	.	.	.	G	.	.	.	.	.	.	.
Dc4.2	.	.	.	.	.	.	G	.	A	G	G	G	T	C	G	A	.	.	G	.	.	.
Dc4.3	T	A	A	T	G	C	G	G	A	G	.	.	.	.	G	.	T	T	.	T	G	T

The highest value of haplotypes and nucleotides diversity found in the Sumatra populations was Lohknga h = 0.6 and π = 0.0078 followed by Panga h = 0.5 and π = 0.0026 (Table 2). Papua populations (Jamursba Medi and Warmon) have a low diversity (h = 0.187 and π = 0.0008). AMOVA results (not mentioned in the table) showed the populations in Sumatra and Papua are still in the one geographical structure (P-Value = 0.0000).

Table 2

Genetic diversity of Sumatran leatherback turtle populations compared with Papua populations

Populations	*n*	*h*	*π*
Sumatera (Panga)	4	0.5	0.0026
Sumatera (Lhoknga)	6	0.6	0.0078
Papua Jamursba Medi*	31	0.187	0.0008
Papua Warmon*	9	0	0

*Dutton et al 2007.

Based on the haplotypes, it is showed that the connectivity patterns of Sumatran leatherback turtles (Figure 2) have migration path to Indian Ocean and South China Sea. Haplotypes that found in Sumatra were same with haplotypes that found 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.

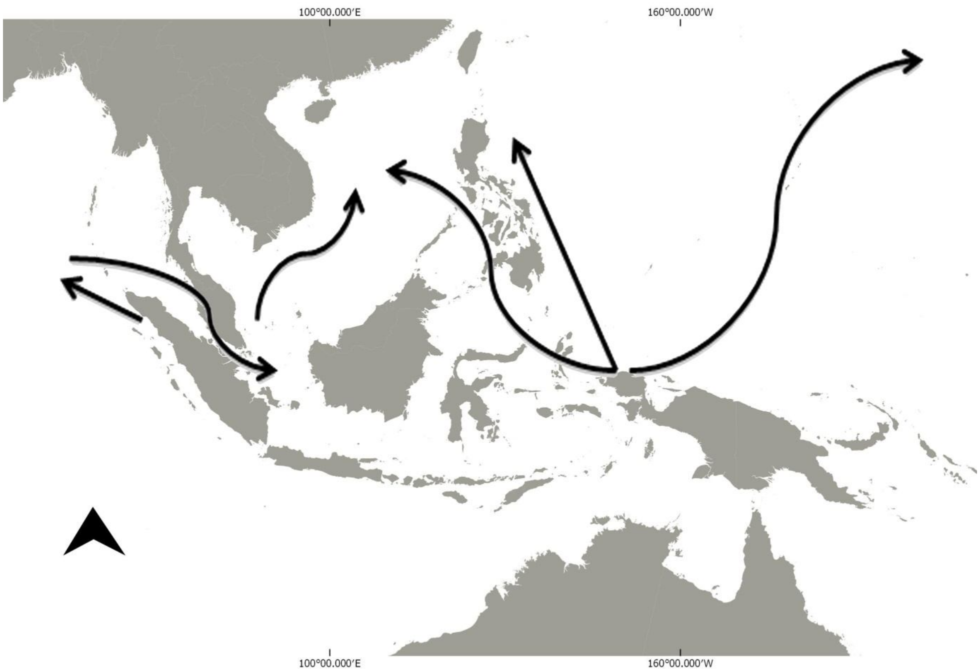

Figure 2. Model of connectivity pattern of *Dermochelys coriacea* in Indonesia (original).

South China Sea is the connecting location between Sumatran and Papuan *D. coriacea*. The connectivity pattern of Sumatran Leatherback turtles was supported by the *D. coriacea* 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 2011). The availability of jellyfish in Malacca Strait and South China Sea (Omori & Nakano 2001) and ocean flow were also important factors for the movement of *D. coriacea*. In addition, according to some public reports, *D. coriacea* 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 *D. coriacea* (Shrive & Hurlburt 1997). Indonesian *D. coriacea* populations were centralized in the two large islands (Papua and Sumatra). Both of these islands are interpretations of the *D. coriacea* populations in the Pacific and Indian Ocean. Therefore, we found genetic mixing between the two populations indicated that the populations of *D. coriacea* in the Pacific and India disable to separate genetically.

This study shows the protection and conservation of the *D. coriacea* population in Sumatra is necessary. Genetic conservation is needed, through the high genetic diversity of *D. coriacea* in Sumatra. The protection of species and the protection of habitats (foraging and nest) must be managed properly. In addition, the connectivity path that were traversed by this species will also be necessary to be protected. The information need to be socialized to the fishermen, so they can be careful in fishing in the connectivity path of *D. coriacea* to avoid by catch.

During this time, the protection of *D. coriacea* species in Indonesia, particularly in Sumatra is still lacking. Due to the absence of data related to this species in Sumatra, so the species protection practices are still lacking. However, with the results of this study, it is expected the stakeholders can take a good policy for the protection of this species.

D. coriacea 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 *D. coriacea* can be identified based on breeding territory. The structure of the *D. coriacea* population divided into two major regions, Atlantic and Pacific-Indian (Bowen & Karl 2007). The identity of Indonesia *D. coriacea* proves that Pacific-Indian region was inseparable as well as the ranges of these species.

Molecular approaches in analyzing the spread of *D. coriacea* are indispensable (Lee 2008). The results of genetic identification has been studied by Dutton et al (1999, 2007, 2013), Vargas et al (2008), Molfetti et al (2013), Prosdocimi et al (2014), Phillott & Gamage (2014) showed the haplotype diversity of *D. coriacea* in the world. All of the studies showed that phylogeogrraphy of leatherback turtle has a genetic identity related to their nesting habitat.

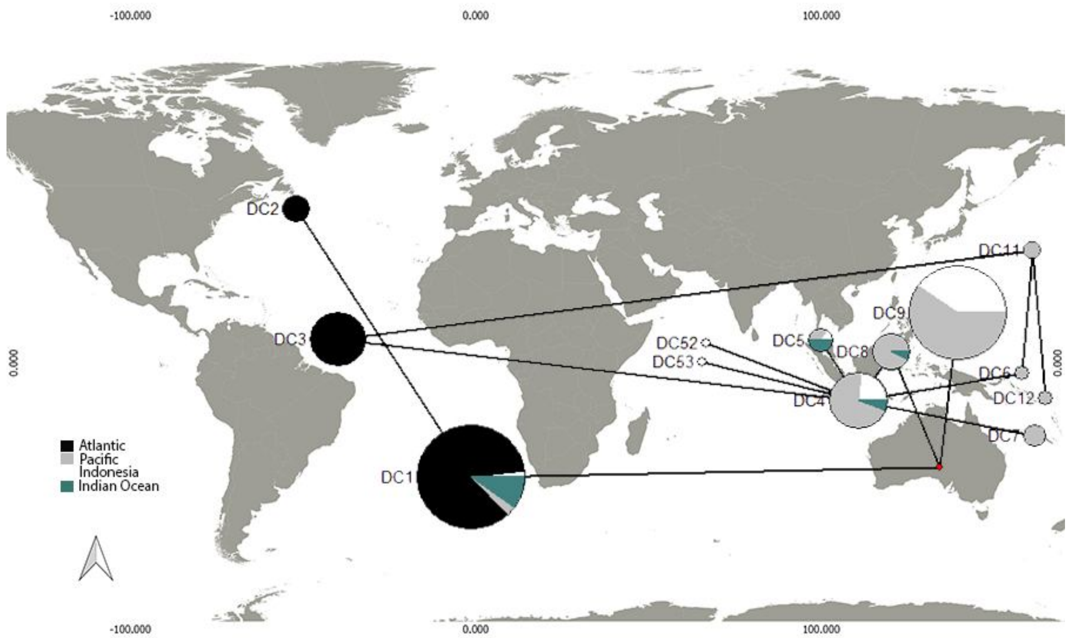

Figure 3. Distribution of leatherback turtle haplotypes around the word using MJ network (original).

D. coriacea populations in Indonesia (Papua and Sumatra) are the combination of *D. coriacea* populations from Pacific and Indian Oceans. Haplotypes diversity that found in Indonesia was similar with Pacific and the Indian Ocean (Figure 3). 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. Figure 3 shows that haplotypes that found in Atlantic came from Pacific-Indian haplotype. It shows that *D. coriacea* in the world come from the Pacific-Indian Ocean region. Then the distribution spread to the Atlantic, before finally separated genetically.

Conclusions. *D. coriacea* populations in Sumatra have excellent potential to increase the population of *D. coriacea* in the world. This population has a high genetic diversity and a good level of connectivity with populations from Pacific (Papua). However, the low data related this species in Sumatra make the protection of this population is still very poor. Through this research, is expected to increase attention to protect this species, particularly in Sumatra. Therefore, we need further studies to obtain data related to population and ecology of this species in Sumatra.

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