 ISSN: 1907-5626

IDENTIFICATION OF THE STOCK/POPULATION OF GREEN TURTLE (*Chelonia mydas*) IN THE SUKAMADE (EAST JAVA) NESTING BEACH

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ABSTRACT

Defining the genetic structure of a particular population of marine turtle is an essential ecological aspect to promote their conservation and enhancement because the resources-protect schemes should be made to the each population unit. Mitochondrial DNA (mtDNA) has been proven effective for detecting population structure in nesting population. We use this method to assess the stock/population of green turtle (*Chelonia mydas*) in the Sukamade nesting beach. Three haplotypes, i.e. C3, C5, and the new one that we called S1 were found. Haplotype (hd) and nucleotide diversities (π) were calculated to be 0.538 ± 0.115 and 0.00381, respectively. The closest genetic distance was 0.003 (between C3 and C5), and the longest was 0.011 (between C3 and S1). Comparison between the genetic distances that found in this research and those defined for the Australasian region by Moritz *et al* (2002) is presented as a *phylogenetic tree*. Pairwise Fst using molecular distances following the model of Tamura-Nei for nucleotide substitution, as well as two other tests, i.e. pairwise Fst using haplotype frequencies, and the Exact test strongly indicates that the nesting population of Sukamade beach is genetically distinct as compared to the other nesting population within the Australasian region.

Key words: Green turtle, Chelonia mydas, Nesting site, PCR, haplotype, mtDNA

INTRODUCTION

Sukamade beach (8°33' – 8°38' S and 113°50' – 113°58'E) has long been known as important turtle nesting beaches in Java. Nowadays, it certainly the last place in the region which is visited all year round by a significant number of female green turtle (*Chelonia mydas*). Total turtles visiting the beach per year are predicted to be more than 500 females. In the past, the beaches were home for at least four species of turtles, namely the green, hawksbill (*Eretmochelys imbricata*), olive ridley (*Lepidochelys olivacea*), and the leatherback turtle (*Dermochelys coriacea*). Nevertheless, threats for turtle population using this nesting site are significant, and as a result, steady decline of the populations is evident.

The success of turtle management strategies is contingent on understanding of their population dynamic. This knowledge of population dynamics is largely obtained from long-term mark-recapture studies of females tagged while nesting on the beaches. It has been shown that that breeding female turtles display high fidelity to the same nesting beaches (Hendrickson 1958; Carr & Ogren 1960; Carr 1967), and thus hypothesized that mature nesting female turtles were selecting their natal beach to deposit eggs. Studies in the southern Great Barrier Reef (sGBR) demonstrate that green turtles also display fidelity to resident feeding grounds throughout their adult lives (Limpus *et al* . 1992). FitzSimmons *et al.* (1999) inferred that male green turtles, like females, are philopatric to natal regions. Detection of subpopulations of sea turtles is a very essential ecological aspect to promote their conservation and enhancement because the resource-protect schemes should be made to the each population unit. Mitochondrial DNA (mtDNA) has proved particularly effective for detecting population structure in marine turtles. Analysis of mtDNA structure in Atlantic green turtle population supported the natal homing hypothesis, as geographically distant were found to have heterogeneous mtDNA frequencies (Bowen & Avise 1996).

The objective of this work is to detect the mtDNA structure of nesting green turtle in the Sukamade nesting beach, which eventually can be used as genetic marker for this particular turtle management unit.

METHODS OF RESEARCH

This work is complementary to the previous work done by a group of Australian scientists in 2002, who analyzed the mtDNA from 27 green turtle rookeries throughout Australasia region but missing this important turtle rookery. DNA was extracted from skin tissue of nesting females and was ensured that the progeny from a given female was only sampled once. Samples were stored in a NaCl saturated solution of 20% DMSO, and transported to Udayana University.

DNA was extracted from small amounts of tissues (typically 0.1 g), using PureLink™ Genomic DNA Purification Kit from Invitrogen®, and stored at -20°C for subsequent polymerase chain reaction (PCR). Successful DNA isolation was confirmed by running 4 µL of genomic DNA in a 1% Agarose gel. A 740 bp segments of the mtDNA control region were amplified using

LTEi9 (GGGAATAATCAAAAGAGAAGG-3') and H950 (GTCTCGGATTTAGGGGTTT-3') primers (Alberto Abreu-Grobois, Pers. Comm.) The RT-PCR was done using SuperScript™ III One-Step RT-PCR System with Platinum@ Taq DNA Polymerase (Invitrogen®) and Rmix (0,2 mM dNTP, 1,6 mM MgSO4, dan buffer).

Sequencing (forward and reverse) was conducted at the sequencing facility of the Repfon Glamor of Malaysia. Sequences were aligned using Clustal_X (Thompson *et al* 1997) and population parameters (haplotype and nucleotide diversities) estimated in Arlequin 2000 (Schneider *et al*, 2000). Comparison with 27 Australasian mtDNA structure as published by Moritz *et al* (2002) was done by Mega 4.0, which also employed to generate the phylogenetic tree. Calculation of variation from each sequence, transition, transvertion, and polymorphic sites were counted by using DNAsp 4.10.

RESULTS AND DISCUSSION

Haplotypes composition and frequency — A total of 23 mtDNA fragments could be amplified out of the overall (25) samples. From these 23 samples that sent to Eijkman, 14 were successfully sequenced. The length of PCR products varied with average 826 bp. Sequences that could be properly read were about 810 bp. For this particular purpose, i.e. to compare sukamade mtDNA structure with the mtDNA structure of breeding green turtle in the 27 Australasian rookeries as defined by Moritz *et al* (2002), 384 bp sequences were used. Results showed 11 polymorphic sites with 11 transitions as shown in **Table-1**. Three different haplotypes were found, i.e. C3, C5, and a new haplotype that we called S1. The frequencies of these three haplotypes were presented in **Table-2**.

Table-1. Polimorphic sites from the mtDNA sequences of three haplotypes of green turtle nesting on Sukamade beach. Numbering was based on position on 384 bp length.

Position				2	2	2	2	2	3	3	3
	6	6	9	2	5	7	8	9	1	2	7
Haplotypes	0	9	1	1	2	0	0	5	7	3	2
C3	C	T	G	G	G	C	C	C	T	C	A
C5	T	.	.	.	.	.	.	.	.	.	.
S1	.	.	.	.	A	.	.	.	.	T	G

Table-2. The frequency of haplotypes found on females turtle on the Sukamade nesting beach (N=14)

Haplotypes	Total samples	Percentages (%)
C3	9	64.3
C5	1	7.1
S1	4	28.6
Total	14	100

Haplotypes diversity and Genetic Distance — Analysis using DNAsp on 14 sequences showed the *haplotype diversity* (hd) of 0.538 ± 0.115, and the nucleotide diversity (π) was 0.00381. *Genetic distance* from the three haplotypes sequences was presented in **Table-3.**

Table-3. Genetic distance from the three haplotypes found in Sukamade nesting beach.

Haplotypes	C3	C5	SM1
C3			
C5	0.003		
S1	0.011	0.008	

Genetic distance showed the genetic difference among two haplotypes. From **Table-3**, the closest genetic distance was 0.003 (between C3 and C5), means there are only three base pairs were different among 1000 base pairs. In this case, for 384 bp that being used in this particular work, the difference was only one base pair. Comparison between the genetic distances that found in this research and those defined for the Australasian region by Moritz et al (2002) is presented as a *phylogenetic tree* in **Figure-1**.

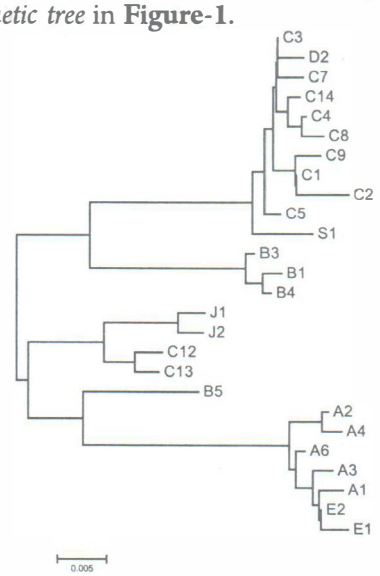

Figure-1. Phylogenetic tree showing the relative position of Sukamade nesting population to the other Australasian nesting populations. Scale bar showing the genetic distance of 0.005.

Three different tests which using Arlequin 3.1 showed that the haplotypes composition of Sukamade nesting turtles were different to the other nesting population found in the Australasian region (**Table 4**), and Sukamade population cannot be included in any turtle management units (**Table-5**) as defined by Moritz *et al* (2002).

CONCLUSION

The finding of this work strongly suggest that Sukamade nesting population is genetically distinct as compared to the other 27 nesting populations within the Australasian region as defined by Moritz *et al* (2002). However, more sample size which is taken in differ-

ISSN : 1907-5626

Table-4. Analysis by Arlequin 3.1 to compare Sukamade nesting population with the other 27 nesting populations within the Australasian region as defined by Moritz *et al* (2002).

No.	Sea/ Ocean	Country	Region	Nesting Location	Sukamade		
					1*	2**	3***
1	SW Pacific	Australia	NGBR	Bramble Cay	+	+	+
2		Australia	NGBR	Raine Is No 8 Sandbank	+	+	+
3		Australia	Coral Sea	Coral Sea Platform	+	+	+
4		Australia	SGBR	Heron Island	+	+	+
5		Australia	SGBR	Lady Musgrave island	+	+	+
6		Australia	SGBR	Northwest Island	+	+	+
7		New Caledonia		New Caledonia	+	+	+
8	NW Pacific	Micronesia	Elato Atoll	Elato Atoll	+	+	+
9		Micronesia	Ngulu Atoll	Ngulu Atoll	+	+	+
10		Micronesia	Yap	Ulithi Atoll	+	+	+
11		PNG	PNG	Long Island	+	+	+
12	S China Sea	Malaysia	Peninsular	Paka Island	+	+	+
13		Malaysia	Peninsular	Redang island	+	+	+
14		Malaysia	Sarawak	Sarawak Turtle Islands	+	+	+
15	Sulu Sea	Malaysia	Sabah	Mal Turtle Islands	+	+	+
16		Philippines	Tawi-Tawi	Phi Turtle Islands	+	+	+
17	Celebes Sea	Indonesia	Berau	Sangalaki Island	+	+	+
18		Malaysia	SE Sabah	Sipidan Island	+	+	+
19	Arafura Sea	Indonesia	Aru	Enu Island	+	+	+
20		Australia	GOC	Bountiful Island	+	+	+
21		Australia	GOC	Groote Eylandt	+	+	+
22		Australia	GOC	Port Bradshaw	+	+	+
23	Timor Sea	Australia	Ash. Reef	Ashmore Reef	+	+	+
24		Australia	Scott Reef	Sandy Island	+	+	+
25	E Indian Ocn	Indonesia	W Java	Pangumbahan	+	+	+
26		Australia	NW Shelf	Northwest Cape	+	+	+
27		Australia	NW Shelf	Lacepedes	+	+	+

Notes:
1* Computing conventional F-Statistics from haplotype frequencies
2** Comparisons of pairs of population samples, Distance method: Tamura & Nei
3*** Exact Test of Sample Differentiation Based on Haplotype Frequencies

ent time/seasons should give a better picture for this particular population.

ACKNOWLEDGEMENT

This work is part of a bigger project entitled: Tracking and genetic analysis of sea turtles in the Eastern Part of Indonesia, a collaboration between Udayana University and WWF Indonesia. The overall objective of this project is to underpin the creation of MPAs network in the Coral Triangle region that protect the migration routes, feeding sites and breeding sites of sea turtles. Support had been also obtained from Dr IGNK Mahardika (Udayana University), Ms Wahidah (SEAFDEC Malaysia), Ms Dita Cahyani and Ms Kiki Dethmers (University of Charles Darwin, Australia) during the lab work and data analysis.

Table-5. Analysis by Arlequin 3.1 to compare Sukamade nesting population with the other 17 management units within the Australasian region as defined by Moritz *et al* (2002).

No	Management Units (MUs)	Sukamade		
		1*	2**	3***
1	North West Shelft	+	+	+
2	Coral_Sea	+	+	+
3	Gulf_Carpentaria	+	+	+
4	NGBR	+	+	+
5	SGBR	+	+	+
6	Ashmore reef	+	+	+
7	Scoot_reef	+	+	+
8	Micronesia	+	+	+
9	Aru	+	+	+
10	Berau	+	+	+
11	Java	+	+	+
12	Peninsula Malaysia	+	+	+
13	Sarawak	+	+	+
14	SE_Sabah	+	+	+
15	New_Caledonia	+	+	+
16	PNG	+	+	+
17	Sulu_Sea	+	+	+

Notes:
1* Computing conventional F-Statistics from haplotype frequencies
2** Comparisons of pairs of population samples, Distance method: Tamura & Nei
3*** Exact Test of Sample Differentiation Based on Haplotype Frequencies

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