



Spatio-temporal patterns of mitochondrial DNA variation in hawksbill turtles (*Eretmochelys imbricata*) in Southeast Asia



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ABSTRACT

Mitochondrial DNA (mtDNA) polymorphisms provide useful information that can be used to estimate the phylogeographic relationships, historical demography, and migratory events of widely distributed animals. In this study, the spatio-temporal patterns of mtDNA polymorphisms were assessed in Indo-Pacific hawksbill turtles from Malaysian nesting rookeries and foraging aggregations sampled during 1996–2014. Clear genetic differences were observed between turtles from the Sabah Turtle Islands rookery in the Sulu Sea, and the rookeries of Melaka and Redang Island in the South China Sea off the Malay Peninsula; however, no temporal genetic changes were found to have occurred on a decadal time scale. Despite the descriptive evaluation of a few samples, Johor nesting turtles possessed different haplotypes from those at the proximate Melaka rookery, indicating the importance of conserving this small nesting population. Although continuous sampling efforts are needed to determine the relationships between specific rookeries and foraging aggregations, the presence of multiple haplotypes in Malaysian foraging aggregations, in combination with Lagrangian drifter buoy data, suggests that there have been migrations to foraging grounds in Southeast Asia from various rookeries. This study provides basic information for the conservation and management of hawksbill turtles in Southeast Asia.

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1. Introduction

The hawksbill turtle [*Eretmochelys imbricata* (L.)] is a marine reptile globally distributed in tropical areas including Southeast Asia. Because of the exploitation of shells used in the *bekko* industry and eggs for food, in addition to bycatch, and the loss of nesting and foraging grounds, the number of hawksbill turtles has declined globally (Meylan and Donnelly, 1999). As a result, the hawksbill turtle is listed as Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List (IUCN, 2014). Southeast Asian hawksbill turtles are no exception and populations have declined (Shanker and Pilcher, 2003). Egg collection is one of the major threats to hawksbill turtles in Southeast Asian rookeries (Chan, 2006; Shanker and Pilcher, 2003); however, legislation banning or regulating the commercial sale of hawksbill turtle eggs is insufficient. For example, in Malaysia there are legislations in the states of Sabah and Sarawak, but not in Peninsular Malaysia (WWF–Malaysia, 2009). As a result, conservation efforts, including the establishment of sanctuaries at nesting sites in Southeast Asia, have recently commenced (Chan, 2006, 2010).

To provide an important basis for conservation and management, it is necessary to investigate migration patterns and population genetic structures of hawksbill turtles. Due to the relatively wide distribution and abundance of rookeries (i.e., nesting grounds), studies of sea turtles have primarily focused on species such as green turtles [*Chelonia mydas* (L.)] and loggerhead turtles [*Caretta caretta* (L.)] (e.g., Boyle et al., 2009; Dethmers et al., 2006; Encalada et al., 1996, 1998; Hatase et al., 2002; Nishizawa et al., 2013; Shamblin et al., 2014). Hawksbill turtles have been studied mainly in the Caribbean and Atlantic, and previous studies demonstrated the genetic differentiation among hawksbill turtles in rookeries that reflects natal philopatry (Bass et al., 1996; Monzón-Argüello et al., 2010a), contemporary migration from rookeries to foraging grounds (Blumenthal et al., 2009; Bowen et al., 2007; Monzón-Argüello et al., 2011; Vilaça et al., 2013), and phylogeography (LeRoux et al., 2012). However, there have been only a few studies on Southeast Asian hawksbill turtles; therefore, filling these geographic and interspecific gaps in knowledge is required.

Mitochondrial DNA (mtDNA) haplotypes of Indo-Pacific hawksbill turtle foraging aggregations in the western Pacific indicates the presence of multiple clades (Nishizawa et al., 2010; Okayama et al., 1999), and the recently reported haplotypes found in nesting hawksbill turtle populations in Southeast Asia supports the existence of distinct clades (Wahidah and Syed Abdullah, 2009). However, the limited number of

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sampling sites and small sample sizes obtained from nesting populations and foraging aggregations restricts further analysis. In addition, although understanding the changes in the genetic structures of sea turtle populations in relation to population size fluctuations and possible recruitment from other rookeries is important for sea turtle conservation, changes occurring on a decadal time scale have only been investigated in a few studies (green turtles: Bjørndal et al., 2005; loggerhead turtles: Shamblin et al., 2011). Thus, continuous sampling efforts over tens of years at multiple sites in the Indo-Pacific region provides insight into the pattern of genetic differentiation among rookeries and temporal genetic variation, further enabling global or ocean-wide analyses, such as mixed-stock analysis, that links foraging grounds to rookeries and estimates contemporary migration patterns (e.g., Atlantic green turtles and loggerhead turtles; Monzón-Argüello et al., 2010b, 2012).

In this study, the spatio-temporal differences were examined among mtDNA sequences of hawksbill turtles from different Malaysian nesting rookeries. In Malaysia, major nesting rookeries of hawksbill turtles are located at Sabah Turtle Islands, Melaka, and Redang Island (Chan, 2006; Mortimer et al., 1993). The nesting rookery at Sabah Turtle Islands has the highest concentration of nesting hawksbill turtles in Southeast Asia (Limpus, 1994). Samples collected from these rookeries during 1996–2014 were analyzed to investigate (1) the genetic differentiation among hawksbill turtles at these rookeries, and (2) the temporal genetic variation among hawksbill turtles within these rookeries. In addition, a small number of samples from the Johor rookery and foraging grounds in Malaysia were also collected. Potential migrations were discussed by investigating the differences between the genetic composition of rookeries and their proximate foraging grounds, and by assessing ocean surface currents (i.e., Lagrangian drifter buoy data) that are strongly related to hatchling drift patterns (Monzón-Argüello et al., 2010b; Scott et al., 2014). Sampling sites were located both on the Malay Peninsula in the South China Sea, and in the Sulu or Celebes Seas; thus, this study provides basic information that can improve the conservation and management of hawksbill turtles in Southeast Asia, and adds information to the global mtDNA haplotype dataset for sea turtles.

2. Material and methods

2.1. Samples and molecular analysis

Samples of nesting hawksbill turtles were collected at Redang Island during 1996–1999 (N = 8) and 2011–2014 (N = 11); at Melaka in 1998 (N = 10) and 2014 (N = 6); at Sabah Turtle Islands in 1998 (N = 24), 2003 (N = 28), and 2014 (N = 60); and at Johor in 1998 (N = 3) (Fig. 1). On Redang Island and Sabah Turtle Islands, individual nesting hawksbills were identified by tags. One nesting turtle sampled in 1998 also nested again and was resampled in 2011. In other rookeries, because nesting turtles were not tagged, samples were collected from hatchlings emerging from nests deposited within 10 consecutive days of each other. This method avoided duplicate sampling because the inter-nesting interval of hawksbill turtles in Southeast Asia is between 12 and 20 days (Chan and Liew, 1999; Chan et al., 1999). One hatchling was sampled per clutch. Due to matrilineal inheritance, hatchlings possess the same mtDNA haplotype as their mother. Short mtDNA sequences from samples collected in 2003 and earlier were previously analyzed by Joseph (2006), but these samples were reanalyzed in the present study to obtain longer mtDNA sequences.

In addition, between 2009 and 2011 samples were collected from hawksbill turtles at several Malaysian foraging grounds: Sabah Tun Sakaran Marine Parks (N = 8), Pulau Sipadan (N = 5), Pulau Tiga (N = 2), and Melaka (N = 8) (Fig. 1). Hawksbill turtles were hand-captured by snorkelers or divers (Eckert et al., 1999), using the rodeo method (Limpus and Reed, 1985), or by drift nets. Once captured, each turtle was lifted into the boat. Blood samples were obtained from the dorsal cervical sinus (Dutton, 1996) and preserved in lysis buffer (100 mM Tris-HCL, 100 mM EDTA, 10 mM NaCl, 1% SDS; pH 8.0), at a 1:10 blood:buffer ratio (Dutton, 1996).

Genetic analyses were conducted at the Universiti Malaysia Terengganu. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) protocol (Bruford et al., 1992). An 800-bp segment of the mitochondrial control region was amplified from genomic DNA

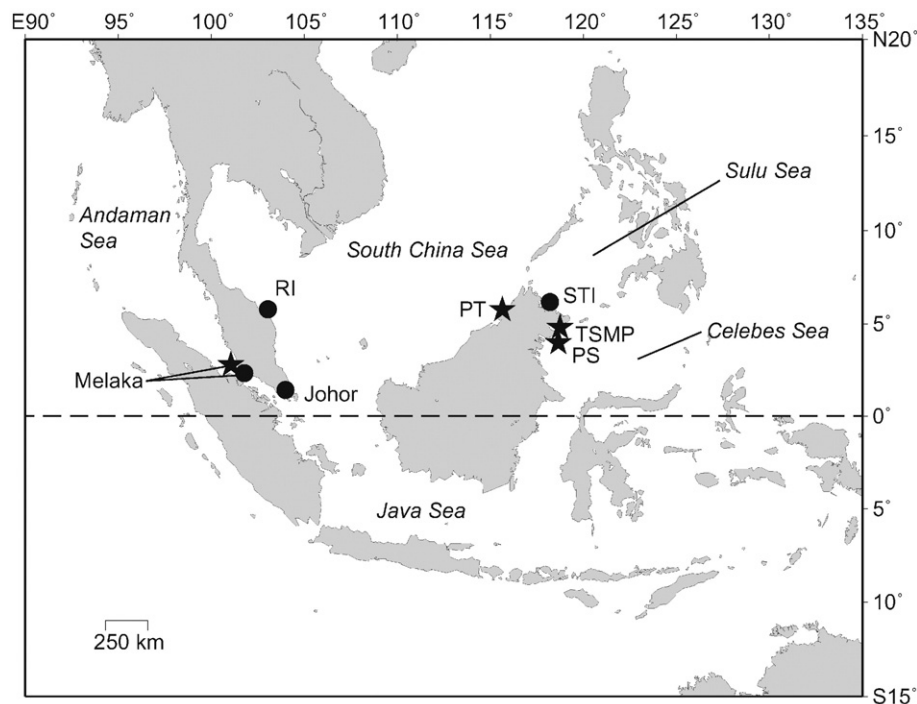


Fig. 1. Geographic locations of the nesting rookeries (circles) at Redang Island (RI; N = 18), Melaka (N = 45), Johor (N = 3), and Sabah Turtle Islands (STI; N = 132), and foraging grounds (stars) at Tun Sakaran Marine Park (TSMP; N = 8), Pulau Sipadan (PS; N = 5), Pulau Tiga (PT; N = 2), and Melaka (N = 8). The number of samples collected from the Melaka and STI rookeries includes those collected by Wahidah and Syed Abdullah (2009).

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