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ORIGINAL PAPER

# Comparison of the rookery connectivity and migratory connectivity: insight into movement and colonization of the green turtle (*Chelonia mydas*) in Pacific–Southeast Asia

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## Abstract

Integration of the rookery connectivity, which includes historical connections among rookeries, and migratory connectivity assessing dispersal or migratory routes of marine animals are important for understanding dispersal and/or migration and its effect on the formation of genetic population structure. The migratory nature and long-distance movement of sea turtles have been reported, while natal philopatry has been suggested by genetic differentiation among rookeries within a relatively narrow geographic scale. Therefore, we hypothesize that contemporary long-distance movement has a limited effect on colonization in new rookeries. This study compared the genetic relationships among rookeries and between the rookeries and foraging grounds of green turtles (*Chelonia mydas*) in Southeast Asia. Mitochondrial control region sequences of 333 turtles from 11 rookeries were newly determined, and combination with previously reported Indo-Pacific rookeries indicated the presence of a genetic barrier in the Torres Strait and Celebes Sea (i.e. Philippines–Sulawesi). On the other hand, an analysis of newly collected 107 turtles from seven foraging sites and mixed stock analyses indicated contemporary movement across this historical genetic barrier, from Micronesian rookeries to foraging grounds in the Celebes Sea (i.e. Sipadan Island and Tun Sakaran Marine Park). Isolation by distance was generally supported for relationships among rookeries, and the high migratory connectivity did not result in a lower genetic distance between rookeries than predicted from geographic distance. Differences between rookery connectivity and migratory connectivity in green turtles in Southeast Asia are likely due to migration to natal regions after long-distance movement.

## Introduction

Understanding a population's genetic structure can help elucidate the genetic diversity and historical geological and climatic events that formed the population (Avise 2000). In addition, the population genetic structures of marine animals are influenced by contemporary events such as dispersal of animals and oceanic surface currents that result in the geographic displacement of an animal's breeding place (Chen

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et al. 2004; Lourie and Vincent 2004; Kool et al. 2011). A widely accepted theory under restricted dispersal is isolation by distance, which is an increase in genetic differences with geographic distance (Wright 1943). However, genetic differences in marine animal populations are hypothesized to vary depending on their dispersal (Palumbi 2003; Bradbury and Bentzen 2007). Identifying genetic differences among rookeries, or rookery connectivity, is particularly important for endangered species, because it provides not only inferences of dispersal, but also the basic information necessary to set conservation priorities and designate "management units" (Moritz et al. 2002).

While rookery connectivity indicates dispersal and/or natal philopatry of animals, dispersal routes or migratory routes are not covered. The dispersal routes or migratory routes of animals (i.e. migratory connectivity) can be measured by techniques such as mark–recapture (Limpus and Chaloupka 1997) and biotelemetry (Cooke et al. 2004; Hussey et al. 2015). Genetic information can also be used to identify migratory connectivity as genetic tags (Pella and Masuda 2001). For comprehensive understanding of dispersal and/or migration of marine animals, both rookery connectivity and migratory connectivity have to be revealed. Comparing these connectivities enables us to assess whether contemporary movement relates to displacement of breeding location (i.e. contemporary dispersal) or tentative movement, followed by a return to the original breeding location (i.e. natal philopatry).

The population genetic structures of marine animals in the seas off Southeast Asia have received attention because of the high marine biodiversity in the area (Roberts et al. 2002). Complex surface current systems driven by inflows from the Pacific Ocean and monsoon winds (Chen et al. 2004; Gordon 2005; Hu et al. 2000), wide continental shelves and land bridges formed by low sea levels during the Pleistocene (Voris 2000) may play a role in the formation of the genetic population structures of marine organisms. In previous studies, genetic breaks have been identified between eastern and western populations of seahorses in Borneo–Philippines (Lourie and Vincent 2004) and between northern and southern populations of shrimp in the Flores and Java Seas (Barber et al. 2000, 2002). Regional groupings of fish based on genetic population structures have also been suggested in some populations in the South China Sea (Ablan 2006; Chen et al. 2004). However, integrative approach combining both rookery connectivity and migratory connectivity has not been applied yet.

Sea turtles have been monitored because of their migratory nature and endangerment (IUCN 2016). Natal philopatry of sea turtles was suggested by the fact that genetic population differentiation is observed on a relatively narrow geographic scale (reviewed by Bowen and Karl 2007). This was partly supported by observations that tagged individuals usually nested at the rookeries where they were first tagged (Dethmers et al. 2006). While the designation of management units based on genetic differences among rookeries has been attempted in sea turtles (e.g. Moritz et al. 2002; Dethmers et al. 2006; Dutton et al. 2014b), long-distance movement of sea turtles has to be considered for their conservation (e.g. Bowen et al. 1995; Parker et al. 2011; Nishizawa et al. 2014). Dethmers et al. (2006) suggested a genetic barrier existed between the Pacific and Southeast Asia populations of green turtle (*Chelonia mydas*) and isolation by distance among their rookeries of green turtles, but the correlation between genetic distance and geographic distance was relatively low. This might be due to colonization after long-distance movement despite general natal philopatry. Historical introgression and fragmentation have been estimated by haplotype-based analyses (e.g. evaluation of haplotype distinction followed by investigation of shared haplotypes or clades) or bifurcation trees based on pairwise genetic distances (Dethmers et al. 2006; Encalada et al. 1996; Dutton et al. 2014a). However, for further investigating dispersal and/or migration and its effect on the formation of population genetic patterns of green turtles in this region, both rookery connectivity and migratory connectivity have to be combined.

To understand rookery connectivity and migratory connectivity of green turtles in Pacific–Southeast Asia, we examined both the rookeries and foraging aggregations. First, genetic differentiation among rookeries of green turtle in Southeast Asia was investigated in detail. Samples collected from Vietnamese and Malaysian rookeries were combined with the data of previous studies (Dutton et al. 2014b; Read et al. 2015; Jensen et al. 2016a), and were used to identify the management units of Southeast Asia and the Pacific region. Second, historical connections among rookeries were investigated based on genetic distance. The genetic barrier of the genetic population structure was explored by the grouping of rookeries. Here, we applied a new approach based on genetic linkages and network analysis (Fortunato 2010). The relationship between genetic distance and geographic distance was examined. Third, linkages between rookeries and foraging aggregations that reflect migratory connectivity were estimated based on haplotype frequency, known as mixed stock analysis (MSA) (Pella and Masuda 2001; Bolker et al. 2007). Then, we addressed (1) whether green turtles show contemporary movement across the genetic barrier, and (2) whether contemporary movement of green turtles results in lower genetic distance than predicted by geographic distance.

## Materials and methods

### Samples from rookeries

Green turtles at eight rookeries located on the coasts of the Andaman Sea, the South China Sea and the Sulu Sea of Malaysia were sampled in 2014–2015 (Fig. 1). Samples collected at Redang Island ($N = 56$) and Sarawak ($N = 122$) in 2014 have already been reported (Joseph and Nishizawa 2016), but samples collected at Redang Island in 2015 ($N = 12$) were added. The sampling procedure was performed according to Joseph and Nishizawa (2016). In short, blood samples were collected from the dorsal cervical sinus of hatchlings and were preserved in a lysis buffer solution.

In addition, green turtle samples collected at 12 sites in 1998, 1999 and 2003 (Joseph 2006) were re-analysed. In total, samples at 14 sites were analysed. Because only a few samples were collected at four sites (Penarik, Setiu, Rhu Kudung and Ma Daerah) on the coast of the Malay Peninsula and the Terengganu mainland, they were combined into one group called Terengganu. Therefore, a total of 11 groups were defined (Fig. 1, Table 1).

### Samples from foraging grounds

Foraging green turtles in Malaysian waters were captured either by SCUBA diving, snorkelling (Eckert et al. 1999), the rodeo method (Limpus and Reed 1985) or the netting technique (Eaton et al. 2008) in 2009–2016. Green turtles were sampled from a total of seven sites (Fig. 1) but were sampled mainly from three sites: Brunei Bay, Sipadan Island and Tun Sakaran Marine Park (TSMP). Samples collected at Brunei Bay in 2011–2014 have already been reported (Joseph et al. 2016), but samples collected in 2016 were added. Blood samples were collected and preserved in the same manner as the samples from rookeries. All captured turtles were released immediately after sampling, morphometric measuring and flipper-tagging. Sipadan Island and Redang Island also provide nesting rookeries for the green turtles in Malaysia. Therefore, to avoid catching nesting females, we caught mostly juvenile samples from these sites. Except for Brunei Bay, most of the green turtle samples caught from the foraging grounds consisted of small juveniles [CCL (curved carapace length) < 65.0 cm; 73%] and sub-adults ($65.0 \leq \text{CCL} < 85.0$ cm; 24%), according to the definition of Sterling et al. (2013).

### Laboratory procedures

The samples were analysed at the Molecular Laboratory of University Malaysia Terengganu. The DNA extraction and polymerase chain reaction (PCR) procedures used were described previously by Joseph et al. (2016). An approximately 800 bp control sequence of mitochondrial DNA was amplified using primers LCM15382 and H950 g (Abreu-Grobois et al. 2006). All successfully amplified samples were purified and sent to First Base (Kuala Lumpur, Malaysia) for sequencing. Sequences were truncated to a ~ 770 bp region that has been widely evaluated in recent studies of sea turtles (e.g. Dutton et al. 2014b; Read et al. 2015; Jensen et al. 2016a). Haplotypes were identified by searching against the GenBank database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) and the database of the Southwest Fisheries Science Centre, NOAA Fisheries Service (https://swfsc.noaa.gov).

### Analysing the relationship among rookeries

At Redang Island and Sabah Turtle Island Park (TIP), more than ten samples were collected during each sampling year. Genetic differences among these sampling years were determined by an exact test (50,000 steps in a Markov chain with a 10,000-step dememorization) and frequency-based conventional pairwise $F_{ST}$ test (10,000 permutations) using Arlequin v3.5 (Excoffier and Lischer 2010). Haplotype compositions of TIP, Sarawak, and Sipadan in this study were also compared to those collected during 1991–1993 in Jensen et al. (2016a), derived from re-analysis of samples from Dethmers et al. (2006). Because there were generally no significant differences (see Results and Online Resource 1), samples from each group were aggregated across sampling years. Genetic differentiation among Southeast Asian 11 groups of rookeries, in addition to Indonesian rookery in Berau (Jensen et al. 2016a), was determined by an exact test and pairwise $F_{ST}$ test. Jensen et al. (2016a) also provided haplotype data from the Malay Peninsula including Redang Island. However, they included data from several rookeries, despite significant genetic differences among rookeries in the Malay Peninsula in this study (see "Results"); therefore, haplotype data from the Malay Peninsula collected by Jensen et al. (2016a) were not included in this study. In multiple comparisons, statistical significance was determined based on the false discovery rate, and adjusted $p$ values were determined according to Benjamini and Hochberg (1995).

After this initial definition of management units in Southeast Asian rookeries, haplotype data of rookeries in the southern Pacific Ocean and the eastern Indian Ocean (Dutton et al. 2014b; Read et al. 2015; Jensen et al. 2016a) were added to the 12 groups of rookeries for further analysis. In total, 31 management units were used (see "Results" and Fig. 1). Significant differences among these were determined by an exact test and pairwise $F_{ST}$ test using the same settings as above.

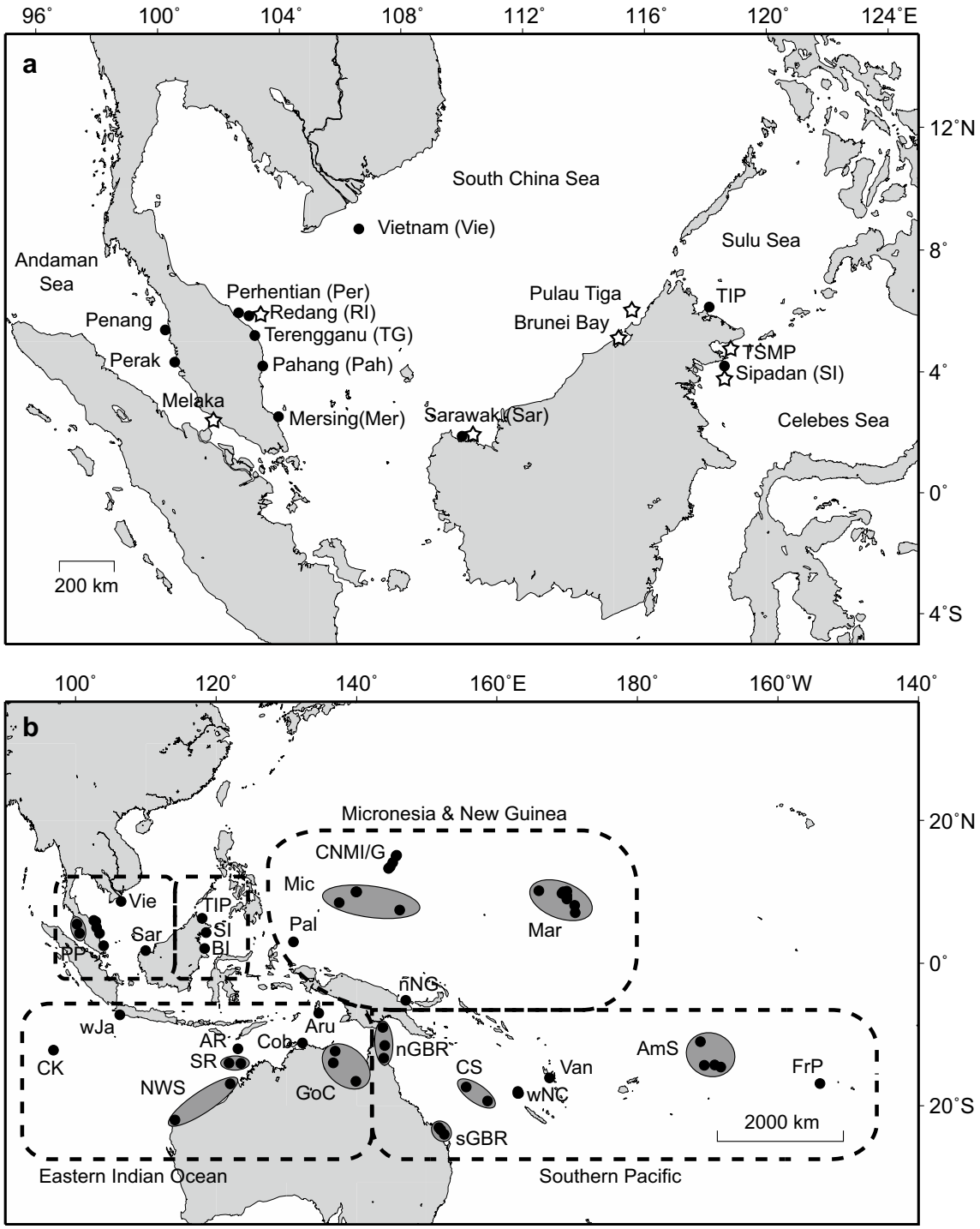


**Fig. 1** Geographic locations of **a** sampling rookeries (circles) and foraging grounds (stars) in Southeast Asia and **b** 31 management units (MUs) as sources in mixed stock analysis. Dashed lines indicate regional grouping of MUs. Abbreviations undefined in the text and figure are as follows: *PP* Penang and Perak, *BI* Berau, *nGBR* northern Great Barrier Reef, *CS* Coral Sea, *sGBR* southern Great Barrier Reef, *wNC* western New Caledonia, *nNG* northern New Guinea, *Van* Vanuatu, *Mic* Micronesia, *Mar* Marshall Islands, *Pal* Palau, *CNMI/G* Commonwealth of the Northern Mariana Islands and Guam, *AmS* American Samoa, *FrP* French Polynesia, *GoC* Gulf of Carpentaria, *AR* Ashmore Reef, *SR* Scott Reef, *wJa* west Java, *NWS* North West Shelf, *Cob* Cobourg Peninsula, *CK* Cocos "Keeling" Island

**Table 1** Haplotype composition of rookeries in Southeast Asia

| Rookery | Penang | Perak | Vietnam | Perhentian | Redang | Terengganu | Pahang | Mersing | Sarawak | TIP | Sipadan | Berau [b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | 2015 | 1998, 2014, 2015 | 2003 | 1999, 2015 | 1998, 2003, 2014[a], 2015 | 1998, 1999 | 1998, 2015 | 2015 | 1991[b], 1998, 2014[a] | 1993[b], 1998, 2003, 2014 | 1993[b], 1998 | 2000 |
| CmP49.1 (AB819808) | 19 | 16 | 10 | 6 | 60 (28[a]) | 6 | 15 | 1 | 32 (26[a]/4[b]) | 6 (3[b]) | 51 (47[b]) | 9 |
| CmP49.3 (KJ502572) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 (8[b]) | 4 (2[b]) | 0 |
| CmP49.5 (KM923921) | 0 | 0 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CmP49.7 (KJ502573) | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 |
| CmP49.9 (KX057742) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CmP57.1 (KJ502588) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1[a]/0[b]) | 124 (39[b]) | 20 (19[b]) | 0 |
| CmP57.2 (KJ502567) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 (11[b]) | 5 (5[b]) | 7 |
| CmP82.1 (KJ502584) | 0 | 0 | 0 | 0 | 12 (3[a]) | 1 | 0 | 0 | 7 (5[a]/2[b]) | 0 | 1 (1[b]) | 0 |
| CmP87.1 (KJ502589) | 0 | 0 | 0 | 1 | 15 (6[a]) | 0 | 0 | 0 | 104 (87[a]/16[b]) | 0 | 0 | 0 |
| CmP91.1 (KF311762) | 0 | 0 | 0 | 0 | 7 (0[a]) | 0 | 0 | 6 | 0 | 0 | 3 (3[b]) | 3 |
| CmP103.1 (KJ502568) | 0 | 0 | 0 | 6 | 22 (7[a]) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CmP104.1 (KJ502569) | 0 | 0 | 0 | 7 | 18 (10[a]) | 3 | 1 | 0 | 3 (2[a]/0[b]) | 0 | 0 | 0 |
| CmP133.1 (KP893544) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1[a]/0[b]) | 0 | 0 | 0 |
| CmP20.1 (AB819806) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 (1[b]) | 0 |
| CmP40.1 (KF311750) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 (1[b]) | 14 (13[b]) | 9 |
| CmP75.1 (KJ502574) | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| CmP227.1 (KX057741) | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CmP228.1 (KX057743) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0[a]/0[b]) | 0 | 0 | 0 |
| CmP106.1 (KJ502576) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1[b]) | 0 |
| CmP67.1 (KF311758) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 22 | 19 | 14 | 22 | 134 (56[a]) | 12 | 27 | 7 | 149 (122[a]/22[b]) | 178 (62[b]) | 103 (92[b]) | 29 |

Counts are noted as the total number including previous studies, but numbers in the parentheses indicate those in previous studies

[a]Joseph and Nishizawa (2016)

[b]Jensen et al. (2016a)

To identify a genetic barrier, clustering of management units was performed through (1) maximization of genetic variance due to differences among clusters, known as spatial analysis of molecular variance (SAMOVA), using the SAMOVA program (Dupanloup et al. 2002) and (2) maximization of the modularity measure (Newman and Girvan 2004) based on links between management units, known as 'community detection' in network analysis, using the igraph package (Csardi and Nepusz 2006) in R v3.2.4 (R Core Team 2016).

The SAMOVA program was run repeatedly for different numbers of groups ($K = 2$–10), and the $\Phi_{ST}$, $\Phi_{CT}$ and $\Phi_{SC}$ values were plotted against the number of groups. The Tamura–Nei model (Tamura and Nei 1993) was used to calculate genetic distance. The Tamura–Nei model has been widely used to evaluate the mitochondrial DNA of sea turtles (e.g. Dethmers et al. 2006; Dutton et al. 2014b; Shamblin et al. 2012).

Community detection was performed based on the spin-glass model and simulated annealing (Reichardt and Bornholdt 2006), which results in relatively accurate detection (Fortunato 2010). Pairwise $\Phi_{ST}$ values in the assumption of the Tamura–Nei model and conventional pairwise $F_{ST}$ values based on haplotype frequency were calculated among these 31 management units using Arlequin. Then, $(1 - \Phi_{ST})$ or $(1 - F_{ST})$ was used as the weight of a link (i.e. strength of connection) between each pair of management units. For network visualization, weak links were removed based on the thresholding value of $(1 - \Phi_{ST})$ or $(1 - F_{ST})$, which was 0.60 or 0.70, respectively, as determined from their histograms (see Online Resource 2). The relationship among management units based on $\Phi_{ST}$ and $F_{ST}$ was also visualized by principal coordinate analysis (PCoA) using GenAlEx v6.5 (Peakall and Smouse 2012).

The isolation by distance was tested by investigating the relationship between genetic distance (i.e. pairwise $\Phi_{ST}$ or $F_{ST}$) and the pairwise geographic distance of management units. When several rookeries consisted of one management unit, their central point became a representative location. Pairwise geographic distances were calculated as the shortest distance between management units using the WGS84 ellipsoid method implemented in the geosphere package (Hijmans 2016) in R. As focusing only on the mean probability distribution of genetic distance is not appropriate, the quantile regression was applied using the quantreg package (Koenker 2016) in R. In this analysis, we estimated the relationships between pairs within groups because of the possible discrepancy in genetic distance values within groups versus between groups.

### Mixed stock analysis

The contributions of nesting populations to foraging grounds of Brunei Bay, Sipadan Island, and TSMP were estimated by Bayesian mixed stock analysis using the BAYES program (Pella and Masuda 2001). MSA for multiple foraging aggregations, known as 'many-to-many' MSA (Bolker et al. 2007), was not performed because of its long computational time. In addition, the validity of the BAYES estimation was in accordance with an estimation by mark–recapture (Jensen et al. 2016a). A total of 31 management units of green turtles in Southeast Asia, the southern Pacific Ocean and the eastern Indian Ocean defined above were used as candidate nesting populations. These populations were geographically classified into five regions: the Southern Pacific, Micronesia and New Guinea, Malay Peninsula and the South China Sea, the Sulu Sea and Celebes Sea, and the Eastern Indian Ocean (Fig. 1, Online Resource 3). Therefore, we ran five Markov Chain Monte Carlo (MCMC) chains, and each chain began with 95% of the mixed sample that was initially provided by each region. The contribution from these regions, in addition to each management unit, was estimated using a regional group option in BAYES. Each chain contained 50,000 samples, and the first 25,000 were discarded as burn-in steps. The convergence of MCMC sampling was assessed using the Gelman–Rubin shrink factor (Gelman and Rubin 1992), which indicates a lack of convergence if the value is greater than 1.2. When the value is greater than 1.2, the number of samples of each chain was increased up to 200,000 with burn-in steps on half of them. MSAs were conducted (1) with an uninformative Dirichlet, assuming the same size of all populations, and (2) with weighting by the population size based on Dutton et al. (2014b), Read et al. (2015) and Jensen et al. (2016a). Haplotype compositions among foraging grounds of Brunei Bay, Sipadan Island, TSMP, and previously investigated Mantanani in the South China Sea (Jensen et al. 2016b) were compared by exact test implemented in Arlequin in the same manner as comparisons among rookeries.

## Results

### Population differentiation and management units

From newly analyzed samples in 11 groups of rookeries, we detected 18 haplotypes (Table 1), 8 and 12 of which were reported by Joseph and Nishizawa (2016) and Jensen et al. (2016a), respectively. Three haplotypes have not been described previously and were registered to GenBank as CmP49.9 (KX057742), CmP227.1 (KX057741) and CmP228.1 (KX057743). Haplotype CmP227.1 was observed in several specimens at Penang and Perak, rookeries on the

western coast of the Malay Peninsula. Haplotype CmP49.5, which has only been reported from a foraging ground (Jensen et al. 2016b), was observed in rookeries of Vietnam and Perhentian Island, Terengganu. Comparison among years detected no significant differences except an exact test on Sipadan samples on 1993 (Jensen et al. 2016a) and 1998 (this study) (Online Resource 1). Pairwise $F_{ST}$ test on this pair did not indicate significant difference, and all haplotypes detected from Sipadan rookery in this study are reported by Jensen et al. (2016a); therefore, samples from each group were aggregated across sampling years and studies (Jensen et al. 2016a; Joseph and Nishizawa 2016).

Exact tests of haplotype compositions indicated that no significant differences between Penang and Perak, but significant differences were found between the other pairs of rookeries (Table 2). No significant difference between Penang and Perak was also supported by pairwise $F_{ST}$ test (Table 2). Therefore, Penang and Perak were treated as one management unit. In contrast to results of exact tests, pairwise $F_{ST}$ tests did not indicate significant differences among several pairs of rookeries other than Penang and Perak (Table 2), but these pairs could be identified by unshared haplotypes (i.e. CmP49.5, CmP75.1, CmP227.1); so, rookeries other than Penang and Perak were treated as different management units. As a result, 11 management units were identified in Southeast Asian rookeries. In combination with previous studies (Dutton et al. 2014b; Read et al. 2015; Jensen et al. 2016a), no significant differences were found between some pairs of Southeast Asia and Indian Ocean or Pacific Ocean rookeries (i.e. Penang–Perak and Cocos Keeling, Mersing and Vanuatu, Mersing and Aru in exact tests, Penang–Perak and Cocos Keeling, Mersing and Vanuatu, Vietnam and Cocos Keeling, Berau and west Java in pairwise $F_{ST}$ tests; Online Resource 4), possibly due to the relatively small sample sizes and dominance of the shared haplotypes of CmP49.1 or CmP91.1. However, because of their geographic separation, these were identified as different management units, composed of 31 management units in total.

## Rookery connectivity

In SAMOVA, changes in the $\Phi_{ST}$, $\Phi_{CT}$ and $\Phi_{SC}$ values in relation to the number of groups were evaluated, and a sharp decrease in $\Phi_{SC}$ and an increase in $\Phi_{CT}$ from $K=2$ to $K=3$ was found (Online Resource 5), indicating that $K=3$ was likely. Community detection of the network analysis based on pairwise $\Phi_{ST}$ resulted in two groups of management units, while the community detection based on pairwise $F_{ST}$ resulted in three groups (Online Resource 6). These groupings indicated the partitions on the PCoA plots and the distinction of Southeast Asia and the Indian Ocean from the Pacific, despite several variations in methodology (Fig. 2). The Ashmore Reef grouped together with the Pacific rookeries in the network analysis, but it served as a connection between the Pacific and Southeast Asia and the Indian Ocean (Fig. 2, Online Resource 6). In the community detection based on $F_{ST}$, one group consisted of various management units connected to each other by relatively weak linkages (Online Resource 6).

The relationship between genetic distance and pairwise geographic distance indicated a discrepancy between genetic distance values of $\Phi_{ST}$, but not $F_{ST}$, within groups and between groups (Fig. 3). Quantile regression estimates ranging from 0.1 to 0.9 indicated that the slope was significantly higher than 0 in $F_{ST}$, but the confidence interval (CI) of the slope was close to 0 at low quantiles in $\Phi_{ST}$ (Online Resource 7).

## Migratory connectivity

From seven foraging grounds, we detected 22 haplotypes (Table 3), 12 of which were reported by Joseph et al. (2016). Two haplotypes had not been described previously and were registered in GenBank as CmP229.1 (KX057744) and CmP230.1 (KX057745).

Whether prior distribution is informative or not, the MSA results indicated the presence of contributions from TIP to all three foraging grounds (Fig. 4). In addition, CIs of the estimation also supported significant contributions from Micronesia to the Sipadan foraging ground (i.e. CI > 0%). Significant contributions to Brunei Bay from the Terengganu mainland and Sarawak were estimated when uninformative prior was assumed, but not when informative prior was assumed. However, contributions from these management units showed relatively high median values. In fact, there were higher contributions from rookeries in the Malay Peninsula and South China Sea and the Sulu Sea and Celebes Sea to Brunei Bay and rookeries in the Sulu Sea and Celebes Sea and Micronesia and New Guinea to Sipadan and the TSMP foraging grounds (Fig. 5). The shrink factor was less than 1.2 in Brunei Bay and TSMP when the numbers of samples in each chain were 200,000 and 100,000, respectively, while the other estimations resulted in shrink factors < 1.2 when the number of samples in each chain was 50,000.

Comparisons in haplotype composition among foraging aggregations showed significant differences between South China Sea and Celebes Sea, but no significant differences within seas (i.e. Brunei Bay and Mantanani, and Sipadan and TIP).

## Comparison between rookery connectivity and migratory connectivity

Sipadan Island has both a foraging ground and rookery. While the migratory connectivity from rookeries of TIP and Micronesia to Sipadan foraging ground was estimated, pairwise $F_{ST}$

**Table 2** $p$ values of exact tests (below diagonal) and pairwise $F_{ST}$ values with $p$ values (above diagonal) that compare 12 rookeries in Southeast Asia

| Rookery | Penang | Perak | Vietnam | Perhentian | Redang | Terengganu | Pahang | Mersing | Sarawak | TIP | Sipadan | Berau | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Penang | – | −0.04052<br>$p=0.99990$ | 0.10468<br>$p=0.05178$ | **0.32613**<br>**$p<0.00001$** | **0.13865**<br>**$p=0.00010$** | **0.18455**<br>**$p=0.00465$** | **0.22304**<br>**$p=0.00158$** | **0.69859**<br>**$p<0.00001$** | **0.50471**<br>**$p<0.00001$** | **0.56809**<br>**$p<0.00001$** | **0.1205**<br>**$p=0.00109$** | **0.28697**<br>**$p<0.00001$** | a |
| Perak | 0.88308 | – | 0.08747<br>$p=0.07217$ | **0.29845**<br>**$p=0.00010$** | **0.12566**<br>**$p=0.00119$** | **0.15581**<br>**$p=0.01129$** | **0.20229**<br>**$p=0.00446$** | **0.67002**<br>**$p=0.00010$** | **0.49278**<br>**$p<0.00001$** | **0.5584**<br>**$p<0.00001$** | **0.10958**<br>**$p=0.00307$** | **0.26266**<br>**$p=0.00010$** | a |
| Vietnam | **0.02066** | **0.01534** | – | **0.20574**<br>**$p=0.00208$** | **0.10402**<br>**$p=0.00594$** | 0.10851<br>$p=0.06366$ | **0.17692**<br>**$p=0.01277$** | **0.57526**<br>**$p=0.00020$** | **0.45773**<br>**$p<0.00001$** | **0.52076**<br>**$p<0.00001$** | **0.09335**<br>**$p=0.01436$** | **0.20523**<br>**$p=0.00089$** | a |
| Perhentian | **<0.00001** | **<0.00001** | **0.00204** | – | **0.03911**<br>**$p=0.03792$** | 0.04965<br>$p=0.12989$ | **0.21585**<br>**$p=0.00020$** | **0.37868**<br>**$p<0.00001$** | **0.36328**<br>**$p<0.00001$** | **0.42124**<br>**$p<0.00001$** | **0.15376**<br>**$p<0.00001$** | **0.15985**<br>**$p<0.00001$** | a |
| Redang | **<0.00001** | **<0.00001** | **0.00008** | **0.00696** | – | 0.01304<br>$p=0.24908$ | **0.12739**<br>**$p<0.00001$** | **0.32749**<br>**$p<0.00001$** | **0.26733**<br>**$p<0.00001$** | **0.39100**<br>**$p<0.00001$** | **0.07449**<br>**$p<0.00001$** | **0.12495**<br>**$p<0.00001$** | a, b |
| Terengganu | **0.00534** | **0.00546** | **0.00818** | **0.04140** | **0.01418** | – | **0.13005**<br>**$p=0.03614$** | **0.42565**<br>**$p=0.00040$** | **0.38539**<br>**$p=0.00010$** | **0.44722**<br>**$p<0.00001$** | 0.06230<br>$p=0.04722$ | **0.12367**<br>**$p=0.01148$** | a |
| Pahang | **0.00016** | **0.00118** | **0.00120** | **<0.00001** | **<0.00001** | **0.00044** | – | **0.49792**<br>**$p=0.00010$** | **0.43964**<br>**$p<0.00001$** | **0.49236**<br>**$p<0.00001$** | **0.13163**<br>**$p<0.00001$** | **0.20863**<br>**$p<0.00001$** | a |
| Mersing | **<0.00001** | **0.00002** | **0.00024** | **<0.00001** | **<0.00001** | **0.00048** | **0.00004** | – | **0.55790**<br>**$p<0.00001$** | **0.56021**<br>**$p<0.00001$** | **0.36234**<br>**$p<0.00001$** | **0.31732**<br>**$p=0.00030$** | a |
| Sarawak | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | – | **0.52174**<br>**$p<0.00001$** | **0.35503**<br>**$p<0.00001$** | **0.38519**<br>**$p<0.00001$** | a, b, c |
| TIP | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | **<0.00001** | – | **0.30555**<br>**$p<0.00001$** | **0.38353**<br>**$p<0.00001$** | a, c |
| Sipadan | **0.00340** | **0.00156** | **0.00222** | **<0.00001** | **<0.00001** | **0.00036** | **<0.00001** | **0.00022** | **<0.00001** | **<0.00001** | – | **0.07636**<br>**$p=0.00267$** | a, c |
| Berau | **<0.00001** | **<0.00001** | **0.00030** | **<0.00001** | **<0.00001** | **0.00018** | **<0.00001** | **0.00192** | **<0.00001** | **<0.00001** | **<0.00001** | – | c |

Significant differences after false discovery rate adjustment by Benjamini and Hochberg (1995) in which threshold vales are 0.04924 (below diagonal) and 0.04470 (above diagonal) are indicated in bold

[a]This study

[b]Joseph and Nishizawa (2016)

[c]Jensen et al. (2016a)

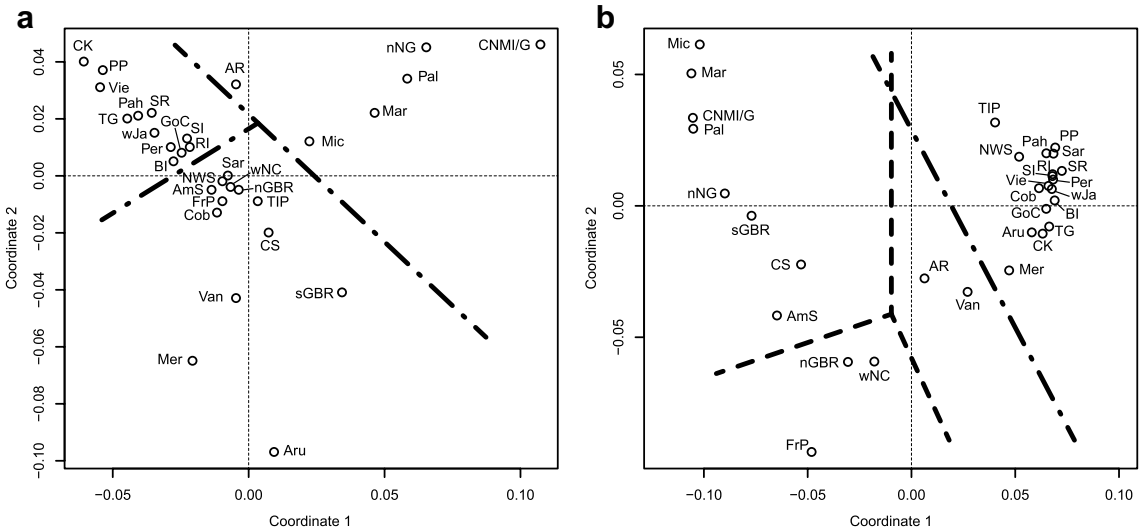


**Fig. 2** Principal coordinate plot based on pairwise values of **a** $F_{ST}$ and **b** $\Phi_{ST}$. Chained lines indicate partitions based on community detection in network analysis, while dashed lines indicate partitions based on SAMOVA. Abbreviations are same as in Fig. 1

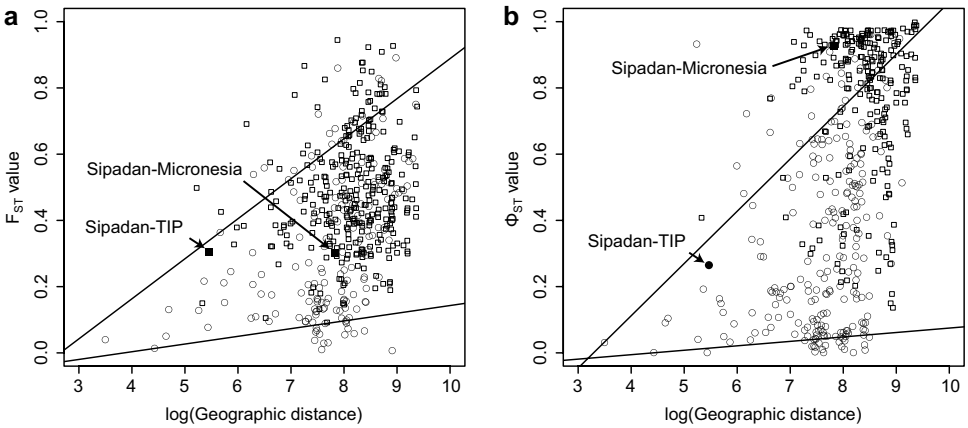


**Fig. 3** Relationship between geographic distance and genetic distance of **a** $F_{ST}$ and **b** $\Phi_{ST}$. Values within groups are represented by circles, while values inter-groups are represented by squares. Relationships between regions where contemporary movement is estimated are indicated by filled marks. Solid lines indicate 0.1 and 0.9 quantile regressions for relationships within groups

between rookeries of TIP and Sipadan or between Micronesia and Sipadan fell within a range of quantile regression estimates from 0.1 to 0.9 (Fig. 3). Pairwise $\Phi_{ST}$ between TIP and Sipadan also fell within this range, and the pairwise $\Phi_{ST}$ between Micronesia and Sipadan showed higher values outside of this range (Fig. 3). If the contemporary movement from rookeries of TIP and Micronesia results in colonization at Sipadan, low genetic distance from Sipadan rookery to rookeries of TIP and Micronesia is predicted. However, this hypothesis was not supported.

## Discussion

### Rookery connectivity

By comprehensive sampling of rookeries in Southeast Asia, particularly in Malaysia, we identified management units in the Malay Peninsula. Haplotype CmP49.1 was commonly observed in all management units in Southeast Asia. However, to the best of our knowledge, the

**Table 3** Haplotype composition of seven sites of foraging grounds in Southeast Asia

| Foraging ground | South China Sea | | | | | Celebes Sea | |
|---|---|---|---|---|---|---|---|
| | Melaka | Redang | Pulau Tiga, Sabah | Sarawak | Brunei Bay | Sipadan | TSMP |
| Year | 2010, 2011 | 2009, 2010 | 2010 | 2010, 2014 | 2011–2014, 2016 | 2009, 2010 | 2009 |
| CmP49.1 (AB819808) | 1 | 2 | 0 | 1 | 8 (7) | 2 | 7 |
| CmP49.3 (KJ502572) | 0 | 1 | 0 | 0 | 2 (2) | 5 | 3 |
| CmP49.7 (KJ502573) | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| CmP57.1 (KJ502588) | 0 | 0 | 1 | 0 | 14 (14) | 10 | 20 |
| CmP57.2 (KJ502567) | 0 | 0 | 0 | 0 | 2 (1) | 2 | 6 |
| CmP82.1 (KJ502584) | 1 | 1 | 0 | 0 | 1 (1) | 0 | 0 |
| CmP87.1 (KJ502589) | 0 | 1 | 0 | 0 | 8 (6) | 0 | 0 |
| CmP91.1 (KF311762) | 0 | 0 | 0 | 0 | 2 (2) | 1 | 2 |
| CmP104.1 (KJ502569) | 0 | 0 | 0 | 0 | 3 (2) | 0 | 0 |
| CmP20.1 (AB819806) | 0 | 0 | 0 | 0 | 3 (3) | 8 | 9 |
| CmP40.1 (KF311750) | 0 | 0 | 0 | 0 | 2 (2) | 1 | 2 |
| CmP75.1 (KJ502574) | 0 | 0 | 0 | 0 | 1 (1) | 0 | 0 |
| CmP19.1 (KM986629) | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| CmP20.2 (KF311744) | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| CmP32.1 (KF311749) | 0 | 0 | 0 | 0 | 1 (0) | 2 | 0 |
| CmP61.1 (KF311755) | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| CmP77.1 (KF311759) | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| CmP89.1 (KJ502590) | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| CmP154.1 (KM923922) | 0 | 0 | 0 | 0 | 1 (1) | 0 | 0 |
| CmP221.1 (KM262220) | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| CmP229.1 (KX057744) | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| CmP230.1 (KX057745) | 0 | 0 | 0 | 0 | 1 (0) | 0 | 0 |
| Total | 2 | 6 | 1 | 3 | 49 (42) | 36 | 52 |

Data from Brunei Bay include those reported in Joseph et al. (2016) and are shown in parentheses

population genetic composition on the western coast of the Malay Peninsula (i.e. Penang and Perak) was identified for the first time, and its differences from those of other rookeries in Southeast Asia indicated that the western coast of the Malay Peninsula formed a management unit. In addition, significant differences were observed between geographically close rookeries, such as Redang Island and Perhentian (geographic distance 33 km), Redang Island and the Terengganu mainland (82 km), and Redang Island and Pahang (190 km). Difference between Redang Island and the Terengganu mainland might not be rigorous because it was not supported by pairwise $F_{ST}$ tests. However, no specimens collected from Redang Island had haplotypes CmP49.5, CmP75.1, or CmP49.7, which were observed in Perhentian, the Terengganu mainland and Pahang, respectively, despite high sampling efforts in Redang Island. These results indicated precise natal philopatry of green turtles in this region, as indicated previously in other regions (Cheng et al. 2008; Nishizawa et al. 2011).

Both genetic variance and linkages among rookeries indicated the genetic barrier between the Torres Strait and Celebes Sea (i.e. Philippines–Sulawesi). The results suggested that the land bridge at the Torres Strait during the Pleistocene restricted gene flow, as previously indicated by Dethmers et al. (2006). On the other hand, separation at Philippines–Sulawesi, supported by the grouping of Sipadan and Berau with other Southeast Asian rookeries, is in contrast with the east–west genetic separation of marine animals in this region in Borneo–Philippines reported previously (Lourie and Vincent 2004). While separation at Borneo–Philippines can be attributed to the land bridge (Lourie and Vincent 2004), isolation of the Celebes Sea from the Pacific Ocean during the Pleistocene is not plausible (Voris 2000; Kuhnt et al. 2004); therefore, the distinction at Philippines–Sulawesi indicated that the restricted gene flow was caused by factors other than the land barrier. One possible factor is the effect of geographic distance (i.e. isolation by distance). A significantly positive slope in the relationship between $F_{ST}$ and geographic distance supported isolation

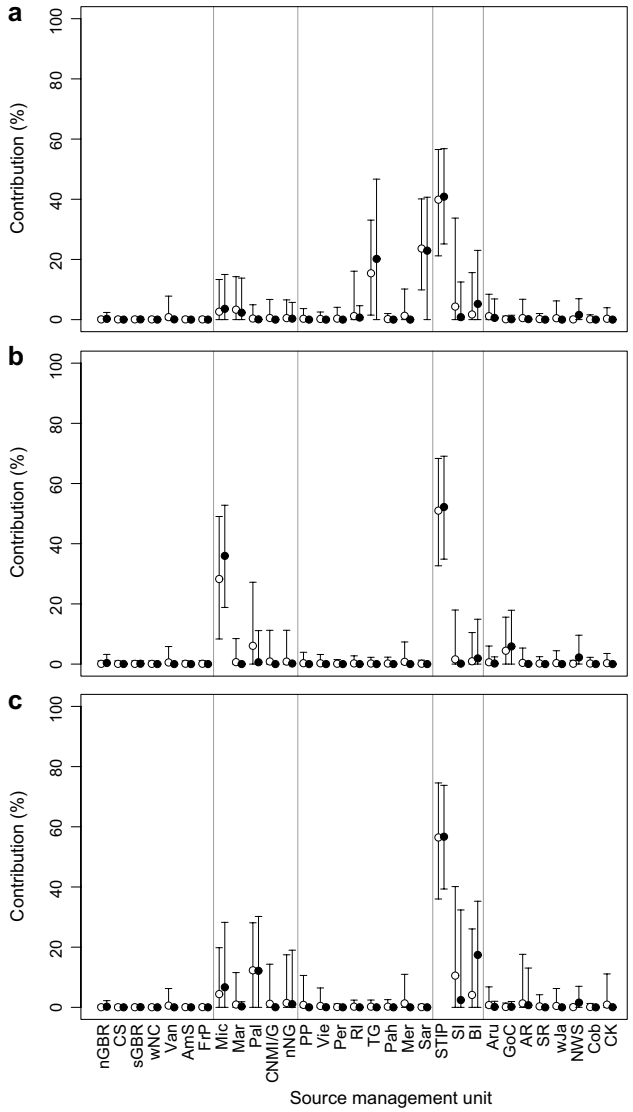


**Fig. 4** Estimated contributions from management units (MUs) to foraging grounds at **a** Brunei Bay, **b** Sipadan Island, and **c** TSMP. Mean values based on uninformative priors (hollow circles) and informative priors (filled circles) are illustrated with 95% confidence intervals. Abbreviations of MUs are same as in Fig. 1

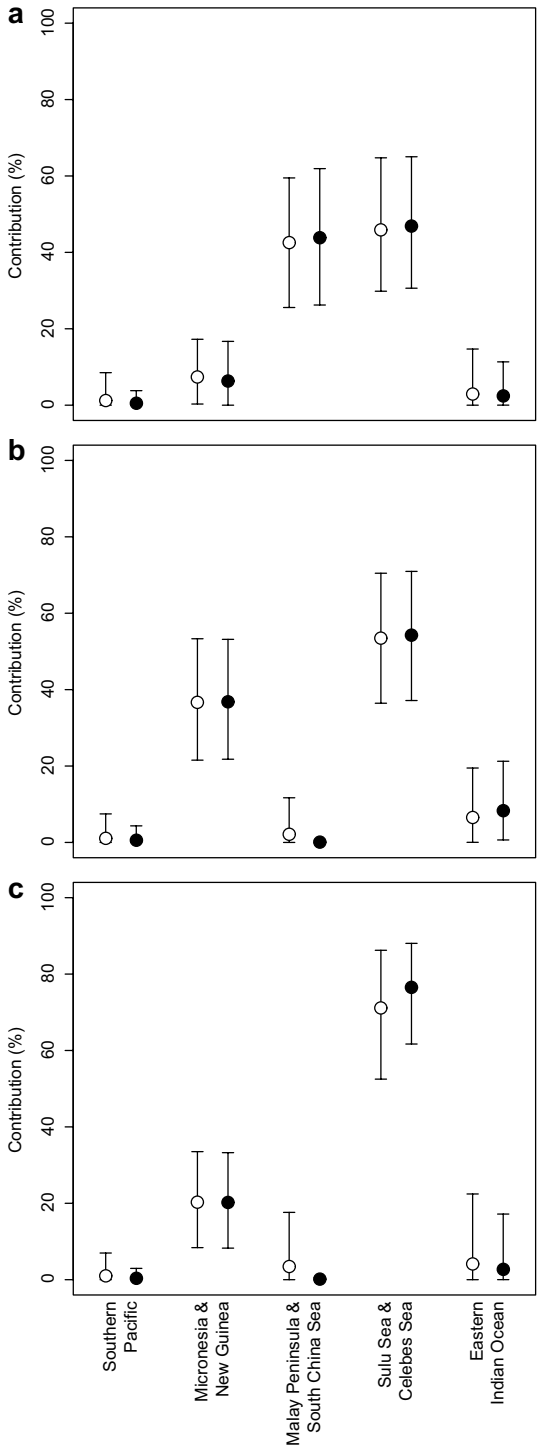


**Fig. 5** Estimated contributions from regional groups to foraging grounds at **a** Brunei Bay, **b** Sipadan Island, and **c** TSMP. Results based on uninformative priors (hollow circles) and informative priors (filled circles) are shown in the same manner as in Fig. 4

by distance. The relationship between $F_{ST}$ and the geographic distance between groups was not outside of those estimated from pairs within groups. Therefore, $F_{ST}$ indicated that the genetic separation could be attributed to the effect of geographic distance. On the other hand, the relationship between $\Phi_{ST}$ and geographic distance is not in accordance with that of $F_{ST}$. The $\Phi_{ST}$ between groups tended to be higher, whereas the $\Phi_{ST}$ within groups tended to be lower. The discrepancy between pairwise $\Phi_{ST}$ and $F_{ST}$ has been found in marine animals including sea turtles (Shamblin et al. 2012; Ashe et al. 2015). While $\Phi_{ST}$ reflects the lineages of haplotypes, $F_{ST}$ is based on the frequency of haplotypes. The differences between lineages of haplotypes among rookeries, likely due to a genetic barrier at the Torres Strait, supported high $\Phi_{ST}$ between groups, although the coexistence

of haplotypes from different lineages at one rookery creates a low $\Phi_{ST}$ (Shamblin et al. 2012).

## Migratory connectivity

Despite the genetic barrier among rookeries at Philippines–Sulawesi, the contemporary movement across this barrier was detected because of the contribution of remote rookeries in Micronesia–Polynesia to the aggregations in the Celebes Sea. The inflow of the North Equatorial Current from the Pacific to the Celebes Sea (Masumoto et al. 2001, Gordon 2005) explains the contribution from Micronesia–Polynesia rookeries to the Celebes Sea, because oceanic currents influence the composition of juvenile feeding aggregations (e.g. Bass et al. 2006; Blumenthal et al. 2009; Monzón-Argüello et al. 2010). The movement from the Pacific to the Celebes Sea is also supported by mark–recapture and satellite telemetry data showing that a green turtle was recaptured in or tracked to the Celebes Sea after tagging at rookeries in Palau (Klain et al. 2007) and Micronesia (Kolinski et al. 2014). Movement of green turtles from Micronesia to foraging grounds in southwestern Japan has previously been reported by MSA (Nishizawa et al. 2013) and mark–recapture and satellite tracking (Kolinski et al. 2014), but this study confirmed that migratory routes of green turtles from one rookery are various.

In addition, movement from the Sulu Sea rookery to the South China Sea and the Celebes Sea foraging aggregations was predicted, while weak genetic linkages between the TIP rookery and the other rookeries were suggested by the $F_{ST}$. This is in contrast to the Lagrangian drifter buoys (Nishizawa et al. 2016, discussed based on data available at http://www.aoml.noaa.gov/envids/) and a simulation (Kool et al. 2011) that indicated the presence of inflow from the South China Sea to the Sulu Sea. Contemporary movement across this potential restriction can be achieved by active swimming of sea turtles (Okuyama et al. 2011; Putman and Mansfield 2015). Alternatively, the outflow of surface water from the Sulu Sea to the Celebes Sea and the South China Sea may be possible during some seasons (Chen et al. 2004, 2006) and may promote the movement of green turtles from the Sulu Sea rookery to both the Celebes Sea and South China Sea.

The rookery along the South China Sea, Sarawak and Malay Peninsula also contributes to the South China Sea aggregation. These results confirmed the insights of Joseph et al. (2016) and Jensen et al. (2016b). Additional sampling from rookeries in the Malay Peninsula in this study identified haplotypes CmP75.1 and CmP49.5. These haplotypes were reported previously in the South China Sea foraging aggregations (Joseph et al. 2016; Jensen et al. 2016b) but were not found in nesting rookeries. Detection of these haplotypes from rookeries in the Malay Peninsula confirmed the contemporary links to the South China Sea aggregation. Furthermore, CmP75.1 is specific to the Terengganu mainland, and CmP49.5 is specific to Vietnam and Perhentian; therefore, the results indicate a possible link between these rookeries and the South China Sea aggregation. On the other hand, restriction on the movement from the South China Sea to the Celebes Sea was suggested by both the result of MSA and difference in haplotype compositions among foraging aggregations. There may be regionally limited contributions to foraging grounds of green turtles from rookeries in the South China Sea, in contrast with contributions to various foraging grounds from Micronesia.

Despite a small sample size, the green turtles foraging at Melaka, Redang Island, and Pulau Tiga had haplotypes that were also observed in Brunei Bay. In addition, two individuals foraging at Sarawak had haplotype CmP49.7, which was observed in the Pahang rookery. The results confirmed the movement of turtles from the Malay Peninsula and Sulu Sea to the South China Sea. One exception is haplotype CmP89.1, which was observed at Redang Island. Haplotype CmP89.1 was not observed in Southeast Asian rookeries but was observed in the Gulf of Carpentaria in Australia (Jensen et al. 2016a). However, it is still unclear whether the movement from the Gulf of Carpentaria to the South China Sea is common, because CmP83.1 (Jensen et al. 2016a), a dominant haplotype at the Gulf of Carpentaria rookery, was not observed.

## Integration and conclusion

This study observed contemporary movement across a genetic barrier at Philippines–Sulawesi, particularly from Micronesian rookery to Sipadan foraging ground, but the genetic distance between rookeries in Micronesia and Sipadan was not decreased. In addition, the genetic distance between rookeries in TIP and Sipadan is not small with respect to the geographic distance. The results do not support the effect of contemporary movement on rookery colonization. This could be attributed to the strong effect of philopatry on natal regions for nesting (e.g. Dethmers et al. 2006; Cheng et al. 2008; Nishizawa et al. 2011), suggesting that rookery colonization has been formed mainly by error in natal philopatry after they go back to their natal regions, leading to isolation by distance. Alternatively, this pattern of contemporary movement may have occurred recently. However, simulation supported a current flow at least from Micronesia to the Celebes Sea during the Last Glacial Maximum in the Pleistocene (Kuhnt et al. 2004), indicating that the pattern of contemporary movement may be conservative.

On the other hand, Ashmore Reef may be a genetic intermediate location of the Southeast Asia–Indian Ocean group and Pacific group. Genetic closeness of the Ashmore Reef to the Pacific rookeries was suggested because the rookery contained haplotypes within a lineage observed

mainly in the Southeast Asia–Indian Ocean group as well as in the Pacific group (Dethmers et al. 2006). The Ashmore Reef shares haplotype CmP20.1 with Pacific rookeries, and haplotype CmP83.1 with Indian Ocean rookeries (Jensen et al. 2016a). The genetic closeness of the Pacific and Ashmore Reef rookeries, compared with the geographically closer Aru, Cobourg Peninsula and Gulf of Carpentaria, possibly indicates the effect of contemporary movement. Despite limited resolution due to the use of short sequences, Dethmers et al. (2010) indicated a possible contribution of Papua New Guinea to the Ashmore Reef foraging ground. Current flow from the Pacific to the Ashmore Reef is plausible even during the Last Glacial Maximum in the Pleistocene (Kuhnt et al. 2004).

In conclusion, this study confirmed the importance of comparing rookery connectivity and migratory connectivity to understand the effect of contemporary movement on colonization of rookeries. Comparisons of the genetic relationships among and between rookeries and foraging grounds revealed contemporary movement of green turtles across a historical genetic barrier, but migratory connectivity of green turtles does not result in lower genetic distances of corresponding rookeries than those predicted by geographic distance. This indicates that rookery connectivity does not reflect migratory connectivity of migratory marine animals, at least in green turtles in some regions of Southeast Asia, probably because of the effect of natal philopatry. The discrepancy has implications for conservation of this endangered marine animal. Conservation at nesting rookeries is important because contemporary movement does not likely result in colonization at new preferable sites. As has been proposed previously (e.g. Dutton et al. 2014b; Jensen et al. 2016b), mixture at foraging sites is a potential risk factor because of heavy exploitation and habitat destruction at foraging grounds, and it may have negative effects on remote rookeries.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Sampling permits for this research were: Sabah Parks (TS/PTD/5/4 Jld. 35[45] and TS/PTD/5/4 Jld. 49[54]), Sabah Wildlife Department (JHL.600-6/1/2 Jld.6), Malaysian National Security Council (MKN [R] 269/2 Jld.26 [37]), Sarawak (NCCD.907.4.4 [Jld. 9]—67, NCCD.907.4.4 [Jld.10]—181 and Park Permit 142/2014) and Peninsular Malaysia (JTLM 620-2/1/1[3] and Prk.ML.34/18 Jld 26[30]).

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